ORIGINAL ARTICLES

Relationship between Histopathology Grading (Nottingham System)
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Relationship between Histopathology Grading (Nottingham System) and Expression of HER2/Subtype Luminal B in Breast Carcinoma with Lymph Node Metastases

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ABSTRACT

Background
Molecular subtypes of breast carcinoma, histological grade, and lymph node metastases are significant in determining the therapy and prognosis of the patient. The luminal B subtype comprises 15%-20% of breast carcinoma and has a more aggressive phenotype, higher histological grade, proliferative index, and a worse prognosis. This study aimed to evaluate the relationship between histopathological grading and expression of HER2 with lymph node metastases in luminal B subtype breast carcinoma.

Methods
We analyzed 279 invasive breast carcinoma luminal-B subtypes from the anatomic pathology laboratory of Dr. Hasan Sadikin Bandung between January 2017 and March 2023. Histological grade using the Nottingham system, expression of HER2 using immunohistochemistry examination, and lymph node metastases status were obtained from anatomic pathology records. The relationship between histological grade and expression of HER2 with lymph node metastases was examined with chi-square tests.

Result
In the current study, we included 279 subjects, dominated by patients whose age at diagnosis was less than 50 years old. *The Chi-square* analysis showed no statistically significant relationship in tumor size between patients with metastasis and those without metastasis, $P=0.74$. Most of them (55.9%) reported a tumor size of more than 5 cm (T3). Almost half of the participants (47.7%) suffered from the third histopathological grade. The Chi-square analysis showed a significant relationship between histopathological grade and lymph node metastases, $p = 0,000052$ ($p<0,05$) and expression HER2 status and lymph node metastasis $p = 0,014$ ($p<0.05$)

Conclusion
This study found that patients present with larger tumors classified as T3 and moderate to poor grade III and grade II histological grades. The statistical analysis showed no significant relationship in the age of diagnosis between patients with metastasis and those without. However, the Chi-square analysis revealed a significant relationship between the histopathological grade and HER2 expression with lymph node metastasis.

Keywords: Breast carcinoma Luminal B subtype, HER2, Histopathology grading, Lymph node metastases

BACKGROUND

Breast carcinoma represents the most commonly diagnosed malignant tumor in women globally.[1,2] In 2020, 2.3 million new breast carcinoma cases were diagnosed worldwide, overtaking lung carcinoma as the most common carcinoma with 680,000 deaths.[1] Breast carcinoma accounts for 24.5% of all female carcinoma. Nearly half of the breast carcinoma patients (45.4%) were diagnosed in Asia.[2]

A statement of the St. Gallen International Expert Consensus includes treatment algorithms based on the classification of breast carcinoma subtypes according to the immunohistochemistry of estrogen (ER), progesterone (PR), HER2, Ki67 expression divided into five intrinsic molecular subtypes of breast carcinoma (Luminal A, Lumina B, HER2-positive (non-luminal), triple-negative).[2-5] The luminal B subtype is associated with high-grade carcinoma and poor outcomes. Interestingly, patients with the lumina B subtype vary in their prognoses and respond differently to thera-peutic strategies. ER and HER2 are important treatment targets in breast carcinoma. Based on the immunohistochemical classification, the luminal B subtype is divided into luminal B (HER2-) and luminal B (HER2+) subtypes according to HER2 status, each associated with different therapeutic strategies.[3,6,7]

The choice of adjuvant systemic therapy is based on the patient's age, tumor size, histological grade, lymph involvement, hormone receptor status, and HER2 status. The only predictive markers with an associated targeted therapy are the estrogen receptor (ER) and HRE2. Approximately 15% of patients with breast carcinoma who have HER2 overex-pressing or amplified tumors are treated with a combination of trastuzumab, a monoclonal antibody targeting HER2, and adjuvant chemo-therapy.[4]

Nottingham histological grading has been reported as one of the strongest predictors of the disease outcome in breast carcinoma patients. It is principally carried out for invasive adenocarcinomas. Nottingham grading system yields a simple and routinely applicable analysis.[5] After the histological grade, the size of the primary tumor is also a robust prognostic entity, being a part of the TNM staging system.[1] In some literature, the histological grade was associated with a molecular subtype of breast carcinoma. Grade I was associated with Luminal A, while grade III was associated with luminal B, HER2, and TNBC.[8] Molecular subtype was associated with lymph node metastases.

However, there are still some inconsistent results concerning the relationship between histology grading and molecular subtype with lymph node metastases in breast carcinoma. In Indonesia, only a limited number of studies have investigated the relationship between histological grade and the expression of HER2 with lymph node metastases in breast carcinoma.[8] In the present study, we aimed to analyze the relationship between histological grade and expression of HER2 with lymph node metastases in breast carcinoma luminal B.

METHODS

Breast carcinoma specimens were obtained from the Anatomic Pathology Laboratory of Dr. Hasan Sadikin Bandung Hospital from January 2017 to March 2023. Histological grade, expression of HER2, and lymph node status were obtained from the laboratory information system. Histological grade was assessed according to the Nottingham system. The inclusion criteria of this cross-sectional study were determined as follows: (a) patients with invasive breast carcinoma Luminal B subtype; (b) patients with available histological grade and lymph node status. Overall, 279 samples were included in the study.

Histological grade evaluation using the Nottingham system, the determination of the histological grade was performed by one pathologist based on specific criteria. We obtained the following parameters for each patient: age at diagnosis, tumor size (T), lymph node status, immunohistochemical profile of the hormonal receptors ER and PR, and immunohistochemical profile of HER2 in the invasive malignant cells. ER and PR expression were considered positive when the tumor cell nucleus was ≥1%.[5] HER2 was considered positive if the membrane intensity was >10% of the tumor cells and homogeneously stained.[9] The tumor size measurement was retrieved from ultrasound reports of the breast before the biopsy or using reports from other radiological modalities. After size assessment, tumors were grouped into ≤2 cm, >2 but ≤5 cm, and >5 cm. The lymph node metastasis status was determined either using radiological modalities or from the evaluation of axillary lymph nodes obtained at mastectomy. The number of lymph nodes and the number of positive for metastasis were determined.[8]

Statistical analysis

The Statistical Package for Social Sciences software (SPSS) version 21.0 (IBMCorp., Armonk, NY, USA) was used for statistical analyses. Demographic character-istics (the age at diagnosis, tumor size, histopathological grade, and nodal status) of the subjects were noted and the relationship between histological grade and expression of HER2 with lymph node metastases was analyzed using Chi-square tests, and p less than 0.05 were concluded as statistically significant.

RESULTS
Descriptive Statistics

Table 1. Data on the characteristics of research subjects of luminal B subtype breast carcinoma at Dr. Hasan Sadikin Hospital, Bandung from January 2017 to March 2023.

Characteristics	n=279	
	n	%
Age at diagnosis		
Mean ± Std	49.2 ± 9.4	
Age at diagnosis (groups)		
<50 years old	151	54.1
≥50 years old	128	45.9
Tumour Size		
T1 (<2 cm)	41	14.7
T2 (2-5 cm)	63	22.6
T3 (>5 cm)	156	55.9
T4#	19	6.8
Histopathological Grade		
1 (first)	15	5.4
2 (second)	131	47
3 (third)	133	47.7
Expression Her 2 Status		
Positive	93	33.3
Negative	186	66.7
Lymph Node		
Metastasis	142	50.9
Non-Metastasis	137	49.1

Note: *Any size of the tumor that has invaded the chest wall and/or skin. In the current study, we included 279 subjects, with the majority ages at diagnosis being less than 50 years old (54.1%) and the average age at diagnosis being 49.2 years old (M=49.2, SD=9.4). Among those patients, most of them (55.9%) reported a tumor size of more than 5 cm (T3). Almost half of the participants (47.7%) suffered from the third histo-pathological grade. Over half of the patients (50.9%) were diagnosed with lymph node metastases. Two-thirds of patients (66.7%) were diagnosed with positive expression of HER 2. The descriptive analysis of the participants in detail can be seen in Table 1.

Lymph Node Metastasis

The statistical analysis showed no statistically significant relationship between the ages of patients diagnosed with metastasis and those without, with p=0.14. Moreover, the Chi-square test showed no statistically significant relationship in tumor size between the metastasis status, with p=0.74. Results suggested that the most common tumor size observed and metastasis status (54.9% vs. 56.9%, metastasis vs. non-metastasis) was categorized as T3, denoting tumors larger than 5 cm. This result suggested that a substantial proportion of patients in both groups had tumors of similar size, regardless of metastasis status (Table 2).

Table 2. Comparison of Characteristics of KGB Metastasis and Non-KGB Metastasis Research Subjects.

Characteristics	Metastasis n=142	Non-metastasis n=137	Statistics Test
Age at diagnosis			
Mean ± Std	48.4 ± 9.6	50.4 ± 9.2	p=0.14
Age at diagnosis (groups)			
<50 years old	83 (58.5%)	68 (49.6%)	p=0.14
≥50 years old	59 (41.5%)	69 (50.4%)	
Tumour Size			
T1 (<2 cm)	21 (14.8%)	20 (14.6%)	
T2 (2-5 cm)	31 (21.8%)	32 (23.4%)	
T3 (>5 cm)	78 (54.9%)	78 (56.9%)	p=0.74
T4#	12 (8.5%)	7 (5.1%)	

Note: A statistically no significant p-value (>0.05) indicates results from an Unpaired T-test or Chi-Square test. #Any tumor size that has invaded the chest wall and/or skin should be noted.

The relationship between Histopathological Grade and Lymph Node Metastasis

The Chi-square analysis showed a significant relationship between histopathological grade and lymph node metastasis, with *p*=0.000052 *(p<0.05)*. Table 3

suggested that the third histopathological grade was dominant among the metastasis group (60.6%). On the other hand, the second grade of histopathological was the most common in the non-metastasis group (59.9%).

Table 3. Histopathological grade relationship with lymph node metastasis in breast carcinoma luminal B subtype.

Characteristics	Metastasis n=142	Non-metastasis n=137	Statistics Test
Histopathological Grade			
1 (first)	7 (4.9%)	8 (5.8%)	
2 (second)	49 (34.5%)	82 (59.9%)	*p*=0.000052*
3 (third)	86 (60.6%)	47 (34.3%)	

Chi-Square test, *p-value <0.05 means statistically significant.

The relationship between Lymph Node Metastasis and Expression Her 2 Status

The Chi-square analysis showed a significant relationship between expression HER2 status and lymph node metastases, with

p=0.014 *(p<0.05)*. Table 4 suggested that among the non-metastasis group, the proportion of positive expression of HER-2 status was higher (73.7%) than the metastasis group (59.9%).

Table 4. Expression of HER2 relationship with lymph node metastasis in breast carcinoma luminal B subtype.

Characteristics	Metastasis n=142	Non-metastasis n=137	Statistics Test
Expression HER 2 Status			
Positive	85 (59.9%)	101 (73.7%)	*p*=0.014*
Negative	57 (40.1%)	36 (26.3%)	

Note: Chi-Square test, *p value <0.05 means statistically significant

DISCUSSION

The study found that most breast carcinoma patients were under 50 (54.1%), with an average age of diagnosis at 49.2 (9.4) years old. This concurs with studies by Chirag et al in 2021 and Muhammad et al in 2020, which also found a high prevalence of breast carcinoma in women under 50.[10,11] The data from this study also supports the American Cancer Society's 2017 statement that breast carcinoma rarely occurs in women under 25 years old. Age is a significant risk factor for breast carcinoma, and the incidence of breast cancer is increasing in young adult women under 50 years old.[12] This could be due to various factors, including increased population, awareness, and reporting, as well as other risk factors such as early parity, family history of breast carcinoma, mutations in the BRCA genes, and p53 mutations.[8]

In this study, most patients (55.9%) had tumor sizes larger than 5 cm (T3). This finding was discordant from a study conducted by Chirag et al in 2021, which found that 65.7% of cases had tumor sizes between 2 and 5 cm, while only 13.6% had tumor sizes greater than 5 cm. The size of the primary tumor is associated with prognosis. As the size of the carcinoma increases, the chance of nodal and distant metastasis also increases, leading to

decreased 5-years survival rates and a poor prognosis. A recent Pakistani study conducted in 2017 by Naqvi et al revealed that 88.1% of tumors were more than 2 cm in size. Another study conducted in Pakistan in 2017 found that increasing tumor size from less than 2 cm to more than 5 cm increased the chance of lymph node involvement from 37% to 90%, highlighting the link between large tumor sizes and local and distant metastasis, as well as poor outcomes.[1,13]

Out of all the cases examined in the study, over half of the patients (50.9%) were diagnosed with lymph node metastasis. This is concordant with a study conducted by Toshiaki in 2020, which also found that more than half (50%) of patients had positive lymph node metastases. Another study by Chirag et al reported that 55.3% of cases showed positive results for lymph node metastasis.[6,14]

Based on statistical analysis, there was no significant relationship in the ages of patients diagnosed with and without metastasis, with p=0.14. Additionally, the Chi-square analysis found no significant relationship in tumor size between the groups with and without metastasis. A 2021 study in India also discovered that there was no significant variation in molecular subtypes,

tumor size, or nodal metastasis according to Chi-square statistics.[14]

The relationship between Histopathological Grade and Lymph Node Metastasis (Table 3)

The Chi-square analysis shows a significant relationship *p*=0.000052 (p<0.05) between histopathological grade and lymph node metastasis. Table 3 indicates that the third histopathological grade is the most dominant among the metastasis group (60.6%). On the other hand, the second grade of histopathological is the most common in the non-metastasis group (59.9%). However, a study by Muhammad et al in 2020 showed that the molecular subtype luminal B is more commonly found in grade 2, which differs from this study.[11] Meanwhile, another study by Kadivar et al found that the TNBC molecular subtype was mainly found in grade 3, with a percentage of 63.5%.[10,11] Several studies in Indonesia have concluded that there is a consistent hypothesis test with this study, although there are differences in the proportion of molecular subtypes based on histopathological grading. For instance, in Firdaus et al in 2016 at RSUP Dr. M. Djamil Padang stated that there was a relationship between histopathological grading and molecular subtypes of breast carcinoma patients with a value of p=0.032 (p<0.05).[8] Meanwhile, Setyawati et al, in Yogyakarta, 2018, also stated a significant relationship between histopathological grading with molecular subtypes of breast carcinoma with p=0.013 (p<0.05). The study showed that 67.4% of Her-2 positive cases had lymph node metastasis on histopathology, followed by luminal B tumors (59%).[8]

Tumor grading determines the abnormality of tumor cells and tissue seen under a microscope. It assesses how different tumor cells look from normal breast cells and how quickly they grow. A scoring system evaluates three tumor characteristics/gland formation, nucleus pleomorphism, and number of mitoses-to reflect the aggressiveness of tumor cells. The higher the grade, the more aggressive the tumor. Grading is the primary prognostic factor reported in the results of histopathological examinations for breast carcinoma. It is associated with a 10-years life expectancy, with grade 1 having the best prognosis (85%), grade 2 having a moderate prognosis (60%), and grade 3 having the worst prognosis, often with metastases.[13,15]

Several studies conducted in Saudi Arabia, the USA, Iraq, and Indonesia indicated that HER-2-positive tumors are more likely to have positive lymph nodes upon presentation. Inc et al also identified the relationship between high Ki67 expression and lymph nodal metastasis in patients. Our study yielded similar results, although it was not statistically significant. The most frequently observed histological type of carcinoma was invasive ductal carcinoma, which accounted for 75.3% of cases. This type of carcinoma is also commonly found in other parts of the world. In our study, 4 0.4% of specimens were moderately differentiated, while 60.6% were poorly differentiated.[14]

The relationship between expression HER2 Status and Lymph Node Metastasis (Table 4)

The Chi-square test showed a significant relationship between histopathological grade and lymph node metastasis. The chi-squared value was 6.0 with a p-value of less than 0.05. As per Table 4, the percentage of people with positive HER2 expression was higher (73.7%) in the non-metastasis group compared to the metastasis group (59.9%). Therefore, this study suggests a relationship between HER2 expression and metastatic status. Individuals with positive HER2 expression have a lower chance of metastasizing. Interestingly, some theories show that the Luminal B subtype with negative expression of Bcl-2 and positive expression of HER-2 is associated with poor prognosis and low survival rates. [8] Additionally, a recent study conducted in August 2022 indicates that the Luminal B subtype is the most heterogeneous intrinsic subtype with varying gene expression profiles and different breast lymphatic pathways. Luminal A and Luminal B HER2- subtypes differ in the expression of proliferation-related genes. In luminal breast carcinoma, estrogen plays a crucial role in metastasis. In addition, high proliferation may increase the likelihood of metastasis.[8,13,16,17]

Studies have shown that the HER-2 subtype of breast carcinoma often involves larger tumors and tends to grow more aggressively.[18] This study found that grade 2 tumors had the highest percentage of the HER-2 molecular subtype, which differs from other studies that found it more common in high-grade tumors (grade 3). This could be due to the significant relationship between HER-2/neu expression and high histological grading. HER-2/neu is a receptor on the cell surface that sends growth signals to the nucleus, and overexpression of this receptor is associated with a poor prognosis. Differences in molecular

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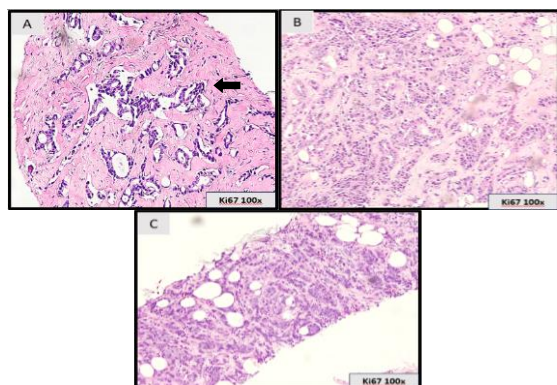characteristics with the luminal subtype can also contribute to a poor prognosis.[8]

Figure 1. A. Macroscopic appearance (100 times) Invasive carcinoma of no special type Grade, I the arrow indicates more tubular area than solid, with relatively monomorphic cell nuclei and low mitotic (<5/10HPF). B. Invasive carcinoma of no special type Grade, II. C. Invasive carcinoma of no special type Grade III.
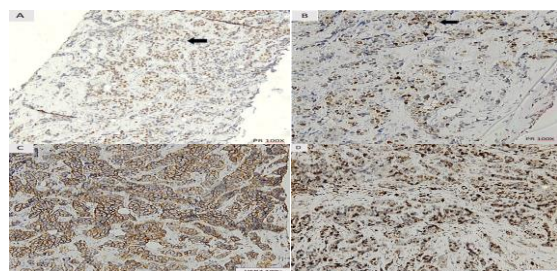

Figure 2. A case of Breast carcinoma with luminal B subtype HER2 positive. A. ER-positive, the arrow indicates a positive ER colored in the nucleus. B. PR positive, the arrow indicates a positive PR colored in the nucleus. C. HER2 Positive 3. D. Ki67 positive high proliferation.
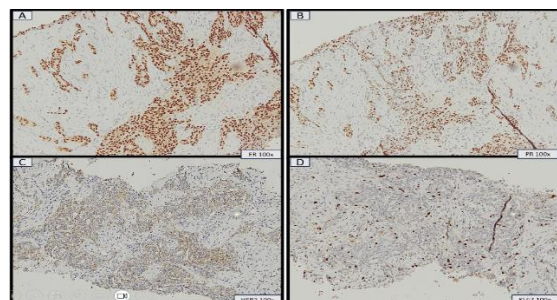

Figure 3. A case of Breast carcinoma with luminal B subtype HER2 negative. A. ER Negative. B. PR Negative. C. HER2 Negative. D. Ki67 positive high proliferation.

CONCLUSION

After analyzing the results of this study, it can be inferred that a majority of breast carcinoma cases are diagnosed in women under the age of 50. It is common for patients to present with larger tumors (T3 category) and moderate to poor histological grades (Grade III and Grade II). The statistical analysis indicated no significant relationship in the ages of diagnosis between patients with metastasis and those without. However, the Chi-square analysis demonstrated a significant relationship between histopathological grade and HER2 expression with lymph node metastasis in Subtype Luminal B Breast Carcinoma .

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