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Ki-67 Labeling Index Based on Clinicopathological Characteristics in Triple Negative Breast Carcinoma (TNBC)

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Abstract : Nowadays, breast cancer has been known as heterogenous disease with various biological features which require different therapy strategies. Breast cancers with *lack or no expression of estrogen (ER), progesterone receptors (PR), and human epidermal growth factor* receptor 2 (HER-2) are defined as triple negative breast cancer (TNBC). To date, Ki-67 has been used in order to characterize cell proliferation. This study is to analyze Ki-67 labeling index (LI) expression based on clinicopathological features of TNBC. This descriptive study with cross-sectional design was performed. Data about demographics were extracted from patients' medical record and histopathologic feature and immunohistochemistry was done. Results of analysis data were presented in frequency tables. Mean and standard deviation Ki-67 LI were also obtained. In this study, most patients aged 35-49 years old with mean of 46,8 years old. Most of them had bigger tumour size, had already metastasized to lymph node, had no distant metastases and higher stage. Ki-67 LI value in this study quite high with mean of 43,89%. %. If TNBC patients were young, had distant metastases, higher mitotic count (≥15/10 HPF) and higher grade, they tended to have higher Ki-67 LI. Patients with metaplastic carcinoma of no special type and carcinoma with medullary features also tended to have higher Ki-67 LI. Meanwhile tumour size, metastases to lymph node and clinical stage were not in accordance with Ki-67 LI.
Keywords : TNBC, Clinicopathological, Immunohistochemistry, Ki-67 LI.

Introduction

To date, breast cancer has been known as heterogenous disease with various biological characters which need different therapy strategies.[1] If immunohistochemical staining with estrogen receptor (ER), progesterone receptor (PR), and HER-2 are performed and all shows negative, then this tumour will be defined as triple negative breast cancer (TNBC).[2] This TNBC is ineffective with hormonal therapy or molecularly

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targeted anti-HER2 so the only effective treatment is chemotherapy.[1-3] *TNBC* generally has bigger size, higher grade, has already metastasized to lymph node at initial diagnosis, and biologically more aggressive compared to other types of breast cancer.[1,3,4]

Recently, International Ki-67 in Breast Cancer Working Group emphasize the potency of Ki-67 in prognosis, response prediction towards chemotherapy and as biomarker of therapy effectiveness.[5] Breast cancer with high Ki-67 expression has better respond to chemotherapy.[4] Ki-67 is a protein related to cellular proliferation and expressed in all cell cycle phase (S, G1, G2, and M phases), but not G0.[6]

The research about TNBC in Indonesia especially in Medan is still limited. Till now there's no standard operating procedure (SOP) or agreement yet about Ki-67 cut-off levels used in clinical practice. Moreover, there is little research on the clinical significance of Ki-67 in TNBC. Hence, researchers were interested to study the expression Ki-67 labeling index (LI) based on clinicopathological characteristics in TNBC.

Materials and method

This descriptive cross-sectional study was carried out in 55 TNBC patients in Haji Adam Malik Hospital/Department of Anatomical Pathology, Medical Faculty USU Medan and Department of Oncology/Surgical Haji Adam Malik Hospital Medan from February 2017 to July 2018. The research was done after getting permission from Ethical Committee of Medical Faculty USU Medan. Studied population are paraffin blocks from patients histopathologically diagnosed with TNBC. Clinical data such as age, tumour size and clinical stage were obtained from medical records and histopathological review of slide were done based on Bloom and Richardson methods modified by Elston Ellis (subtypes and grading histology). Then, TNBC tumours were further stained with Ki-67 (clone MIB-1, Dako Denmark A/S, dilution 1:75-1:150).

Ki-67 LI was defined as percentages of nuclear immunostained cells in at least 300 tumour cells.[7] Ki-67 was interpreted in hot spot areas. Ki-67 LI was recorded as numeric and ordinal scale. As ordinal scale, Ki-67 LI was categorized into 5 groups. Absent or present in ≤ 10% positive tumour cells was taken as score 1, 11-25% positive tumour cells was taken as 2, 26-50% was 3, 51-75% was 4, and 76-100% was 5. Ki-67 LI was quantitatively double blind evaluated by researchers. The data obtained in the research was processed with help of statistical software and presented in frequency tables. Mean and standard deviation of Ki-67 LI was also obtained.

Results and Discussion

Clinicopathological characteristics of studied patients are shown in Table 1. The mean age was 46,8 years old (range 26-63 years). Most of them had tumour size more than 5cm (58.2%), had already metastasized to ipsilateral axillary lymph node level I, II (N1) (52.7%) and had no distant metastases (96.4%). Most patients had stage IIB and IIIB (both 34.5%). About 78.2% patients with TNBC were diagnosed as IC-NST, 9.1% carcinoma with medullary features, 7.3% ILC, 3.6% metaplastic carcinoma of no special type, and only 1.8% mucinous carcinoma. Most of patients had mitotic counts ≤7/10 HPF and histology grade 2.

Ki-67 LI value in this study quite high with mean of 43.89%. If TNBC patients were young (<35 years old), had distant metastases, higher mitotic count (≥15/10 HPF) and higher grade, they tended to have higher Ki-67 LI. Patients with metaplastic carcinoma of no special type and carcinoma with medullary features also tended to have higher Ki-67 LI. Meanwhile tumour size, metastases to lymph node and clinical stage were not in accordance with Ki-67 LI (Table 2).

Table 1. Clinicopathological characteristics of TNBC

Clinicopathological characteristics	Total (n)	Percentage (%)
Age (years)		
– < 35	2	3.6
– 35 – 49	36	65.5
– 50 – 64	17	30.9
– ≥65	-	-
Clinical staging		
• **Tumour size (T)**		
– T1 (0,1cm < T ≤ 2cm)	-	-
– T2 (2cm < T ≤ 5cm)	3	5.5
– T3 (T > 5cm)	32	58.2
– T4 (tumour size expanding to chest wall and/ or to skin)	20	36.4
• **Lymph node metastases (N)**		
– N0	25	45.5
– N1	29	52.7
– N2	1	1.8
– N3	-	-
• **Metastasis (M)**		
– M0	53	96.4
– M1	2	3.6
• **Stage**		
– I	-	-
– II	20	
○ IIA	1	1.8
○ IIB	19	34.5
– III	33	
○ IIIA	14	25.5
○ IIIB	19	34.5
○ IIIC	-	-
– IV	2	3.6
Subtypes histology		
– Invasive Carcinoma, No of Special Type (IC-NST)	43	78.2
– Invasive lobular carcinoma (ILC)		
– Tubular carcinoma	4	7.3
– Cribriform carcinoma	-	-
– Mucinous carcinoma	-	-
– Carcinoma with medullary features	1	1.8
– Carcinoma with apocrine differentiation	5	9.1
– Carcinoma with signet ring cell	-	-
– Invasive micropapillary carcinoma	-	-
– Metaplastic carcinoma of no special type	-	-
	2	3.6
Mitotic counts (per 10 HPF)		
– Score 1 (≤ 7)	20	36.4
– Score 2 (8-14)	18	32.7
– Score 3 (≥ 15)	17	30.9
Grade histology		
– Grade 1	-	-
– Grade 2	32	58.2
– Grade 3	23	41.8
Total	55	100

Table 2. Ki-67 LI based on clinicopathological characteristics of TNBC

Variables	*Ki-67 LI*					Total
	≤10% (%)	**11-25% (%)**	**26-50% (%)**	**51-75% (%)**	**76-100 (%)**	
Age (years)						
– < 35	0	0	0	0	2 (100)	2
– 35 – 49	19 (52.8)	0	5 (13.9)	4 (11.1)	8 (22.2)	36
– 50 – 64	6 (35.3)	1 (5.9)	0	4 (23.5)	6 (35.3)	17
Tumor size						
– T2	0	0	0	1 (33.3)	2 (66.7)	3
– T3	17 (53.1)	1 (3.1)	3 (9.4)	3 (9,4)	8 (25)	32
– T4	8 (40)	0	2 (10)	4 (20)	6 (30)	20
Lymph node metastases (N)						
– N0	12 (48)	1 (4)	2 (8)	2 (8)	8 (32)	25
– N1	12 (41.4)	0	3 (10.3)	6 (20.7)	8 (27.6)	29
– N2	1 (100)	0	0	0	0	1
Metastasis (M)						
– M0	25 (47.2)	1 (1.9)	5 (9.4)	8 (15.1)	14 (26.4)	53
– M1	0	0	0	0	2 (100)	2
Stage						
– IIA	0	0	0	0	1 (100)	1
– IIB	9 (47.4)	0	1 (5.2)	3 (15.8)	6 (31.6)	19
– IIIA	8 (57.1)	1 (7.15)	2 (14.3)	1 (7.15)	2 (14.3)	14
– IIIB	8 (42.1)	0	2 (10.5)	4 (21.1)	5 (26.3)	19
– IV	0	0	0	0	2 (100)	2
Subtype histology						
– *IC-NST*	18 (41.9)	1 (2.3)	5 (11.6)	7 (16.3)	12 (27.9)	43
– *ILC*	4 (100)	0	0	0	0	4
– Mucinous carcinoma	1 (100)	0	0	0	0	1
– Carcinoma with medullary features	2 (40)	0	0	1 (20)	2 (40)	5
– Metaplastic carcinoma of no special type	0	0	0	0	2 (100)	2
Mitotic counts (per 10 HPF)						
– Score 1 (≤ 7)	20 (100)	0	0	0	0	20
– Score 2 (8-14)	5 (27.8)	1 (5.55)	5 (27.8)	6 (33.3)	1 (5.55)	18
– Score 3 (≥ 15)	0	0	0	2 (11.8)	15 (88.2)	17
Grade histology						
– II	18 (56.3)	0	3 (9.35)	5 (15.6)	6 (18.75)	32
– III	7 (30.4)	1 (4.4)	2 (8.7)	3 (13.0)	10 (43.5)	23
Total	25	1	5	8	16	55

There were 55 cases of TNBC samples in Haji Adam Malik General Hospital/Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara Medan. This triple negative is still a problem in breast cancer therapy due to ineffective of hormonal and Her-2 therapy. In these cases, chemotherapy to date is still an option for the clinicians in TNBC treatment.

In this study, about 65,5% patients aged 35-49 years old with mean 46,8 years old. The results of many studies varied widely. Some said that TNBC is commonly found in younger age, but some is older age.[3,8,9] Compared to literatures mentioned above, this study results had the youngest mean age of TNBC. These

different results might be occurred because of different races and lifestyle from the patients that lived in the countries.

TNBC generally had bigger size, had already metastasized to lymph node at initial diagnosis and higher clinical stage.[1,3,4] In this study, we found that most of patients had T3 (58.2%), N1 (52.7%), M0 (96,4%), and clinical stage III (33 cases) such as IIIB (34.5%). This study is in accordance with Yuan *et al.* but opposite with Wang *et al.*[10,11] Wang *et al.* (2018) found that approximately 60% of cases didn't metastasize to lymph node.[11] This study results were varied widely because of various sample sized, different races, and patients' desire to seek treatment. In Indonesia, patients often came for treatment when late stage because of low health education and dependence on traditional medicine (herbal). This caused most of patients come with more severe illness.

In this study, from the most common diagnoses to least common found in TNBC were IC-NST, *carcinoma with medullary features, ILC, metaplastic carcinoma of no special type*, and *mucinous carcinoma. This researches were in accordance with* Hashmi *et al.* (2014) and Wang *et al.* (2016).[12,13] Various literatures were also found that TNBC generally had higher grade than non-TNBC.[1,3,4] This study results were in accordance with other studies.[8,10,11]

In cancer, cell proliferation is one of the most important prognostic factors. This correlates with overall patient survival rate. Until now, there are some markers related to proliferation that often been used.[14] Ki-67 is considered as the most suitable markers because this protein is expressed in all cell cycles except G0, both in normal or malignant cells.[4,6,14] Compared to other proliferation markers, Ki-67 LI is an accurate, easy, and cost-efficient examination. Because of that, Ki-67 LI is considered the most ideal diagnostic tools.[14] This study showed that *mean Ki-67 levels was not much different than* Hashmi *et al.* (2014).[12] Our study also found that most of patients had Ki-67 LI $\leq$ 10%. This result was in contrast to Munzone *et al.* (2012) and Bakhit *et al.* (2016).[1,15] Munzone *et al.* (2012) revealed that about 88,7% patients had *Ki-67 LI* $\geq$20.[15] Higher Ki-67 LI was correlated with higher risk of recurrency.[16] Compared to other studies, Ki-67 LI value was quite high. Maybe this occurred because there were difference in races.

The difference in interpreting Ki-67 LI was due to several things. First, the use of cut-point Ki-67 LI in each center was varies greatly. Although other centers sometimes used same antibody, there were many variations in Ki-67 examination between laboratory centers, such difference in staining methodology, scoring interpretation, and the selection of tumour areas to be assessed.[17,18] Second, there were difference in Ki-67 LI protein expression during cell cycle because this protein peaked during phase M and lower during phase G1 and S. This different expression was one of the limiting factors in assessing percentages immunostained cells. Third, technical aspects in tissue fixation, processing, immunostaining and counterstaining probably modified the intensity of staining and proportion of immunostained cells.

If TNBC patients were young (<35 years old), had distant metastases, higher mitotic count ($\geq$15/10 HPF) and higher grade, they tended to have higher Ki-67 LI. This results were in line with Hao et al. and Cserni et al.[13,19] Besides that, we also found that patients with metaplastic carcinoma of no special type and carcinoma with medullary features tended to have higher Ki-67 LI. This results was in accordance with Montagna et al.[20] However, tumour size, metastases to lymph node and clinical stage were not in accordance with Ki-67 LI in our study. In contrary, Li et al. revealed that the higher clinical stage, the higher Ki-67 LI.[21] Munzone et al. also found that the higher Ki-67 LI, the bigger tumour size ($p<0.01$).[15]

Our study had several limitation. First, samples in this study were not evenly distributed. For example stage I dan some histology subtypes were not found. Second, Ki-67 staining were useful to determine prognostic in TNBC. Due to time constraints and difficult to get TNBC cases, this study were only be done with cross-sectional design. We expected that further prospective research will be done.

Conclusion

TNBC is a heterogenous breast cancer. If TNBC patients were young (<35 years old), had distant metastases, higher mitotic count and higher grade, they tended to have higher Ki-67 LI. Patients with metaplastic carcinoma of no special type and carcinoma with medullary features also tended to have higher Ki-67 LI. Meanwhile tumour size, lymph node metastases and clinical stage were not in accordance with Ki-67 LI. We suggested that Ki-67 LI should be examined in every TNBC cases.

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