

ORIGINAL PAPER

HER2-LOW BREAST CANCER: A NEW DIAGNOSTIC CHALLENGE FOR PATHOLOGISTS – INSIGHTS FROM A SINGLE-CENTER RETROSPECTIVE COHORT

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The introduction of antibody-drug conjugates, particularly trastuzumab deruxtecan, has fundamentally changed the clinical significance of low HER2 expression in breast cancer. Consequently, accurate discrimination between human epidermal growth factor receptor 2 (HER2) immunohistochemistry (IHC) score 0 and HER2-low (IHC 1+ or IHC 2+/ISH-negative) has become increasingly important in routine pathological practice.

This retrospective single-center observational study included consecutive patients diagnosed with invasive breast carcinoma between January 2023 and December 2024. Histopathological and immunohistochemical slides were re-evaluated according to current American Society of Clinical Oncology/College of American Pathologists recommendations. Cases were reclassified into HER2 0, HER2-low, and HER2-positive categories. Clinicopathological variables, including patient age, histological subtype, hormone receptor status, Ki-67 proliferation index, histological grade, and biological subtype, were analyzed using descriptive statistics.

Seventy-eight patients were analysed. Following reassessment, HER2-low tumors accounted for 33.3% (26/78) of all invasive breast cancers. Fifteen tumors initially classified as HER2 0 were reclassified as HER2-low. All HER2-low tumors were invasive carcinoma of no special type. Luminal B represented the predominant molecular subtype (65.4%), followed by triple-negative breast cancer (30.8%) and luminal A tumors (3.8%). Estrogen receptor expression was observed in 69.2% of HER2-low tumors, while progesterone receptor expression was detected in 61.5%.

Reclassification of HER2 expression substantially increased the number of patients classified as HER2-low, emphasizing the importance of precise immunohistochemical assessment, particularly in distinguishing IHC score 0 from IHC score 1+. These findings highlight the growing clinical relevance of standardized HER2 assessment in identifying patients who may become eligible for HER2-targeted antibody-drug conjugate therapy.

Key words: breast cancer, immunohistochemistry, pathology, core needle biopsy, trastuzumab deruxtecan, predictive biomarkers, HER2-low, HER2 reclassification.

Introduction

Invasive breast carcinoma is the most frequently diagnosed malignancy among women worldwide. According to data from the Polish National Cancer Registry, 21,554 new cases of breast cancer were diagnosed in Poland in 2022, accounting for 23.6% of all cancers in women, while 6,611 women died from the disease during the same year [1]. Despite substantial advances in diagnostic techniques and systemic treatment, breast cancer remains one of the leading causes of cancer-related mortality among women, and epidemiological projections indicate a continued increase in disease incidence [2, 3].

The diagnosis of breast cancer is primarily established using core needle biopsy, which provides adequate tissue for histopathological diagnosis and assessment of prognostic and predictive biomarkers using immunohistochemical and molecular methods. Biomarker evaluation is essential for selecting patients for targeted therapies, as it predicts both disease behavior and treatment response. Appropriate tissue fixation in accordance with the recommendations of the Polish Society of Pathologists, together with accurate interpretation of immunohistochemical findings, is fundamental for optimal therapeutic decision-making in patients with breast cancer [4]. Routine pathological evaluation includes assessment of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and the Ki-67 proliferation index [5]. In equivocal HER2 immunohistochemical cases, assessment of *HER2* gene amplification using fluorescence *in situ* hybridization (ISH/FISH) is recommended.

For more than two decades, anti-HER2 therapy with trastuzumab has been the standard treatment for patients with HER2-positive breast cancer. Eligibility for this treatment has traditionally been based on HER2 protein overexpression and/or *HER2* gene amplification. Tumors demonstrating strong HER2 protein expression (immunohistochemistry [IHC] 3+) or *HER2* gene amplification were classified as HER2-positive, whereas tumors with IHC scores of 0, 1+, or 2+ without gene amplification were collectively regarded as HER2-negative and therefore ineligible for HER2-targeted therapy. Recommendations for HER2 testing have been periodically updated by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) in 2007, 2013, 2018, and most recently in 2023, providing the current international standard for HER2 assessment in breast cancer [6–11].

A major paradigm shift occurred following the development of antibody-drug conjugates (ADCs), particularly trastuzumab deruxtecan (T-DXd, Enhertu), which demonstrated clinically meaningful efficacy in patients with breast cancers lacking HER2 overex-

pression or gene amplification [12–14]. Consequently, tumors exhibiting low HER2 expression – defined as IHC 1+ or IHC 2+ without *HER2* gene amplification – have emerged as a clinically relevant subgroup.

Importantly, HER2-low is not considered a distinct biological subtype of breast cancer nor an independent diagnostic category according to current ASCO/CAP recommendations. Rather, it represents a clinically relevant therapeutic category defined by low HER2 protein expression, which may identify patients eligible for treatment with HER2-directed ADCs [14–19]. These developments have highlighted the importance of accurate assessment of predictive biomarkers, particularly in distinguishing HER2 IHC score 0 from HER2-low tumors.

The introduction of effective HER2-directed therapies for patients with low HER2 expression has created a need to determine the prevalence of HER2-low breast cancer and to characterize its clinicopathological features in different patient populations. Establishing the frequency of HER2-low tumors in routine clinical practice is essential for estimating the number of patients who may potentially benefit from novel targeted therapies and for evaluating the clinicopathological characteristics associated with this therapeutic category [15].

The primary objective of this retrospective, single-center observational study was to reassess HER2 expression in core needle biopsy specimens from patients with invasive breast carcinoma and to determine the prevalence of HER2-low tumors according to current diagnostic criteria.

The secondary objectives were:

1. To determine the prevalence of HER2-low breast cancer in the study cohort;
2. To evaluate the proportion of tumors reclassified from HER2 0 to HER2-low following reassessment of HER2 immunohistochemical expression according to current recommendations;
3. To characterize the clinicopathological features of HER2-low breast cancer, including histological subtype, ER and PR expression, Ki-67 proliferation index, histological grade, and molecular subtype.

The study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [20].

Material and methods

Research material

The study material consisted of core needle biopsy specimens in which breast cancer had been diagnosed between 1 January 2023 and 31 December 2024 at the Pathomorphology Center, National Medical Institute of the Ministry of the Interior and Administration (PIM MSWiA), Warsaw, Poland. As part of the pres-

Table I. Inclusion and exclusion criteria used in the study

INCLUSION CRITERIA	EXCLUSION CRITERIA
Histopathologically confirmed invasive breast carcinoma	Missing FISH result in HER2 IHC 2+ cases
Available histopathological and immunohistochemical slides	Incomplete diagnostic documentation
Adequate material for HER2 assessment	Material unsuitable for HER2 evaluation

FISH – fluorescence in situ hybridization, IHC – immunohistochemistry

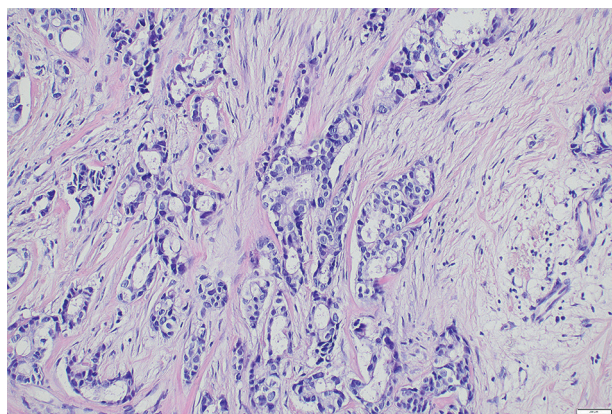


Figure 1. Invasive breast carcinoma of no special type (NST; HE, 10×)

ent study, all specimens underwent repeat microscopic evaluation. Clinical and pathological data were obtained from histopathological records. Histopathological documentation, together with hematoxylin and eosin (HE)-stained slides and HER2 immunohistochemically stained slides, was made available under a collaboration agreement between the National Medical Institute of the Ministry of the Interior and Administration and Lazarski University.

Initially, 80 consecutive patients diagnosed with invasive breast carcinoma were enrolled in the study. The patient eligibility criteria are presented in Table I.

The inclusion criteria were: histopathologically confirmed invasive breast carcinoma, availability of histopathological and immunohistochemical slides enabling assessment of HER2 expression, and complete diagnostic documentation.

Two cases with an HER2 IHC score of 2+ were excluded because the *HER2* gene amplification results, assessed by FISH at an external institution, were unavailable. Consequently, the final analysis included 78 patients.

Histopathological material

The histopathological material re-evaluated in the present study consisted of 16-gauge core needle biopsy specimens obtained from clinically suspicious breast lesions. As part of the routine diagnostic workflow at the Department of Pathomorphology, PIM MSWiA, the tissue specimens had been fixed in 10% neutral buffered formalin (pH 7.2–7.4) for 24–48 hours immediately after collection and subse-

quently processed using standard histopathological procedures.

In each case, HE-stained sections and immunohistochemical studies were performed to assess predictive biomarkers. Histopathological diagnosis was established according to the current World Health Organization (WHO) Classification of Breast Tumours, and WHO classification of tumours of the breast 6th edition 2026. Histological grade was determined using the Nottingham grading system (Elston-Ellis/Bloom-Richardson) [16]. Figure 1 presents invasive breast carcinoma of no special type (NST) ductal.

In cases requiring differentiation between invasive carcinoma of NST ductal and invasive lobular carcinoma, additional immunohistochemical staining using an anti-E-cadherin antibody was performed.

Furthermore, immunohistochemical expression of the predictive biomarkers ER, PR, HER2, and Ki-67 was assessed, and breast cancers were classified into the following biological subtypes: luminal A (ER+/PR+, HER2–), luminal B (ER+/PR+, HER2 0 or low, HER2-positive (ER–/PR–, HER2+), and triple-negative breast cancer (TNBC; ER–/PR–/HER2–).

Immunohistochemical method

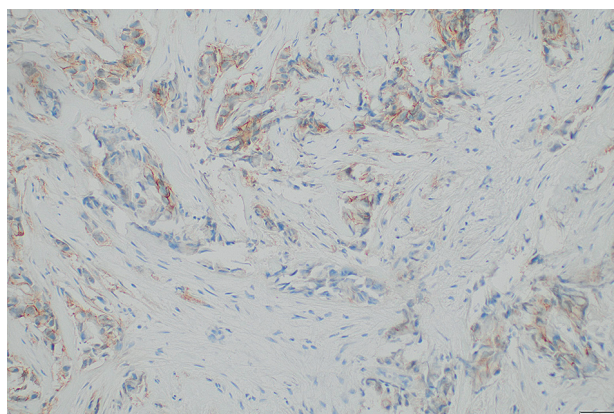
Immunohistochemical staining was performed using antibodies against ER, PR, Ki-67/MIB-1, and HER2. Antibody clone 4B5 (Roche/Ventana) has become a widely accepted global diagnostic standard (CDx, companion diagnostics). This is a rabbit monoclonal antibody directed against a specific intracellular domain of the 185 kDa c-erbB-2 oncoprotein. The 4B5 clone antibody is characterized by high affinity and epitope specificity, which minimizes the risk of non-specific cross-reactions. Technical standardization plays a key role in the diagnostic process, involving the analytical validation of clone 4B5 through its close integration with closed systems, such as the BenchMark capillary platforms (Roche/Ventana). Full process automation enables the reduction of manual errors. The immunohistochemistry immunohistochemistry test result is interpreted by a qualified pathologist using a semi-quantitative scale (0 to 3+), in accordance with the CAP recommendations for breast cancer biomarker assessment, as presented in Table II [17].

HER2 reclassification was performed by the study team after establishing uniform interpretation criteria. Following re-evaluation of each invasive breast carci-

Table II. Assessment of HER2 expression by immunohistochemistry according to the College of American Pathologists recommendations [17]

IHC SCORE	ASCO/CAP INTERPRETATION	ASSESSMENT CRITERIA
0	HER2-negative	No membranous staining or incomplete, faint/barely perceptible membranous staining in $\leq 10\%$ of tumour cells
1+	HER2-negative	Incomplete faint membranous staining in $> 10\%$ of tumour cells
2+	Equivocal (requires FISH)	Weak to moderate complete membranous staining in $> 10\%$ of tumor cells or intense complete membranous staining in $\leq 10\%$ of tumour cells
3+	HER2-positive	Intense, complete membranous staining in $> 10\%$ of tumour cells

ASCO – American Society of Clinical Oncology, CAP – College of American Pathologists, FISH – fluorescence in situ hybridization, IHC – immunohistochemistry

**Figure 2.** Immunohistochemistry HER2 1+/low (10^x)

noma specimen, HER2 protein expression was reassessed immunohistochemically. The entire biopsy section was examined to identify areas of invasive carcinoma, and HER2 expression was assessed with respect to its localization and staining intensity.

Cases showing incomplete weak membranous staining in more than 10% of tumour cells were classified as HER2 1+/low. Figure 2 presents immunohistochemistry HER2 1+/low. Cases with no membranous staining or staining present in less than 10% of tumour cells were classified as HER2 0. Cases demonstrating strong, circumferential membranous staining in more than 10% of tumour cells were classified as HER2 3+.

Cases with diagnostic uncertainty were reviewed jointly during consensus meetings, and the final HER2 classification was established by consensus, with participation from the supervising pathologists.

The HER2 status categories used in the present analysis are presented in Table III, which illustrates the three categories with clinically predictive value [9, 12, 17].

Statistical analysis

The following variables were analysed: patient age, histological tumour type, histological grade, ER and PR expression, Ki-67 proliferation index, HER2 status, and clinicopathological subtype of breast cancer.

Table III. HER2 status categories applied in the study

CATEGORY	DEFINITION
HER2 0	IHC score 0
HER2 1+/low	IHC score 1+ or IHC score 2+ without HER2 gene amplification by FISH
HER2-positive	IHC score 3+ or IHC score 2+ with HER2 gene amplification by FISH

FISH – fluorescence in situ hybridization, IHC – immunohistochemistry

The results are presented using descriptive statistics as absolute numbers and percentages. Data were compiled and analysed using Microsoft Excel.

Results

Initially, 80 consecutive women diagnosed with invasive breast carcinoma were enrolled in the study. Two patients were excluded from the analysis because FISH results were unavailable for cases with an HER2 IHC score of 2+. Consequently, the final analysis included 78 patients.

The patients ranged in age 34–94 years, with a median age of 58 years. They were divided into three age groups: 20–40 years, 41–60 years, and over 60 years.

The predominant histological type was invasive carcinoma of no special type, NST (ductal), diagnosed in 66 patients (82.5%). This tumour type was identified in 31 patients aged 41–60 years, 30 patients older than 60 years, and 5 patients aged 20–40 years. Invasive lobular carcinoma was diagnosed in 12 patients (15.0%), including 4 women aged 41–60 years and 8 women older than 60 years. Tubular carcinoma and mucinous carcinoma were each identified in a single case, occurring in the 20–40-year and > 60 -year age groups, respectively. No cases of breast cancer were diagnosed in women younger than 20 years.

The distribution of histological tumour types according to age group among all 80 patients initially enrolled, prior to the exclusion of two cases without available FISH results, is presented in Table IV.

In the initial immunohistochemical assessment, 63 patients (80.8%) were classified as HER2-negative

Table IV. Histological types of breast cancer according to age group

HISTOLOGICAL TYPE	20–40 YEARS	41–60 YEARS	> 60 YEARS	TOTAL, N (%)
Invasive carcinoma of NST	5	31	30	66 (82.5)
Invasive lobular carcinoma, NOS	0	4	8	12 (15.0)
Tubular carcinoma	1	0	0	1 (1.25)
Mucinous carcinoma	0	0	1	1 (1.25)
Total	6	35	39	80 (100)

NOS – not otherwise specified, NST – no special type

Table V. Distribution of HER2 expression before and after reclassification

HER2 IHC STATUS	BEFORE RECLASSIFICATION, N (%)	AFTER RECLASSIFICATION, N (%)
HER2 0	52 (66.7)	37 (47.4)
HER2 1+/low	11 (14.1)	26 (33.3)
HER2 3+	15 (19.2)	15 (19.3)
Total	78 (100)	78 (100)

IHC – immunohistochemistry

Table VI. Clinicopathological characteristics of patients with HER2 1+/low breast cancer

CLINICOPATHOLOGICAL CHARACTERISTIC	HER2-LOW CASES (N)	ER+	PR+	KI-67 < 50%	KI-67 ≥ 50%	G2	G3	20–40 YEARS	41–60 YEARS	> 60 YEARS
Histological type										
NST	26	18	16	12	14	15	11	1	12	13
Lobular carcinoma, NOS	0	0	0	0	0	0	0	0	0	0
Tubular carcinoma	0	0	0	0	0	0	0	0	0	0
Mucinous carcinoma	0	0	0	0	0	0	0	0	0	0
Molecular subtype										
Luminal A	1	1	1	1	0	1	0	0	1	0
Luminal B	17	17	15	9	8	10	7	0	8	9
Triple-negative	8	0	0	0	8	3	5	1	5	2
Non-luminal	0	0	0	0	0	0	0	0	0	0

NOS – not otherwise specified, NST – no special type

(HER2 0 and HER2 1+), whereas 15 patients (19.2%) demonstrated HER2 overexpression (HER2 3+).

Following HER2 reclassification incorporating the HER2 1+/low category, 37 patients (47.4%) were classified as HER2 0 (Figure 1), and 26 patients (33.3%) as HER2 1+/low (Figure 2). Strong HER2 overexpression (HER2 3+) was confirmed in the remaining 15 patients (19.3%). Cases with HER2-ultralow expression were included in the HER2 0 category.

The distribution of HER2 status before and after reclassification is presented in Table V.

All 26 patients with HER2 1+/low expression were diagnosed with invasive carcinoma of no special type, NST (ductal). Regarding molecular subtype, luminal B was the most common, accounting for 17 of 26 cases (65.4%), followed by triple-negative breast cancer in 8 of 26 cases (30.8%). Only one patient (3.8%) was classified as having the luminal A subtype.

The distribution of histological grade and the expression of the remaining predictive biomarkers, including ER, PR, and Ki-67, is presented in Table VI.

Histological grade G2 was observed in 15 patients (57.7%), and G3 in 11 (42.3%). Estrogen receptor expression was detected in 18 of 26 patients (69.2%), and PR expression in 16 of 26 patients (61.5%). A Ki-67 proliferation index of < 50% was observed in 12 patients (46.2%), whereas a Ki-67 index of ≥ 50% was found in 14 patients (53.8%).

The clinicopathological characteristics of the HER2-low subgroup are summarized in Table VI.

In summary, breast cancers classified as HER2 1+/low occurred predominantly in older, postmenopausal women, with a median age of 71 years. They accounted for 33.3% of the study cohort (26 of 78 cases) and 41.3% of cases without *HER2* gene amplification (26 of 63 patients).

All patients in the HER2-low subgroup were diagnosed with invasive carcinoma of NST. Estrogen receptor expression was detected in 69.2% of patients, while PR expression was observed in 61.5%. The luminal B subtype was the most common clinicopathological subtype, occurring in 17 of 26 patients. Triple-negative breast cancer was diagnosed in 8 patients, whereas the luminal A subtype was identified in one patient.

Discussion

Precision medicine is based on the identification of the histopathological type and histological grade of a tumour, assessment of immunohistochemical marker expression, and molecular testing, on the basis of which individualized therapy is selected for each patient. HER2 receptor status is one of the key predictive factors in breast cancer and plays an important role in individualizing treatment strategies. Depending on HER2 status, patients have traditionally been divided into two groups: HER2-negative cases, showing no expression or weak immunohistochemical HER2 expression without *HER2* gene amplification, and HER2-positive cases, showing strong HER2 expression and *HER2* gene amplification. Patients with HER2-positive breast cancer have been eligible for anti-HER2 targeted therapy combined with chemotherapy, aimed at blocking tumour cell proliferation. It is estimated that approximately 20% of patients benefited from this type of treatment.

A breakthrough in breast cancer therapy was the identification of a subgroup of patients with low immunohistochemical HER2 receptor expression and no *HER2* gene amplification, who may be eligible for innovative therapies with antibody-drug conjugates, including T-DXd, the efficacy of which has been confirmed in studies such as DESTINY-Breast04 [12]. This drug is an immunoconjugate composed of an antibody directed against the HER2 receptor and a topoisomerase I inhibitor. After binding to the HER2 receptor, the complex is internalized and the active metabolite is released, leading to DNA damage in tumour cells. In addition, T-DXd demonstrates a so-called “bystander effect”, which enables cytotoxic activity against neighbouring tumour cells with lower HER2 expression.

Clinical trials are ongoing to evaluate the efficacy of antibody-drug conjugates, including trastuzumab deruxtecan. This has been reflected, among others, in the publication by Denkert *et al.* [18], who demonstrated that HER2 1+/low tumours may differ from HER2 0 tumours in terms of biological and clinical features. Their findings suggest that this group of tumours is not merely an intermediate stage between HER2-negative and HER2-positive cancers. These results emphasize the importance of precise HER2 expression assessment, particularly in distinguishing IHC 0

from IHC 1+/low cases, which may currently have important diagnostic and therapeutic implications. Distinguishing a complete absence of receptor expression (IHC 0) from low HER2 expression (HER2 1+/low) has therefore gained clinical relevance. Correct identification of cases showing low HER2 expression may be important in qualifying selected patients for treatment with ADCs. The significance of the HER2-low category is particularly evident in patients who, according to the previous diagnostic approach, were classified as HER2-negative and were not eligible for HER2-targeted therapy.

The identification of the HER2 1+/low category represents an important diagnostic and clinical challenge in contemporary pathology. The 2023 ASCO/CAP update emphasizes the importance of precise differentiation between IHC 0 and IHC 1+/low categories [9]. Marszałek *et al.* [10, 11] also indicate that, in the era of therapies targeting HER2 1+/low tumours, accurate assessment of borderline cases and differentiation between IHC 0 and IHC 1+/low have become particularly important, as these distinctions may influence therapeutic options for patients with breast cancer. This position is also reflected in other published studies. Solarewicz *et al.* [19] emphasize that contemporary breast cancer classification should evolve with respect to the assessment of HER2 expression, and that distinguishing the HER2 1+/low category may allow more precise evaluation of this predictive factor in the context of eligibility for antibody-drug conjugate therapy. This category is not a separate biological subtype of breast cancer, but rather a predictive category used in the context of qualification for treatment with antibody-drug conjugates.

Individually targeted therapy must meet specific conditions. First, it should be based on widely accepted, mandatory, and reproducible microscopic criteria. Second, the group of patients who may benefit from treatment based on the expression of a given biomarker should be strictly defined. The criteria for pathological assessment, based on the CAP recommendations [17], are widely accepted and included in the ASCO/CAP guidelines. A major issue in HER2-low diagnostics remains the reproducibility of immunohistochemical assessment. Shaaban *et al.* [14] indicate that the greatest difficulties concern distinguishing between IHC 0 and IHC 1+/low categories, and that interobserver agreement among pathologists in this group of cases is lower than in classical HER2-positive tumours. Therefore, current recommendations emphasize the importance of appropriate training for pathologists and the use of standardized criteria for slide interpretation. In diagnostically challenging cases, repeat assessment of the material and consensus-based interpretation of the result are recommended.

The reliability of immunohistochemical assessment is also influenced by pre-analytical and analytical factors.

Press *et al.* [21] showed that improper fixation of tissue material may interfere with HER2 expression assessment. In addition, the use of different antibodies, diagnostic platforms, and staining protocols may affect the obtained results. Ivanova *et al.* [22] emphasize the importance of standardizing immunohistochemical procedures and implementing quality control systems to improve the reproducibility and reliability of HER2 testing [22].

In the analysed group of 78 patients, HER2 1+/low was identified in 26 cases after reclassification, accounting for 33.3% of all evaluated cases. Reassessment of the slides resulted in the reclassification of 15 cases initially assessed as HER2 0. These findings confirm observations reported in the literature, indicating significant diagnostic difficulties in distinguishing HER2 0 from HER2 1+/low. A similar study based on the reassessment of histopathological material, performed by Wang *et al.* [23], showed that 18.6% of cases were initially classified as HER2 0 and subsequently reclassified as HER2-low. Viale *et al.* [24] reported that more than 30% of HER2 0 cases were reclassified as HER2-low. Although the reported percentages of HER2-low cases vary between studies, they indicate that a substantial number of patients may potentially meet eligibility criteria for treatment with T-DXd.

The HER2 1+/low rate of 33% obtained in the present study was lower than that reported in some publications including larger patient populations, where the proportion of HER2 1+/low tumours was approximately 50–60%. In the study by Pous *et al.* [25], HER2-low was identified in 57% of cases, while in the study by Tsai *et al.* [26], it accounted for 60% of breast cancers. Differences between studies may result from variability in pathological interpretation, especially in the assessment of IHC 0 vs. IHC 1+/low. It should also be noted that discrepancies between studies may be related to the fact that older studies did not yet apply the currently used HER2 classification. Other contributing factors may include the method of tissue sampling, tissue processing, and the reagents used. HER2 receptor expression may also be influenced by ethnicity, access to medical care, and the extent of tumour progression. The variability of published results highlights the complexity of this issue and the need for further detailed observations and studies aimed at standardizing HER2 classification.

Based on the histopathological analysis performed in the present study, all cases in the HER2 1+/low group were diagnosed as invasive carcinoma of no special type, NST (ductal). The most common biological subtype was luminal B (65.4%), followed by triple-negative breast cancer (30.8%) and luminal A (3.8%). Estrogen receptor expression was detected in 69.2% of patients, while PR expression was detected in 61.5%. These findings are consistent with observations from other authors indicating that HER2 1+/low is more fre-

quently observed in tumours expressing hormone receptors. Chen *et al.* [27] found that ER expression was more frequent in HER2 1+/low tumours than in HER2 0 cancers (78.2% vs. 58.6%). The literature emphasizes that the HER2 1+/low category is often identified luminal B HER2 0. A similar tendency was observed in our material, where most HER2-low cases belonged to the luminal B subtype and showed hormone receptor expression. These observations suggest that low HER2 expression may coexist with hormone receptor expression, which may potentially be relevant for the biological characterization of the tumour and therapeutic decision-making.

The importance of precise HER2 assessment is further supported by the possibility of receptor status conversion during the course of malignant disease. Schrijver *et al.* [28] demonstrated that HER2 expression may change between the primary tumour and metastatic lesions, with both loss and acquisition of HER2 expression being observed. The high analytical sensitivity of clone 4B5 proved crucial for identifying patients with low receptor expression (IHC 1+ or IHC 2+ without ISH amplification) and ultra-low expression (IHC 0 with faint, barely perceptible membranous staining) [29]. This phenomenon supports the rationale for reassessing HER2 status and hormone receptor expression in cases of disease progression or the development of metastatic lesions.

The findings of the present study, in which slide reassessment led to the reclassification of 15 cases from HER2 0 to HER2-low, are consistent with observations reported in the literature and indicate the importance of careful evaluation of borderline cases. This highlights the role of HER2 diagnostic standardization and multidisciplinary collaboration in the process of qualifying patients for modern molecularly targeted therapies.

Conclusions

The performed HER2 reclassification demonstrated that the HER2-low category accounted for 33.3% of the analyzed breast cancer cases and enabled the identification of additional patients who may potentially meet the eligibility criteria for treatment with ADCs. The obtained results emphasize the importance of standardized assessment of HER2 expression and strict adherence to pre-analytical and analytical procedures in pathological diagnostics.

Disclosures

1. Institutional review board statement: Not applicable.
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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