

Predictive factors for triple-negative breast carcinoma

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Summary

Objective: To determine the clinicopathologic factors and Ki67 expression predicting triple-negative breast cancer (TNBC). **Subject and method:** A descriptive study on 169 patients with breast cancer at the 108 Military Central Hospital from October 2015 to September 2020. The status of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki67 expression were examined by immunochemistry. **Result:** The mean age of the patients was 53.8 ± 12.2 years. The rate of tumor size larger than 2cm was 54.4% (92/169). The percentage of cases with tumor grade 2 was highest at 64.5% (109/169). The rate of axillary lymph node metastasis was 37.9%. TNBC accounted for 16.6%. The statistically significant correlation was observed between TNBC and location, tumor size, histological grade and $Ki67 \geq 14\%$. **Conclusion:** Our study suggested that the right side, histological grade 3 and $Ki67 \geq 14\%$ were independent predictive factor of TNBC.

Keywords: Triple-negative, breast cancer, immunohistochemistry, clinicopathologic, predicting factors.

1. Background

Breast cancer is one of the most common cancers and is the leading cause of death among women in the world as well as in Vietnam. Although there are many methods and techniques for diagnosing breast cancer, histopathological diagnosis is always considered the gold standard.

In 2019, the World Health Organization (WHO) released the fifth edition of classification of breast tumors with some important additions. Immunohistochemistry and molecular biology have been applied in classification. Breast cancer is classified into molecular subtypes, based on the expression of some immunohistochemical markers: ER, PR, HER2, and Ki67 [6].

Triple-negative breast cancer (TNBC) is defined by the absence of expression of ER, PR and HER2. It accounts for 15-20% of all breast cancers. TNBC tends to be more aggressive than other types of breast cancer. It is also associated with a poor

prognosis due to its clinical behavior and lack of molecular targets for cancer therapy [1], [6]. Advanced clinical stage, larger tumor size, angiolymphatic invasion, axillary node involvement, positive sentinel lymph node, higher cancer burden, surgical treatment with mastectomy, and recurrence were related to a significant decrease in overall survival and/or disease-free survival and increased risk of mortality and/or recurrence in TNBC. The 10-year overall survival and disease-free survival were around 61 and 65%, respectively [3]. With Vietnamese TNBC patients, overall survival was 84.63 months on average [10].

In Vietnam, there have been some studies on histopathological and immunohistochemical characteristics of breast carcinoma, but studies on TNBC have still been limited. This study aims to determine the clinicopathologic characteristics and Ki67 expression in predicting TNBC.

2. Subject and method

2.1. Subject

169 patients were enrolled in our study. All of them were operated and diagnosed with breast carcinoma at the 108 Military Central Hospital from October 2015 to September 2020.

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2.1.1. Inclusion criteria

Patients were treated by mastectomy and axillary lymph node resection.

Histopathological diagnoses were invasive breast carcinomas and immunohistochemical staining with markers ER, PR, HER2, Ki67.

Specimens, blocks are enough qualify to study.

2.1.2. Exclusion criteria

Small biopsy specimen.

Recurrent breast carcinoma or metastatic cancer from other organs.

Patients were treated with preoperative chemotherapy.

2.2. Method

A cross-sectional, descriptive study.

Specimens were routinely processed and fixed overnight in 10% neutral buffered formalin, paraffin embedded. Four to five micrometer thick tumor sections were stained with Haematoxylin-Eosin.

Immunohistochemical staining: Immunohistochemical staining slides using BenchMark ULTRA machine of Ventana (USA) with ER, PR, HER2, Ki67 markers (Dako Company, Denmark).

Age, location, tumor size, the status of axillary lymph node metastasis were collected through medical records and pathology reports.

Histological types, grades of tumor and the expression of immunohistochemical markers were evaluated by experienced pathologists from the Department of Pathology.

2.3. Research content

Age, location, tumor size, the status of axillary lymph node metastasis.

Histological types were classified according to WHO 2019.

Histological grades were assessed according to Nottingham modification of the Bloom-Richardson system.

To evaluate the expression of immunohistochemical markers: Evaluating ER, PR results according to the standards of Allred and the manufacturer Dako; evaluating HER2 results according to ASCO/CAP standards.

The expression of Ki67 is divided into two groups: < 14% and ≥ 14%, according to the Saint Gallen Consensus Meetings (2011).

2.4. Statistical analysis

Patients were subdivided into two groups based on the immunohistochemical results (TNBC and other subtypes). Age variable was reported as mean with standard deviation (SD), and categorical variables were presented as numbers with percentages. A Chi-square test or Fisher’s exact test were used to compare categorical variables between two groups. Multivariate logistic regression analyses were performed to identify which factors were associated with TNBC. Odds ratios (OR) with a relative 95% confidence interval (95% CI) were calculated to determine the relevance of all potential predictors of TNBC. The analysis was performed using SPSS software (version 26.0) and statistical significance was accepted for p<0.05.

3. Result

3.1. Clinicopathological and immunohistochemical characteristics of the patients

Table 1. Patients' clinicopathological and immunohistochemical characteristics

Features		Number of patients	Percentage (%)
Total cases		169	100
Age (years)	< 50	60	35.5
	≥ 50	109	64.5
	Mean ± SD	53.8 ± 12.2	

Features		Number of patients	Percentage (%)
Location	Left	93	55.0
	Right	76	45.0
Tumor size (cm)	≤ 2	77	45.6
	> 2	92	54.4
Histological grade	1	13	7.7
	2	109	64.5
	3	47	27.8
Histological types	Invasive ductal carcinoma (IDC)	152	89.9
	Papillary carcinoma	4	2.4
	Mucinous carcinoma	4	2.4
	Medullary carcinoma	7	4.1
	Metaplastic carcinoma	1	0.6
	Lobular carcinoma	1	0.6
Lymph node metastasis	No	105	62.1
	Yes	64	37.9
Ki67	< 14%	51	30.2
	≥ 14%	118	69.8
ER	Positive	111	65.7
	Negative	58	34.3
PR	Positive	66	39.1
	Negative	103	60.9
HER2	Positive	64	37.9
	Negative	105	62.1
Triple-negative breast carcinoma		28	16.6

The mean age of the patients was 53.8 ± 12.2 (30-82 years). Majority of the patients were ≥ 50 years at diagnosis (64.5%).

The left breast tumors were more common (55%).

The common rate of more than 2 cm tumor was 54.4%.

The percentage of cases with histologic grade 2 was highest at 64.5%.

Triple-negative breast carcinoma accounted for 16.6%.

3.2. Predictive factors for triple-negative breast carcinoma

Table 2. The relationship between some clinicopathological characteristics and TNBC

Features		Triple-negative breast cancer				p
		No		Yes		
		n	Percentage %	n	Percentage %	
Age (years)	< 50	50	35.5	10	35.7	0.98
	≥ 50	91	64.5	18	64.3	
Location	Left	83	58.9	10	35.7	0.024
	Right	58	41.1	18	64.3	
Tumor size (cm)	≤ 2	69	48.9	8	28.6	0.048
	> 2	72	51.1	20	71.4	

Features		Triple-negative breast cancer				p
		No		Yes		
		n	Percentage %	n	Percentage %	
Histological types	IDC	129	91.5	23	82.1	0.133
	Other types	12	8.5	5	17.9	
Histological grade	1 and 2	112	79.4	10	35.7	<0.001
	3	29	20.6	18	64.3	
Lymph node metastasis	No	87	61.7	18	64.3	0.797
	Yes	54	38.3	10	35.7	
Ki67	< 14%	50	35.5	1	3.6	0.001
	≥ 14%	91	64.5	27	96.4	

The rate of TNBC was significantly higher in the group of tumors with tumor size larger than 2cm, on the right side, histological grade 3, and Ki67 expression ≥ 14% (p<0.05).

Table 3. Multivariate analysis of predictive factors for TNBC

Factors	p	OR	95% C.I	
			Lower	Upper
Location (right side)	0.037	2.86	1.07	7.68
Tumor size (> 2cm)	0.358	1.69	0.55	5.17
Histological grade (grade 3)	<0.001	6.21	2.30	16.72
Ki67 (≥ 14%)	0.016	12.92	1.62	103.25

To identify the independent predictive factors associated with TNBC, a logistic regression analysis was performed (Table 3). The results revealed that location (right side), histological grade (grade 3) and Ki67 (≥ 14%) were independent predictive factors of TNBC.

4. Discussion

4.1. Clinicopathological and immunohistochemical characteristics of the breast carcinoma

Age: 169 patients were enrolled in our study. Most of the patients (64.5%) were more than 50 years at diagnosis, and the mean age of the study participants was 53.8 years. This corresponds to what was reported by Dang Cong Thuan (2018) with the data was 66.3% [4]. On the contract, Usman Malami Aliyu (2020) reported the rate was higher in patients under 50 years of age [1].

Location: The left breast was more common (55%). This was consistent with the study conducted by Nguyen Van Chu (2016) [7].

Tumor size: The higher rate of more than 2cm tumor was 54.4%. Other authors also published similar results such as Azizun-Nisa et al (2008) [2], Usman Malami Aliyu et al (2020) [1] with the rate of 52.7% and 50.6%, respectively. Patients can easily detect tumors with a size of more than 2cm, so the percentage of tumors in this size range accounted for the highest percentage.

Histological grade: Tumor grade 2 had the highest rate of 64.5%, followed by tumor grade 3 accounting for 27.8%, tumor grade 1 had the lowest rate with 7.7%, consistent with the results of some local studies and international studies.

Table 4. Comparison of histological grades between studies

Authors	Histological grade (%)		
	1	2	3
Dang Cong Thuan (2018) [4]	11.8	60.3	27.9
Ta Van To (2004) [9]	12.2	71.4	16.4

Authors	Histological grade (%)		
	1	2	3
Azizun-Nisa (2008) [2]	6.7	55.3	38
Usman Malami Aliyu (2020) [1]	23.9	50.6	25.5
Our study	7.7	64.5	27.8

Histological grade was an important prognostic factor in breast cancer. It was related not only to recurrence rate, mortality rate, but also to disease-free survival and overall survival of patients with breast carcinoma after surgery. The 5-year survival rate gradually decreased by grade 1, grade 2, grade 3 with a statistically significant difference [10].

Expression of some immunohistochemical markers in breast carcinoma

The positive rates for ER, PR, HER2 in our study were 65.7%, 39.1% and 37.9%, respectively. Comparing with the results of some studies conducted by Vietnamese authors and foreign authors presented in the following table:

Table 5. Positive ER, PR, HER2 staining results across studies

Authors	ER (+) (%)	PR (+) (%)	HER2 (+) (%)
Dang Cong Thuan (2018) [4]	42.4	41.3	34.8
Nguyen Van Chu (2016) [7]	52.7	50.3	26.7
Usman Malami Aliyu (2020) [1]	68.5	77.7	57.5
Edlira Pajenga (2016) [8]	69.1	66.4	41
Our study	65.7	39.1	37.9

Ki67 expression: Ki67 ($\geq 14\%$) was positive in 69.8%. This data is slightly higher than the result of the study conducted by Dang Cong Thuan (59.8%) and Nguyen Van Chu (58.4%) [7].

The difference in results could be due to the small number of patients in our study. Furthermore, evaluating the grade of tumors, the expression of immunohistochemical markers was based on microscopic observations, so there were differences because of the subjective assessments of the pathologists. In addition, it was possible that the biological characteristics of patient groups in other regions may be different.

4.2. Predictive factors for triple-negative breast carcinoma

Table 2 showed the relationship between some clinicopathological characteristics and TNBC. TNBC accounted for a higher proportion in the right breast (64.3%), tumor size larger than 2cm (71.4%), histological grade 3 (64.3%), Ki67 expression $\geq 14\%$ (96.4%) compared with non TNBC. The difference was statistically significant ($p < 0.05$).

Tran Thi Hoai (2022) studied 70 TNBC patients and found that TNBC was also seen mainly in women over 50 years old (60%), invasive ductal breast cancer (80%), histological grade 3 (33.9%) [10].

Amira Elwan et al. (2020) conducted a study on 80 TNBC patients. The results showed that the mean age at presentation was 42.6 years. Most of the cases were grade 3 (56.3%) and 82.5% showed lymph node metastasis, invasive ductal breast cancer was the most common histopathological type (65%), high Ki67 expression (66.3%) [5].

In our study, the results showed that the right side, histological grade 3 and Ki67 $\geq 14\%$ were independently predicted TNBC. Histological grade is related not only to recurrence rate, mortality rate, but also to disease-free survival and overall survival of breast cancer patients after surgery. The 10-year survival rate for tumors with histological grade 1 is 85%, while histological grade 3 is 43%. Ki67 expression is closely related to cell proliferation and growth. Currently, Ki67 has become one of the most commonly used biomarkers for assessing cell proliferation. In breast cancer, measuring the expression intensity of Ki67 expression by immunohistochemistry staining is a

routine approach for prognostic evaluation. High Ki67 expression is associated with tumors with poor prognosis [7]. Histological grade 3 and high Ki67 expression in TNBC are consistent with the aggressive clinical presentation of this subtype. Therefore, in patients with these characteristics, clinical doctors should follow up and treat as with TNBC when immunohistochemical staining results for ER, PR and HER2 are not available.

5. Conclusion

In summary, triple-negative breast carcinoma occurred in 16.6% of the patients studied and was more common with right side, histological grade 3 and Ki67 expression ($\geq 14\%$). Further studies in Vietnam with larger samples should be performed to evaluate the predictive factors for triple negative breast cancer.

References

1. Aliyu UM, Musa AA (2020) *Assessment of breast cancer immunohistochemistry and tumor characteristics in Nigeria*. World J Clin Oncol 11(11): 935-944.
2. Azizun-Nisa, Bhurgri Y, Raza F, Kayani N (2008) *Comparison of ER, PR and HER-2/neu (C-erb B 2) reactivity pattern with histologic grade, tumor size and lymph node status in breast cancer*. Asian Pac J Cancer Prev 9(4): 553-556.
3. Costa R, Oliveira F, Araújo A, & Vieira SC (2021) *Prognostic factors in triple-negative breast cancer: A retrospective cohort*. Revista da Associação Médica Brasileira (1992) 67(7): 950-957.
4. Dang Cong Thuan et al (2018) *HER2 status and relationship with prognostic factors in invasive breast carcinoma*. Journal of medicine and pharmacy, Hue university of medicine and Pharmacy 8(4): 13-22.
5. Elwan A, Abdelrahman et al (2021) *Clinicopathological features and treatment challenges in triple negative breast cancer patients: A retrospective cohort study*. Turk patoloji dergisi 37(2): 121-129.
6. Lakhani S, Ellis I, Schinitt S et al (2019) *WHO classification of tumours of the breast*. 5th ed, International Agency for Research on Cancer, Lyon.
7. Nguyen Van Chu (2016) *Studying application of molecular classification in breast cancer by immunohistochemical method*, Doctoral Thesis in Medicine, Hanoi Medical University.
8. Pajenga E, Rexha T, Çeliku S, Ugrinska A, Bejtja G. (2016) *Hormonal receptor, human epidermal growth factor and its association with breast cancer tumor characteristics in Albania*. Cent Eur J Public Health 24(3): 171-175.
9. Ta Van To (2004) *Research on morphology, immunohistochemistry and their prognostic value in breast carcinoma*. Doctoral thesis of Medicine, Hanoi Medical University.
10. Tran Thi Hoai, Le Hong Quang, Nguyen Ngoc Tu, Doan Thi Hong Nhat (2022) *Treatment results of stage I-II triple-negative breast cancer in Nghe An Oncology Hospital*. Vietnam Medical Journal 518: 321-325.