R E S E A R C H   A R T I C L E

Basal-Like Subgroup is Associated with Younger Age, Increased Expression of Androgen Receptor, and Worse Prognosis, while Non-basal-like Subtype is Associated with Higher BMI in Triple-Negative Breast Cancer Patients

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Abstract

BACKGROUND: Triple-negative breast cancer (TNBC) represents a heterogenous disease which differ in characteristic, treatment response and prognosis. We aim to perform in-depth analysis on the clinicopathologic feature and the prognostic value of basal-like and non-basal-like TNBC patients in an Indonesian tertiary hospital.

METHODS: We retrospectively included patients diagnosed with TNBC between 2014-2017. Clinical variables were collected from medical record. Expression of epidermal growth factor receptor (EGFR), cytokeratin 5/6 (CK5/6), p53 mutant and androgen receptor (AR) were examined by using immunohistochemistry (IHC).

RESULTS: We included 67 subjects, 67.1% were basal-like and the remaining 32.9% were non-basal-like, with mean age of 51 years old, 59.7% subjects had BMI <25 and 40.3% subjects had BMI ≥25; 16.4%, 65.7%, and 17.9% subjects

presented with early stage, locally advanced stage, and distant metastasis respectively; T<5 cm was found in 29.9% subjects, while 70.1% subjects had T≥5; 67.2% subjects presented with N-, while 32.8% subjects were N+. The most common histological type was infiltrating ductal (82% of subjects). P53 mutant and AR expressions were positive in 44.8% and 15% subjects, respectively. Basal-like subtype presented with younger age at and had higher expression of AR, while non-basal-like subtype is associated with BMI ≥25 ($p<0.05$). Basal-like subjects had shorter overall survival (23.9 months (95% CI: 21.9-25.9) *vs.* 26.1 months (95% CI: 23-29.2).

CONCLUSION: Basal-like subtype is associated with worse prognosis, younger age at diagnosis and increased expression of AR, while non-basal-like subtype is associated with higher BMI in Indonesian TNBC.

KEYWORDS: TNBC, subtype, basal-like, young age, Indonesia

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Introduction

Breast cancer is still the most common type of malignancy in female with annual incidence of 200,000 cases worldwide

with estimated incidence of 18.6/100,000 females annually in Indonesia.(1,2) Triple-negative breast cancer (TNBC), lacks obvious therapeutic target and is historically associated with poor prognosis.(3) Genetic profiling has improved our understanding on the heterogeneity of breast cancer,

including TNBC, thus allowing us to deconstruct TNBC into a spectrum of subtypes which differ in cellular origin, tumor characteristic, response towards therapy, and most importantly, prognosis.(4) Triple-negative breast cancer can be broadly classified into basal-like and non-basal-like subtypes according to cytokeratin 5/6 (CK5/6) and epidermal growth factor receptor (EGFR) expression. Basal-like subtype is generally associated with more aggressive tumor characteristic, albeit better response towards systemic chemotherapy.(3) Nevertheless, genetic variation exists in TNBC, proven by its wide intrinsic molecular variation and varied survival rate reported by many studies.(5)

Several biomarkers have been extensively studied to help clinician to stratify prognosis and determine potential treatment modalities for TNBC patients, including androgen receptor (AR) and p53 mutant. Expression of AR and p53 mutant have been associated with worse prognosis and worse response towards systemic chemotherapy in TNBC.(6-8) However, these studies usually consider TNBC as a single disease, thus expression pattern and clinical significance of these biomarkers among TNBC subtypes is not yet adequately studied. This paper aims to perform in-depth analysis on the clinicopathologic feature and prognostic value of basal-like and non-basal-like TNBC patients in an Indonesian tertiary hospital. To the best of our knowledge, this is the first of such study involving Indonesian patients.

Methods

This was a retrospective study conducted at Dr. Sardjito Hospital, Yogyakarta, Indonesia. We consecutively included stage I-IV TNBC patients diagnosed between 2014-2017. Out of 272 detected patients, 205 patients were excluded due to incomplete data, leaving 67 patients included in this study. Clinical data was retrieved from medical records. This study has been approved by the institutional review board of the Ethics Committee Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/ Dr. Sardjito Hospital, Yogyakarta, Indonesia with approval numbers: KE/FK/0751/EC/2018 (first approval) and KE/0286/03/2020 (latest update).

Immunohistochemistry Analysis
Tumor samples were obtained from formalin-fixed paraffin-embedded (FFPE) tissue stored at the Department of Anatomical Pathology, Dr. Sardjito General Hospital, Yogyakarta; Waskhita Laboratory, Yogyakarta; Panti Rapih General Hospital, Yogyakarta; and CITO Laboratory,

Yogyakarta. Block paraffin samples were cut 4 μm in thickness to analyze the expression of p53 mutant, EGFR, CK5/6 and AR by using immunohistochemistry method. Antibody used in this study were Rabbit monoclonal Ab p53 mutant (Clone Ab 32049, dilution 1:50, Abcam, Massachusets, USA), mouse monoclonal Ab EGFR (Clone NCL-L-EGFR, dilution 1:20, Novocastra, Illinois, USA), mouse monoclonal Ab CK5/6 (Clone D5 & 16B4, RTU, Cell Marque, California, USA), and mouse monoclonal Ab AR (Clone NC1-AR-318, dilution 1:50, Novocastra, Illinois, USA), with chromogen DAB. In this study, the membranous expression of CK 5/6 and EGFR in more than 10% of the tumor cells, the nuclei expression of AR in more than 5% of the tumor cells, and nuclei expression of p53 mutant in more than 1 % of the tumor cells were considered positive.(9)

Pathology Assessment
All samples were evaluated independently by 2 senior pathologists. If there was any disagreement between the 2 pathologists, discussion was allowed and consensus had to be made between them. Basal-like characteristic was defined as tumor specimen which expressed EGFR and/or CK 5/6.

Statistical Analysis
Statistical analysis was performed using SPSS version 24 software (IBM Corporation, Armonk, New York, USA). Association between tumor subtype and clinicopathologic feature was analyzed using chi-square test or Fisher's exact test if assumptions for chi-square test were violated. All of the reported p-values are two-sided, with significance level set at $p<0.05$. Survival analysis was performed using Kaplan Meier curve. Overall survival (OS) was defined as duration between diagnosis until event (death) or the end of follow-up (30 months). Cutoff for OS analysis was set at 30 months.

Results

Out of 67 included subjects, 45 were classified into basal-like subgroup dan 22 were classified into non-basal-like. The mean age of the subjects was 51 years old, while the median age was 50.16. Subjects were divided into 2 groups according to its median value, 33 (49.3%) subjects were below 50 years old and 34 (50.7%) subjects were more than or equal to 50 years old. Subjects in basal-like subgroup was significantly younger (57.8% of basal-like subgroup patients

were classified into <50 years old category vs 42.2% in non-basal-like group, $p<0.05$). The mean and median BMI in this study were 24. Forty (59.7%) subjects had BMI <25 and 27 (40.3%) subjects had BMI ≥25. Non-basal-like subgroup has significantly higher BMI (63.7% of non-basal-like patients had BMI ≥25 vs. 28.9% in basal-like group, $p<0.05$). Eleven (16.4%) subjects presented with early stage (I-IIA), 44 (65.7%) subjects presented with locally advanced stage (IIB-IIIC), while 12 (17.9%) subjects presented with stage IV disease. Twenty (29.9%) subjects presented with tumor size (T) <5 cm while 47 (70.1%) subjects presented with T ≥5 cm. Forty-five (67.2%) subjects presented with no nodal involvement (N), while 22 (32.8%) subjects had at least 1 nodal metastasis. The most commonly found histological type was infiltrating ductal, which was found in 55 (82%) subjects, lobular type was found in 6 (9%) subjects, while

the remaining 6 (9%) subjects had variable histological type. There was no significant difference in disease stage, tumor size, nodal status, as well as histological type between basal-like and non-basal-like subjects. P53 mutant was positive in 30 (44.8%) subjects with no significant difference between basal-like and non-basal-like subjects. Androgen receptor expression was found to be significantly higher in basal-like subgroup ($p<0.05$), with all 9 (15%) AR expressing subjects were found in basal-like subgroup (20% of basal-like subjects) (Table 1, Figure 1).

Out of 67 patients included, 34 patients (50.7%) were alive at 30-months follow up, while 33 patients (49.3%) passed away. Twenty-seven patients passed away in the basal-like group, while 6 patients passed away the non-basal like group (60% vs. 21.4%, respectively). The mean of OS in the basal-like group was 23.9 months (95% CI: 21.9-25.9

Table 1. Clinicopathologic profile of basal-like and non-basal-like TNBC.

Parameter	Basal-Like (n=45)	Non-Basal-Like (n=22)	p-value
CK5/6			
Positive	40	0	
Negative	5	22	
EGFR			
Positive	28	0	
Negative	17	22	
AR			0.025*
Positive	9	0	
Negative	36	22	
p53 Mutant			0.983
Positive	20	10	
Negative	25	12	
Type			0.476
Ductal	37	18	
Lobular	3	3	
Other	5	1	
Stage			0.275
Early	9	2	
Locally Advanced	29	15	
Metastatic	7	5	
Tumor Size			0.373
<5 cm	15	5	
≥ 5 cm	30	17	
Nodal Status			0.498
Positive	16	6	
Negative	29	16	
Distant Metastasis			0.51
Present	38	17	
Absent	7	5	
Age			0.046*
<50 y.o	26	7	
≥50 y.o	19	15	
BMI			0.006*
<25	32	8	
≥25	13	14	

* Chi-squared test and fisher exact test were used to analyze this table.
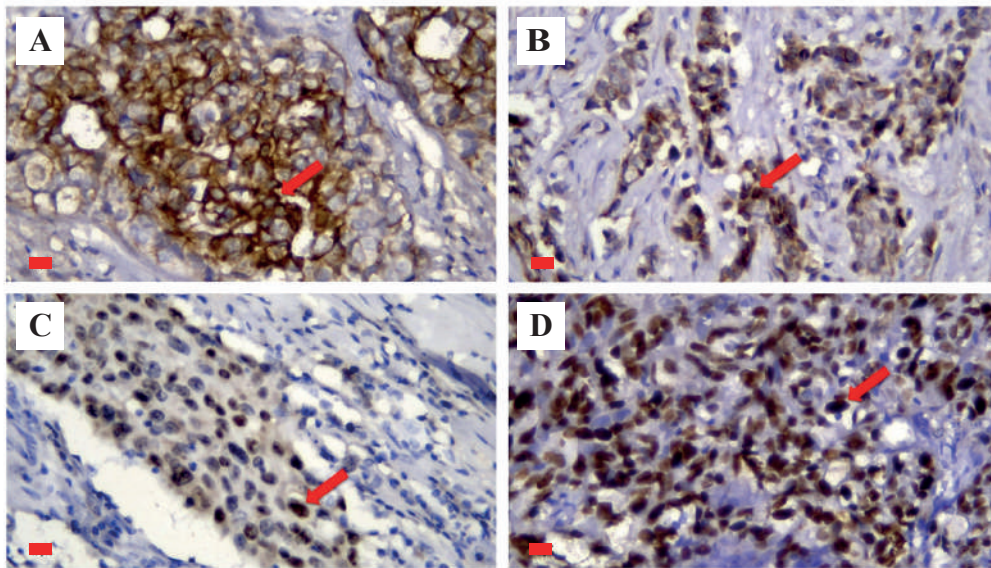
Figure 1. Immunohistochemistry staining result. A: CK 5/6 (red arrow points at positively stained in cytoplasmic membrane and cytoplasm of tumor cell); B: EGFR (red arrow points at positively stained in cytoplasmic membrane and cytoplasm of tumor cell); C: AR (red arrow points at positively stained in nucleus of tumor cell); D: p53 mutant (red arrow points at positively stained in nucleus of tumor cell). Red bar: 10μm.

months), significantly shorter than non-basal-like subgroup of 26.1 months (95% CI: 23-29.2 months; *p*<0.05) (Figure 2, Table 2). Median OS of basal-like subgroup was 25.4 months (95% CI: 17.4-33.3 months), while median OS in non-basal-like subgroup was not yet reached.

Discussion

Studies showed that TNBC has general symptoms found in many diseases, and has diverse histopathological features. Some markers indicate poor prognosis, such as CK5/6 and EGFR, which were also found in the basal-like group. Aligned with other reports, our study also found that basal-like subtype had poor prognosis with shorter OS and found in the younger patients.[9] The underlying mechanism is thought to be associated with the important role of EGFR in tumor cell proliferation, migration, invasiveness, as well as cellular survival.[10]

Basal-like subtpe can be further classified into subgroups of differing prognosis according to expression of numerous biomarkers, including AR dan p53 mutant. Interestingly, in this study we observed increased expression of AR in basal-like subgroup (20% in basal-like *vs.* 0% in non-basal-like; 15% in all study subjects). Androgen receptor is expressed in roughly 28% (12-53%) of all TNBC cases. (11-15) The wide variability of reported AR expression in TNBC is considered due to the absence of standardized method of determining AR positivity. Although the exact role of AR in TNBC is not yet fully understood, several studies consider AR as a negative prognostic factor.(8,16,17) Detrimental effect of AR is hypothesized by inducing cancer cell migration and invasiveness.(6) Vanderbilt classification considered AR+ TNBC as a separate entity (luminal androgen receptor (LAR) subtype) which had lower response rate towards systemic chemotherapy. (18) However, addition of androgen antagonist, including bicalutamide abiraterone acetate (AA) and enzalutamide, to

Table 2. Mean overall survival and survival rate at 30-months of basal-like and non-basal-like TNBC.

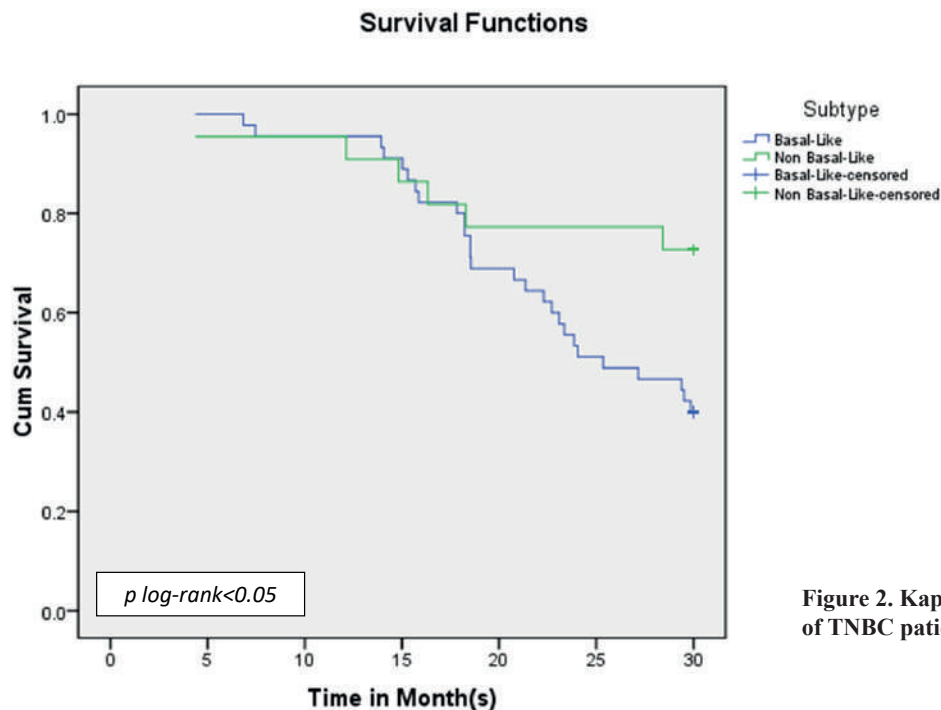| Subtype	Mean Survival (Months)			Survival Rate at 30-Months (n (%))	
	Estimate	95% Confidence Interval		Alive	Deceased
		Lower Bound	Upper Bound		
Basal-Like	23.933	21.971	25.896	18 (40)	27 (60)
Non-Basal-Like	26.109	23.024	29.194	16 (72.7)	6 (27.3)
Overall	24.648	22.967	26.328	34 (50.7)	33 (49.3)

Figure 2. Kaplan-meier overall survival curve of TNBC patients according to subtypes.

AR+ TNBC patients have shown positive clinical response, which might serve as potential treatment modality. (13,19,20) To understand which patient might gain the most benefit from anti-androgen therapy, previous study stratified TNBC into 3 risk groups according to AR and EGFR expression. The best prognosis was observed in AR+ EGFR- (represents LAR subtype) which may benefit the most from anti-androgen therapies; worse prognosis was observed in AR- EGFR+ (represents the basal-like subtype) which may benefit the most from chemotherapy regimens; and intermediate prognosis in AR+ EGFR+ and AR- EGFR- TNBC.(16) Expression of p53 mutant is known to be prevalent in all of breast cancer and has been reported to be associated more aggressive tumor characteristic, including expression of basal-like markers (EGFR and CK5/6). (21) However, in this study we observed no significant association between basal-like subtype and p53 mutant expression. Expression of p53 mutant in our study was found in 30 subjects (44.8%), which is considerably lower than previously reported by other studies (64-82%).(7,22-25) This discrepancy is thought to be influenced by race and genetic as p53 mutant is known to be expressed higher in African-American women and in early onset breast cancer (≤35 years old), as well as in women with familial history of breast cancer, which is considerably different with our cohort of Indonesian patients with mean age of 51 years old. (26,27) The only clinical feature with significant difference between basal-like and non-basal-like subtype in our study is BMI, which higher proportion of subject with BMI ≥25

is observed in non-basal-like subtypes (63.7% *vs.* 28.9%, *p*<0.05). Higher BMI is known to be associated with luminal breast cancer due to the interaction between adipose tissue with hormonal receptors. As such, proportion of patients with BMI <25 is usually higher in TNBC when compared to luminal type.(28) However, there are other roles of adipose tissue in carcinogenesis of breast cancer, including increased pro-inflammatory cytokines, increased mammalian target of rapamycin (mTOR) activation, and alteration of tumor microenvironment, which might explain the increased risk of developing TNBC in women with obesity.(29) The higher BMI observed in our non-basal-like subjects might suggest different process of metabolic carcinogenesis between these 2 subtypes.(30)

This study has several limitations. Our study was a retrospective cohort study with limited sample size, thus further prospective study with bigger sample size and longer follow up period to validate its result is required. We performed analysis of overall survival at 30 months, which is considered as short-term compared to the more commonly reported of 5-years overall survival.

Conclusion

In a cohort of 67 Indonesian TNBC patients, basal-like subtype is associated with shorter overall survival, younger age at diagnosis and higher frequency of AR expression, while non-basal-like subtype is associated with higher BMI.

Acknowledgements

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