Correlation between BRCA1 expression and the advanced stage of triple-negative breast cancer

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Abstract. Triple-negative breast cancer (TNBC) is highly aggressive and has a poor prognosis. TNBC is commonly reported in young women and often relapses quickly, exhibiting aggressive characteristics. It is also linked to a loss of function of BRCA1. Patients with BRCA mutations require different treatments because this tumor type is sensitive to platinum-based chemotherapy regimens and inhibitors of the poly (ADP ribose) polymerase. The present study aimed to investigate the prognostic significance of BRCA1 expression in Indonesian patients with TNBC. The study included 57 patients with TNBC. Epidermal growth factor receptor and cytokeratin 5/6 immunostaining were used to classify TNBC into basal-like and non-basal-like subtypes. The BRCA1 expression was also determined using immunohistochemistry. Pearson's Chi-square analysis and Fisher's exact test were used to examine correlations between variables. Kaplan-Meier method was used to analyze the survival rate. Patients with TNBC had an average age of 55.18±10.014; most of them were ≥50 years-old, had high-grade tumors (75.4%), and were in the advanced stages of cancer (82.5%). The majority had no specific type of cancer (78.9%), received non-platinum-based therapies (64.9%), had basal-like subtypes (72.9%), and were still alive (56.1%). Negative BRCA1 expression was higher (52.6%) than positive expression (47.4%) and correlated with advanced cancer stage (P=0.035). However, the BRCA1 expression was not correlated with other clinicopathological variables and the types of therapy. Survival analysis showed that the stage and BRCA1 expression acted as insignificant prognostic factors in patients with TNBC (P=0.091 and P=0.150). In the present study, negative BRCA1 expression was correlated with advanced stage but did not act as a prognostic factor in Indonesian patients with TNBC.

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Introduction

Triple-negative breast cancer (TNBC) refers to any breast cancer that does not express the genes for the estrogen receptor, progesterone receptor and HER2. TNBC accounts for 10-15% of all breast cancers. These cancers tend to be more common in women aged <40 years, who are African American, or who have a BRCA1 mutation. This tumor differs from other breast cancer subtypes because it grows and spreads faster, has limited treatment options, and has a worse outcome (1,2). A previous study by the authors found that the frequency of TNBC cancers was high (29.3%), with large size, high-grade, and 70% with lymph node metastasis (3).

TNBC is unresponsive to endocrine therapy or other available targeted agents. Cytotoxic chemotherapy remains the primary treatment for TNBC disease, along with surgery and/or radiotherapy (4-6). Novel drug developments in TNBC include antibody-drug conjugates, immune checkpoint inhibitors, PARP inhibitors, and androgen receptor-targeted agents (7).

TNBC is partly a basal-like subtype, with increased expression of basal cytokeratins, such as cytokeratin (CK) 5/6, CK 17 and epidermal growth factor receptor (EGFR). Basal-like cancer occurs mainly in young women, often relapsing rapidly, with aggressive characteristics such as high-grade, high-proliferation indexes, p53 mutation, EGFR overexpression, c-MYC amplification, loss of phosphatase and tensin analog tumor suppressor gene, and the loss of function of BRCA1 (8-10). High recurrence and poor response to chemotherapy in TNBC are probably due to the presence of basal-like cancer (11).

The identification of BRCA mutations in patients with TNBC can have a significant effect on treatment. The BRCA mutation status of patients with TNBC may predict the response to treatment with inhibitors of poly (ADP ribose) polymerase (PARP) (12,13). Identification by immunohistochemistry (IHC) is a simple and reliable method to access the expression of BRCA1 protein in tumor tissues. The present study aimed to investigate the prognostic and predictive value of BRCA1 expression by using the IHC method in TNBC.

Materials and methods

Data collection. A total of 57 samples of patients with TNBC received from Sardjito General Hospital (Yogyakarta, Indonesia) from January 2015 to December 2020 were used in the present retrospective study. Samples included patients who underwent breast surgery with axillary dissection and had never received neoadjuvant therapy. Clinicopathological data were collected from the medical records. The present study was conducted after obtaining permission from the ethics committee of Faculty of Medicine, Public Health and Nursing, University Gadjah Mada/Sardjito General Hospital (approval no. KE/FK/1291/E1; date, December 2021; Yogyakarta, Indonesia) and patient consent for sample collection was waived by the ethics committee.

IHC examination. Out of 57 samples, only 48 sample cases were found eligible to be stained. Tissues were fixed in 10% Neutral Buffered Formalin (NBF) for 24 h at room temperature. The paraffin-embedded sections were cut into a 3-μm slice and subjected to deparaffinization in xylene, rehydrated in series grade of ethanol, and incubated with 3% H_2O_2 for 20 min. SNIPER Reagent (BioCare Medical) were used as blocking agent for 20 min at room temperature. The sections were incubated with the primary antibodies of EGFR and CK 5/6 and observed under light microscope at x400 magnification (CX33; Olympus Corporation) to classify TNBC into basal-like and non-basal-like. The expression of CK 5/6 and EGFR was considered positive when stained in >10% of the tumor cells and defined as negative when stained in <10% of the tumor cells. Based on CK 5/6 and EGFR IHC results, TNBC was divided into basal-like TNBC when deemed positive for either or both CK 5/6 (1:100; cat. no. 6057682) and EGFR (1:100; cat. no. 6067929; both from Novocastra Laboratories Ltd.) and non-basal-like TNBC when both CK 5/6 and EGFR were negative (14) (Fig. 1). All samples were stained by IHC using a primary monoclonal antibody against the BRCA1 mutation (clone MS110; CM; 1:100; cat. no. 345A.C; BioCare Medical, LLC) to identify the expression of BRCA1 in TNBC tissues. The primary antibody was incubated for 60 min at room temperature, followed by incubation with the secondary antibody for 30 min at room temperature. Chromogen DAB was used to visualize the BRCA1 protein expression. BRCA1 was positive if the expression was in the tumor nuclei. A nuclear stain that appeared brownish and accounted for <20% of the nucleus is considered negative, while nuclear staining that accounted for >20% is considered positive, according to the American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update 2013 (15) (Fig. 2). The BRCA1 expression was determined using ImageJ software version 1.54 (National Institutes of Health) with a median cut-off of 30%.

Statistical analysis. Statistical analysis was performed with SPSS Version 26 (IBM Corp.) and presented in ± standard deviation. Pearson's Chi-square test was used to analyze the correlation between several variables, including age, grade, stage, histological type, type of therapy and TNBC subtype. Fisher's exact test was used to analyze stage. Follow-up survival was started in January 2019 and completed in March 2023. Survival was analyzed using Kaplan-Meier (not followed by the log-rank test) and $P<0.05$ was considered statistically significant.

Table I. Characteristics of the TNBC samples (total n=57).

Characteristics	Number, (%)
Age, years	
<50	17 (29.8)
≥50	40 (70.2)
Histological grade	
Poorly	43 (75.4)
Low	14 (24.6)
Stage	
Advanced	47 (82.5)
Early	10 (17.5)
Status	
Dead	25 (43.9)
Alive	32 (56.1)
BRCA1 expression	
Positive	27 (47.4)
Negative	30 (52.6)
Therapy	
Platinum-based	20 (35.1)
Non-platinum-based	37 (64.9)
Histological type	
NST	45 (78.9)
Specific	12 (21.1)
TNBC subtype	
Basal-like	35 (72.9)
Non-basal-like	13 (27.1)

TNBC, triple-negative breast cancer.

Results

There were 100 cases of TNBC reported between 2015 and 2020 with complete clinicopathological data available. However, only 57 cases were accompanied by data on survival and therapy. Of the 57 cases studied, the mean patient age was 55.18±10.014 (32-83). Patients aged ≥50 years were more frequent (70.2%) compared with patients aged <50 years (29.8%). More patients were high-grade, advanced staged, alive, received non-platinum-based therapy, and non-specific type. The BRCA1 expression was detected in 44 cases (77.19%). The median value of the BRCA1 expression was 30, and it was used as a cut-off to categorize BRCA1 expression into negative and positive. The number of negative BRCA1 expression cases was 52.6%. Of the 48 cases studied, 72.9% were basal-like subtypes. The characteristics of the samples are presented in Table I.

Fisher's exact test analysis revealed a correlation between the expression of BRCA1 and the disease stage, as demonstrated in Table II. A negative expression of BRCA-1 was

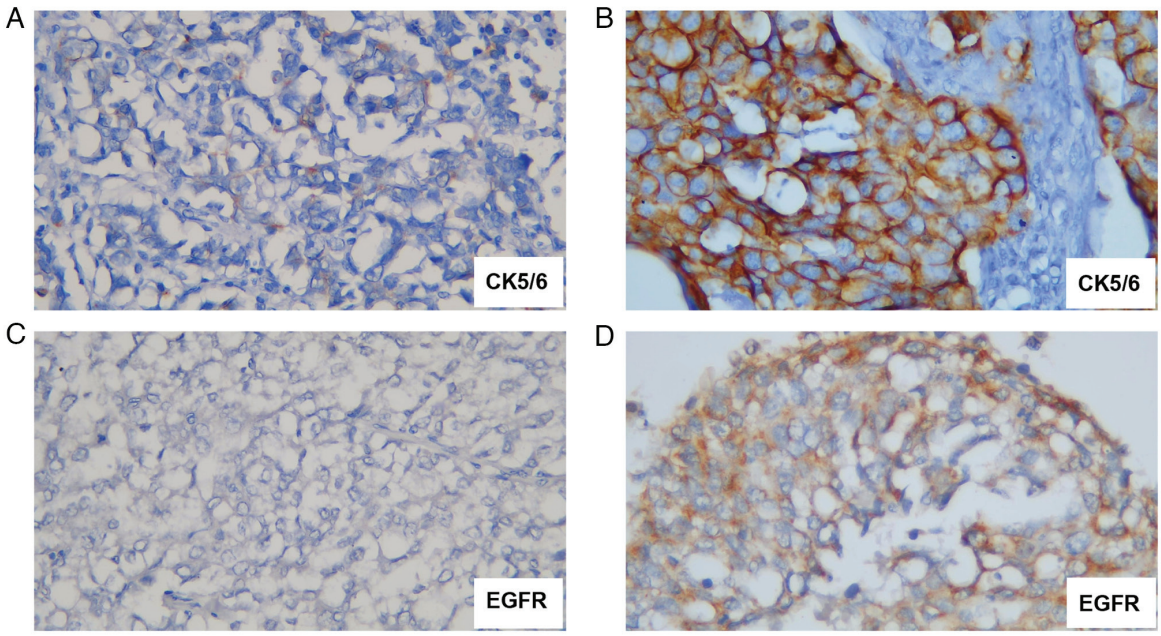

Figure 1. Application of CK5/6 and EGFR staining to differentiate between basal-like and non-basal-like triple-negative breast cancer. (A and B) Sample A did not exhibit any CK5/6 staining, whereas sample B displayed positive staining for CK5/6 in the cytoplasmic membrane. (C and D) Similarly, sample C did not display any EGFR staining, whereas sample D exhibited positive staining for EGFR in the cytoplasm. CK, cytokeratin; EGFR, epidermal growth factor receptor. Magnification, x400.

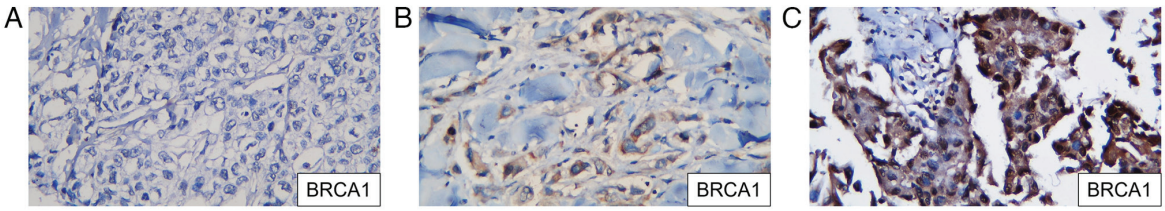

Figure 2. BRCA1-staining pattern. (A) Negative staining was displayed for sample A, indicating a low presence of the BRCA1 protein. (B) Sample B was positively stained, indicating the presence of the BRCA1 protein. (C) Sample C showed intense staining, indicating a high level of BRCA1 protein expression. Magnification, x400.

significantly associated with a more advanced disease stage (P=0.035). However, there was no correlation between the expression of BRCA-1 and other clinicopathological characteristics, such as the type of therapy.

The survival analysis of the BRCA1 expression is presented in Figs. 3 and 4. The mean survival time for patients was 100.79 weeks (minimum 17 weeks and maximum 265 weeks). The results of the survival analysis demonstrated that neither BRCA1 expression (P=0.150; Fig. 3) nor stage (P=0.091; Fig. 4) were significant prognostic factors in patients with TNBC.

Discussion

In the present study, the mean age of patients with TNBC at diagnosis was 55.18±10.014 years. The number of patients aged >50 years was high (70.2%). Previous studies concluded that TNBC more frequently occurred at the age of ≤40 years, especially among African-Americans (16-18), and mostly has a poorer prognosis than other breast cancer subtypes (1-3). The present study found that 75.4% of TNBC cases were high-grade and 82.5% were advanced stage. In total, 60-80% of TNBC cases are basal-like subtypes with poor prognoses because they tended to recur and were resistant to therapy (19-21). Our cases were also dominated by basal-like subtypes (72.9%); of these cases, 63% were high-grade, 74% were advanced-stage, and 52.6% succumbed.

The prevalence of BRCA mutations differs across various ethnic groups. Previous studies on BRCA1/2 mutations in TNBC predominantly focused on Caucasian populations. Studying within the Asian population is crucial, as Asian patients with breast cancer manifest the disease at a younger age compared with their Caucasian counterparts. The frequency of BRCA1/2 mutations in Korean patients with non-familial high-risk breast cancer and familial breast cancer was 17.8 and 21.7%, respectively (22). The prevalence of BRCA1/2 mutations in patients with familial breast cancer and early-onset breast cancer in China ranged from 8-13.5% and from 8.7-11.4%, respectively (23). A study of Japanese patients with familial breast cancer indicated that 15-31.8% expressed mutations in the BRCA1/2 genes (24).

Several methods are available for detecting BRCA1 and BRCA2 dysfunction. Identification through IHC is a simple and reliable method to assess the expression of the BRCA1

Table II. Correlation between the BRCA1 expression and several characteristics of TNBC cases.

Characteristics	Expression level of BRCA1		P-value
	Positive (%)	Negative (%)	
Age, years			0.583 (0.444-4.291)
<50	9 (15.7)	8 (14.1)	
≥50	18 (31.6)	22 (38.6)	
Histological grade			0.125 (0.115-1.396)
Poorly	18 (31.7)	25 (43.8)	
Low	9 (15.8)	5 (8.7)	
Stage			0.035
Advanced	19 (33.3)	28 (49.1)	
Early	8 (14.1)	2 (3.5)	
Therapy			0.396 (0.537-4.795)
Platinum-based	11 (19.3)	9 (15.8)	
Non-platinum-based	16 (28.1)	21 (36.8)	
Histological type			0.392 (0.157-2.052)
NST	20 (35.2)	25 (43.8)	
Specific	7 (12.3)	5 (8.7)	
Status			0.325 (0.204-1.697)
Dead	10 (17.6)	15 (26.3)	
Alive	17 (29.8)	15 (26.3)	
TNBC subtype			0.978 (0.274-3.542)
Basal-like	16 (33.3)	6 (12.5)	
Non-basal-like	19 (39.6)	7 (14.6)	

TNBC, triple-negative breast cancer.

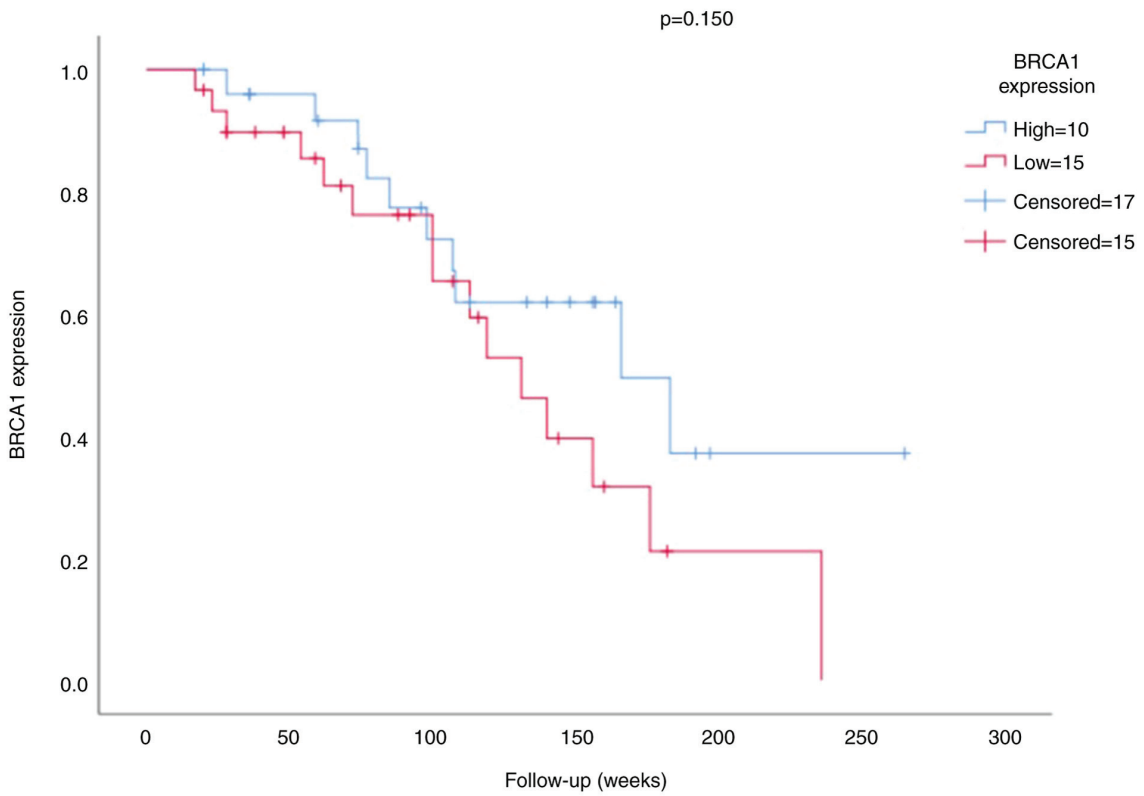

Figure 3. Survival analysis of the BRCA1 expression.

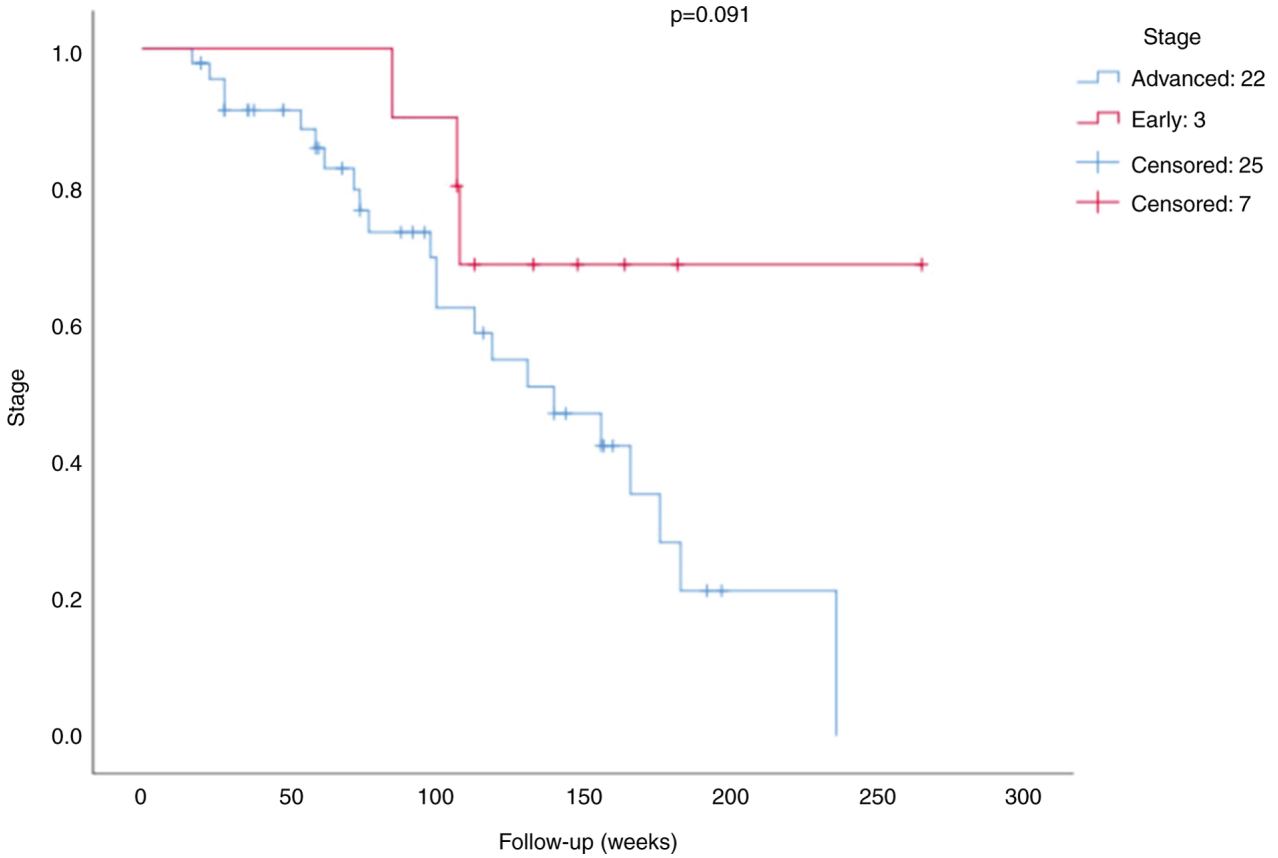

Figure 4. Survival analysis of stage in patients with triple-negative breast cancer.

protein in tumor tissues. Using the IHC method, cancer with positive BRCA1 expression in the present study was 47.4%. BRCA1-positive expression reported by other studies was 17.5% (25) and 20.5%, (15) possibly due to differences in the method and cut-off value of the BRCA1 expression. Previous research used the cystoscope method, and BRCA1 was considered positive if the score was ≥4 (25). Meanwhile, other groups used the cut-off value research of 20% and found that a cut-off of 20% is improved for avoiding missing variants and has a lower false positive rate compared with a cut-off of 10% (0.14 vs. 6.82%) (15,26).

In the present study, a negative BRCA1 expression was correlated with advanced-stage cancer, but not with other clinicopathological characteristics. Altered BRCA1 expression was significantly associated with high-grade and advanced-stage breast carcinoma; however, there was no correlation of the BRCA1 expression with clinicopathological parameters (15,25). A previous study proved that reduced BRCA1 expression was associated with high-grade tumors, negative hormone receptors and HER2 status (27). Differences in the number of samples, method and type of antibody used influence these controversial results.

In the present study, basal-like and non-basal-like subtype cancers tended to have a positive BRCA1 expression and were not statistically significant. This result differed from those of a previous study wherein positive BRCA1 expression correlated with basal-like tumors (27). In relation to mutation, basal-like breast cancer did not improve the estimate of BRCA1 mutation risk (12).

Chemotherapy in TNBC can be platinum- or non-platinum-based, including taxane, and anthracycline, among others. The number of patients treated with non-platinum-based treatment was higher (64.9%) than that of patients treated with platinum-based treatment (35.1%). The therapy type did not correlate with the BRCA1 expression. However, the mutation status of BRCA1 in patients with TNBC was considered essential as it influences the treatment choice. Patients with TNBC with BRCA1 mutation respond well to platinum-based therapy and PARP inhibitors (12,13,28). It was necessary to investigate the relation between the BRCA1 expression at the protein level and its mutation status considering that protein detection is markedly simpler, cheaper and visible in different laboratories in developing countries, such as Indonesia.

The disease stage did not act as a prognostic factor for patients with TNBC in the present study. It was recently concluded that the advanced stage was related to significant overall and disease-free survival reduction (29). Another study confirmed that young patients with TNBC have a higher pathological stage and worse long-term survival than young patients with other breast cancer subtypes (30). The present study has several limitations that need to be addressed in future studies. Only 57 cases were included due to difficulty in obtaining survival and therapy data and challenges in reaching the patients. Transportation costs could constrain the distance from the patient's house; therefore, check-ups were irregular. In addition, limited therapy options for TNBC and national health insurance are occasionally

not easily accessible. Therefore, further study using a more significant number of cases is needed to elaborate on the prognostic significance of the disease stage in Indonesian patients with TNBC.

The BRCA1 expression in the present study did not act as a prognostic factor for TNBC cancer. A 20% cut-off was employed for the BRCA1 expression, considering the lack of a standard consensus on the cut-off point (15). The present study focuses on the Indonesian population as it is currently an underexplored topic, especially within the Indonesian demographic. Consequently, the current study can significantly impact future research using samples from the Southeast Asian region, specifically the Indonesian population. Comprehensive research must therefore be conducted to determine the standard value of BRCA1 expression, especially when later BRCA1 protein expression can be applied to determine the treatment choice in patients with TNBC.

In conclusion, the present study concluded that a negative BRCA1 expression was correlated with the advanced stage of Indonesian patients with TNBC, albeit it was not a prognostic factor.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

TCG, EKD and II conceived the research. EKD and II wrote the manuscript, with significant contributions from SLA and RGB. EKD and II confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. KE/FK/1291/E1; date, December 2021) by the ethics committee. and patient consent for sample collection was waived by the ethics committee Faculty of Medicine, Public Health and Nursing, University Gadjah Mada/Sardjito General Hospital (Yogyakarta, Indonesia) and patient consent for sample collection was waived by the ethics committee.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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