RESEARCH ARTICLE

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Prognostic Values of CD8+, PARP, and EGFR on Overall Survival in Patients with Triple-Negative Breast Cancer

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Abstract

Objective: This study aimed to investigate the associations of *CD8+*, *PARP,* and *EGFR* expressions with two-year survival in patients with triple-negative breast cancer (TNBC). **Methods:** A retrospective cohort study was conducted in a national cancer center. All patients aged 18 years diagnosed with TNBC (2013-2017) were included and followed for 24 months or until the patients were deceased. Kaplan-Meier survival function and Cox proportional hazard model were applied for the analyses. **Results:** The study population was followed for 24 months (2,692 person-months, N = 126). At the end of the follow-up, 27 patients were deceased. The two-year mortality rate was 10 per 1,000 person-month. Kaplan-Meier graphs showed that after approximately one year of follow-up, poorer survival was seen in patients with low *CD8+*, positive PARP, and positive EGFR. The adjusted analysis found staging as the main predictor of overall survival in TNBC (HR = 7.20, 95% CI= 2.07 – 25.00). **Conclusions:** Patients with low *CD8+*, positive PARP, and positive *EGFR* expressions seem to be associated with poorer overall survival in TNBC. After approximately one year of follow-up, higher survival was observed in patients with high *CD8+*, negative *PARP*, and negative *EGFR*. Staging remains the main predictor of TNBC survival. Therefore, early detection and treatment of TNBC are essential to improve survival.

Keywords: TNBC- *CD8+*- PARP- EGFR- overall survival- histopathology- immunohistochemistry

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Introduction

Female breast cancer has become the most common cancer worldwide, with more than 2 million new cases in 2020 [1]. Of all breast cancer cases, 15-20% is contributed by triple-negative breast cancer (TNBC), which is marked by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression [2]. Patients with TNBC had lower overall survival and 5-year disease-free survival than patients with non-TNBC at the same cancer stage [3-5]. Moreover, compared to other subtypes of breast cancer, TNBC was associated with an increased risk of metastasis [5], particularly to the lung and central nervous system [6].

The tumor microenvironment, including inflammatory and immune cells, has essential roles in breast cancer as it might promote or suppress tumor growth [7]. Therefore, understanding the expression of these histological markers is essential to predict and subsequently improve treatment outcomes in TNBC.

Tumor-infiltrating lymphocyte (TIL) is one element of the tumor-immune microenvironment (TIME) linked to prognostic relevance in patients with breast cancer. Previous research has suggested that TIL density in TIME corresponds with the survival of patients with ER-negative and HER2-negative subtype breast cancer, which is observed to be achieved by innate and adaptive immune molecular processes [8,9]. Aside from the fact that molecular mechanisms can already explain why TNBC patients with high *CD8+* scores have aberrant cytokine expression that plays a role in cancer apoptosis, thus increasing TNBC survival, *CD8+* expression in TNBC still yields conflicting and variable results.

Survival in TNBC patients is also known to be influenced by DNA repair mechanisms, one of which is played by *PARP* expression. *PARP* plays a role in repairing single-strand DNA breaks through the base excision repair (BER) pathway, yet it is known that positive *PARP* expression describes tumor cells with mutations in BRCA1/BRCA2 which have a broken function of repairing damaged double-strand DNA due to reduced function of homologous recombinant (HR) [10].

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Studies have proven that *PARP* expression is significantly associated with BRCA1 status in basal-like and TNBCs; hence it is envisaged that *PARP* testing is able to replace the currently expensive BRCA mutation testing to facilitate the selection of BC patients who are eligible for *PARP* inhibitor therapy [11].

Furthermore, it is known that the epidermal growth factor receptor (*EGFR*), which expression is upregulated in TNBC, can also stimulate breast cancer cell proliferation and development as an initiator of signal transduction pathways. *EGFR* expression is reported to be present in at least 50% of TNBC cases, implying a high potential for targeted therapy [12].

Several molecular studies had shown the interaction between *CD8+*, *PARP*, and *EGFR*. Chu et al. (2020) [13] reported in their study that *EGFR*, as one of the receptor tyrosine kinases (RTKs), simultaneously interacts with c-MET, promoting the phosphorylation of *PARP1* at the Tyr907 residue, hence contributing to *PARP*-inhibitor (*PARPi*) resistance. The interaction between *PARP* and *CD8+* is further compiled by a study conducted by Pantelidou et al. (2019) [14], finding that *PARP* inhibitor (olaparib) was known to be able to induce *CD8+* T cell infiltration and activation in vivo; thus, *PARPi*-resistance in TNBC would deplete the employment of *CD8+* T cells in TIME. Furthermore, TNBC with higher *EGFR* expression was also known to have a lower fraction of immune cells: *CD8+* T cells, CD4+ T cells, and M1 macrophages and pro-cancer immune cells, Th2 and M2 macrophages, resulting in a low level of cytolytic activity in TNBC [15].

The study of the association between *CD8+*, *PARP*, and *EGFR* expressions remains scarce and is poorly established. Hence, understanding the expression and interaction between these molecular activities as well as their association with overall survival may provide a better understanding and rationale of the therapy of choice in TNBC.

Materials and Methods

We conducted a retrospective cohort study at Dharmais National Cancer Center Hospital, Jakarta, Indonesia. We included all patients aged ≥18 diagnosed with TNBC and who underwent treatment in our institution between 2013 and 2017. Patients were followed for 24 months. The exclusion criteria were an incomplete medical record, loss to follow-up within two years of initial diagnosis, or having another primary cancer than breast cancer. Data were retrieved from patients' medical records. If two-year follow-up data was unavailable in the medical record, a phone interview was made to determine the survival. Informed consent was obtained from all patients.

Staging of TNBC was defined according to the American Joint Committee on Cancer Breast Cancer Staging System [16] and the treatment response was determined based on the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) guideline [17].

Immunohistochemical Staining for CD8+, PARP, and EGFR

Immunohistochemistry analysis for *CD8+*, *PARP*, and *EGFR* was performed using formalin-fixed, paraffin-embedded tissue blocks.

For *CD8+* expression analysis, the paraffin-embedded tissue blocks were sliced into 4 µm sections and quantitatively analyzed by viewing each section with at least five high-power fields (x 40 objective and x 10 eyepiece) and the most abundant TILs to determine the percentage of expression: (1) Each slide was examined to identify the 5 hpfs (x 40) with the most abundant TILs, (2) TILs were counted as a percentage against the tumor tissue background in those 5 hpfs with most abundant TILs, using the automated image analyzer, and (3) The percentage of *CD8+* TILs, in this case, was calculated based on the median percentage of those 5 hpfs. Counting was utilized using an automated image analyzer. Additionally, densities were quantified in designated regions of interest at magnifications ranging from 200x to 400x. The median field was used to calculate the percentage of TILs in each case. The patients were categorized further into high (*CD8+* TILs ≥ 10%) and low (*CD8+* TILs < 10%) [18].

The polymer Envision detection system, the Dako EnVision TM kit (Dako, Copenhagen, Denmark), was used for immunohistochemical staining for *PARP* expression. Tissue sections ranging in thickness from 3-5 mm were deparaffinized in xylene and rehydrated in graded alcohol. Slides were incubated in 3% hydrogen peroxide for 10 minutes to inhibit endogenous peroxidase. Dako target antigen retrieval solution (pH 6.0) was utilized. The slides were subsequently incubated with polyclonal rabbit anti-human antibody against *PARP*. The reaction was pictured by incubating the sections with diaminobenzidine (DAB) for 15 minutes, which later continued with Mayer's hematoxylin. *PARP1* nuclear expression was evaluated based on positive cells distribution. Positive *PARP* was defined as 1+ to 3+ nuclear staining in 5% of tumor cells [19].

EGFR immunohistochemical staining was conducted after overnight heating at 37°C that was applied on 4 µm thick formalin-fixed paraffin sections. Following deparaffinization, rehydration, and antigen retrieval were carried out in a microwave oven by heating the slides for 15 minutes in a single step with EDTA. (pH 8.0). Normal horse serum was administered for 30 minutes after rinsing with Tris-buffered saline to inhibit nonspecific antibody binding. The sections were then incubated with the primary antibody overnight at 4°C. For visualization, a three-step technique was used utilizing diaminobenzidine as a chromogen. Finally, the sections were counterstained with hematoxylin and mounted. A 1:100 dilution of mouse anti-human monoclonal antibody against *EGFR* was employed [20].

The scoring system developed by Putti and colleagues (2002) was used to evaluate *EGFR*. The extent of *EGFR* immunoreactivity was scored as 0 for less than 5% positive cells, 1 for 5% to 9% positive cells, 2 for 10% to 50% positive cells, and 3 for more than 50% positive cells. The intensity of *EGFR* immunoreactivity was assigned a value of 1 for weak staining, 2 for moderate staining,

and 3 for strong staining. The overall staining score for each case was calculated by multiplying the extent of the immunoreactivity score by the intensity score. Cases with an overall score ≥1 were considered positive [20].

Statistical analysis

Descriptive analyses are presented as mean (standard deviation) or median (Q1 – Q3) depending on the data distribution. Categorical variables are presented as proportions. Survival analyses were performed using the Kaplan-Meier survival function and Cox proportional hazard model. A log-rank test was applied to assess the difference between survival functions in the Kaplan-Meier graph. Some Cox proportional hazard models were applied to determine the effect of each independent variable on survival. *CD8+*, *PARP*, and *EGFR* were the main exposures. Therefore, they were included in the final model regardless of the P-value in the univariate analysis. Other variables with a P-value <0.125 in the univariate analysis were also included in the final model. The proportionality assumption for each model was examined using commands stphplot and stcoxkm. The hazard ratio (HR) and its 95% confidence interval (CI) are presented. Statistical significance was determined if the P value <0.05.

Results

A total of 126 women with TNBC were included in the present study. The mean age was 46 ± 10.9 years. Half of the study population was in stage IIIB or IV. Additionally, patients who were overweight or obese made up approximately half of the study population as well. Low *CD8+* expression was found in 71% of patients, positive *PARP* in 6%, and positive *EGFR* in 46% of patients. Clinical characteristics are shown in Table 1.

The study population was followed for 24 months (2,692 person-months). A total of 27 patients were deceased. The two-year mortality rate was 10 per 1,000 person-month. Median survival was not reached during the 24 months of follow-up (Figure 1).

Association between 2-year mortality and clinical characteristics, CD8+, PARP, and EGFR

Univariate analyses showed that advanced staging and a high Ki67 index were associated with a higher mortality rate (P <0.001 and 0.035, respectively). White blood cells and platelets before chemotherapy were positively associated with a mortality rate (HR for white blood cells = 1.11 [1.02 - 1.21]; HR for platelets = 1.00 [1.00 - 1.01]). No association between mortality rate and age, BMI, or comorbidities was observed (Table 2).

Neither *CD8+*, *PARP*, nor *EGFR* was statistically associated with the mortality rate (Table 2). However, after approximately one year of follow-up, higher survival was observed in patients with high *CD8+*, negative *PARP*, and negative *EGFR* (Figure 2-4). Adjusted analysis showed staging as the main predictor for 2-year mortality (HR = 7.20, 95% CI = 2.07 - 25.00, P = 0.002). *CD8+*, *PARP*, and *EGFR* were not associated with 2-year mortality in the adjusted analysis (Tables 3 and 4).

Table 1. Patients' Characteristics

Variable	n	%
Demographic variables		
Age (years)	46.0 (10.9) [a]	
Body mass index (BMI) (kg/m^2)	24.6 (4.1) [a]	
Underweight	7	5.6
Normal weight	58	46
Overweight	50	39.7
Obesity	11	8.7
Comorbidities		
Diabetes	13	10.3
Hypertension	24	19.1
Clinical variables		
Staging		
I	1	0.8
II	48	38.1
IIIA	13	10.3
IIIB	47	37.3
IV	17	13.5
Hemoglobin (g/dL)	12.2 (11-13.5) [b]	
White blood cell (x 10^3/uL)	7.8 (6.6-9.8) [b]	
Platelets (x 103/uL)	322.5 (265-380) [b]	
Chemotherapy regiment		
Doxorubicin-based	70	55.5
Taxane-based	50	39.7
Mixed	6	4.8
Chemotherapy type		
Neoadjuvant	42	33.3
Adjuvant	81	64.3
Palliative	3	2.4
Treatment response (N = 44)		
Complete response	4	9.1
Partial response	29	65.9
Stable disease	1	2.3
Progressive disease	10	22.7
Histopathology characteristics		
Ki67	60 (14-80) [b]	
Low	32	25.4
High (≥20%)	94	74.6
CD8+		
<10%	89	70.6
10-40%	29	23
>40%	8	6.4
PARP		
Negative	118	93.6
Positive	8	6.4
EGFR		
Negative	68	54
Positive	58	46

[a], Data are shown in mean (standard deviation); [b], Data are shown in median (Q1-Q3)

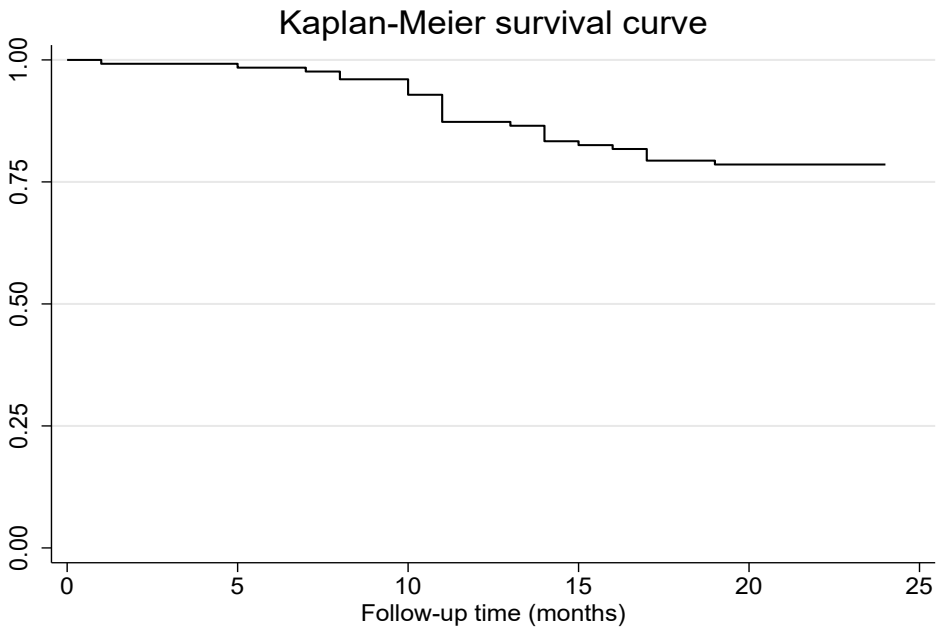

Figure 1. Kaplan-Meier Overall-Survival Curve of All Patients

Immunohistochemistry Staining of CD8+, PARP, and EGFR

Immunohistochemical staining of *CD8+*, *PARP*, and *EGFR* was performed on all subjects and the grouping was done accordingly. Figures 5, 6, and 7 represent the results of each staining.

Discussion

Breast cancer is the most common type of cancer in Indonesia, accounting for the majority of new occurrences each year (Globocan, 2020). The clinical burden of TNBC remains high, owing to high mitotic characteristics, higher tumor grade, and limited therapeutic options due to the absence of hormone receptors and HER2. In addition, TNBC is also more common in younger people when compared to other breast cancer subtypes.

In this retrospective cohort study, TNBC was found at an average age of 46 ± 10.9 years, with half of the subjects diagnosed at an advanced stage (stage IIIB or IV). This is in line with a previous study by Thakur et al. (2018) [21] in India which found that the peak age prevalence of TNBC was in the age group of 40-55 years with a mean age of 50 years. The study by Thakur et al. (2018) [21] also cited a study done by Singh et al. (2021) [10] which found that the TNBC subtype was prevalent among 31.57% of

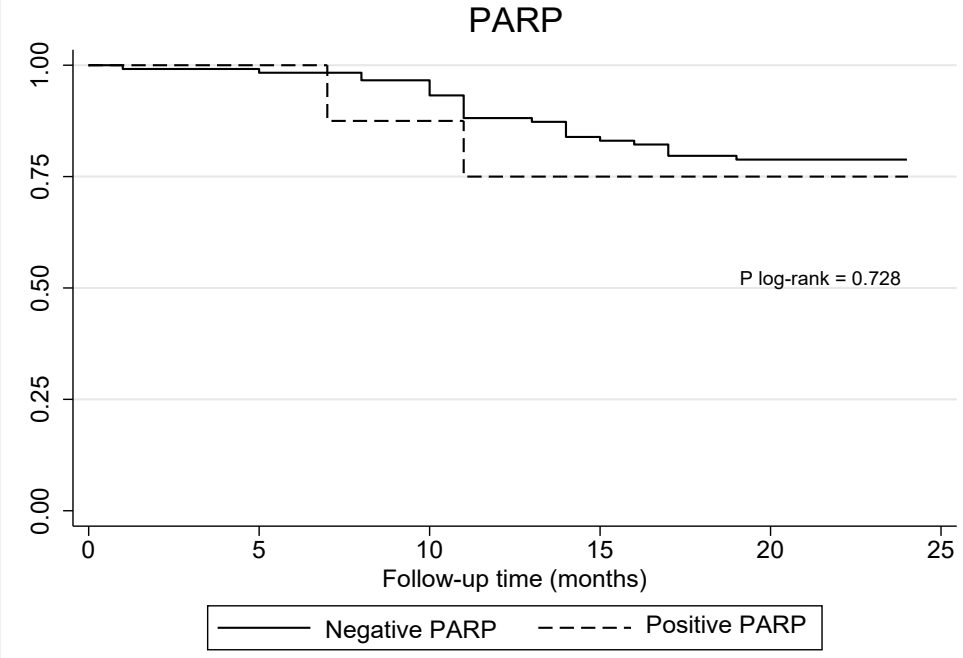

Figure 2. Kaplan-Meier Overall-Survival Curve by PARP

Table 2. Association between Clinical Characteristics and 2-Year Mortality

Variable	2-year mortality		Univariate analysis	P
	No	Yes	HR (95% CI)	
Demographic variables				
Age (years)	46.4 (10.5)	44.3 (12.3)	0.98 (0.95 - 1.02)	0.371
Body mass index (BMI) (kg/m^2)	24.8 (3.9)	24.9 (5.0)		
Underweight	6 (6.1)	1 (3.7)	ref	ref
Normal weight	44 (44.4)	14 (51.8)	1.64 (0.21 - 12.44)	0.635
Overweight	41 (41.4)	9 (33.3)	1.17 (0.15 - 9.27)	0.879
Obesity	8 (8.1)	3 (11.1)	1.83 (0.19 - 17.64)	0.6
Comorbidities				
Diabetes	10 (10.1)	3 (11.1)	1.07 (0.32 - 3.56)	0.911
Hypertension	20 (20.2)	4 (14.8)	0.68 (0.24 - 1.97)	0.481
Clinical variables				
Staging				
I	1 (1.0)	0 (0)	ref	ref
II	48 (48.5)	0 (0)		
IIIA	10 (10.1)	3 (11.1)		
IIIB	37 (37.4)	10 (37.0)	8.98 (2.70 - 29.84)	**<0.001**
IV	3 (3.0)	14 (51.9)		
Hemoglobin (g/dL)	12.2 (11-13.5)	12.3 (10.7-13.8)	1.07 (0.85 - 1.35)	0.578
White blood cell (x 10^9/L)	7.6 (6.5-9.5)	9.3 (6.8-13.3)	1.11 (1.02 - 1.21)	**0.013**
Platelets (x 10^9/L)	314 (264-373)	368 (306-413)	1.00 (1.00 - 1.01)	**0.008**
Chemotherapy regiment				
Doxorubicin-based	55 (55.6)	15 (55.6)	ref	ref
Taxane-based	39 (39.4)	11 (40.7)	1.04 (0.48 - 2.27)	0.919
Mixed	5 (5.0)	1 (3.7)	0.76 (0.10 - 5.77)	0.792
Chemotherapy type				
Neoadjuvant	24 (24.2)	18 (66.7)	ref	ref
Adjuvant	74 (74.8)	7 (25.9)	0.16 (0.07 - 0.38)	**<0.001**
Palliative	1 (1.0)	2 (7.4)	1.82 (0.42 - 7.88)	0.42
Histopathology characteristics				
Ki67	60 (10-80)	70 (40-80)	1.01 (1.00 - 1.02)	0.097
Low	30 (30.3)	2 (7.4)	ref	ref
High	69 (69.7)	25 (92.6)	4.72 (1.12 - 19.91)	**0.035**
CD8+				
Low	67 (67.7)	22 (81.5)	ref	ref
High	32 (32.3)	5 (18.5)	0.51 (0.19-1.33)	0.168
PARP				
Negative	93 (93.9)	25 (92.6)	ref	ref
Positive	6 (6.1)	2 (7.4)	1.29 (0.30-5.43)	0.731
EGFR				
Negative	56 (56.6)	12 (44.4)	ref	ref
Positive	43 (43.4)	15 (55.6)	1.54 (0.72-3.29)	0.266

Hazard ratios (HR), 95% confidence intervals (95% CI), and P values were calculated using Cox proportional hazard. No assumptions of Cox proportional hazard were violated; Bold fonts indicate statistical significance.

Table 3. Association of CD8+, PARP, and EGFR with the 2-Year Mortality Rate

	Adjusted HR	95% CI	P
High CD8+ (vs low CD8+)	0.5	0.19 - 1.31	0.158
Positive PARP (vs. negative PARP)	1.12	0.26 - 4.82	0.877
Positive EGFR (vs. negative EGFR)	1.56	0.72 - 3.36	0.258

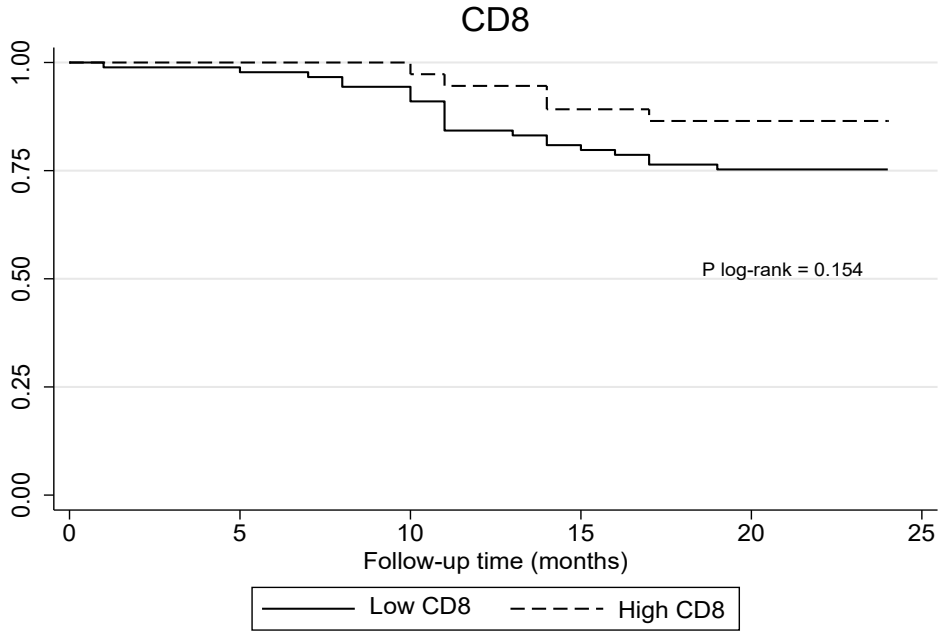

Figure 3. Kaplan-Meier Overall-Survival Curve by CD8+

Table 4. Multivariate Analysis of Factors Associated with the 2-Year Mortality Rate

	Adjusted HR	95% CI	P
Stage IIIB / IV (vs. stage I-IIIA)	7.2	2.07 - 25.00	0.002
Ki67 (vs low Ki67)	3.53	0.80 - 15.46	0.095
White blood cell (x 109/L)	1	0.92 - 1.11	0.848
Platelets (x 109/L)	1	0.99 - 1.00	0.503
High CD8+ (vs low CD8+)	0.51	0.19 - 1.37	0.178
Positive PARP (vs. negative PARP)	0.86	0.19 - 3.70	0.837
Positive EGFR (vs. negative EGFR)	1.53	0.69 - 3.33	0.288

Fully adjusted Cox proportional hazard model

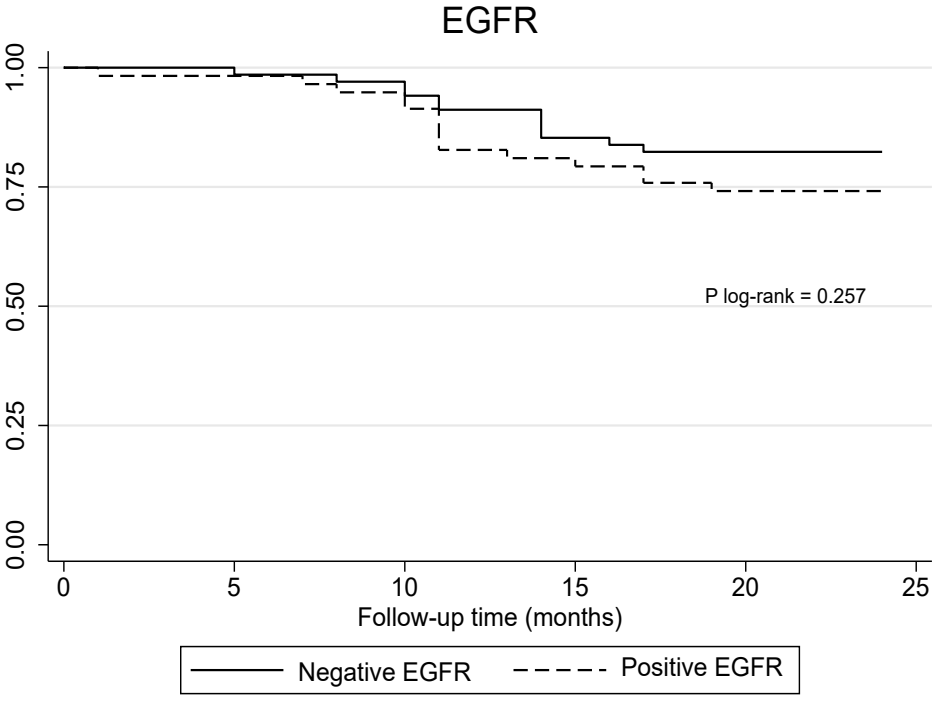

Figure 4. Kaplan-Meier Overall-Survival Curve by EGFR

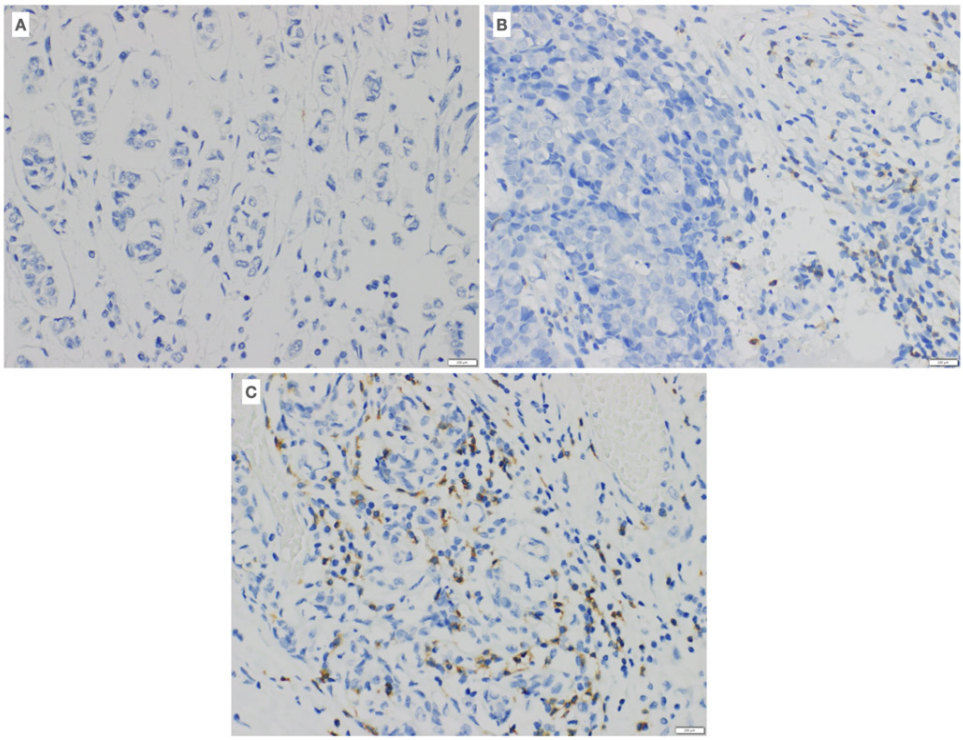

Figure 5. CD8+ Staining on Breast Cancer Tissues. Results were Grouped into Three Categories: (A) Negative CD8+ staining on breast cancer tissues, (B) CD8+ percentage of 10-40%, and (C) CD8+ percentage of above 40%. These categories were later categorized further into low (CD8+ < 10%) and high (CD8+ ≥ 10%).

younger women and was found to be the highest among all races (Indian, Chinese, Hispanic, non-Hispanic White, and African-American). The evidence of higher staging in patients of reproductive age shows that TNBC subtype breast cancer carries a higher clinical and economic burden since TNBC patients cannot benefit from hormonal or anti-HER2 therapy which challenges therapeutic strategy. Our study also proved and confirmed that staging, among other clinical aspects, took a major role as a key predictor in TNBC, making it crucial in daily practice to find and diagnose breast cancer as early as possible to lessen the clinical and economic burden of breast cancer, particularly in the TNBC subtype.

Another notable clinical finding in our study was that nearly half (48.4%) of our study sample was overweight or obese (BMI ≥ 25 kg/m^2). Although BMI had no statistically significant effect on overall survival in our analysis, it is worth noting that, consistent with prior research, there is a clear correlation between overweight or obesity status and the incidence of TNBC. Obesity or overweight is known to enhance the initiation, progression, and aggressive biology of TNBC through insulin on Akt/mTOR (mammalian target of rapamycin) signaling and glycolysis; obesity-mediated tissue inflammatory cytokines, namely leptin, and activation of signaling pathways that promote invasion and metastasis; and obesity, immune cell switching, and a

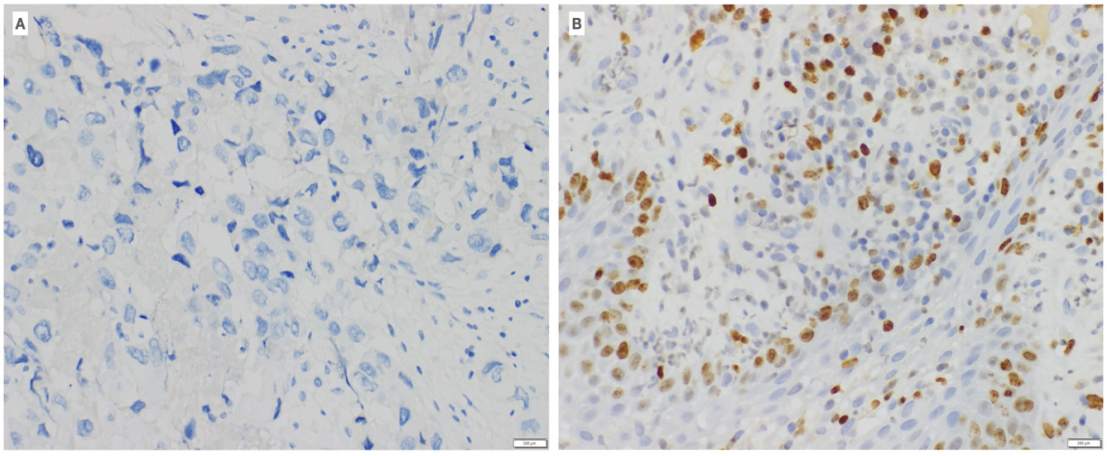

Figure 6. Immunohistochemical Staining for *PARP* Expression. *PARP1* nuclear expression was evaluated based on positive cells distribution and subsequently grouped into: (A) Negative *PARP* expression and (B) Positive *PARP* expression.

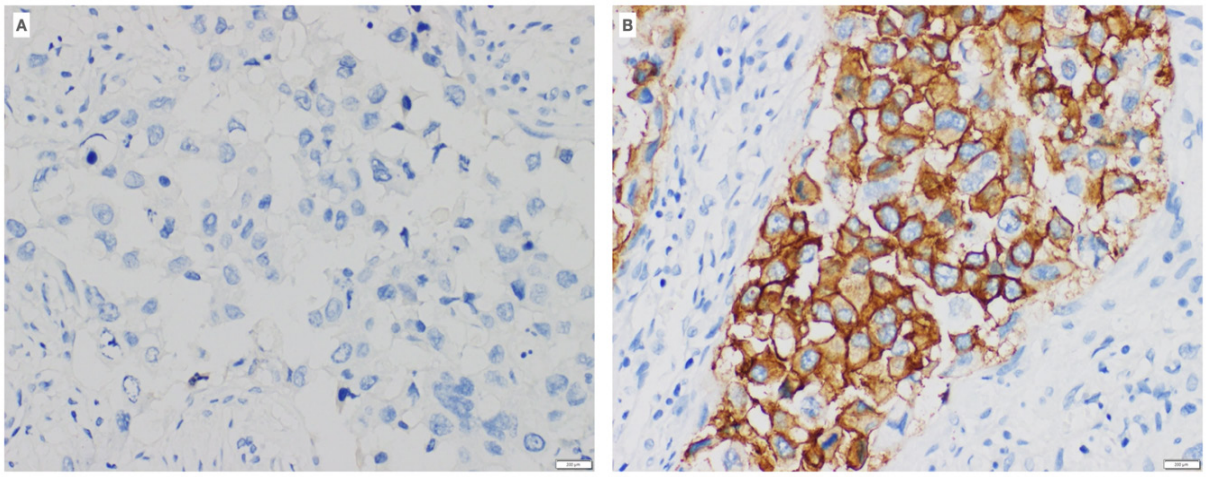

Figure 7. *EGFR* Immunohistochemical Staining on Breast Cancer Tissues. The scoring system by Putti et al. was used to determine *EGFR* expression which later grouped into: (A) Negative *EGFR* expression and (B) Positive *EGFR* expression, which determined by the intensity of EGFR immunoreactivity's value of 1 for weak staining, 2 for moderate staining, and 3 for strong staining.

protumorigenic tissue microenvironment [22].

This study also showed that TNBC patients with high *CD8+* seemed to have a higher overall two-year survival. Moreover, TNBC patients with negative *EGFR* and *PARP* tended to have higher survival after a year of follow-up.

Our study confirms previous findings by Matsumoto et al. (2016) [23], Vihervuori et al. (2019) [24], and Oshi et al. (2020) [8], proving that *CD8+* expression was associated with favorable survival in TNBC patients. Moreover, a recent study involving all breast cancer subtypes highlighted that high *CD8+*expression was associated with better 10-year overall survival in TNBC but not other breast cancer subtypes [8]. This is achieved through the molecular mechanism of TIL in TIME via cytotoxic T cells, helper T cells, B lymphocytes, macrophages, natural killer (NK) cells, and dendritic cells [9] which density is higher in TNBC patients. The higher the density of TILs detected in TNBC, the higher the expression of interferon (IFN)-α dan IFN-γ [8], which promotes an increase in cancer cell death.

Higher *PARP* expression has been suggested to be associated with lower disease-free survival [25] and poorer overall survival [26] in the general breast cancer population. Additionally, a meta-analysis found higher *PARP* expression in TNBC than in other subtypes of breast cancer [25]. The present study found no difference in survival among TNBC patients with positive *PARP* expression compared to those with negative *PARP* in the first year of follow-up. However, in the second year of follow-up, patients with positive *PARP* seemed to have worse survival although not statistically significant. This poorer survival rate is related to the single-stranded DNA damage repair mechanism via the base excision repair (BER) pathway. Furthermore, high *PARP* expression defines tumor cells with BRCA1/BRCA2 mutations, further promoting DSBs accumulation due to ineffectual homologous recombination [10]. In comparison with wild-type BRCA patients, the negative consequences of BRCA mutation not only increased the incidence of BC but also led to pathological progression with higher tumor grade. However, larger sample sizes and longer follow-up times should be considered in future research to explore the role of *PARP* in overall survival among patients with TNBC.

EGFR has been known to promote cell proliferation, growth, and survival [7]. While previous studies showed the role of *EGFR* in promoting cell proliferation and invasion in TNBC [27], the present study adds that *EGFR* seemed to be associated with poorer prognosis in TNBC, particularly after a year of follow-up. Our finding was in line with a meta-analysis reporting *EGFR*-overexpression was associated with poorer overall survival in women with early stage of TNBC [27]. This occurs because cancer cell proliferation and progression are controlled by signaling pathways initiated by the *EGFR* receptor and mediated by PI3 kinase, Ras-Raf-MAPK, JNK, and PLCγ. At the cellular level, the ligand not only initiates cell proliferation but also alters adhesion and motility and prevents cell apoptosis, while at the physiological level, the ligand promotes invasion and angiogenesis [12]. In a study published in 2020, Chu et al [13]. found that c-MET, as one of the receptor tyrosine kinases (RTK), interacts with *PARP1* to increase the enzymatic activity of *PARP* and decrease its affinity for *PARP* inhibitors.

EGFR might be able to be used to determine TNBC prognosis, but studies in other populations are needed to confirm our findings. It is also necessary to have a consensus on cut-off values for *EGFR*-positive to reduce heterogeneity, given that previous studies have used various cut-off values. Moreover, although most studies investigating *EGFR* inhibitor targeted therapy on TNBC did not show promising results [28, 29], identifying TNBC patients with *EGFR* overexpression might predict which patients are more likely to benefit from the targeted treatment.

Although it was profoundly regarded that the expressions of *CD8+*, *PARP*, and *EGFR* individually and simultaneously correlated with the survival of TNBC patients, the statistical analysis of our study showed

otherwise: the effects of *PARP*, *CD8+*, and *EGFR* were attenuated, particularly after staging was included in the multivariate analysis. This emphasizes staging as the main predictor of survival in TNBC regardless of how the expression *CD8+*, *PARP*, and *EGFR* are. Hence, identifying patients with TNBC in the early stage is indeed a greater necessity.

Our findings add to a body of evidence that *CD8+*, *EGFR*, and *PARP* might have important prognostic values in TNBC. Nonetheless, this study also found that the expression of *CD8+*, *EGFR*, and *PARP* yields a potential targeted therapy as well as immunotherapy in TNBC. However, there were some limitations in our study. Firstly, the TNBC stage and treatment regimen varied in this study and thus might affect the results. Secondly, the follow-up time was relatively short, and the median survival was not reached. Finally, as the patients were recruited from a tertiary hospital, the external validity might be limited.

In conclusions, the present study suggests higher *CD8+* expression is associated with favorable survival in TNBC patients. Our study also indicates poorer overall survival in patients with positive *PARP* and *EGFR* expression, particularly after a year of follow-up. Staging remains the main predictor of overall survival in TNBC.

Author Contribution Statement

RIP, SJH, and NS designed the concept of the study. All authors involved in the definition of intellectual content, literature search, clinical and experimental studies, data acquisition, data analysis, and statistical analysis. RIP, BB, and YHP prepared and edited the manuscript while SJH and NS reviewed it. All authors have read and approved to the published version of the manuscript, and each author believes that the manuscript represents honest work.

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Conflict of interest

The authors declare no conflicts of interest.

Ethics approval statement

The evaluation of research ethics was conducted by the Ethics Commission of Dharmais National Cancer Center Hospital, Indonesia, under the reference number 018/KEPK/IV/2017, adhering to the principles outlined in the Declaration of Helsinki. Researchers ensure the confidentiality of all data collected during the study.

Patient consent statement

Informed consent was obtained according to national regulations.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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