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Triple-negative breast cancer in Thai patients: Experience in King Chulalongkorn Memorial Hospital

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Background : *Triple-negative breast cancer (TNBC) is defined by the lack of expression of ER, PR and Her-2 receptors and exhibits aggressive behaviors and poor clinical outcomes. Eighty percent of TNBCs are classified as basal-like tumor and share clinical behaviors that are consistent with this subtype of breast tumors.*

Objectives : *To characterize the epidemiological features of triple-negative breast cancers (TNBC) occurring in Thai patients in terms of demographics and pathological hallmarks including the expression patterns of ER, PR, Her-2, CK5/6, CK17 and EGFR as well as clinical outcomes.*

Methods : *In 166 TNBCs, adequate tissue samples were obtained from seventy cases, and patient/tumor characteristics as well as the survival outcomes were reviewed retrospectively.*

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Results : *The mean age was 48.04 years (range 26 – 79 years); 89% of the tumors were invasive ductal carcinomas, and 72.4% were high-grade tumors. A high proliferative index (Ki-67 > 30%) was found in 81.4% of the samples. Positive staining for CK5/6 and CK17 were found in 51.4% and 55.7% of the tumors, respectively; 58.6% of the tumors had EGFR expression. After we analyzed the expressions of ER, HER-2, CK5/6 and EGFR to define the basal phenotype, 72.9% were categorized into the basal-like group. At a median of 67.5 months, 32.5% of the tumors recurred and 30.1% of the patients had died. The 5-year disease-free survival and overall survival estimates were 69% and 74%, respectively. In multivariate Cox proportional hazards models, the basal-like subtype was not independently correlated with DFS or OS in TNBCs (HR 1.72, [95% CI, 0.13 - 22.96], P = 0.68; for DFS and HR 2.34, [95% CI, 0.17 - 31.75], P = 0.52 for OS).*

Conclusions : *TNBCs occurred at younger age in these Thai patients than in previous studies. We also demonstrated higher tumor grades, higher proliferation indices and inferior outcomes of TNBCs in Thai patients. Additionally, the presence of basal-like characteristics was not an independent predictor of the survival outcome.*

Keywords : *Basal-like subtype, triple-negative breast cancer, Thai.*

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เหตุผลของการทำวิจัย : *มะเร็งเต้านมชนิดทริปเปิลเนกาติพ วินิจฉัยได้จากการที่ตรวจไม่พบการแสดงของตัวรับสัญญาณเอสโตรเจน, โปรเจสเตอโรน และเฮอร์ทู ในชิ้นเนื้อทางพยาธิวิทยา มักมีความสัมพันธ์กับการดำเนินโรคที่รุนแรงกว่ามะเร็งเต้านมชนิดอื่น ๆ ประมาณร้อยละ 80 ของมะเร็งกลุ่มนี้ เมื่อตรวจชิ้นเนื้อด้วยวิธีพิเศษจะจัดอยู่ในกลุ่มย่อยเบซอลไลค (basal like phenotype)*

วัตถุประสงค์ : *เพื่อศึกษาลักษณะทั่วไป ลักษณะทางชิ้นเนื้อพยาธิวิทยาพิเศษ เช่น ER, PgR, Her-2, CK5/6, CK17 และ EGFR รวมทั้งการดำเนินโรคในผู้ป่วยมะเร็งเต้านมชนิดทริปเปิลเนกาติพในประเทศไทย*

วิธีการทำวิจัย : *ผู้ป่วยมะเร็งเต้านมชนิดทริปเปิลเนกาติพ 166 ราย มีผู้ป่วยที่มีชิ้นเนื้อทางพยาธิวิทยาที่เหมาะสมในการศึกษาต่อทั้งหมด 70 รายเก็บข้อมูลในเรื่องของลักษณะทั่วไป ลักษณะทางชิ้นเนื้อพยาธิวิทยาการดำเนินโรคและอัตราการรอดชีวิต*

ผลการศึกษา : *ช่วงตั้งแต่เดือนสิงหาคม พ.ศ. 2549 ถึงเดือนสิงหาคม พ.ศ. 2553 พบว่าอายุเฉลี่ยของผู้ป่วย คือ 48.6 ปี, ร้อยละ 89.0 ของผู้ป่วยมีลักษณะทางพยาธิวิทยาเป็นมะเร็งเต้านมชนิด ductal, ร้อยละ 72.4 ของผู้ป่วยพบว่ามีพยาธิวิทยาเป็นแบบ high grade, ร้อยละ 81.4 พบว่ามีค่า Ki-67 มากกว่าร้อยละ 30.0, ร้อยละ 51.4 และร้อยละ 55.7 ของผู้ป่วยพบว่ามีการแสดงออกของ CK5/6 และ CK17, ร้อยละ 58.6 ของผู้ป่วยพบว่ามีการแสดงออกของ EGFR หลังจากที่ใช้ค่าการแสดงออกของเอสโตรเจน, เฮอร์ทู, CK5/6 และหรือ EGFR ช่วยในการวินิจฉัยมะเร็งเต้านมกลุ่มย่อยเบซอลไลค พบว่าร้อยละ 72.9 ของผู้ป่วยทั้งหมดถูกจัดให้อยู่ในกลุ่มย่อยเบซอลไลคเมื่อติดตามการดำเนินโรคเฉลี่ยนาน 67.48 เดือน พบว่าร้อยละ 32.5 และร้อยละ 30.1 ของผู้ป่วยมีการกลับเป็นซ้ำของโรคและเสียชีวิต ระยะเวลาที่โรคไม่ลุกลามและระยะเวลารอดชีวิตในช่วง 5 ปีในผู้ป่วยมะเร็งเต้านมชนิดทริปเปิลเนกาติพทั้งหมด คือ ร้อยละ 69.0 และร้อยละ 74.0 ตามลำดับ*

สรุป : *มะเร็งเต้านมชนิดทริปเปิลเนกาติพในผู้ป่วยไทย พบมากในผู้ป่วยอายุน้อยเมื่อเทียบกับการศึกษาอื่น แต่พบลักษณะทางพยาธิวิทยาชนิด high grade และมีค่าการแบ่งตัวที่มาก (high proliferation index) เหมือนกับการศึกษาอื่น ๆ การตรวจพบมะเร็งเต้านมชนิดทริปเปิลเนกาติพกลุ่มย่อยเบซอลไลคไม่สัมพันธ์กับการดำเนินโรค*

คำสำคัญ : *มะเร็งเต้านมชนิดทริปเปิลเนกาติพ, กลุ่มย่อยเบซอลไลค (Basal like phenotype).*

Breast cancer is a complex and heterogeneous disease. Several breast cancer subgroups have been identified by assessing altered expression of the standard breast markers estrogen receptor, progesterone receptor and Her-2 receptor: hormonal receptor positive, Her-2 positive, and triple negative. Additionally, recent advanced gene expression profiling allowed invasive ductal carcinoma to be divided into five intrinsic subtypes: Luminal A, Luminal B, Her-2 positive, basal-like and normal breast-like. Each subgroup exhibits different clinical and pathological features, prognoses and clinical outcomes. [1 - 3] Triple-negative breast cancer (TNBC), defined by a lack of estrogen, progesterone and HER-2 receptor expression accounts for 10 - 20% of breast cancers. Various epidemiological studies have demonstrated a higher prevalence of TNBC among African-American premenopausal women.[4] Furthermore, most TNBCs overlap with basal-like tumors.

TNBC shares some pathological features with breast myoepithelial tissue, such as expression of CK5/6 and CK17. [5 - 8] The association between EGFR expression and basal-like characteristics has been reported in different ethnicities, although its significance remains poorly understood. [9, 10] Recently, Nielsen TO, *et al*. [5] proposed a panel of five antibodies to receptors implicated in breast cancer, including ER, HER-2, cytokeratin 5/6 and EGFR, to classify basal-like tumors instead of using gene expression analysis. These five markers can classify the basal-like subgroup with 76% sensitivity and 100% specificity.[5] Several studies supported the application of this panel of proteins in clinical practice. Recently, these comprehensive immunohistochemical biomarkers have been investigated in various cohorts to validate that this novel method can be used to identify the basal-like subtype in an accessible way. [11]

In terms of clinical outcomes, TNBCs have been shown to correlate with a higher recurrence rate, particularly in the first 3 years after diagnosis, and shorter survival outcomes despite better responses to chemotherapy.[12 - 15] Because of the absence of hormone and HER-2 receptors, traditional hormone therapies and targeted agents such as trastuzumab are not effective against this subtype of cancer. Therefore, a better understanding of the features of TNBC may lay the foundation for customized drug development to better meet patients' needs. In particular, little is known about this aggressive subtype in Thailand. Thus, the objective of this study was to characterize the epidemiological information of TNBC in Thai women in terms of demographic features, pathological features and clinical outcomes. To assess pathological features of these tumors, we investigated the frequency of specific basal markers such as CK5/6, CK17 and EGFR. In addition, we compared these clinical features among different ethnicities.

Material and Methods

Patients

A total of 166 Thai women, diagnosed with TNBC between August 2006 and August 2010 and treated at King Chulalongkorn Memorial Hospital, were retrospectively reviewed. Patients with histopathologically and immunohistochemically confirmed TNBC who had frequent follow-up data were included into the study. Tumors were considered triple

receptor negative if nuclear staining of ER and PR were <1% of normal and membrane staining of HER-2 was found in < 0% of the cells using IHC or if no gene amplification was found using fluorescence *in situ* hybridization. Adjuvant and neoadjuvant chemotherapies with anthracycline-based, taxane-based, anthracycline and taxane-based or non-anthracycline and non-taxane-based regimens were administered depending on the patients' and physicians' preferences. Patients received adjuvant radiation therapy if indicated. The institutional review board (IRB) of King Chulalongkorn Memorial Hospital and Chulalongkorn University approved this retrospective study (COA No.172/53).

Immunohistochemistry staining and scoring

Seventy-one formalin-fixed, paraffin-embedded tissues were evaluated and the representative areas for each invasive tumor cells from representative paraffin blocks were processed for the tissue cores (0.5 mm in diameter). Immunohistochemistry was performed on the tissue microarray. Optimal antigen retrieval procedure and dilution applied for each antibody are demonstrated in Table 1. Antibodies were used to detect ER (dilution 1:300, clone SP1, Neomaker), PR (dilution 1:200, clone Y85, Cell Marque), HER-2 (dilution 1:1,200, polyclonal, Dako), CK5/6 (dilution 1:50, clone D5/16B4, Zymed), CK17 (dilution 1:50, clone CK-E3DAKO, EGFR (dilution 1:50, clone H11, DAKO). After routine deparaffinization in xylene, it was then rehydrated with a descending series of alcohol concentrations. The slide was incubated with 3% hydrogen peroxide to block endogenous peroxidase activity for 5 minutes followed by washing with *Phosphate buffered* saline (PBS). Subsequently, the sections were incubated with 3% normal horse serum for 20 minutes to block non-specific background. The slides were incubated with primary MoAbs using the primary antibodies (ER, PR, HER-2, CK5/6, EGFR, CK17 (dilution shown in Table 1) for 60 minutes. Then they were washed with PBS buffer, the tissue was incubated with the secondary antibody for 30 minutes at 37°C, followed by DAB chromogen incubation for 10 minutes, and then counterstained with Mayer's hematoxylin.

Table 1. Antibody panel used in this study.

Antibody	Clone	Dilution	Source
ER	SP1	1:300	Neomarker
PR	Y85	1:200	Cell Marque
HER-2	Polyclonal	1:1,200	Dako
CK5/6	D5/16 B4	1:50	Zymed
CK17	CK-E3	1:50	Dako
EGFR	Monoclonal mouse, clone H11	1:50	Dako

The staining results were assessed by a pathologist. Negative expression of the estrogen and progesterone receptors was defined as complete absence of staining (0%), whereas negative expression of HER-2 was defined as an absence of membrane staining in < 0% of tumor cells. As for CK5/6, CK17 and EGFR, this study used a three-point scoring system (0, 1, and 2). Immunoreactivity was considered positive, any weak or strong staining of the cytoplasm and/or membrane of an invasive carcinoma cell was observed (>10% of tumor cells). The staining results are listed in Table 2.

Basal-like cancers were identified when the IHC staining pattern showed negative expression of both ER and HER-2 and positive expression of CK 5/6 and/or EGFR. [5]

Statistical analysis

The baseline characteristics of each patient such as age, ECOG, disease stage, tumor characteristics and treatment options (i.e., surgery, radiation and systemic therapy), were collected. Additionally, we investigated the frequency of specific basal markers such as CK5/6, CK17 and EGFR. After IHC staining, TNBCs were categorized into 1 of the 2 groups according to their basal-like expression: basal-like positive and basal-like negative. Patient characteristics, including age, pathological stage (early stage; stage I-II, locally advanced stage; stage III and advanced stage; stage IV), tumor grade, tumor size, lymph node involvement, lymphovascular invasion, and adjuvant chemotherapy were compared between the two groups using Fisher's exact test.

Overall survival (OS) was measured from the date of surgery to the date of death or loss of follow up. Disease-free survival (DFS) was measured from the date of surgery to the date of first reported local or distant recurrence, loss of follow up or death from any cause. Patients who died before experiencing the relevant events were considered censored at their dates of last follow up. The Kaplan-Meier product limit method was used to estimate the 5-year OS and DFS with 95% confidence intervals (CIs) of all patients and those with or without basal-like subtype and other clinical characteristics; groups were compared using log-rank statistics.

Cox proportional hazards models were fit to determine the association of patients and clinical characteristics with their survival outcomes. Cox multivariate models based on both statistical significance and clinical relevance included age (≤ 50 years, >50 years), grade (II, III), tumor size (T1-2, T3 - 4), lymph node involvement (N0 - 1, N2 - 3), lymphovascular invasion (negative, positive), and adjuvant chemotherapy (anthracycline-based, anthracycline and taxane based). Results are expressed as HRs and 95% CIs. All statistical analyses were performed using SPSS software package (Version 16.0). P <0.05 was considered statistically significant.

Table 2. IHC staining and scoring for CK5/6, CK17 and EGFR.

Score	Nuclear and/or cytoplasmic staining	Result
0	No staining seen (<10%)	Negative
1	Staining in 10 - 20% of tumor cells	Weak positive
2	Strongly staining in > 20% of tumor cells	Positive

Results

From August 2006 to August 2010, one hundred sixty-six patients, were diagnosed with TNBC and treated at King Chulalongkorn Memorial Hospital. The mean age was 48.04 years (range 26 - 79). Eighty-nine patients (53.6%) were less than 50 years old at the time of diagnosis, and twenty (14.1%) had family history of breast cancer. In terms of clinical manifestations, 96.2% of the patients presented with breast masses, whereas 6% and 10% presented with breast discharge and breast tenderness, respectively. Fine-needle aspiration was used for the diagnosis in 37.5%. In terms of the pathological features, 146 out of 164 patients (89%) demonstrated invasive ductal carcinomas, 105 out of 145 patients (72.4%) had high-grade (grade III) tumors, 82.6% had T1 - 2 tumors and 62% had node-negative tumors. A high proliferative index (Ki-67 >30%) was found in 79 out of 97 patients (81.4%); 65 out of 100 patients (65%) had confirmed TP53 expression. Other baseline characteristics are listed in Table 3.

Table 3. Patients' baseline characteristics.

Patient Characteristics	**TNBCs (n = 166)**
Age (years)	48.04 (26 - 79)
<40 years	47 (28.3 %)
40 - 49 years	42 (25.3 %)
50 - 59 years	51 (30.7 %)
60 - 69 years	19 (11.4 %)
≥ 70 years	6 (3.6 %)
ECOG	
0	97 (58.4 %)
1	69 (41.6 %)
Family history of breast cancer: yes	20/162 (14.1 %)
Stage of disease	
Early stage	124 (74.7 %)
Locally advanced stage	37 (22.3 %)
Advanced stage	5 (3 %)
Pathologic features (n = 164)	
Invasive ductal carcinoma (IDC)	146 (89 %)
IDC and DCIS	13 (8 %)
Histology grading (n = 145)	
I	3 (2.1 %)
II	37 (25.5 %)
III	105 (72.4 %)

Table 3. (Con) Patients' baseline characteristics.

Patient Characteristics	TNBCs (n = 166)
Tumor size (n = 161)	
T1	47 (29.2 %)
T2	86 (53.4 %)
T3	24 (14.9 %)
T4	4 (2.4 %)
Nodal involvement (n = 163)	
N0	101 (62%)
N1 (1 - 3 nodes)	34 (20.8%)
N2 (4 - 9 nodes)	22 (13.5%)
N3 (≥ 10 nodes)	6 (3.7 %)
Lymphovascular invasion (n = 87)	
Yes	36 (41.4 %)
No	51(58.6 %)
KI-67 (n = 97)	
< 30%	18 (18.6 %)
≥ 30%	79 (81.4 %)
TP53 (n = 100)	
Positive	65 (65 %)
Negative	35 (35 %)
Margin (n = 136)	
Negative	108 (79.4 %)
Positive	6 (4.4 %)
Closed margin	22 (16.2 %)
Type of surgery (n = 151)	
Wide excision	53 (35.1 %)
Simple mastectomy and MRM	98 (64.9%)
Sentinel LN biopsy	5/150 (3.3 %)
Axillary LN dissection	115/150 (76.7 %)
Adjuvant radiotherapy	94/153 (61.4 %)
Chemotherapeutic treatment (n = 157)	
No treatment	4 (2.5 %)
Neoadjuvant treatment	46 (29.3 %)
Adjuvant treatment	107 (68.2 %)
Anthracycline-based CMT	
Yes	125 (75.3 %)
No	41 (24.7 %)

Table 3. (Con) Patients' baseline characteristics.

Patient Characteristics	TNBCs (n = 166)
Regimen : AC (Doxorubicin and cyclophosphamide)	100/125 (80 %)
Taxane-based CMT	53 (31.9 %)
Yes	113 (68.1 %)
No	42/53 (79.2%)
Regimen: Paclitaxel (q week and q 3 weeks)	
Non-anthracycline and taxane-based CMT	
Regimen: CMF iv and oral regimen	29 (17.5 %)
Yes	137 (82.5 %)
No	(3.07 - 166.3 months)
Follow up time (month) Mean 67.48 months	
First recurrence (events) (n = 161)	
Yes	54 (33.5 %)
No	107 (66.5 %)
Site of recurrent	Total events = 79
Locoregional	32 (40.5%)
Bone	13 (16.5 %)
Lung	20 (25.3 %)
Liver	9 (11.4 %)
Brain	5 (6.3 %)
Death (events) (n = 166)	
Yes	50 (30.1 %)
No	116 (69.9 %)

ECOG = Eastern Cooperative Oncology Group Score, DCIS = Ductal carcinoma in-situ,
LN = lymph node, CMT = chemotherapy, CMF = Cyclophosphamide, methotrexate and fluorouracil

Only 70 cases had tumor tissues with sufficient quality for measuring the basal-specific markers CK5/6, CK17 and EGFR. Positive staining of CK5/6 was found in 36 out of 70 patients (51.4%), and positive expression of CK17 was identified in 39 out of 70 patients (55.7%) as shown in Figure 1A and 1B. Forty-one out of 70 patients (58.6%) showed positive EGFR expression (Figure 1C).

After determining the core basal phenotype using the expression patterns of ER, HER-2, CK5/6 and EGFR, we noted a significant overlap between the TNBC and basal-like groups. Fifty-one out of 70 cases (72.9%) were considered basal-like subgroup. Additionally, we report a higher rate of grade III (80.4% *vs*. 72.2%) and highly proliferative tumors (Ki-67>30%; 91.9% *vs*. 83.3%) as well as an increased rate of

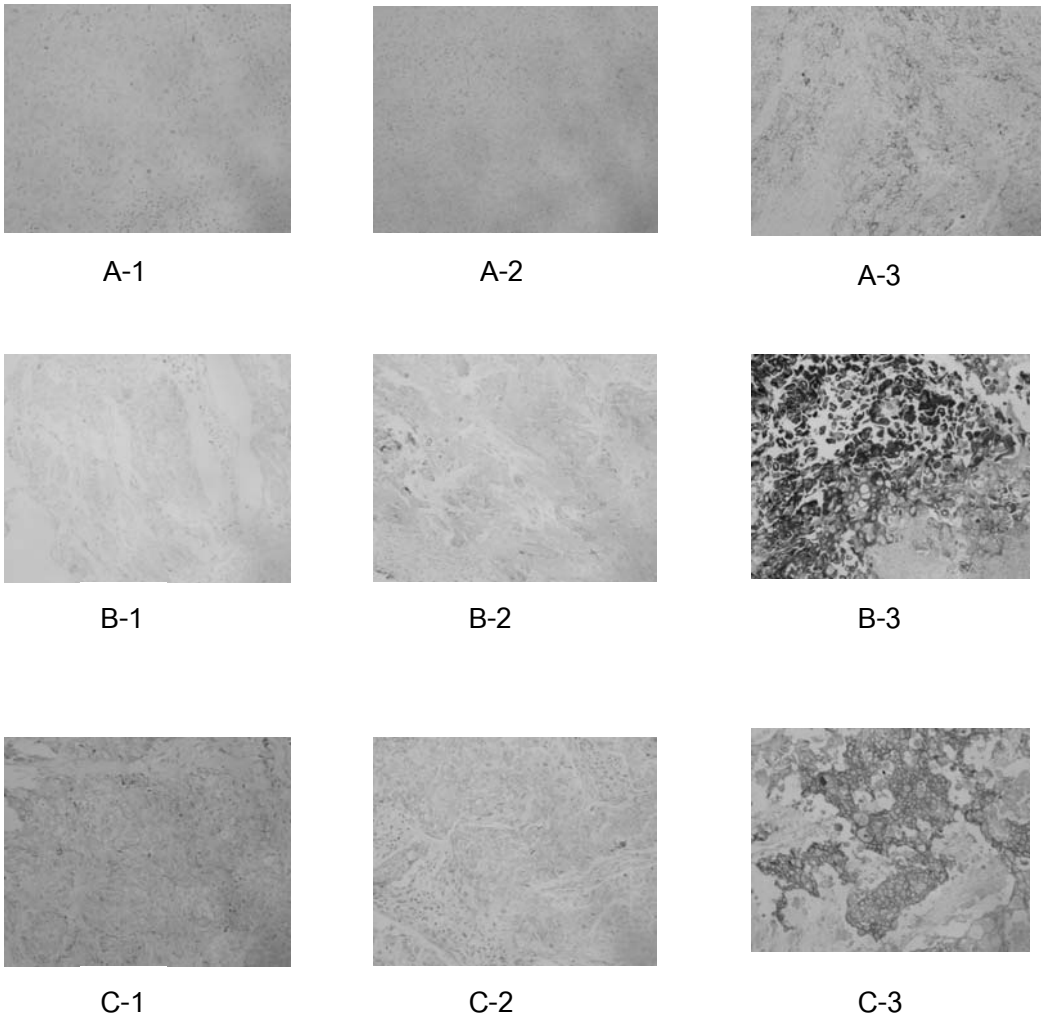

Figure 1. CK5/6 (A) (magnification, × 100), CK17 (B)(magnification, × 200 and EGFR (C) (magnification, × 200), immunohistochemistry staining results. (1 = negative, 2 = Weak positive, 3 = positive)

lymphovascular invasion (45.5% *vs*. 27.3%) in basal-like tumors compared with non-basal-like cancer; however, the differences between groups were not statistically significant.

In terms of treatment options, breast-conserving surgery was performed in 35.1% of the patients. Almost all patients (97.5%) received adjuvant or neoadjuvant chemotherapy. Anthracycline-based chemotherapy was administered in 75.3% of the patients, and a taxane-based regimen was administered in 31.9% of the patients.

Through 31 December 2014, the mean follow-up time was 67.5 months (range 3.1 - 166.3), and 54 patients had recurrent diseases (32.5%). The most common type of relapse was multiple organs relapse (20/54; 37%), and the common sites of relapse were the chest wall, axillary lymph nodes, lung, bone, liver and brain. Furthermore, we identified a higher rate of relapse events in patients with basal-like tumors compared with non-basal-like tumors (36% vs 26.3%, P = 0.54). Fifty out of 166 patients (30.1%) were reported to have died. We additionally identified a

higher rate of death events in patients with basal-like tumors compared with non-basal-like tumors (33.3% vs 26.3%, $P = 0.57$). The 5-year disease-free survival and overall survival estimates were 69% and 74%, respectively. The 5-year DFS and 5-year OS estimates according to patient and tumor characteristics are summarized in Table 4.

Table 4. Survival estimates by patients characteristics.

	Patients (n)	Events (n)	5-year overall survival estimate (95% CI)	*P*	Events (n)	5-year disease-free survival estimate (95% CI)	*P*
All Patients	166	50	0.74 (0.71, 0.77)		54	0.69 (0.65, 0.73)	
Age, years							
≤50	95	34	0.67 (0.62, 0.72)		37	0.63 (0.58, 0.68)	
>50	70	16	0.82 (0.77, 0.87)	0.1	17	0.78 (0.73, 0.83)	0.06
Stage of disease							
Early stage	124	25	0.84 (0.81, 0.88)		32	0.78 (0.74, 0.82)	
Locally advanced	37	20	0.47 (0.38, 0.56)		22	0.40 (0.32, 0.48)	<0.0001
Advanced stage	5	5	0.00	<0.0001			
Nuclear Grade							
II	37	10	0.75 (0.68, 0.82)		10	0.75 (0.68, 0.82)	
III	105	34	0.72 (0.68, 0.76)	0.62	37	0.66 (0.61, 0.71)	0.33
Tumor Size							
T1 - 2	133	37	0.76 (0.72, 0.80)		43	0.71 (0.67, 0.75)	
T3 - 4	28	12	0.59 (0.49, 0.69)	0.06	9	0.67 (0.57, 0.77)	0.46
Lymph Nodes							
N0	101	16	0.87 (0.84, 0.90)		21	0.81 (0.77, 0.85)	
N1	34	13	0.67 (0.59, 0.75)		15	0.65 (0.57, 0.73)	
N2-3	28	19	0.35 (0.26, 0.44)	<0.0001	16	0.32 (0.22, 0.42)	<0.0001
Lymphovascular Invasion							
Negative	51	5	0.89 (0.84, 0.94)		10	0.80 (0.74, 0.86)	
Positive	36	19	0.50 (0.42, 0.58)	<0.0001	20	0.48 (0.39, 0.57)	<0.0001
Adjuvant chemotherapy							
Anthracycline	79	15	0.81 (0.76, 0.86)		19	0.76 (0.71, 0.81)	
Anthracycline/Taxane	47	20	0.59 (0.52, 0.66)	0.014	21	0.57 (0.50, 0.64)	0.02

CI = confidence interval

The 5-year disease-free survival estimates were 66% and 79% in patients whose tumors were basal-like and in those whose tumors were non-basal-like, respectively ($P = 0.48$). The 5-year overall survival estimates were 69% and 79% in patients with basal-like tumors and in those with non-basal-like tumors ($P = 0.56$), respectively. The basal-like subtype was not an independent predictor of survival outcomes. The 5-year DFS and 5-year OS estimates according to patient characteristics and basal-like subtype are summarized in Table 5. The Kaplan-Meier curves for the DFS and the OS according to basal-like phenotype are shown in Figure 2A,B.

Table 5. Survival estimates by patients' characteristics and basal-like subtype.

	Patients (n)	Events (n)	5-Year Overall Survival Estimate (95% CI)	*P*	Events (n)	5-Year Disease -Free Survival Estimate (95% CI)	*P*
All Patients	70	22	0.72 (0.67, 0.77)		23	0.70 (0.64, 0.76)	
Basal-like subtype							
Yes	51	17	0.69 (0.62, 0.76)		18	0.66 (0.59, 0.73)	
No	19	5	0.79 (0.70, 0.88)	0.56	5	0.79 (0.70, 0.88)	0.48
Age, years							
Age ≤ 50	40	14	0.65(0.56, 0.74)		15	0.63 (0.55, 0.71)	
Age > 50	29	6	0.79(0.71, 0.87)	0.1	6	0.79 (0.71, 0.87)	0.09
Stage of disease							
Early stage	54	10	0.82 (0.77, 0.87)		12	0.78 (0.72, 0.84)	
Locally advanced	15	8	0.47 (0.34, 0.60)		8	0.47 (0.34, 0.60)	
Advanced stage	2	2	0.00	<0.001	1	0.00	<0.001
Nuclear Grade							
II	14	4	0.71 (0.59, 0.83)		4	0.71 (0.69, 0.83)	
III	51	12	0.77 (0.71, 0.83)	0.9	13	0.74 (0.68, 0.80)	0.7
Tumor Size							
T1 - 2	59	15	0.75 (0.69, 0.81)		17	0.71 (0.65, 0.77)	
T3 - 4	11	5	0.55 (0.40, 0.70)	0.1	4	0.60 (0.44, 0.76)	0.4
Lymph Nodes							
N0	41	5	0.88 (0.93, 0.83)		6	0.85 (0.79, 0.91)	
N1	23	10	0.57 (0.47, 0.67)		10	0.57 (0.47, 0.67)	
N2 - 3	6	4	0.33 (0.14, 0.52)	<0.001	4	0.20 (0.02, 0.38)	<0.001
Lymphovascular Invasion							
Negative	20	1	0.95 (0.90, 1.00)		1	0.95 (0.90, 1.00)	
Positive	13	9	0.31 (0.18, 0.44)	<0.001	9	0.31 (0.18, 0.44)	<0.001
Adjuvant chemotherapy							
Anthracycline	35	5	0.86 (0.80, 0.92)		6	0.83 (0.77, 0.89)	
Anthracycline/Taxane	22	12	0.46 (0.35, 0.57)	<0.001	12	0.46 (0.35, 0.57)	<0.001

CI = confidence interval

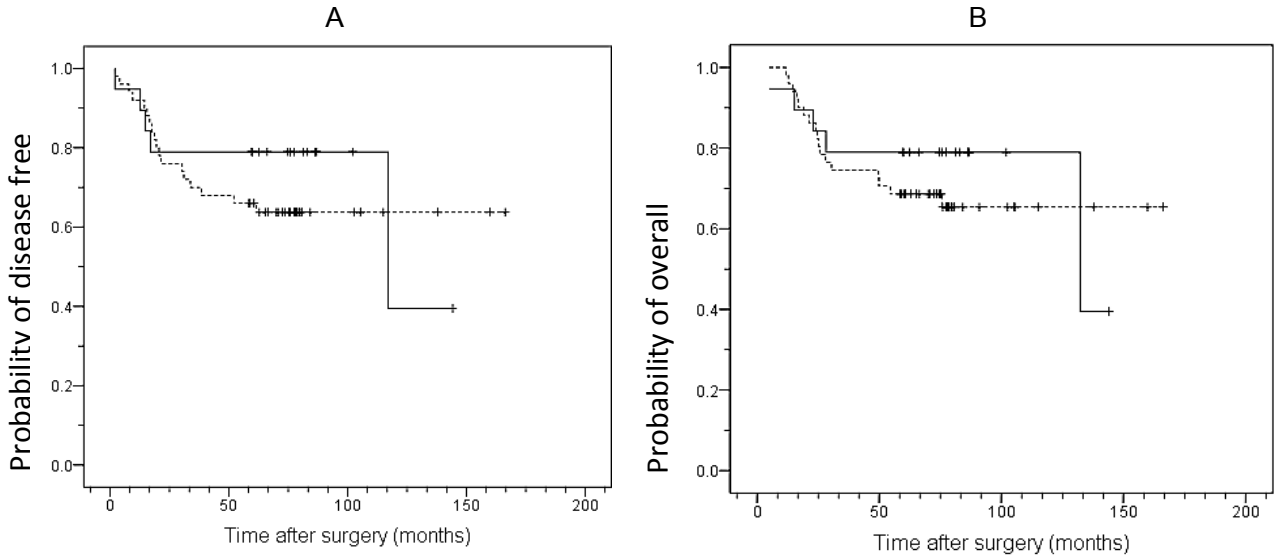

Figure 2. Disease free survival (A) and overall survival (B) by basal-like subtype in triple negative breast cancer patients.

According to multivariable Cox proportional hazards models, the basal-like subtype was not independently correlated with DFS or OS in TNBC (HR 1.72, [95% CI, 0.13 - 22.96], *P* = 0.68; as for DFS and HR 2.34, [95% CI, 0.17 - 31.75], *P* = 0.52 for OS) after adjustment for various important risk factors such as age, tumor grade, tumor size, nodal involvement, lymphovascular invasion, and treatment with adjuvant chemotherapy. The univariate Cox proportional hazards models of DFS and OS are reported in Table 6, and the multivariable Cox proportional hazards models of DFS and OS that were adjusted for basal-like subtype, grade, stage, lymphovascular invasion, and adjuvant chemotherapy are summarized in Table 7.

Table 6. Univariable Cox proportional hazards model.

Factor	Overall Survival			Disease-Free Survival		
	Hazard Ratio	95% CI	*P*	Hazard Ratio	95% CI	*P*
Basal-like subtype: No vs Yes	0.74	0.27 - 2.02	0.56	0.70	0.26 - 1.89	0.48
Age: ≤ 50 years vs >50 years	2.09	0.82 - 5.34	0.12	2.14	0.84 - 5.42	0.11
Grade: 2 vs 3	0.91	0.29 - 2.82	0.87	0.83	0.27 - 2.55	0.74
Tumor: T1- 2 vs T3 - 4	0.45	0.17 - 1.23	0.12	0.64	0.22 - 1.89	0.42
Node: N0 - 1 vs N2 - 3	0.16	0.06 - 0.41	0.000*	0.18	0.06 - 0.49	0.001*
Lymphovascular invasion: No vs Yes	0.04	0.01 - 0.34	0.003*	0.03	0.004 - 0.27	0.001*
Adjuvant chemotherapy						
Anthracyclines vs Anthracyclines/Taxanes	0.2	0.07 - 0.56	0.02*	0.21	0.08 - 0.54	0.001*

CI = confident interval, **P* = significant

Table 7. Multivariable Cox proportional hazards model.

Factor	Overall Survival			Disease-Free Survival		
	Hazard Ratio	95% CI	*P*	Hazard Ratio	95% CI	*P*
Basal-like subtype: No vs Yes	2.34	0.17 - 31.75	0.52	1.72	0.13 - 22.96	0.68
Age: ≤ 50 years vs >50 years	0.44	0.07 - 2.73	0.37	1.01	0.2 - 5.18	0.99
Grade: 2 vs 3	3.67	0.49 - 7.27	0.20	2.19	0.35 - 13.87	0.41
Tumor: T1 - 2 vs T3 - 4	0.12	0.01 - 2.01	0.14	0.43	0.04 - 5.32	0.51
Node: N0 - 1 vs N2 - 3	0.57	0.23 - 2.49	0.65	1.01	0.11 - 9.53	0.99
Lymphovascular invasion: No vs Yes	0.03	0.001 - 0.80	0.04*	0.03	0.001 - 0.63	0.03*
Adjuvant chemotherapy						
Anthracyclines vs Anthracyclines/Taxanes	1.93	0.07 - 56.62	0.7	1.83	0.07 - 49.35	0.72

CI = confident interval, **P* = significant

Discussion

The aims of the present study were to characterize epidemiological information of TNBC in Thai women, to investigate the frequency of specific basal markers such as CK5/6, CK17 and EGFR in this subgroup, and to assess the associations between the basal-like subtype, clinical characteristics and outcomes. Of the total 166 patients with TNBC in our series, we found that the mean age was 48.04 years (range 26 - 79); 53.6% were diagnosed at an age less than 50. Approximately, three-fourths presented with high-grade (grade III) tumors, four-fifths had early T stage (T1 - 2) tumors and two-thirds were node-negative. A high proliferative index (Ki-67 >30%) was also found in nearly four-fifths of the entire population. The 5-year disease-free survival and overall survival estimates in our cohort were 69% and 74%, respectively. In the subset of 70 patients analyzed for basal markers, we found that the prevalence of the basal-like subtype was 72.9%. At a median follow-up time of 67.5 months, the 5-year OS and DFS estimates for those whose tumors were basal-like *vs*. non-basal-like were 69% and 66% *vs*. 79% and 79% (OS; $P = 0.56$, DFS; $P = 0.48$), respectively.

Recent studies have demonstrated the differences in breast cancer incidences among distinct ethnicities. Several studies showed that white women had a higher incidence of breast cancer compared with African American women, but a lower overall mortality. According to the Carolina Breast Cancer study, a large population-based, case-controlled study in North America, Carey *et al*. found that the TNBC subtype was more prevalent among premenopausal African American women (39%) compared with postmenopausal African American women (14%) and non-African American women (16%) of any age. Thus, TNBCs were more than twice likely to occur in African Americans.[4] Recently, a discrepancy in the prevalence of TNBCs has been described in various ethnicities. A higher incidence was described in African American or African women compared with European white, Hispanic or Asian

women.[4] In Thailand, Chuthapisith S, *et al*. reported that the prevalence of TNBCs was approximately 15%[16], which was similar to some Asian populations. Therefore, the tumor biology within each ethnicity may be an important factor contributing to these differences. Additionally, a clear correlation has been established between TNBC and young age at diagnosis. In our study, 28.3% were diagnosed with breast cancer at an age under 40 years old, and the mean age at diagnosis was 48.04 years old, which was similar to the African American women from Carey's study (the mean age was 46 years old). In contrast with other studies in the US, which included more white patients, Lund MJ, *et al*. reported that the mean age of TNBC patients was in the postmenopausal period, 56.2 years old.[17] Like the white population, some Chinese and Singapore-based studies described a similar mean age of diagnosis [18, 19], whereas Japanese, Korean and Malaysian studies reported different results.[20 - 22] Nevertheless, the molecular similarities among the TNBCs in Thai and African American women should be intensively studied using advanced technologies that can efficiently identify comprehensive information in order to tailor the therapy to the targeted mutation.

In terms of pathological features, TNBCs were larger in size, had higher tumor grades and had increased proliferation indices than other subtypes. Similar to other studies, 72.4% and 81.4% of the patients in our study presented with high-grade (grade III) tumors and highly proliferative tumors (Ki-67 > 30%), respectively.[13, 23] In addition, approximately 70.8% of our patients had tumors that were more than 2 cm in diameter, which is in agreement with several studies.[23, 24] Regarding the nodal status, although some studies demonstrated that TNBCs correlated with a higher incidence of axillary nodal metastases [12], only one-third of our patients had axillary metastases. Numerous lines of evidence have shown that tumors with altered TP53, which can be characterized by IHC, are generally associated with ER-negative cancer.[4, 9, 25] Positive TP53 expression was defined in 65% of our patients, consistent with previous studies from the United Kingdom. Rakha EA, *et al*. reported that 56% of the TN subgroup, compared with 22% of non-triple-negative subgroup, showed TP53 expression.[9] CK5/6 and EGFR were more frequently expressed in the TN subtype (60% CK5/6 positive and 52% EGFR positive) than the HER-2 subtype (20% CK5/6 positive and 46.7% EGFR positive).[26] Here, our results similarly showed that 51.4% and 58.6% of the TN tumors were positive for CK5/6 and EGFR, respectively. Therefore, our results support the idea that the essential pathological features of TNBCs in Thai women do not differ from those in other populations.

Although gene expression profiling is considered to be the standard method for defining basal-like breast cancer, this technique is not feasible or cost-effective for clinical applications. Hence, several research groups have proposed routine IHC staining for markers such as ER, PR, HER-2, basal cytokeratins (CK5/6, CK14, CK17) and EGFR to determine the presence of the basal-like subtype. Currently, whether to recommend using IHC staining for the detection of the basal-like phenotype is still questionable, and some IHC markers have not been standardized for clinical practice. Using the markers outlined in Nielsen's study, we could define the basal-like phenotype in 72.9% of the Thai TNBCs,

which is comparable to other previous studies.[9 , 27] Furthermore, we found a higher rate of grade III tumors (80.4% *vs*. 72.2%), highly proliferative tumors (Ki-67 >30%, 91.9% *vs*. 83.3%) and lymphovascular invasion (45.5% *vs*. 27.3%) in tumors with the basal-like phenotype compared with the non-basal-like phenotype; however, the differences between these two groups were not statistically significant. Regardless, our findings reveal an overlap between the triple-negative and basal-like subtypes.

TNBCs have been demonstrated to have worse prognoses compared with non-TN cancers, especially in the first 5 years. Several studies have reported that patients with TNBCs have a significantly decreased relapse-free survival and OS compared with non-TNBCs.[12,13, 28] Despite the fact that women in this subgroup seem to respond better to cytotoxic treatment and have higher rates of pathologically complete responses (pCR) to therapy, OS is still worse than women with other subtypes, especially those who do not achieve a pCR to therapy. After a 5.5-year follow up, our study similarly showed inferior 5-year DFS and 5-year OS estimates, especially in Thai women with locally advanced and advanced TNBC (5-year DFS; 40% *vs*. 0%, 5-year OS; 47% and 0% in locally advanced and advanced subgroups, respectively). Previous studies reported that the poorer survival outcomes in the basal-like subtype compared with the non-basal-like subtype was independent of tumor size, grade and lymph node status;[28, 29] whereas these adverse prognosis factors are not associated with inferior outcomes in other subtypes.[12, 30] Here, we also identified the 5-year DFS and OS estimates were 66% versus 79% and 69% versus 79% in patients whose tumors were basal-like and in those whose tumors were non-basal-like, respectively (P = 0.48 for DFS, P = 0.56 for OS). Although, the basal-like subtype we defined here was not an independent predictor of lower survival outcomes, a large prospective cohort with long-term follow up might help in defining this inconsistent prognosis in Thai TNBCs.

To our knowledge, this is the first large study of Thai women with TNBC. Moreover, the results from this study can inspire Thai researchers to further investigate the biology of TNBCs, which may provide both an understanding of tumor cell heterogeneity and possible therapeutic strategies for this challenging subgroup. Finally, advanced knowledge in the molecular characterization of TNBCs in the Thai population would aid personalized treatment to better fitting in with our patients' needs.

Our retrospective single-center study has several limitations. First, although 166 TNBC patients were reviewed in our study, the small sample size (only 70 cases) used to test for the basal-like phenotype might have affected the results for the frequency of this subtype. Further investigations should be completed in a large independent cohort population. Second, novel comprehensive immunohistochemical biomarkers have been recently discovered in various cohorts in order to categorize the basal-like subtype in a more effective way[11]; therefore, using the present surrogate basal markers, as in our study, might not be suitable for classifying the TNBC basal-like subtype in the future. Exploration using innovative validated basal markers may be valuable to identifying the subgroups with the worst outcomes and who could benefit most from novel targeted treatments.

Conclusion

TNBC presented at a younger age in Thai women than in previous studies of Western and Asian women. The TNBCs analyzed here were of high grade, had high proliferation indices and had high TP53 expression. Approximately, 72.9% of the TN cases fell into the basal-like subgroup. Despite the fact that patients with basal-like tumors had more relapse events than those with non-basal-like tumors, these patients had similar 5-year DFS and OS estimates. Lastly, the basal-like subtype was not an independent predictor of survival outcome in Thai women with TNBC.

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