

RESEARCH ARTICLE

Clinicopathologic Features of Breast Carcinomas Classified by Biomarkers and Correlation with Microvessel Density and VEGF Expression: A Study from Thailand

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Abstract

Background: To correlate breast cancer subtypes with prognostic factors, microvessel density (MVD), vascular endothelial growth factor (VEGF) expression and clinical features. **Materials and Methods:** One hundred cases of primary breast carcinoma were classified using biomarkers on tissue microarray as: luminal A [estrogen receptor (ER)+, HER2-, Ki-67 \leq 14%], luminal B [ER+, HER2+ or ER+, HER2-, Ki-67 $>$ 14%], HER2, triple negative basal-like (TNB) [any basal cytokeratins (CKs, 5, 14, 17) and/or endothelial growth factor receptor (EGFR) expression], and TN without such markers [TNN, null], and assessed for p53, vimentin, VEGF and CD31 immunoperoxidase. **Results:** Of the 100 cases (mean age, 51 years; mean tumor size, 3.2cm; 56% with nodal metastasis; 89 invasive ductal carcinomas, not otherwise specified, 4 invasive lobular carcinomas, 3 metaplastic carcinomas, and 4 other types) there were 39 luminal A, 18 luminal B, 18 HER2, 15 TNB and 10 TNN. The positivities of basal-like markers in the basal-like subtype were 78.3% for CK5, 40% for CK14, 20% for CK17, 46.7% for EGFR. There was no significant difference in age distribution, tumor size, degree of tubular formation, pleomorphism, lymphovascular invasion, nodal metastasis, MVD, VEGF expression and survival among subgroups. TNs demonstrated significantly higher tumor grade, mitotic count, Ki-67 index, p53 and vimentin and decreased overall survival compared with nonTN. **Conclusions:** The distribution of breast cancer subtypes in this study was similar to other Asian countries with a high prevalence of TN. The high grade character of TN was confirmed and CK5 expression was found to be common in our basal-like subtype. No significant elevation of MVD or VEGF expression was apparent.

Keywords: Breast carcinoma - biomarker classification - triple negative - Ki-67 - MVD - VEGF

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Introduction

Breast cancer is the most common cause of cancer-related death among women in Thailand. Since the introduction of the molecular subtypes of breast carcinoma in 2000, breast cancer has been classified based on gene expression profiling into 5 subtypes including: luminal A [estrogen receptor(ER)+, HER2-], luminal B [ER+, HER2+], HER2, triple negative (TN, ER-and HER2-) basal-like (TNB), and TN without basal-like markers (TNN, null/normal breast-like) (Perou et al., 2000; Sorlie et al., 2001; Sotiriou et al., 2003). The TN group does not benefit from hormonal therapies or treatment targeted against HER2 receptors. The majority of TNB subtype consistently harbored genes usually found in normal basal/myoepithelial cells of breast including high-molecular-weight 'basal' cytokeratins (CKs, 5, 14 and 17), vimentin, p-cadherin, α B crystalline, fascin and caveolins 1 and 2 (Nielsen et al., 2004; Irvin and Carey, 2008). Recent

studies of basal-like subtype breast cancer revealed p53 expression immunohistochemically or TP53 gene mutation, displayed high levels of proliferation related genes and EGFR expression (Nielsen et al., 2004; Kim et al., 2006). The TNB subtype has an intimate association with BRCA1 function (Foulkes et al., 2003), often affects younger patients, has aggressive clinical behavior and poor prognosis. Now there are some remarkable advances in the treatment of breast cancer with combination of chemotherapies, hormonal, and modern targeted therapies which signify the importance of the diagnosis of certain subtypes (Nielsen et al., 2004; Livasy et al., 2006; Reis-Filho and Tutt, 2008). The relation of basal-like subtype with epidermal growth factor receptor (EGFR) (Nielsen et al., 2004; Kim et al., 2006) and/or angiogenesis (vascular endothelial growth factor, VEGF) if present could lead to the role of targeted therapy.

Since the use of immunohistochemical markers to classify basal-like subtype (Nielsen et al., 2004) there

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have been many studies from many countries using immunomarkers to classify breast cancers according to molecular subtypes (Kim et al., 2006; Reis-Filho and Tutt, 2008; Thike et al., 2010). In a recent consensus at St. Gallen, 2011 related to treatment strategies, immunohistochemical staining of Ki-67 has been additionally used to classify ER+ HER2- subgroup into luminal A (ER+, HER2-, Ki-67 \leq 14%), and luminal B (HER2- subgroup) (ER+, HER2-, Ki-67 $>$ 14%) (Goldhirsch et al., 2011). The new classification on breast cancers by using immunohistochemical markers of basal-like markers including EGFR has not been reported in Thai patients before. We use immunomarkers on tissue microarrays (TMA) to classify subtypes, study their clinicopathologic features and correlate each subtype with the prognostic markers, Ki-67 index and p53 expression, microvessel density (MVD) and VEGF expression.

Chaiwun et al reported a prevalence (35.9%) and high grade character of the TN in their study of high-nuclear-grade breast cancer in Thailand (Chaiwun et al., 2010) but the new classification by using immunohistochemical markers on tissue microarrays (TMA) of the general breast cancers has not been reported in Thai patients before. To validate the prevalence of the subtypes, their clinicopathologic features and clinical status a panel of basal-like immunomarkers was used. We also correlate each subtype with the prognostic markers, Ki-67 index and p53 expression, microvessel density (MVD) and VEGF expression.

Materials and Methods

This study was approved by the Institutional Review Board of Faculty of Medicine Siriraj Hospital No. R015332015 and included 100 mastectomy cases with adequate material for study. All cases were obtained during 2002-2004 from the Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University previously enrolled in the study of 'The role of the vascular endothelial growth factor polymorphisms in Thai breast cancer patients (Sa-Nguanraksa et al., 2013).

The histology was reviewed including type, grade (modified Bloom-Richardson classification), lymphovascular invasion (LVI) and nodal status. Three cores of 2 mm diameter from each case plus 6 controls were constructed yielded 6 TMA blocks. Each set was stained for ER, PR, HER2, markers of basal-like subtype (three basal cytokeratins, CK 5, CK14, and CK17, EGFR, CD117 and p63) (Table 1). Prognostic factors as p53, vimentin and Ki-67 immunohistochemistry (IHC) were also performed. The IHC performed were evaluated by TC, TP, and SW without knowledge of clinical outcomes. Cases were classified into: 1) luminal A (ER+ and/or PR+, HER2-, Ki-67 \leq 14%), 2) luminal B (ER+ and/or PR+, HER2+ or ER+ and/or PR+, HER2-, Ki-67 $>$ 14%), 3) HER2 (ER-, PR-, HER2+), 4) TN basal-like (TNB) (ER-, PR-, HER2-, with at least any CK5+, CK14+, CK17+ or EGFR+) and 5) TN without basal-like markers (TNN) (ER-, PR-, HER2-, CK5-, CK14-, CK17-, EGFR-). Expression of VEGF was assessed semiquantitatively using H scores (Table 1). The median of the score was

used as a cut-off level to categorize tumors into low- and high-expressing tumors (Yang et al., 2002). The median of MVD was used as the cut-off level to categorize tumors into low- and high-MVD tumors. Clinical information including mode of treatment and survival status was assessed.

For statistical analysis, the Chi-square test or Fisher's Exact test were used. The age, size of tumor, Ki-67 index, the percentage of VEGF expression (H score) and MVD count were reported using descriptive statistics and Mann Whitney U test to compare the mean of the variables between subtypes. The follow-up period was defined as the time from diagnosis to the last visit/observation or death. Disease-free survival (DFS) was defined as the time between the date of the diagnosis to the date of relapse or August 2011. Overall survival (OS) was defined as the time between the date of the diagnosis to the date of death or August 2011. The log-rank test was used to estimate and compare survival. A p value of <0.05 was considered statistically significant.

Results

Overall characteristics

All patients were women, age ranged from 25-85 years (mean 51, median 50). There were 89 invasive ductal carcinoma (IDC), not otherwise specified (NOS), 4 invasive lobular (ILC), 3 metaplastic carcinoma, 2 mixed-type carcinomas, 1 invasive micropapillary, and 1 carcinosarcoma. The tumor size ranged from 0.24-11.5 cm (mean 3.2, median 3.0). Surgical treatment from 99 patients included modified radical mastectomy (32%), total mastectomy (63%) and breast conserving surgery (5%). The latter two were performed with axillary node dissection (sentinel with/without non-sentinel lymph node dissection). Of 99 cases, 15, 49, 29, and 6% were in stages 1, 2, 3, and 4, respectively; 56% had positive axillary nodes of which 55% had perinodal invasion; 48% had LVI. The follow up time ranged from 2-101 months (mean, 81). 71% were disease-free, 17% had relapse or metastasis and 12% died of disease.

Subtypes of breast cancers classified by hormonal receptors, HER2 and basal cytokeratins and EGFR immunohistochemical studies

There were 39% of luminal A, 18% of luminal B [10% of luminal B (HER2+), 8% of luminal B (HER2-)], 18% of HER2, and 25% of TN (15% TNB, and 10% TNN) subtypes.

Immunohistochemical studies of each subtype (Table 2)

ER, PR and HER2 were expressed in 57, 38 and 28%, respectively. In the hormonal receptor positive subtypes ER was positive in all while PR was positive in 58.9 and 83.3% of luminal A and luminal B, respectively.

Basal-like biomarkers (Table 2)

Basal CKs: (CK 5, CK14, and CK17) When all three were used 80% of TNB could be diagnosed. Among these, CK5 had a greater sensitivity (73%) in detecting basal-like subtype when compared with cytokeratins

Table 1. Antibodies Used and Positive Criteria

Antibody	Clone	Source	Dilution	Positive criteria
ER	SP1	Ventana	Ready to use	≥1% positive cells with intense staining
PR	1E2	Ventana	Ready to use	≥1% positive cells with intense staining
HER2	4B5	Ventana	Ready to use	Score 3+ strong, complete membrane staining in >30% of invasive tumor cells
CK5 *	D5/16 B4	DAKO	1:50	≥1% cytoplasmic and membrane staining
CK14*	LL002	Novocastra	1:100	≥1% cytoplasmic and membrane staining
CK17*	E3	Novocastra	1:100	≥1% cytoplasmic and membrane staining
EGFR*	H11	DAKO	1:25	≥10% membrane staining
CD117	C-kit	DAKO	1:5000	≥10% membrane staining
p63	4A4	DAKO	1:1000	Any nuclear staining
p53	D0-7	DAKO	1:500	≥10% nuclear staining
Vimentin	Vim3B4	DAKO	1:500	At least 10% cytoplasmic staining
Ki-67	MIB1	DAKO	1:300	To be counted from at least 200 tumor cells, expressed in %
VEGF**	VG1***	DBS	1:100	H-score= %positive X intensity (1,2,3) Cut off=median of H score (H=160)
CD31**	JC/70A	DAKO	1:300	Average number of microvessels/individual stained cells from 3 most intense fields (200X) evaluated as MVD.

VEGF=vascular endothelial growth factor, MVD=microvessel density; All except ** were performed using automated immunostainer; *Performed from the Institute of Pathology using automated immunostainer; ** Manual stains with En Vision; ***Recognize VEGF121, 165 and 189 isoforms

Table 2. Subtypes of Breast Cancers with Biomarker Expressions, Prognostic/Predictive Markers, Mvd and Vegf Expression

Antibody	Luminal A		Luminal B		HER2	Triple negative		p			
	no.	(%)	no.	(%)		Basal-like no.	Null no.		(%)		
ER	39	(100)	18	(100)	0	0	0				
PR	23	(58.9)	15	(83.3)	0	0	0				
HER2	0		10	(55.6)	18	(100)	0				
CK5	1	(2.6)	1	(5.6)	4	(22.2)	11	(73.3)	0	<0.001	
CK14	0		1	(5.6)	0		6	(40.0)	0	<0.001	
CK17	0		1	(5.6)	1	(5.6)	3	(20.0)	0	0.046	
EGFR	0		0		5	(27.8)	7	(46.7)	0	<0.001	
CD117	1	(2.6)	0		1	(5.6)	4	(26.7)	0	<0.001	
p63	0		3	(16.7)	3	(16.7)	5	(33.3)	2	(20.0)	0.016
p53	7	(17.9)	8	(44.4)	9	(50)	10	(66.7)	7	(70.0)	0.002
Vimentin	2	(5.1)	0		2	(11.1)	7	(46.7)	1	(10.0)	<0.001
Ki-67(%)	4.11		15.7		8.37		18.07		12.68		0.002
VEGF (%cells)	72		68		68.9		72.33		55.4		
VEGF (mean H score)	148.8		137.2		137.5		139.3		117.4		
VEGF Low	16	(41)	7	(38.9)	11	(61.1)	9	(60.0)	7	(70.0)	
VEGF High	23	(59)	11	(61.1)	7	(38.9)	6	(40.0)	3	(30.0)	0.264
MVD (mean)	73.3	62	60.5		61.3		78				
MVD Low	17	(43.6)	9	(50)	12	(66.7)	7	(46.7)	4	(40.0)	0.462
MVD High	22	(56.4)	9	(50)	6	(33.3)	8	(53.3)	6	(60.0)	

*VEGF=vascular endothelial growth factor, MVD=microvessel density

14 and 17 but a lower specificity when compared with cytokeratin 14 alone or combined CKs since basal CKs could be expressed in subtypes other than basal-like as was present in 1 case of luminal A with minimal quantity of CK5 (>1<10%) and in 1 luminal B with all three CKs. Basal CKs were also found in 4 (22.2%) of HER2, 3 of which had CK5 alone, and the other one with only CK17. No basal CK was found in luminal B.

EGFR: this protein was detected in 7 cases (46.7%) of TNB and 5 cases (27.8%) of HER2; 20% of TNB had positive EGFR alone and 26.7% had a combination of EGFR and one or more basal CKs. Four of 5 (EGFR+) HER2 subtype also expressed CK5.

CD117: it was detected in 4 cases (26.7%) of TNB, 1 luminal A and 1 HER2.

p63: this protein had a low sensitivity (33.3% positive) comparing with basal CKs in detecting TNB; 3 out of 5 positive cases were 2 metaplastic carcinomas and 1

carcinosarcoma. It was also found in other subtypes (2-20%)

Breast cancer subtypes and prognostic biomarkers

Vimentin: it was significantly expressed in TNB (46.7%, compared with 11.1% and 10% in HER2 and TNN, p<0.001).

p53: This protein was expressed in more than 50% of each subtype except for luminal A (21.3%). It was significantly markedly expressed in 70% of TNN (overall p=0.001)

Ki-67 index: A total of 91 cases could be evaluated due to inadequate tissue in some cores. This index was significantly increased in TNB with an average value of 18.07% (SD=13.1, range 4-44.3%) when compared with luminal A (p=0.005) and HER2 subtype (p=0.042), but not significantly increased when compared with luminal B (p=0.337) and TNN (p=0.283). When comparing the

Table 3. Patient Demographics and Tumor Characteristics

		Luminal A	Luminal B	HER2 positive	Triple negative	
					Basal-like	Null
Total cases	100	39	18	18	15	10
Mean age (years)	51	53	48	51	49	56
Age range (years)	(25-85)	(25-85)	(27-62)	(34-84)	(35-61)	(32-77)
				positive cases/total cases (%)		
Family history of breast/ovarian cancer	13/78(17)	4/38(11)	2/9(22)	2/15(13)	4/9(44)	1/6(17)
Histologic subtype						
Invasive ductal	89/100(89)	34/39(87)	18/18(100)	18/18 (100)	14/15(93)	5/10(50)
Invasive lobular	4/100(4)	1/39(3)	-	-	-	3/10(30)
Metaplastic	3/100(3)	2/39(5)	-	-	1/15(7)	-
Others	4/100(4)	2/39(5)	-	-	-	2/10(20)
Histologic grade						
1	9/94(10)	9/39 (23)	-	-	-	-
2	33/94(35)	18/39 (46)	8/18(44)	4/17(24)	3/15(20)	-
3	52/94(55)	11/39 (31)	10/18(56)	13/17(76)	12/15(80)	6/6(100)
Mitotic grade						
1	27/95(29)	20/39(51)	4/18 (22)	1/18(5)	2/15(13)	-
2	22/95(23)	10/39(26)	3/18(17)	5/18(28)	1/15(7)	3/6(50)
3	46/95(48)	8/39(21)	11/18(61)	12/18(67)	12/15(80)	3/6(50)
Tumor size, mean, cm [range]	3.2 [0.2-11.5]	2.9 [0.7-8]	3.3 [1.3-6]	3.4 [0.2-10]	3.7 [1.5-11.5]	3.4 [1-6]
≤2 cm	22/99(22)	9/39(23)	3/18(17)	5/18(28)	4/15(27)	1/10(10)
>2-5 cm	54/99(55)	22/39(56)	11/18(61)	9/18(50)	7/15(47)	5/10(50)
> 5 cm	11/99(11)	2/39(5)	3/18(17)	2/18(11)	1/15(6)	3/10(30)
T4	12/99(12)	5/39(13)	1/18(6)	2/18(11)	3/15(20)	1/10(10)
Lymphovascular invasion	48/100(48)	17/39(44)	11/18(61)	7/18(39)	9/15(60)	4/10(40)
Axillary node metastasis	56/100(56)	24/39(62)	13/18(72)	8/18(44)	8/15(53)	3/10(30)
1 to 3 nodes	28/56(50)	15/24(63)	4/13(31)	2/8(25)	6/8(75)	1/3(33)
≥ 4	28/56(50)	10/24(42)	8/13(62)	6/8(75)	2/8(25)	2/3(67)
with perinodal invasion	31/56(55)	11/24(46)	9/13(69)	6/8 (75)	3/8(38)	2/3(67)
Staging						
1	15/99(15)	4/38 (11)	2/18(11)	5/18(28)	3/15(20)	1/10(10)
2	49/99(49)	21/38(55)	7/18(39)	7/18(39)	7/15(47)	7/10(70)
3	29/99(29)	10/38(26)	8/18(44)	6/18(33)	4/15(27)	1/10(10)
4	6/99(6)	3/38(8)	1/18(6)	-	1/15(6)	1/10(10)
Type of surgery						
Breast conserving	5/99(5)	2/38(5)	1/18(6)	2/18(11)	-	-
Total mastectomy	62/99(63)	25/38(66)	15/18(83)	8/18(44)	7/15(47)	7/10(70)
MRM	32/99(32)	3/38(8)	10/18(56)	8/18(44)	8/15(53)	3/10(30)
Adjuvant therapy						
Hormonal	56/97 (58)	35/37(95)	15/18(83)	2/18(11)	2/14(14)	2/10(20)
Chemotherapy	74/97 (76)	26/37(70)	12/18(67)	15/18(83)	12/14(86)	9/10(90)
Neoadjuvant	13/97 (13)	4/38(11)	2/18(17)	3/18(17)	3/14(21)	1/10(10)
Radiotherapy	40/97 (41)	16/37(41)	8/18(44)	10/18(56)	3/14(21)	3/10(30)
Survival status						
Alive, disease free	71/100 (71)	27/39(69)	13/18(72)	14/18(78)	11/15(73)	6/10(60)
Alive, with disease	17/100 (17)	10/39(26)	3/18(17)	2/18(11)	-	2/10(20)
Dead with disease	12/100 (12)	2/39(5)	2/18(11)	2/18(11)	4/15(27)	2/10(20)

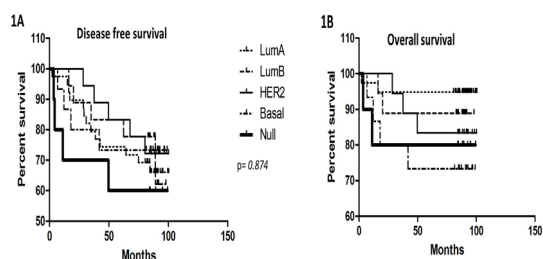


Figure 1. Kaplan-Meier Survival Curves of Various Breast Cancer Subtypes. Disease free survival (1A) and overall survival (1B). No significant differences were found

whole TN with the luminal A it was also significantly increased ($p=0.003$).

Microvessel density and VEGF expression: there was a significant association between MVD & VEGF ($p=0.006$). However, there was no significant difference in MVD among the 5 subtypes ($p=0.462$). The frequency of breast cancer cases of each subtype with MVD values are shown in Table 2. Also there was no significant increase in VEGF positive cells in basal-like subtype as compared with others ($p=0.264$) (Table 2)

There was no correlation of CK5 expression and high VEGF expression ($p=0.796$)

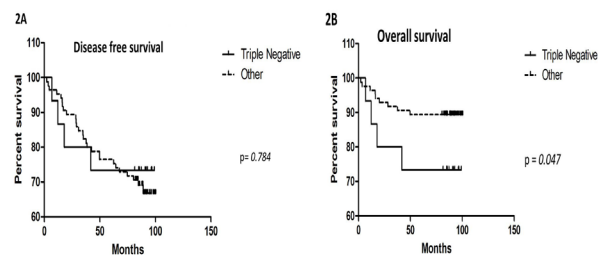


Figure 2. Kaplan-Meier Survival Curves of Triple Negative versus Non Triple Negative. Disease free survival (2A) and overall survival (2B). Only overall survival difference was found

Clinico-pathological parameters (Table 3)

There was no significant difference in the age of the patients among subtypes ($p=0.265$). Four out of 9 patients (44%) in the TNB had a family history of breast/ovarian cancer while others had this history in 13-17%.

Comparison of clinic-pathological data among TNB, TNN and non-TN subtypes: (Table 4)

TN carcinomas had significant high histologic grade ($p=0.038$), and significant high mitotic count ($p=0.03$). There were no significant differences in tumor size, tubule formation, nuclear pleomorphism, LVI, pathological

Table 4. Clinico-Pathologic Data: Comparison between Basal-Like, Null and Non-Triple Negative Subtypes

Feature		Triple negative (n=25)		Non- triple negative (n=75)	p
		Basal-like (n=15)	Null (n=10)		
Age (years)	<50	8	3	44	0.229
	>50	7	7	31	
Tumor size (cm)	<2	6	2	15	0.424
	2<X<5	8	6	50	
Histologic grading	>5	1	2	10	0.038
	I	0	0	9	
	II	3	0	30	
	III	12	6	34	
Mitotic count (grade)	I	2	0	25	0.03
	II	1	3	18	
	III	12	3	30	
Tubule and gland formation	>75%	0	0	7	0.319
	10%-75%	2	0	15	
	<10%	13	6	51	
Nuclear pleomorphism	Mild	0	0	2	0.101
	Intermediate	2	0	30	
	Marked	13	6	41	
Metastatic lymph node	Positive	8	3	44	0.17
	Negative	7	7	30	
LVI	Positive	9	4	35	0.569
	Negative	6	6	39	
Pathologic staging	I	3	1	9	0.859
	II	7	7	35	
	III	4	1	24	
	IV	1	1	4	
VEGF (H-score)	Low	9	7	34	0.24
	High	6	3	41	
MVD score	Low	7	4	38	0.802
	High	8	6	37	

*LVI=lymphovascular invasion, VEGF=vascular endothelial growth factor, MVD=microvessel density

staging, VEGF (count and H score) and MVD.

Correlation of phenotypes and survivals (Figures 1 and 2)

TN had significant decreased OS when compared with all nonTN (p=0.047) but no significant difference among patients of all subtypes (p=0.250); TNB versus others (p=0.08); TNB versus TNN (p=0.794); luminal B and HER2 versus others (p=0.881); HER2 versus luminal A and luminal B (p=0.256).

There was no significant difference in DFS among patients of all subtypes (p=0.874); TN versus all nonTN (p=0.784); luminal B and HER2 versus others (p=0.366)

Discussion

The prevalence of breast cancer subtypes classified by biomarkers could vary not only from the ethnicity (Carey et al., 2006; Thike et al., 2010) but may depend on the method used, biomarker profile, clone of the antibody, laboratory technique and criteria of evaluation. In comparison with the data obtained from the study of 324 Thai breast carcinomas (Chuthapisith et al., 2012) there were 59.3% luminal A, 12.3% luminal B, 13.3% HER2 and 15.1% TN while from the current study of 100 TMA cases when transformed to the comparable data there were

47% luminal A, 10% luminal B, 18% HER2 and 25% TN. The prevalence of HER2 overexpression was quite similar 28.4 and 28% while the prevalences of TN and luminal A differed. These could result from many factors including method use to evaluate, heterogeneity of the hormonal markers and criteria of positivity. However, the high grade nature of the TN were confirmed from both studies. The percentage of HER2 subtype in our study was also comparable to most studies but the TN group differed from some studies. With similar setting our result was similar to the Korean population (luminal A, 44.5%; luminal B, 7.8%; HER2, 17.1%; TNB, 14.7%; TNN, 15.9%) (Kim et al., 2006). TN subtype in the previous reports varied from 10-17% (Reis-Filho and Tutt, 2008). It was 17.6% without racial difference from the study from Malaysia (Tan et al., 2009) and 11% from Singapore (Thike et al., 2010) while in our study it was 25% (TNB,15%). Our prevalence of TN was quite different from that of Singapore despite using the same positive criteria of HER2 and the lower ER cut off value of ours.

Among basal-like markers, similar to previous studies CK5 was the more sensitive but less specific in determining basal-like subtype compared with CK14 (Nielsen et al., 2004; Kim et al., 2006) and could demonstrate 73%TNB in our study. CK17 was the least sensitive. On the contrary CK17 was the most sensitive but CK5/6 was the least one in the study of Thike et al., 2010. The frequencies of CD117 and p63 in TNB and other subtypes were comparable to previous reports (Nielsen et al., 2004; Kim et al., 2006) as well as EGFR immunoreactivity which ranged from 44-72% (Nielsen et al., 2004; Kim et al., 2006). Different EGFR clone may have varying sensitivity thus affecting the differential diagnosis between TNB and TNN. This marker and CK5 were 80% present together in nearly one third of HER2 subtype in our study. Regarding the prognostic /predictive markers, Ki-67, p53 and vimentin were highly expressed in the basal-like comparable to other reports (Nielsen et al., 2004; Kim et al., 2006; Livasy et al., 2006; Han et al., 2010).

Regarding the clinico-pathologic features the results were similar to others, the TN compared with nonTN displayed high grade tumor (Sotiriou et al., 2003; Nielsen et al., 2004; Carey et al., 2006; Kim et al., 2006; Livasy et al., 2006; Irvin et al., 2008; Reis-Filho and Tutt, 2008; Tan et al., 2009; Chaiwun et al., 2010; Thike et al., 2010); no significant correlation was found in patients' age, tumor size, LVI, axillary node status, and staging (Kim et al., 2006). The association between MVD and VEGF expression in our study existed but correlation of these markers among subtypes nor correlation between CK5 and high VEGF expression could not be found probably due to the small sample size. From a few reports on the latter two, the basal-like had higher VEGF expression, correlation with CK5 expression but no significant higher MVD (Ribeiro-Silva, 2006; Lopes et al., 2009). Recently, in a study of 1788 invasive ductal carcinomas from the Nurses' Health Study, VEGF expression was found to correlate with intrinsic subtypes with higher frequency in luminal B, HER2, and basal-like versus luminal A subtypes; it was not significantly related to poor survival

but was significantly associated with increased risks for breast cancer-specific mortality and distant recurrence among women with luminal A tumors (Liu et al., 2011).

Many reports signified poor outcomes in HER2 and TN subgroup (Sorlie et al., 2001; Kim et al., 2006; Dent et al., 2007; Spitale et al., 2009). Our study failed to demonstrate significant difference in survival among 5 subtypes except for OS difference between TN and non-TN groups. We could not find survival difference between the non-basal-like and basal-like tumors similar to the study using immunohistochemical and gene-expression-based classification (Jumppanen et al., 2007). The explanation for this result in our study could be due to the small sample size. More cases should be studied to find the clinical significance of the TNB and TNN and any biomarkers that could predict the clinical outcome other than the ones that we used to classify these two subtypes. Recently calretinin expression had been found to correlate with poor clinical outcome in TNB subtype (Taliano et al., 2013). We did not study this marker in the current study.

In conclusion: biomarkers identified 25% of Thai breast cancers as TN, 52% of which were basal-like. TNB phenotype had high tumor grade, mitotic count and Ki67-index. Besides TNB, EGFR expression could be seen in HER2. No different MVD or VEGF expression or poor clinical outcomes were observed among subtypes. However, TN had decreased overall survival when compared with nonTN. Further study with more samples should be done.

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