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Original article

Histopathological Features and Outcomes of Triple-Positive *versus* Triple-Negative Breast Cancer

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Abstract
To compare and analyse the clinicopathological features and prognosis of patients with triple positive breast cancer (TPBC) and triple negative breast cancer (TNBC). A total of 162 patients with primary breast cancer were diagnosed and treated between 2007 and 2018 at the National Cancer Institute in Misurata, Libya. Further molecular classification into TPBC and TNBC was performed. Statistical analysis was performed using the Kaplan-Meier method, log-rank test, and Cox regression test to compare the clinicopathological features and prognosis between the two groups. The prevalence of TNBC was 46.9%. Compared with the TPBC, the TNBC patients were associated with aggressive tumour grade, such as higher rates of positive lymph nodes (66.2% vs. 33.8%, p=0.004), poorly differentiated tumours (59.7% vs. 40.3%, p=0.002), and positive neural invasion (59.5% vs. 40.5%, p=0.014). Univariate analysis of overall survival (OS) showed that mortality was higher in TNBC compared to TPBC (p-value<0.0001), and patients with TNBC were also associated with short disease-free survival and a high rate of recurrences (p=0.031). Cox regression analysis revealed TNBC (p=0.003) and stage at diagnosis (p=0.013) are independent predictors of short overall survival. TNBC was a distinct subgroup of breast cancer with clinicopathological behaviour. Compared with the TPBC, TNBC was characterized by a more aggressive tumour grade and a poor prognosis.
Keywords. Breast Cancer, Triple Positive Breast Cancer, Triple Negative Breast Cancer, Clinicopathological Features and Outcomes.

Introduction

Breast cancer (BC) is considered the most widespread malignancy present in women and is one of the leading causes of cancer-related mortality [1]. BC is a heterogeneous disease that varies in morphology, biology, behaviour, and response to therapy. Carefully, an understanding of the current molecular classification of BC is very important to predict the benefit of specific therapies such as targeted therapy and chemotherapy [2]. Identifying the molecular type of BC is important to guide different treatment modalities and to alter the patient outcome. Patients with BC have a 90% probability of surviving 5 years. However, a patient's likelihood of surviving 5 years subsides to 20% once it spreads to other body parts [3]. Molecular classification of BC based on the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 neu (HER2 neu). Positive expression of ER/PR and/or HER2 neu defines triple-positive breast cancer (TPBC), while the absence of ER, PR, and HER2 neu expression defines triple-negative breast cancer (TNBC) [4]. Positivity of both ER and HER2 subtypes is effectively treated with specific targeted therapy. While TNBCs lack targeted therapy and are still being treated with systemic chemotherapy drugs [5]. TNBC accounts for 15%–20% of breast cancer patients, and the patients are younger in age, with larger tumour size, higher recurrence rate, and metastasis [6 and 7]. TNBC has a higher response rate than luminal but with shorter disease-free survival (DFS) and overall survival (OS), which makes it one of the most aggressive subtypes of breast cancer [8].
While patients with TPBC were associated with higher tumour grade, larger tumour size, and exhibited worse prognosis [9]. This study aimed to identify the clinicopathological features and patient outcomes of triple-positive breast cancer (TPBC) vs triple-negative breast cancer (TNBC).

Methods

Demographic and Clinicopathological data

The study group consisted of 162 patients with breast cancer diagnosed between 2007 and 2018 at the National Cancer Institute in Misurata, Libya. We used estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 expression data to identify patients with triple-positive breast cancer (TPBC) and triple-negative breast cancer (TNBC). TPBC was defined as ER and PR positivity detected via immunohistochemistry (IHC) assays and a HER2 IHC score of 3+ or HER2 gene amplification detected by fluorescence in situ hybridization (FISH) of tumour tissue. TNBC was defined by the lack of ER and PR expression in IHC assays and a HER2 IHC score of 0 or 1+ or no HER2 amplification via FISH. Demographic and clinicopathological data included patient age, comorbidity, family history, marital status, menopausal status, side of breast cancer, TNM staging, lympho-vascular invasion, neural invasion, surgical margins, histology type, histology grade, type of treatment, and follow-up data. These data were collected from the

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patients' medical records and are shown in (Tables 1 and 2). The mean age of the patients was 45 years (range, 29-80 years), (Figure 1). TNM staging of breast cancer was evaluated according to the American Joint Committee on Cancer (AJCC), TNM staging [10].

Table 1. Demographic and genetic characteristics of TPBC and TNBC patients

Characteristics		Number of patients	TPBC (percent)	TNBC (percent)	p value
Age	< 50 years	110	42.7	57.3	0.083
	≥ 50 years	52	44.2	55.8	
Menopausal status	Pre-menopausal	104	43.3	56.7	0.140
	Post-menopausal	58	46.6	53.4	
Marital status	Married	131	51.1	48.9	0.207
	Single	31	61.3	38.7	
Comorbidity	Yes	41	51.2	48.8	0.461
	No	121	53.7	46.3	
Family history	Positive	12	50.0	50.0	0.529
	Negative	150	53.3	46.7	

TPBC = triple positive breast cancer; TNBC = triple negative breast cancer.

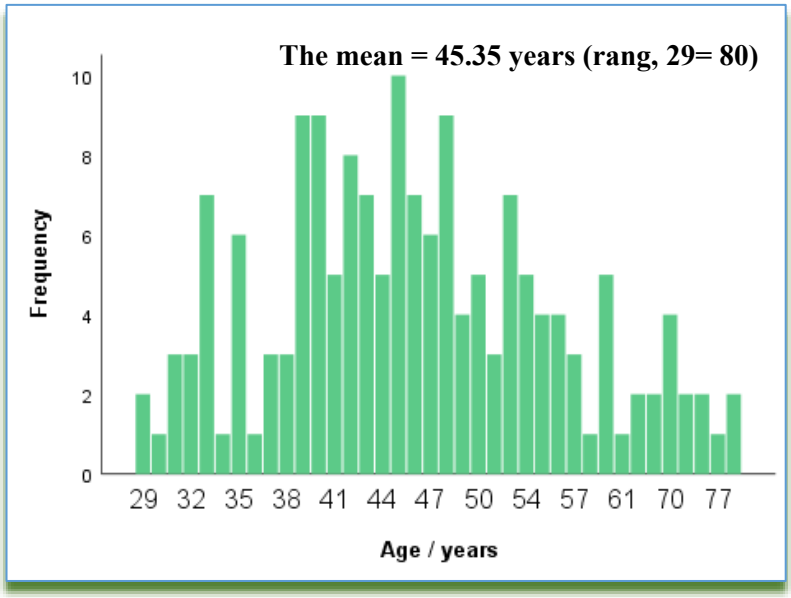

Figure 1. Age distribution of 162 patients with breast cancer

Treatment and follow-up

One hundred and forty-five patients were treated by radical surgery (modified radical mastectomy or breast conserving surgery) with lymph nodes resection, while palliative surgery was performed in 2 patients, and no surgery was performed in 15 patients who had metastases at the time of diagnosis. However, tissue biopsy was performed in these patients for histopathological diagnosis. In the National Cancer Institute in Misurata, the following guidelines were established: Systemic chemotherapy based on FAC protocol (5-fluorouracil, Adriamycin, and cyclophosphamide) for 6 cycles every 3 weeks or 4AC (Adriamycin and cyclophosphamide) plus Taxol protocol was given to all patients with node-positive or high-risk node-negative tumours (no =145). Anti-HER2 therapy (trastuzumab) was given to 67 patients with HER2-positive status. Hormonal therapy was given for all hormone-dependent breast cancer patients using tamoxifen or aromatase inhibitors with or without goserelin according to menopausal status (no= 86). Radiotherapy was given to 141 patients (Table 3). Follow-up of patients was carried out every 3 months during the first 2 years, every 6 months from year 2 to year 5, and annually thereafter. Follow-up included a clinical examination at every visit, plain chest X-ray, pelvic-abdominal ultrasound, and mammography once a year, complete blood cell counts and tumour markers twice a year; other image examinations were performed when needed. Disease recurrence (local and distant) was confirmed by a clinical examination, laboratory results, biopsy, and imaging (Computed Tomography, Magnetic Resonance Imaging, or Positron Emission Tomography) performed when clinical symptoms suggestive of disease recurrence were present. Patients' outcomes were considered as follows: overall survival (OS), duration between the date of pathological diagnosis and the date of death and/or to the date of the end follow up period; disease-free survival (DFS), duration between the date of pathological diagnosis and the date of diagnosis of recurrence (local and/or distant) or death. Patients

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were followed up until death or to the end of the observation period (until December 2023). The median follow-up duration was 63 months (range, 13-161 months). At the end of the follow-up period, 54 patients (33.3%) had disease recurrence, and 57 patients (35.2%) had died of breast cancer.

Table 2. Clinicopathological characteristics of TPBC and TNBC patients

Characteristics		Number of patients	TPBC (percent)	TNBC (percent)	p value
Site	Right	79	54.4	45.6	0.430
	Left	83	51.8	48.2	
Histological type	IDC	149	54.4	45.6	0.208
	Others	13	38.5	61.5	
Tumor size	T1	14	42.9	57.1	0.639
	T2	77	57.1	42.9	
	T3	38	44.7	55.3	
	T4	29	58.6	41.4	
	Tx	4	50.0	50.0	
Lymph Node status	Positive	74	33.8	66.2	*0.004*
	Negative	75	42.7	57.3	
	Unknown	13	38.5	61.5	
Stage group	Stage 1 and 2	85	48.2	51.8	0.127
	Stage 3 and 4	77	58.4	41.6	
Histological grade	Grade 1	9	90.0	10.0	*0.002*
	Grade 2	75	61.3	38.7	
	Grade 3	77	40.3	59.7	
Resection margin	Positive	11	54.5	45.5	0.987
	Negative	124	53.2	46.8	
	Unknown	27	51.9	48.1	
Lymphovascular invasion	Positive	37	67.6	32.4	0.124
	Negative	86	47.7	52.3	
	Unknown	39	51.3	48.7	
Neural invasion	Positive	37	40.5	59.5	*0.014*
	Negative	47	70.2	29.8	
	Unknown	78	48.7	51.3	

TPBC = triple positive breast cancer; TNBC = triple negative breast cancer.

Table 3. Treatment of TPBC and TNBC patients

Treatment strategies		Number of patients	TBBC (n=86)	TNBC (n=76)	p value
Surgery	MRM and ALND	115	55.7	44.3	0.692
	BCS and ALND	30	50.0	50.0	
	Palliative surgery	2	50.0	50.0	
	No surgery	15	40.0	60.0	
Chemotherapy	Adjuvant	145	54.5	45.5	0.184
	Palliative	15	46.7	53.3	
	No	2	0.0	100.0	
Radiotherapy	Yes	141	55.3	44.7	0.107
	No	21	38.1	61.9	
Hormonal therapy	Yes	86	100.0	0.0	<0.0001
	No	76	0.0	100.0	
Anti HER2 therapy	Yes	67	100.0	0.0	<0.0001
	No	95	20.0	80.0	

TPBC= triple positive breast cancer ; TNBC= triple negative breast cancer ; BCS= breast conserving surgery; ALND= axillary lymph node dissection; HER2= human epidermal growth factor 2.

Statistical analysis

Continuous variables were calculated using SPSS 26.0 for Windows (IBM Corp.). The Chi-square (x2) test was used to analyse demographic and clinicopathological variables affecting the prognosis of patients in the TPBC and TNBC groups. Differences in OS and DFS between the two groups were analysed using the Kaplan–Meier method. Multivariate survival analysis for the outcome (OS and DFS) was performed using the proportional hazard Cox model in a backward stepwise manner with the log likelihood ratio (L R) significance test, using standard values for the entry and exclusion criteria. $p<0.05$ was considered to indicate a statistically significant difference.

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Results

Demographic, genetic, and clinicopathological variables of the studied groups (TPBC and TNBC)

A total of 162 breast cancer patients were included in this study. They were divided into two groups according to the results of IHC and/or FISH: the TPBC group and the TNBC group. TPBC had 86 patients (53.1%), and TNBC had 76 patients (46.9%), (Figure 2). Demographic, genetic, and clinicopathological variables for each group (TPBC and TNBC) are summarized in (Tables 1 and 2). The mean age in all patients was 45 years (range, 29-80 years), (Figure 1). While the mean age of TPBC patients was 48.34 years (range=30–80 years) and the mean age for TNBC was 45.71 years (range=29–70 years). Compared with TPBC, patients with TNBC had the following features: higher ratio of positive lymph nodes (66.2% VS 33.8%, p=0.004); higher ratio of poorly differentiated tumours (59.7% VS 40.3%, p=0.002); higher ratio of positive neural invasion (59.5% VS 40.5%, p=0.014). There was no statistically significant difference in terms of other traditional variables, including age, menopausal status, marital status, comorbidity, family history, tumour site, tumour size, histological type, tumour stage, resection margins, lymph-vascular invasion, and surgical treatment between the two groups (Table 1, 2, and 3).

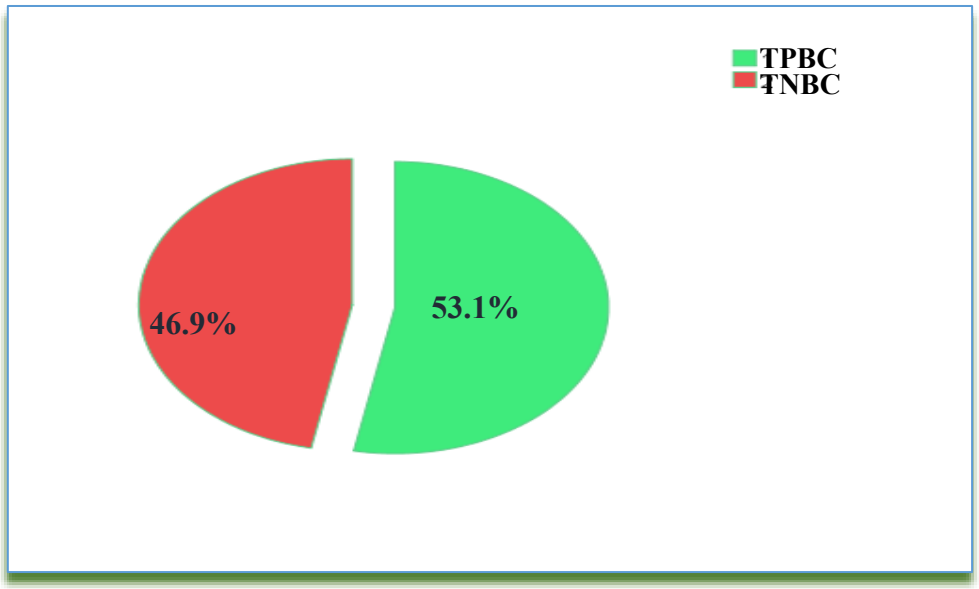

Figure 2. Frequency of TPBC and TNBC patients

Patient outcomes

Locoregional recurrence and metastasis

The median duration of follow-up was 63 months (range 13-161). During this time, a total of 54 patients experienced recurrence or metastasis. The rate of local recurrence in the TNBC group was 62.5% compared to the rate in the TPBC group, which was 37.5%. The rate of metastasis in the TNBC group was 53.8% compared to the rate in the TPBC group, which was 46.2%. This difference was insignificant (p=0.067), (Table 4).

Survival outcomes

During the period of observation, 54 patients (33.3%) had disease recurrence, and 57 patients (35.2%) had died of breast cancer. In the TNBC group, the 5-year OS and DFS rates were 48.7% and 59.2% which differed significantly (p<0.0001, p=0.031, respectively) compared to the rates in the TPBC group were 79.1% and 73.3%. Univariate survival analyses (survival rates) of triple-positive and triple-negative breast cancers are shown in (Table 5). We use the Kaplan-Meier method to estimate survival functions and a log-rank test to compare the survival functions among the two groups. We found that the 5-year OS and DFS were significantly lower in the TNBC group than those of the TPBC group (Figures 3 and 4).

Table 4. Prevalence of recurrence of TPBC and TNBC

Type of recurrence	Number of patients	TPBC (percent)	TNBC (percent)	p value
No recurrence	108	58.3	41.7	0.067
Local	8	37.5	62.5	
Distant	26	46.2	53.8	
Local and distant	3	33.3	66.7	
Metastasis at diagnosis	17	41.2	58.8	

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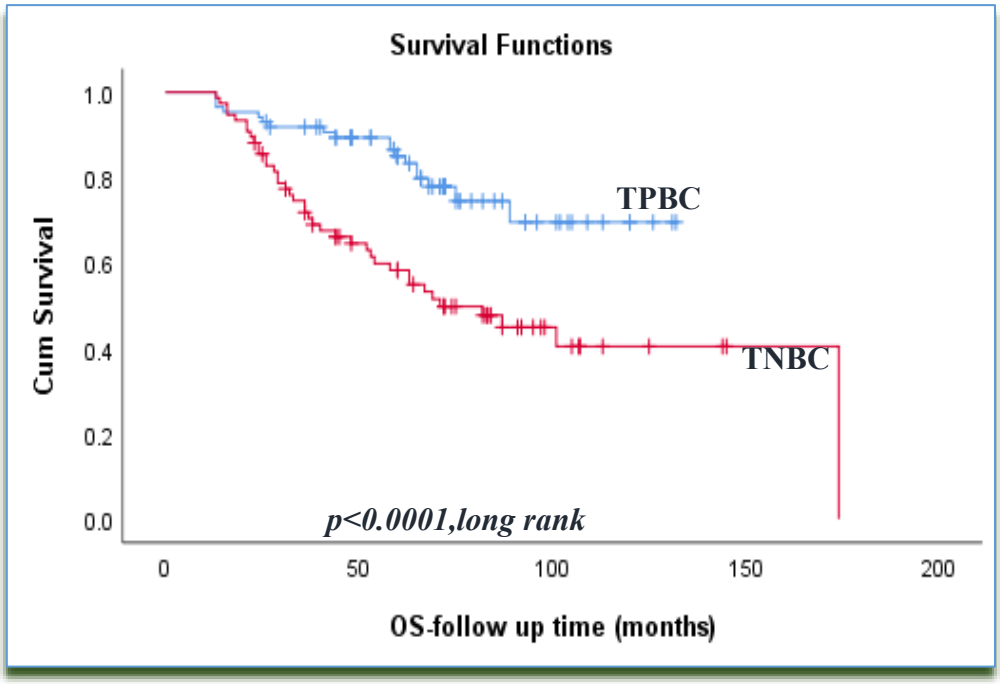

Figure 3. Overall survival curves between TPBC and TNBC patients. Kaplan-Meier survival analysis shows a significant statistical difference in 5-year survival between two groups.

Table 5. Univariate survival analysis of TPBC and TNBC patients.

		Survival analysis			p-value
		Median time (months)	Mean time (months)	Survival rate (present)	
Overall survival					
	All patients	62.00	63.84	64.8	
	TPBC	64.00	66.60	79.1	<0.0001
	TNBC	56.00	60.71	48.7	
Disease-free survival					
	All patients	53.60	56.00	66.7	
	TPBC	55.50	57.37	73.3	0.031
	TNBC	44.00	49.33	59.2	

TPBC = triple positive breast cancer; TNBC = triple negative breast cancer.

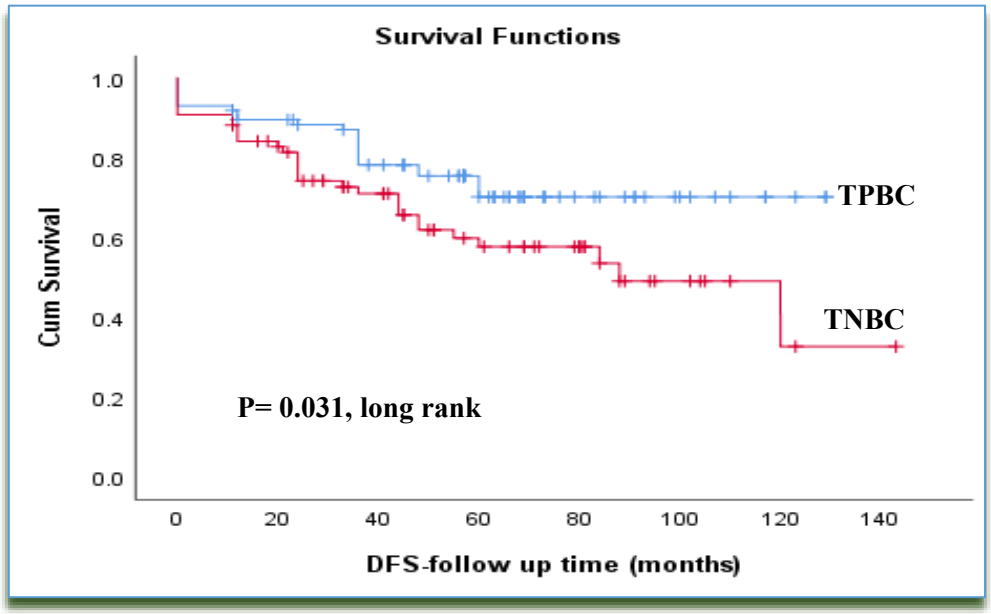

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Figure 4. Disease free survival curves between TPBC and TNBC patients. Kaplan-Meier survival analysis shows TPBC group had the best DFS

The multivariate Logistic regression analysis

Cox regression analysis revealed TNBC (p=0.003) and advanced (p=0.013) are independent of short overall survival as assessed in a multivariate survival (Cox) analysis containing marital status, menopausal status, and histology type variables. For DFS, the same model was used to assess the role of these variables (age at diagnosis, marital status, menopausal status, histology type, and clinical stage) as an independent predictor of DFS. Only the advanced stage proved to be an independent predictor (p = 0.003) (Table 6).

Table 6. Multivariate analysis (Cox proportional hazard model) for prognostic factors for 162 patients with breast cancer. related to DFS and overall survival

Variables	Overall survival model		Disease-free survival model	
	Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)	P value
Age (<50 years / ≥50 years)	1.376 (0.177 -10.664)	0.760	0.685 (0.151-3.103)	0.623
Marital status (married/single)	0.822 (0 420-1.610)	0.568	0.723 (0.373-1.400)	0.336
Menopausal status (pre-menopausal/ post-menopausal)	0.66 (0.089 -4.988)	0.692	1.443 (0.335-6.217)	0.622
Histology type (adenocarcinoma /others)	1.292 (0.502-3.321)	0.595	2.237 (0.984-5.086)	0.055
Clinical Stage (I + II / III + IV)	2.032 (1.164 -3.548)	0.013	2.389 (1.337-4.270)	0.003
TPBC/TNBC	2.487 (1.378 -4.490)	**0.003**	1.742 (0.979-3.099)	0.059

TPBC = triple positive breast cancer; TNBC = triple negative breast cancer.

Discussion

This study was performed to identify clinical and pathological features and compare survival outcomes of patients with TPBC and TNBC. 162 patients who underwent surgery for breast cancer at the National Cancer Institute in Misurata, Libya, were retrospectively investigated. In our study among 162 patients, 46.9% were TNBC, which is approximately higher than results from other previous studies [11-14]. Breast cancer is a heterogeneous disease, with substantial genotypic and phenotypic diversity. TNBC is a special subtype of breast cancer that accounts for approximately 10-30% of all breast cancer subtypes around the world and 10%-17% in Western countries [11 and 12]. The African American women have the highest incidence rate of all, reporting up to 30% [13 and 14].

The incidence has significant regional and ethnic differences, which may be related to some genetic or biological differences [15]. African American women with breast cancer are more likely to be diagnosed at an advanced stage and have breast cancer markers of bad prognosis [16]. The prognostic information for each individual patient is based on the analysis of biological markers in the primary tumour, including hormone status (ER and PR), Her-2 neu and Ki 67, together with other traditional prognostic factors such as age, tumour size, histological grade and lymph node involvement [17]. So, the distinction between TPBC and TNBC has been an increasing clinical interest. TPBC cancers derive benefit from targeted therapy, while there are limited therapeutic options for TNBC patients. TNBC represents a special subgroup of breast cancers with heterogeneous clinical presentation, clinical behaviour, histological grade, and response to therapy as compared to TPBC [18].

TNBC patients present at a younger age with an advanced stage of disease and predominantly higher tumour grade as compared to TPBC patients [19 and 20]. Our study revealed that TNBC was noted in 57.3 % of patients who were <50 years and 56.7% in premenopausal status. The same results were reported by Sajid et al. [20] and others [21 and 22]. Compared with TPBC, TNBC was associated with positive axillary lymph node, with poorly differentiated tumours, and with positive neural invasion, which were consistent with previous reports [12,1 8 and 19]. Moreover, the rate of OS and the rate of DFS in TNBC patients were all much lower than those in TPBC patients. Yuan et al. [13] showed a 5-year OS rate of 88.5% and a 5-year DFS rate of 73.7% in the TNBC, compared to 80.8%, 92.8% in the TPBC, which all differed significantly. Our study showed that the 5-year OS rate and 5-year DFS rate in the TNBC were all significantly lower than those in the TPBC, which was similar to other reports. According to multivariate analysis, TNBC and clinical stage are independent risk factors for OS. The poor prognosis of TNBC could be related to biological behaviours such as younger age, larger tumor size, more advanced clinical stage, higher rate of lymph node metastasis, higher histological grade, earlier recurrence and metastasis, and non-susceptibility to targeted therapy [21 ,22].

Conclusions

The incidence of TNBC was 46.9% in Libyan patients with breast cancer. TNBC was a distinct subgroup of breast cancer with particular clinicopathologic behavior. Compared with TPBC, TNBC was associated with a high grade of malignancy, and lower OS and DFS rates. Further large-scale studies are required to

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emphasize these analysis findings, alongside the constant collection of clinical data, for more comprehensive and precise results in the future.

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Authors' contributions
MK designed the present study, drafted the manuscript, and wrote. AR, MA, MR, and MBS analysed data, reviewed the manuscript, and performed data interpretation and analysis. AJ drafting and proof reading and discussions. EE made the statistical analysis and prepared the figures and tables reviewed the study, interpreted data, and aided in drafting and proofreading of the manuscript. ER, RB, ME, and NA critically reviewed and approved the final version of the manuscript.

Ethical approval
The cohort study was done under research ethics approval by the ethical committee at the National Cancer Institute, Misurata. Written informed consent was obtained from all patients for surgical treatment, pathologic examinations, and investigations performed according to the institutional guidelines of the National Cancer Institute, Misurata, Libya.

Competing interests
The authors declared that they have no competing interests.

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