Strong impact of pathological node-negative on long-term overall survival of patients with triple-negative breast cancer receiving neoadjuvant chemotherapy

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Received July 5, 2020; Accepted February 5, 2021

DOI: 10.3892/mco.2021.2261

Abstract. Triple-negative breast cancer (TNBC) has a high pathological complete response (pCR) rate; however patients without a high pCR are reported to have a poor prognosis. The current study investigated the long-term overall survival of patients with TNBC who received neoadjuvant chemotherapy (NAC) and analyzed various prognostic factors including basal marker and claudin expressions. Between November 2005 and March 2012, the current study retrospectively reviewed the records of 323 patients with breast cancer who received anthracycline followed by taxane as NAC at the Jikei University Hospital Basal marker and claudin expression was determined via immunohistochemistry. The median age of the patients was 53.0 years. Of the 323 patients, 26 (8%) achieved a pCR, including 13 patients (19.7%) with TNBC and 13 (5.1%) with non-TNBC (P<0.001). Of the 66 patients with TNBC, 13 (19.7%) demonstrated recurrence and 8 (12.1%) died after a median follow-up time of 111.5 months [10-year disease-free survival (DFS), 80.3%; 95% confidence interval (CI), 0.68-0.88; 10-year overall survival (OS), 84.8%; 95% CI, 0.72-0.92]. Of the 257 patients with non-TNBC, 45 (17.5%) patients demonstrated recurrence and 26 (10.1%) died (10-year DFS, 82.1%; 95% CI, 0.76-0.87; 10-year OS, 88.6%; 95% CI, 0.83-0.92). There was no statistical difference between the patients with and without TNBC. In the TNBC group, patients with pathological node-negative status survived without distant recurrence. Additionally, negative lymphovascular infiltration was another favorable prognostic factor. Patients with TNBC who received NAC demonstrated comparably high prognoses to non-TNBC patients. Overall, pathological node status after NAC had a strong impact on the prognosis of patients with TNBC.

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Abbreviations: TNBC, triple-negative breast cancer; pCR, pathological complete response; NAC, neoadjuvant chemotherapy; DFS, disease-free survival; OS, overall survival; ER, estrogen receptor; PgR, progesterone receptor; HER2, human epidermal growth factor receptor-2; EGFR, epidermal growth factor receptor-1; CK, cytokeratin; IHC, immunohistochemistry; FFPE, formalin-fixed paraffin embedded tissue

Key words: triple-negative, neoadjuvant chemotherapy, pathological node-negative, survival, breast cancer

Introduction

Breast cancer is one of the most common cancers in women worldwide (1,2). Triple-negative breast cancer (TNBC) is characterized by the lack of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor-2 (HER2) expression. TNBC is reported to account for 10-15% of all sporadic breast cancers. Compared to non-TNBCs, they are generally larger, show higher grade, have lymph node involvement at the time of diagnosis, and are biologically more aggressive (3-5). In previous studies, 20-56% of patients with TNBC were reported to achieve a pathological complete response (pCR) after neoadjuvant chemotherapy (NAC). Despite higher response rates to NAC, the patients with TNBC who did not achieve pCR had a higher rate of distant recurrence and poorer prognosis compared to the non-TNBC group. Disease-free survival (DFS) of patients with TNBC was reported to be 5060% (6-13). However, the median follow-up periods in those reports were relatively short (3-6 years), and the long-term overall survival of patients with TNBC who did not achieve pCR have not been reported. Furthermore, reports of a pooled analysis included various regimens for NAC.

Gene expression profiling has established several distinct breast cancer molecular subtypes, including luminal A and B, HER2-positive, basal-like, and claudin-low (14-16). TNBCs account for 39-54% of basal-like and 25-39% of claudin-low cases. Each breast cancer is associated with different clinical outcomes, biological features, and treatment responses (17).

Epithelial-derived cancers often show high or low expression of claudins. These proteins are the most important structural and functional components of tight junction integral membrane proteins. However, the association between claudin expression and prognosis is unknown (18).

In this study, we retrospectively investigated the long-term overall survival of patients with TNBC who received NAC and their prognostic factors including basal marker and claudin expression.

Patients and Methods

Patients and treatment. We retrospectively reviewed the records of 323 consecutive breast cancer patients who received NAC at the Jikei University Hospital between November 2005 and March 2012. This study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983, and was approved by the Ethics Committee of the Jikei University School of Medicine; patient consent was also obtained. We evaluated the age of patients, clinicopathological characteristics such as clinical tumor size and clinical lymph node status before NAC, ER, PgR, and HER2 expression, pathological tumor size, pathological lymph node status, lymphovascular infiltration, epidermal growth factor receptor (EGFR), cytokeratin (CK) 5/6 as the basal marker, claudin-3 expression, and survival data for the patients. Lymphovascular infiltration was evaluated with the operative sample after NAC. A clinically node-positive axilla was defined as the presence of palpable mass in the nodal basin and, when assessed using ultrasound, magnetic resonance imaging, or computed tomography images, the presence of abnormal lymph nodes. When these had a suspected cancerous appearance on images, positivity was confirmed via fine needle aspiration.

All patients received NAC with four cycles of epirubicin (100 mg/m^2), 5-fluorouracil (500 mg/m^2), and cyclophosphamide (500 mg/m^2), followed by four cycles of docetaxel (100 or 75 mg/m^2). If patients were HER2-positive, trastuzumab was administered concurrently with docetaxel and adjuvant trastuzumab for one-year duration. A five-year adjuvant hormonal therapy was followed if the patients were hormone receptor positive. Patients who underwent breast conserving surgery received whole breast radiotherapy, and regional lymph node radiotherapy was used for patients with ≥4 -positive nodes. Post-mastectomy radiation therapy was administered to patients with initial tumors ≥5 cm or those with ≥4 positive nodes.

Systemic and breast examinations were performed before neoadjuvant chemotherapy, before surgery, and every 12 months postoperatively using chest and abdominal computed tomography, mammograms, breast ultrasonography, brain magnetic resonance imaging, and bone scans.

Pathology assessment. Immunohistochemistry (IHC) was evaluated using core needle samples before NAC and to ensure accuracy we used positive staining tissue as a control. IHC was performed according to the standard protocol on 3 μm sections of formalin-fixed paraffin embedded (FFPE) tissues using CONFIRM anti-ER rabbit monoclonal antibody (SP1; Roche Diagnostics Ltd.) for ER staining and CONFIRM anti-PGR rabbit monoclonal antibody (SP1; Roche Diagnostics Ltd.) for PgR staining. Nuclear staining ≥1% was considered positive. HER2 expression was determined using IHC with a rabbit polyclonal antibody (Agilent Technologies) on 4 μm sections of paraffin-embedded tissue. A staining score of 3$^+$ according to the HercepTest criteria was considered positive; a result of 2$^+$ was considered positive only if confirmed by fluorescence *in situ* hybridization with an amplification ratio of ≥2.0. Furthermore, on 3 μm FFPE tissue sections, the EGFR and CK5/6 expression were determined using IHC with mouse monoclonal antibodies (EGFR; dilution, 1:10; Leica Biosystems, cat. no. EGFR-L-CE, Wetzlar, CK5/6; dilution, 1:50; Agilent Technologies, cat. no. M7273). The expression of claudin-3 was determined using IHC with a rabbit polyclonal antibody (dilution, 1:100; Invitrogen; Thermo Fisher Scientific, Inc. cat. no. 18-7340) on 5 μm FFPE tissue sections. EGFR, CK5/6 and claudin-3 staining results were considered positive if any cytoplasmic and/or membranous invasive carcinoma cell staining was observed. If EGFR and/or CK5/6 were positive, the sample was considered basal marker positive.

pCR was defined as no evidence of residual tumor cells in the breast and in the lymph nodes.

Statistical analysis. We conducted Fisher's exact test to assess the association of clinicopathological characteristics and pCR between the patients with and without TNBC and that between basal marker and claudin expressions and pCR in the TNBC group. DFS was measured from the date of surgery to the date of any recurrence or the last follow-up. Overall survival (OS) was measured from the date of diagnosis to the date of death or the last follow-up. The Kaplan-Meier method was used to generate survival curves and the cumulative incidence of events. Cramér-von Mises test was used to assess the differences in Kaplan-Meier curves. The Cox regression model was used to identify the potential prognostic and predictive indicators. All significant tests were two-sided, a P-value ≤0.05 was considered statistically significant. All analyses were performed using Stata statistical software (Stata SE 10; StataCorp LP).

Results

Patients and tumor characteristics. The median patients age was 53 years (range; 24-75 years). Table I shows the patient details and tumor characteristics. TNBC accounted for 20.4% of the total breast cancer. Age, clinical tumor size and node status before NAC, pathological tumor size and node status, and lymphovascular infiltration were not significantly different between TNBC and non-TNBC patients. Among the 323 patients, 26 patients (8%) achieved a pCR including 13 patients (19.7%) with TNBC and 13 (5.1%) without TNBC. The pCR rate of TNBC was significantly higher than that of the non-TNBC group (P<0.001). The reduction in tumor status was significant in patients with TNBC (P=0.001).

Correlation between pCR and expression of basal marker and claudin in TNBC. Core needle samples before NAC were available for basal marker and claudin staining for 43 patients with TNBC. We observed basal marker positivity in 25 (58.1%) cases of TNBCs and claudin-3 positivity in 21 (48%) cases with TNBC. Table II shows the associations between basal marker and claudin expressions and pCR. The

Table I. Patient and tumor characteristics by subtype.

Characteristics	Triple-negative (n=66)	Non Triple-negative (n=257)	P-value
Age (years)			0.395
<50	22	103	
≥50	44	154	
Clinical tumor size before NAC (cm)			0.625
≤5	49	199	
>5	17	58	
Clinical node status before NAC			>0.999
Negative	38	147	
Positive	28	110	
Clinical stage before NAC			0.285
I	8	18	
II	41	181	
III	17	58	
Estrogen receptor			<0.001
Negative	66	38	
Positive	0	219	
Progesterone receptor			<0.001
Negative	66	95	
Positive	0	162	
HER2			<0.001
Negative	66	178	
Positive	0	79	
Pathological tumor size after NAC (cm)			0.311
≤5	55	226	
>5	11	31	
Pathological node status after NAC			0.249
Negative	47	161	
Positive	19	96	
Lymphovasculer infiltration after NAC			>0.999
Negative	58	226	
Positive	8	31	
pCR			<0.001
Yes	13	13	
No	53	244	
Tumor status			0.001
Downstaging	50	138	
Stable	16	119	
Nodal status			0.297
Downstaging	16	47	
Stable	50	210	

HER2, human epidermal growth factor receptor 2; pCR, pathological complete response; NAC, neoadjuvant chemotherapy. Clinical stage was determined using the 8th edition of the Unio Interanationalis Contra Cancrum.

pCR rate of basal marker-positive tumors was 24% and that of claudin-positive tumors was 25%. Basal marker-negative and claudin-negative tumors had the lowest pCR rate (0%) whereas basal marker-negative and claudin-positive tumors had the highest pCR rate (33.3%). The association between the basal marker and/or claudin expression and pCR rate were not statistically significant (P=0.134).

Survival. After a median follow-up time of 111.5 (range: 6.8-170.2) months, 23 patients showed local recurrence

Table II. Expression of basal marker and claudin, and the association between pathological complete response rate and their expression.

Marker expression	N	pCR, n (%)	P-value
Basal marker			0.712
Positive	25	6 (24.0)	
Negative	18	3 (16.7)	
Claudin			0.708
Positive	21	6 (25.0)	
Negative	22	3 (15.8)	
Basal marker and Claudin			0.134
Basal marker positive, claudin positive	15	3 (20.0)	
Basal marker positive, claudin negative	10	3 (30.0)	
Basal marker negative, claudin positive	9	3 (33.3)	
Basal marker negative, claudin negative	9	0 (0.0)	

pCR, pathological complete response.

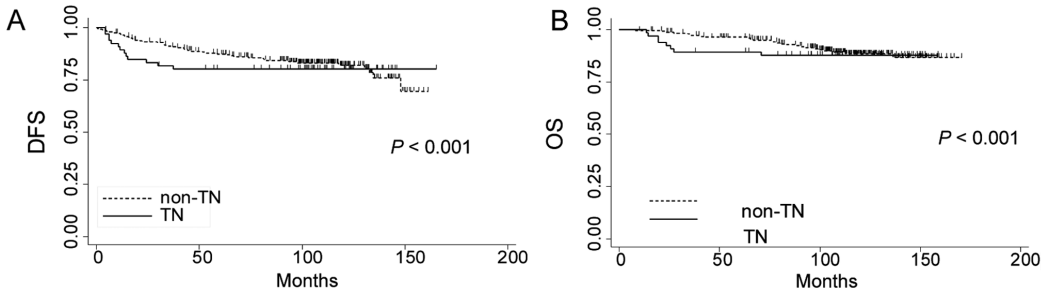

Figure 1. Survival of patients with triple-negative breast cancer. (A) Disease-free survival (P=0.618). (B) Overall survival (P=0.661). TN, triple-negative; DFS, disease-free survival; OS, overall survival.

and 47 patients showed distant recurrence. Breast cancer related-disease occurred in 13 (19.7%) patients with TNBC (5-year DFS: 80.3%, 95% CI: 0.68-0.88; 10-year DFS: 80.3%, 95% CI: 0.68-0.88) and in 45 (17.5%) patients with non-TNBC (5-year DFS: 87.5%, 95% CI: 0.83-0.91; 10-year DFS 82.1%, 95% CI: 0.76-0.87). Fig. 1A shows the cumulative DFS by TNBC versus non-TNBC (Cramér-von Mises P<0.001). Overall, 8 (12.1%) patients with TNBC (5-year OS: 86.8%, 95% CI: 0.74-0.93; 10-year OS: 84.8%, 95% CI: 0.72-0.92) and 26 (10.1%) patients with non-TNBC died (5-year OS: 96.2%, 95% CI: 0.93-0.98; 10-year OS: 88.6%, 95% CI: 0.83-0.92). Fig. 1B shows the cumulative OS by TNBC versus non-TNBC (Cramér-von Mises P<0.001). Table III summarizes the results of univariate and multivariate analyses for survival. In the univariate analysis for DFS and OS, clinical tumor size, node status before NAC, pathological tumor size, pathological node status, and lymphovascular infiltration were statistically significant prognostic factors. In the multivariate analysis for DFS, clinical and pathological tumor size, and lymphovascular infiltration were independent prognostic factors. In the multivariate analysis for OS, pathological node-positive and lymphovascular infiltration were independent worse prognostic factors. Fig. 2 shows the cumulative survival of patients with TNBC. The patients with pathological node-negative status showed significantly good prognosis. (log rank P<0.001). Among TNBC patients with pathological node-positive status, negative lymphovascular infiltration was a significant favorable prognostic factor (Table IV).

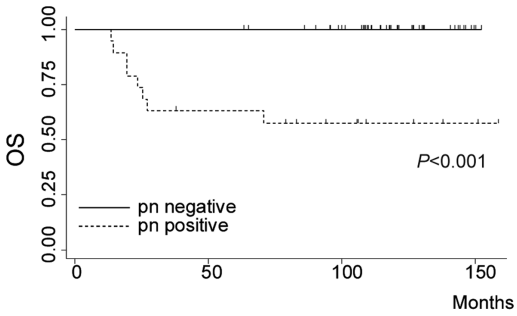

Figure 2. Overall survival based on pathological node status among patients with triple-negative breast cancer (P<0.001). pn, pathological-node; OS, overall survival.

Discussion

Our study showed that the long-term overall survival of patients with TNBC who received NAC containing anthracycline and taxane was favorable compared to that in non-TNBC patients. Especially, patients with TNBC who achieved pathological

Table III. Univariate and multivariate analyses of patient overall survival.

	Disease-free survival				Overall survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
Parameter	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Age <50 vs. ≥50 years	0.91	0.53-1.53			0.91	0.46-1.80		
Clinical tumor size >5 vs. ≤5 cm	3.49	2.08-5.85	2.31	1.30-4.10	3.19	1.62-6.26	2.01	0.96-4.21
Clinical node status positive vs. negative	2.37	1.40-4.02	1.66	0.96-2.88	2.69	1.32-5.43	1.69	0.81-3.50
Estrogen receptor positive vs. negative	0.70	0.41-1.20			0.57	0.29-1.13		
Progesterone receptor positive vs. negative	0.73	0.44-1.23			0.50	0.25-1.02		
HER2 positive vs. negative	1.33	0.75-2.38			1.85	0.91-3.74		
Subtype TN vs. non-TN	1.17	0.63-2.17			1.19	0.54-2.64		
pTumor size >5 vs. ≤5 cm	4.18	2.41-7.27	2.18	1.18-4.03	4.22	2.09-8.54	1.93	0.89-4.16
pNode status positive vs. negative	2.42	1.44-4.07	1.39	0.82-2.42	3.90	1.90-8.01	2.20	1.04-4.66
Pathological response pCR vs. non-pCR	0.38	0.09-1.57			0.33	0.04-2.40		
Lymphovascular infiltration positive vs. negative	2.64	1.43-4.87	4.21	2.05-8.66	6.05	3.05-11.99	4.63	2.29-9.35
Tumor status down vs. stable	0.87	0.52-1.47			0.79	0.40-1.55		
Node status down vs. stable	1.03	0.53-2.00			0.92	0.38-2.23		

HR, hazard ratio; CI, confidence interval; HER2, human epidermal growth factor receptor-2; TN, triple negative; p, pathological; pCR, pathological complete response.

Table IV. Univariate analysis for the overall survival of patients with node-positive triple-negative breast cancer.

	Univariate analysis	
Parameter	Hazard ratio	95% CI
Age <50 vs. ≥50 years	0.98	0.93-1.04
Clinical tumor size ≤5 vs. >5 cm	1.22	0.29-5.11
Pathological tumor size ≤5 vs. >5 cm	7.01	0.85-57.5
Lymphovascular infiltration negative vs. positive	15.68	3.00-81.97
Basal marker expression negative vs. positive	0.24	0.04-1.49
Claudin expression negative vs. positive	0.79	0.13-4.74

CI, confidence interval; pCR, pathological complete response.

node-negative status after NAC survived during the median follow-up of 111.5 months.

A previous study using PAM 50 showed that the new subtype 'claudin-low' had a preponderance for low to absent expressions of E-cadherin and claudin-3, and almost all TNBC cases were either basal-like or claudin-low (16). They showed different prognosis among subtypes. In this study, we evaluated the efficiency of IHC for claudin-3 and basal markers such as EGFR and CK5/6 to predict pCR and prognosis instead of gene profiling. The expression of claudin-3 and basal markers was not associated with prognosis and the response to neoadjuvant chemotherapy. IHC is an inexpensive technique; however, it failed to substitute for the gene profiles.

On the contrary, Lehman and colleagues reported that TNBC could be classified into seven subtypes. These seven TNBC subtypes were characterized based on gene ontologies and differential gene expressions and were labeled as basal-like 1, basal-like 2, immunomodulatory, mesenchymal, mesenchymal stem-like, luminal androgen receptor, and unstable (19). They also reported the survival analysis and chemotherapy response (20), and advocated the implications for NAC according to the seven subtypes (21). In their study, basal-like 1 and basal-like 2 showed different prognosis and response to standard chemotherapy. The basal-like 2 subtype had unique ontologies involving growth factor signaling and new therapeutic applications were required.

In our study, the multivariate analyses showed that pathological node status was an independent prognostic factor

for overall survival but not for disease-free survival. On the other hand, clinical and pathological tumor statuses were independent prognostic factors for disease-free survival but not for overall survival. These contradictory results might stem from the fact that patients who had only locoregional lymph node metastasis or breast recurrence without distant metastasis were alive for long periods, and that such patients had large tumors and negative lymph nodes.

Our study showed that patients with TNBC without any recurrence within 4 years had an excellent prognosis. For patients with TNBC, achievement of a pathological node-negative status was the most desirable result for improving prognosis. Pathological node-positive status or positive lymphovascular infiltration after NAC might be observed because of resistance to chemotherapy and may lead to worse prognosis. Hence, we need to predict chemosensitivity and develop specific treatments. A recent study has shown that adjuvant capecitabine therapy improved the outcomes for patients with TNBC without a pCR after standard NAC with anthracycline and taxane (22). Furthermore, various studies and clinical trials including targeted therapies, such as tyrosine kinase inhibitors, poly ADP-ribose polymerase-1 inhibitors, immune checkpoints, anti-androgens, and histone deacetylase inhibitors, have been conducted to improve the prognosis of patients with TNBC (23-26).

The limitations of this study need to be considered carefully. This is a retrospective study conducted at a single institute and the number of patients was limited, especially in the TNBC group. The strength of this study lies in the use of a single regimen as the NAC and the long follow-up periods for evaluating the survival of patients who received NAC.

In conclusion, patients with TNBC who showed no distant recurrence within 4 years after surgery had a good prognosis and their survival curve crossed with that of the non-TNBC group. For the patients with TNBC, pathological node-negative status and negative lymphovascular infiltration were favorable prognostic factors.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

HN contributed to the conception, design, analysis and integrity of the current study. MK, YT, EN and HT performed the experiments. MS performed the pathological evaluation. NH and MK confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Jikei University School of Medicine, and patient consent was obtained.

Patient consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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