RESEARCH ARTICLE

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The Association between Molecular Subtypes of Breast Cancer with Histological Grade and Lymph Node Metastases in Indonesian Woman

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Abstract

Objective: Breast carcinoma is a heterogeneous disease which is rich in diversity. Molecular subtypes of breast cancer, histological grade and lymph node metastases are strong prognostic and predictive factors. In Indonesia, only a limited number of studies have investigated the correlation between molecular subtypes with histological grade and lymph node metastases. **Methods:** We analyzed 247 invasive breast carcinoma cases from the Anatomic Pathology Installation of Dr. Sardjito General Hospital Yogyakarta between 2012-2015. The slides were stained for estrogen receptors (ER), progesterone receptors (PR), HER2, Ki-67 and CK5/6 for classification into breast cancer subtypes (BCS). Histological grade using the Nottingham system and lymph node status were obtained from anatomic pathology records. The association between histological grade and lymph node status with BCS was examined with Chi-square tests. **Results:** The immunohistochemical features of 247 cases of women with invasive breast carcinoma were examined. There were 102 (41.3%) patients with Luminal A, 34 (13.8%) patients with Luminal B, 48 (19.4%) patients with HER2-positive, and 63 (25.5%) patients with triple negative breast cancer (TNBC). There were 148 (59.9%) patients with negative lymph node status and 99 (40.1%) with positive status. Among 63 TNBC cases, 37 (58.7%) patients were positive for CK5/6 staining (basal-like). Statistically, there were significant differences between histological grade and subtypes (p=0.013). However, no significant differences were found for lymph node metastases (p=0.540). **Conclusion:** Among subtypes, Luminal A has the highest frequency, followed by TNBC, HER2-positive and Luminal B. Histological grade was associated with molecular subtypes of breast carcinoma in Yogyakarta. Grade I was associated with Luminal A, while Grade III was associated with Luminal B, HER2 and TNBC subtypes.

Keywords: Breast carcinoma- molecular subtypes- histological grade- lymph node metastases

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Introduction

Breast cancer is a leading cause of death in women (Ferlay et al., 2015). It is a heterogeneous disease which is comprised of many biologically different entities with distinct pathological features and clinical implications such as histological grades, tumor size, lymph node status and hormone receptor status (Anderson et al., 2006; Azizun-Nisa et al., 2008; Chen et al., 2010; Engstrøm et al., 2013; Layeequr Rahman et al., 2015; Ono et al., 2015). Breast cancer can have different histopathological and biological features which exhibit distinct behaviors that lead to different treatment responses and should be given different therapeutic strategies. Consequently, classifying breast cancer into relevant molecular subtypes is important for therapeutic decision making.

Classical immunohistochemistry (IHC) markers such as ER, PR, HER2 and Ki-67 together with traditional clinicopathological variables including tumor grade and nodal involvement are conventionally used for patient prognosis and management (Elston and Ellis, 1991; Engstrøm et al., 2013; Layeequr Rahman et al., 2015; Lips et al., 2013; Ono et al., 2015; Rakha et al., 2010; Schwartz et al., 2014).

According to the St. Gallen consensus 2011, molecular subtypes of breast cancer can be classified into Luminal A (ER+/PR+/HER2-/low Ki-67); Luminal B (ER+/PR+/HER2-/+/high Ki-67); HER2-overexpression (ER-/PR-/HER2+) and triple negative breast cancers/TNBCs (ER-/PR-/HER2-) (Goldhirsch et al., 2011). Basal-like refers to TNBC that was found positive for basal marker (CK5/6) expression (Elesawy et al., 2014; Zhang et al., 2012).

Many studies have shown that histological grade is significantly associated with the molecular subtypes of breast cancer. Grade I is associated with Luminal A, while

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Grade III is associated with HER2 and TNBC (Ambroise et al., 2011; Choi et al., 2013; El-Hawary et al., 2012; Elesawy et al., 2014; Errahhali et al., 2017; Kadivar et al., 2012; Onitilo et al., 2009; Siadati et al., 2015; Tamaki et al., 2013). However, there are still some inconsistent results concerning the association between lymph node metastases and breast cancer subtypes. In Indonesia, only a limited number of studies have investigated the correlation between histological grade and lymph node status with molecular breast cancer using the five molecular markers (ER, PR, HER2, Ki-67 and CK5/6).

Materials and Methods

Breast carcinoma specimens were obtained from the Anatomic Pathology Installation of Dr. Sardjito General Hospital from January 2012 to December 2017. This study was conducted with approval of the institutional review board at the Universitas Gadjah Mada, Yogyakarta, Indonesia. Histological grade and lymph node status were obtained from medical records. Histological grade was assessed according to Nottingham modification of the Bloom-Richardson system. The inclusion criteria of this cross-sectional study were determined as follows: (a) patients with invasive breast carcinoma; (b) patients with available histological grade and lymph node status; and (c) available formalin-fixed, paraffin embedded (FFPE) samples with good quality. Overall, out of 817 cases found, 267 FFPE were available and 247 samples were included in the study.

Immunohistochemistry

Immunohistochemical staining was performed on 3-µm-thick sections. Five primary antibodies (Biocare Medical LLC, Concord, USA) were used for detection of ER (rabbit monoclonal antibody /CRM 301 A,B,C), PR (rabbit monoclonal antibody/CRM 302 A,C), HER2 (rabbit monoclonal antibody/CME 342 A,B,) Ki-67 (rabbit monoclonal antibody/ CRM 325 A,B) and CK5/6 (rat monoclonal antibody/ CK5/6.007/ACR 105 A,B,C). The dilution for primary antibodies anti-ER and HER2 were 1:100, while 1:200 was used for PR, Ki-67 and CK5/6. Primary antibodies anti-ER, PR and Ki-67 were incubated for 1 hour while CK5/6 and HER2 were incubated overnight.

Histological grade evaluation

Using the Nottingham system, the determination of the histological grade was performed by one pathologist based on specific criteria. ER and PR expression were considered positive when the tumor cell nucleus was ≥1% (Silver et al., 2010). HER2 was considered positive if the membrane intensity was >10% of the tumor cells and homogeneously stained (Wolff et al., 2014). The cut off of Ki-67 was ≥14% for high category (Cheang et al., 2009). CK5/6 was positive if it had a score of 1+, 2+ or 3+ (Livasy et al., 2006).

The classification of molecular subtypes of breast cancer was defined into five subtypes according to the St. Gallen consensus 2011 (Goldhirsch et al., 2011) as follows:

Luminal A: ER and or PR (+); HER2 (-/+); low Ki-67 (<14%)

Luminal B: ER and or PR (+); HER2 (-/+); high Ki-67 (≥14%)

HER2 (+): ER and PR (-); HER2 (+)

TNBC (basal-like): ER(-); PR(-); HER2 (-); CK5/6 (+)

TNBC (non basal-like): ER(-); PR(-); HER2 (-); CK5/6 (-)

Statistical analysis

Associations between molecular subtypes with histological grade and lymph node status were evaluated by Chi-square tests. In the present study, the significance level was set as $p < 0.05$.

Results

Among the 247 patients, the median age was 52 years (range, 24-92 years). The clinicopathological features for each molecular subtype as determined by the expression of ER, PR, HER2, Ki-67 and CK5/6 were shown in Table 1. Out of the 247 patients, there were 102 (41.3%) patients with Luminal A 34 (13.8%) patients with Luminal B, 48 (19.4%) patients with HER2-positive, and 63 (25.5%) patients with TNBC.

Thirty-two (13.0%) patients had Grade I, 47 (19.0%) Grade II and 169 (68.0%) Grade III. There were significant differences across subtypes in histological grade (p=0.013). In comparison, Luminal A had the highest frequency of Grade I (17.6%), while most of the patients in Luminal B (79.4%), TNBC (77.8%) and HER2 (70.8%) had Grade III. Out of all of the 247 patients,

Table 1. Clinicopathological Features for Molecular Subtypes of 247 Invasive Breast Cancer Patients

	Luminal A n (%)	Luminal B n (%)	HER2 n (%)	TNBC n (%)	Total n (%)
Cases (%)	102 (41.3)	34 (13.8)	48 (19.4)	63 (25.5)	247 (100)
Histological grade (p= 0.013)					
I	18 (17.6)	4 (11.8)	2 (4.2)	8 (12.7)	32 (13.0)
II	26 (25.5)	3 (8.8)	12 (25)	6 (9.5)	47 (19.0)
III	58 (56.9)	27 (79.4)	34 (70.8)	49 (77.8)	169 (68.0)
Lymph node metastases (p= 0.540)					
Negative	61 (59.8)	24 (70.6)	28 (58.3)	35 (55.6)	148 (59.9)
Positive	41 (40.2)	10 (29.4)	20 (41.7)	28 (44.4)	99 (40.1)

Chi-square, p value<0.05

Table 2. Clinicopathological Features for 63 Triple Negative Breast Cancer

	Basal-like n (%)	Non basal-like n (%)	Total n (%)
Cases (%)	37 (58.7)	26 (41.3)	63 (100)
Histological grade (p=0.138)			
I	3 (4.8)	5 (7.9)	8 (12.7)
II	2 (3.2)	4 (6.3)	6 (9.5)
III	32 (50.8)	17 (27.0)	49 (77.8)
Lymph node metastases (p=0.457)			
Negative	22 (34.9)	13 (20.6)	35 (55.6)
Positive	15 (23.8)	13 (20.6)	28 (44.4)

Chi-square, p value<0.05

there were 148 (59.9%) patients who had negative lymph node status and 99 (40.1%) had positive status. Results showed lymph node status was not statistically different among subtypes (p = 0.540). The frequency of negative lymph node status was higher than positive lymph node status in each subtypes.

Among 63 TNBC cases, 37 patients (58.7%) were positive for CK5/6 staining (basal-like) (Table 2). There were no significant differences between basal-like and non basal-like in histological grade and lymph node status with p=0,138 and p=0.457, respectively. While basal-like had higher frequency of Grade III (50.8%) than non basal-like, most patients with basal-like subtype had negative lymph node status (34.9%).

Discussion

Distribution of molecular subtypes of breast cancer

The immunohistochemical analysis of five molecular markers in 247 patients in Dr. Sardjito General Hospital showed that Luminal A was the most prevalent subtype. Almost world-wide, research has shown Luminal A as the most frequent subtype. However, patients in China (Zhang et al., 2014) and Pakistan (Khokher et al., 2013) have the highest proportions of TNBC. Compare to the previous studies within Indonesian population, our result is similar to Widodo et al., (2014) in Yogyakarta but different from Firdaus et al., (2016) in Padang, where Luminal B and TNBC had the highest proportion. Differences in ethnicity and genetics may account for these different results. Additionally, different proportions of subtypes in populations were associated with several risk factors for breast cancer such as age, BMI, menopause status, familial history, parity and duration of breastfeeding (Devi et al. 2012), while this study only examined the histological grade and patients' lymph node status.

The frequency of Luminal A (41.1%) in this study was slightly higher than the previous study (38.1%) conducted by Widodo et al., (2014). Some risk factors which might increase the frequency of Luminal A include high BMI, no breastfeeding and early age of menarche (Millikan et al., 2008; Yang et al., 2011). Porter (2008) and Devi et al., (2012) also mentioned multi-factorial influences (westernization) that contributed to increased incidence of Luminal A such as sedentary lifestyle and obesity. Similar to previous results from studies in Japan (Tamaki et al., 2013), Pakistan (Khokher et al., 2013) and Iran (Kadivar et al., 2012), the frequency of Luminal B in this study was the lowest among all subtypes.

The proportion of TNBC in the present study was higher than research in Morocco and America (Bhatia et al., 2014; Errahhali et al., 2017; Ugras et al., 2014). However, studies conducted in some Asian populations, like in China, Pakistan, Egypt, and Korea, were found to have higher proportions of TNBC than our study, ranging from 32-36%, (Choi et al., 2013; Elesawy et al., 2014; Khokher et al., 2013; Liu et al., 2012; Zhang et al., 2012). High frequency of TNBC has been associated with obesity when factoring in premenopause and familial history (Yang et al., 2011).

Association of molecular subtypes of breast cancer with patients' grade and lymph node status

In this study, the majority of patients have Grade III (68.0%). Statistical analysis showed that molecular subtypes were associated with histological Grade (p=0.013). Grade III was mostly found in Luminal B, TNBC and HER2, while Grade I and Grade II were predominated in Luminal A. The majority of previous studies also found that Luminal A was associated with Grade I (Ambroise et al., 2011; Choi et al., 2013; Elesawy et al., 2014; Errahhali et al., 2017; Geethamala et al., 2015; Kadivar et al., 2012; Onitilo et al., 2009; Siadati et al., 2015; Tamaki et al., 2013). Luminal A was associated with good prognosis and higher expression of FOX A1, GATA-3 and Bcl-2, which correlated to the presence of well-differentiated tumors (Asselin-Labat et al., 2007; Dawson et al., 2010; Mehta et al., 2012; Tamaki et al., 2013).

Similar to the present study, the high proportion of Grade III in Luminal B, HER2 and TNBC subtypes was also found in India, America, Iran, Egypt, Morocco, Japan and Korea (Ambroise et al., 2011; Choi et al., 2013; Elesawy et al., 2014; Errahhali et al., 2017; Geethamala et al., 2015; Kadivar et al., 2012; Onitilo et al., 2009; Siadati et al., 2015; Tamaki et al., 2013). Luminal B was found to be more aggressive than Luminal A, which might correlate with increased levels of proliferation genes, HER2 amplification and proliferation alternative pathway activation (Loi et al., 2009; Reis-Filho and Tutt, 2008; Tran and Bedard, 2011; Zhang et al., 2014). HER2 and TNBC are associated with poor prognosis. HER2 subtype is associated with expression of c-Met, survivin, EGFR, HIF-1α, higher levels of oncogene, p-53 mutation and

resistance to chemotherapy (Ayadi et al., 2008; Burstein et al., 2008; Maksimovic, 2009; Siadati et al., 2015; Tamaki et al., 2013). TNBC was also associated high incidents of p-53 mutation, downregulation of Retinoblastoma (Rb) and increased levels of p-16, Glut-1 and CAIX (Choi et al., 2013; Rakha and Reis-Filho, 2009; Si et al., 2014).

TNBC can be classified into basal-like and non basal-like carcinoma using CK5/6, a biomarker that has high specificity and sensitivity to identify basal-like classification (Nielsen et al., 2004). In this study, basal-like breast cancer showed higher frequency of Grade III than non basal-like, similar to Elesawy et al., (2014) and Tamaki et al., (2013). This result may be associated with several characteristics of basal-like carcinoma, such as shorter disease-free survival, high mitosis, p-53 mutation, deregulation of Rb and integrin, overexpression of P-cadherin, fascin, caveolins 1 and 2, alphabeta crystallin and Epidermal Growth Factor Receptor (EGFR) and reduction of PTEN expression (Bauer et al., 2007; Korsching et al., 2002; Rakha et al., 2007).

Lymph node status serves as a strong prognosis and predictive factor. Out of the 247 patients, negative lymph node status (59.9%) was more frequent than positive lymph node status (40.1%). In every subtype, negative lymph node status always had higher proportions than positive lymph node status. There was no statistically significant correlation between subtypes and lymph node status (p=0.540) found in this study. This result was similar to Ambroise et al. (2011), Jones et al., (2013) and Tamaki et al., (2013). However, other studies found some association between subtypes and lymph node status (Bhatia et al., 2014; Ehinger et al., 2017; El-Hawary et al., 2012; Elesawy et al., 2014; He et al., 2015; Onitilo et al., 2009; Si et al., 2014; Siadati et al., 2015). These inconsistent results show that lymph node status cannot be an independent prognosis factor for breast cancer.

Although lymph node status was not associated with subtypes, we found that Luminal patients have higher frequency of negative lymph node compared to non Luminal patients. This result is supported by previous studies which found Luminal patients tend to have negative lymph node, while non luminal cases (HER2 and TNBC subtypes) tend to have positive lymph node (El-Hawary et al., 2012; Howland et al., 2013; Shriver et al., 2014; Widodo et al., 2014). Luminal subtypes have been associated with lower grade and less aggressive tumors (Lee et al., 2010; Widodo et al., 2014). Interestingly, some research contradict to our results. They explained that patients with TNBC tend to be negative for lymph node metastasis because the cancer is likely to develop distant metastases by hematogenous rather than lymphogenous spread (Bhatia et al., 2014; Errahhali et al., 2017; He et al., 2015; Holm-Rasmussen et al., 2015; Si et al., 2014; Ugras et al., 2014).

Among TNBC subtypes, basal-like carcinoma (TNBC with CK5/6+) has higher frequency of negative node status than non basal-like (Table 2). This result is supported by the findings of Honrado et al., (2006) that found basal-like tumors tend to metastasize into visceral organs, mostly into the brain and lungs due to the microenvironment involving important factors that promote basal-like metastasis, such as up-regulation of EMT markers (vimentin, smooth muscle-actin, N-cadherin, and cadherin-11) and overexpression of proteins involved in extracellular matrix remodeling and invasion (SPARC, laminin, and fascin), together with reduction of characteristic epithelial markers (E-cadherin and cytokeratins) (Sarrio et al., 2008).

In conclusion, this study suggests that classical immunohistochemistry-based subtyping is essential for breast carcinoma management. In this study, Luminal A has the highest frequency, followed by TNBC, HER2-positive and Luminal B. Histological grade was associated with molecular subtypes of breast carcinoma in Yogyakarta, while lymph node metastases was not. Grade I was associated with Luminal A, while Grade III was associated with Luminal B, HER2 and TNBC. In retrospect, there are several limitations in this study. For HER2 evaluation, we did not use fluorescence in situ hybridization (FISH) method to confirm the immunohistochemically HER2 2+ score. We also did not study other factors which may affect the histological grade and lymph node status.

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References

Ambroise M, Ghosh M, Mallikarjuna V, Kurian A (2011). Immunohistochemical profile of breast cancer patients at a tertiary care hospital in South India. *Asian Pacific J Cancer Prev*, **12**, 625–9.

Anderson WF, Pfeiffer RM, Dores GM, Sherman ME (2006). Comparison of age distribution patterns for different histopathologic types of breast carcinoma. *Cancer Epidemiol Biomarkers Prev*, **15**, 1899–905

Asselin-Labat ML, Sutherland KD, Barker H, et al (2007). Gata-3 is an essential regulator of mammary-gland morphogenesis and luminal-cell differentiation. *Nat Cell Biol*, **9**, 201–9.

Ayadi L, Khabir A, Amouri H, et al (2008). Correlation of HER-2 over-expression with clinico-pathological parameters in Tunisian breast carcinoma. *World J Surg Oncol*, **6**, 1–8.

Azizun-Nisa, Bhurgri Y, Raza F, Kayani N (2008). Comparison of ER, PR and HER-2/neu (C-erb B 2) reactivity pattern with histologic grade, tumor size and lymph node status in breast cancer. *Asian Pacific J Cancer Prev*, **9**, 553–6

Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V (2007). Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative

phenotype: A population-based study from the California Cancer Registry. *Cancer*, **109**, 1721–8

Bhatia JK, Metzger D, Ashamalla H, Katsoulakis E, Mattes MD (2014). Breast cancer molecular subtype as a predictor of lymph node metastasis. *Int J Radiat Oncol*, **90**, 267–8

Burstein HJ, Storniolo AM, Franco S, et al (2008). A phase II study of lapatinib monotherapy in chemotherapy-refractory HER2-positive and HER2-negative advanced or metastatic breast cancer. *Ann Oncol*, **19**, 1068–74.

Cheang MCU, Chia SK, Voduc D, et al (2009). Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. *J Natl Cancer Inst*, **101**, 736–50.

Chen XS, Ma CD, Wu JY, et al (2010). Molecular subtype approximated by quantitative estrogen receptor, progesterone receptor and Her2 can predict the prognosis of breast cancer. *Tumori*, **96**, 103–10.

Choi J, Jung W, Koo J (2013). Metabolism-related proteins are differentially expressed according to the molecular subtype of invasive breast cancer defined by surrogate immunohistochemistry. *Pathobiology*, **80**, 41–52.

Dawson S, Makretsov N, Blows FM, et al (2010). BCL2 in breast cancer: a favourable prognostic marker across molecular subtypes and independent of adjuvant therapy received. *Br J Cancer*, **103**, 668–75.

Devi CRB, Tang TS, Corbex M (2012). Incidence and risk factors for breast cancer subtypes in three distinct South-East Asian ethnic groups: Chinese, Malay and natives of Sarawak, Malaysia. *Int J Cancer*, **131**, 2869–77.

El-Hawary AK, Abbas AS, Elsayed AA, Elsayed AA, Zalata KR (2012). Molecular subtypes of breast carcinoma in Egyptian women: Clinicopathological features. *Pathol Res Pract*, **208**, 382–6.

Elesawy BH, Abd A, Shawky AE, et al (2014). Immunohistochemistry-based subtyping of breast carcinoma in Egyptian women: A clinicopathologic study on 125 patients. *Ann Diagn Pathol*, **18**, 21–6.

Elston CW, Ellis IO (1991). pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology*, **19**, 403–10.

Engstrøm MJ, Opdahl S, Hagen AI, et al (2013). Molecular subtypes, histopathological grade and survival in a historic cohort of breast cancer patients. *Breast Cancer Res Treat*, **140**, 463–73.

Errahhali ME, Errahhali ME, Ouarzane M, et al (2017). First report on molecular breast cancer subtypes and their clinico-pathological characteristics in Eastern Morocco: series of 2260 cases. *BMC Womens Health*, **17**, 1–11.

Ferlay J, Soerjomataram I, Dikshit R, et al (2015). Cancer incidence and mortality worldwide: Sources, methods and major patterns in Globocan 2012. *Int J Cancer*, **136**, 359–86

Firdaus V, Asri A, Khambri D (2016). Hubungan grading histopatologi dan infiltrasi limfovaskular dengan subtipe molekuler pada kanker payudara invasif di bagian bedah RSUP. Dr. M. *Djamil Padang J Kesehat*, **5**, 165–72.

Geethamala K, Murthy S, Vani B, Rao S (2015). Histopathological grade versus hormone receptor status in breast carcinoma- treasure the past. *Int J Biomed Res*, **6**, 466–71.

Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thu B (2011). Strategies for subtypes - dealing with the diversity of breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Ann Oncol*, 22, 1736–47.

He ZY, Wu SG, Yang Q, et al (2015). Breast cancer subtype is associated with axillary lymph node metastasis: a eetrospective cohort study. *Medicine (Baltimore)*, **94**, 1–7

Holm-Rasmussen EV, Jensen MB, Balslev E, Kroman N, Tvedskov TF (2015). Reduced risk of axillary lymphatic spread in triple-negative breast cancer. *Breast Cancer Res Treat*, **149**, 229–36.

Honrado E, Hardisson D, Calero F, et al (2006). Prognostic significance of basal-like phenotype and fascin expression in node-negative invasive breast carcinomas. *Clin Cancer Res*, **12**, 1533–40.

Howland NK, Driver TD, Sedrak MP, et al (2013). Lymph node involvement in immunohistochemistry-based molecular classifications of breast cancer. *J Surg Res*, **185**, 697–703.

Jones T, Neboori H, Wu H, et al (2013). Are breast cancer subtypes prognostic for nodal involvement and associated with clinicopathologic features at presentation in early-stage breast cancer?. *Ann Surg Oncol*, **20**, 2866–72.

Kadivar M, Mafi N, Joulaee A, Shamshiri A (2012). Breast cancer molecular subtypes and associations with clinicopathological characteristics in Iranian women, 2002- 2011. *Asian Pacific J Cancer Prev*, **13**, 1881–6.

Khokher S, Qureshi MU, Mahmood S (2013). Association of immunohistochemically defined molecular subtypes with clinical response to presurgical chemotherapy in patients with advanced breast cancer. *Asian Pacific J Cancer Prev*, **14**, 3223–28.

Korsching E, Packeisen J, Agelopoulos K, et al (2002). Cytogenetic alterations and cytokeratin expression patterns in breast cancer: integrating a new model of breast differentiation into cytogenetic pathways of breast carcinogenesis. *Lab Invest*, **82**, 1525–33.

Layeequr Rahman R, Crawford SL, Siwawa P (2015). Management of axilla in breast cancer - The saga continues. *Breast J*, **24**, 343–53.

Lee JH, Kim SH, Suh YJ, Shim BY, Kim HK (2010). Predictors of axillary lymph node metastases (ALNM) in a Korean population with T1-2 breast carcinoma: triple negative breast cancer has a high incidence of ALNM irrespective of the tumor size. *Cancer Res Treat*, **42**, 30–6.

Lips EH, Mulder L, De Ronde JJ, et al (2013). Breast cancer subtyping by immunohistochemistry and histological grade outperforms breast cancer intrinsic subtypes in predicting neoadjuvant chemotherapy response. *Breast Cancer Res Treat*, **140**, 63–71.

Liu N, Yu Q, Liu T, et al (2012). P-cadherin expression and basal-like subtype in breast cancers. *Med Oncol*, **29**, 2606–12.

Livasy CA, Karaca G, Nanda R, et al (2006). Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol*, **19**, 264–71.

Loi S, Sotiriou C, Haibe-Kains B, et al (2009). Gene expression profiling identifies activated growth factor signaling in poor prognosis (Luminal-B) estrogen receptor positive breast cancer. *BMC Med Genomics*, **2**, 1–9.

Maksimovic S (2009). Prognostic value HER-2/neu expression in T1 to T3 breast cancer with axillary lymph node metastasis. *Breast Cancer Res*, **11**, 9.

Mehta RJ, Jain RK, Leung S, et al (2012). FOXA1 is an independent prognostic marker for ER-positive breast cancer. *Breast Cancer Res Treat*, **131**, 881–90.

Millikan RC, Newman ÆB, Patricia ÆCTÆ, et al (2008). Epidemiology of basal-like breast cancer. *Breast Cancer Res Treat*, **109**, 123–39.

Nielsen TO, Hsu FD, Jensen K, et al (2004). Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res*, **10**, 5367–74.

Onitilo AA, Engel JM, Greenlee RT, Mukesh BN (2009). Breast cancer subtypes based on ER/PR and Her2 expression: Comparison of clinicopathologic features and survival. *Clin*

Med Res, **7**, 4–13.
Ono M, Tsuda H, Yunokawa M, et al (2015). Prognostic impact of Ki-67 labeling indices with 3 different cutoff values, histological grade, and nuclear grade in hormone-receptor-positive, HER2-negative, node-negative invasive breast cancers. *Breast Cancer*, **22**, 141–52.
Porter P (2008). "Westernizing" women's risk? breast cancer in lowe-income countries. *N Engl J Med*, **358**, 213–6
Rakha EA, El-Sayed ME, Green AR, et al (2007). Prognostic markers in triple-negative breast cancer. *Cancer*, **109**, 25–32.
Rakha Ea, Reis-Filho JS, Baehner F, et al (2010). Breast cancer prognostic classification in the molecular era: the role of histological grade. *Breast Cancer Res*, **12**, 1–12.
Rakha E, Reis-Filho JS (2009). Basal-like breast carcinoma: from expression profiling to routine practice. *Arch Pathol Lab Med*, **133**, 860–8.
Reis-Filho JS, Tutt ANJ (2008). Triple negative tumours: A critical review. *Histopathology*, **52**, 108–18.
Sarrio D, Marı S, Hardisson D, Cano A, Moreno-bueno G (2008). Epithelial-mesenchymal transition in breast cancer relates to the basal-like phenotype. *Cancer Res*, **68**, 989–98.
Schwartz AM, Henson DE, Chen D, Rajamarthandan S (2014). Histologic grade remains a prognostic factor for breast cancer regardless of the number of positive lymph nodes and tumor size: A study of 161 708 cases of breast cancer from the SEER program. *Arch Pathol Lab Med*, **138**, 1048–52.
Si C, Jin Y, Wang H, Zou Q (2014). Association between molecular subtypes and lymph node status in invasive breast cancer. *Int J Clin Exp Pathol*, **7**, 6800–6.
Siadati S, Sharbatdaram M, Nikbakhsh N, Ghaemian N (2015). Correlation of ER, PR and HER-2/Neu with other prognostic factors in infiltrating ductal carcinoma of breast. *Iran J Pathol*, **10**, 221–6.
Silver DP, Richardson AL, Eklund AC, et al (2010). Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. *J Clin Oncol*, **28**, 1145–53.
Tamaki M, Kamio T, Kameoka S, Kojimahara N, Nishikawa T (2013). The relevance of the intrinsic subtype to the clinicopathological features and biomarkers in Japanese breast cancer patients. *World J Surg Oncol*, **11**, 1–13.
Tran B, Bedard PL (2011). Luminal-B breast cancer and novel therapeutic targets. *Breast Cancer Res*, **13**, 1–10.
Ugras S, Stempel M, Patil S, Morrow M (2014). Estrogen receptor, progesterone receptor, and HER2 status predict lymphovascular invasion and lymph node involvement. *Ann Surg Oncol*, **21**, 3780–6.
Widodo I, Dwianingsih EK, Triningsih E, Utoro T, Soeripto R (2014). Clinicopathological features of Indonesian breast cancers with different molecular subtypes. *Asian Pac J Cancer Prev*, **15**, 6109–13.
Wolff AC, Hammond MEH, Hicks DG, et al (2014). Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *Arch Pathol Lab Med,* **138**, 241–56.
Yang XR, Chang-claude J, Goode EL, et al (2011). Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies. *J Natl Cancer Ins*, **103**, 250–63.
Zhang M, Liu H, Teng X, et al (2012). The differences in CXCR4 protein expression are significant for the five molecular subtypes of breast cancer. *Ultrastruct Pathol*, **36**, 381–6.
Zhang MH, Man HT, Zhao XD, Dong N, Ma SL (2014). Estrogen receptor-positive breast cancer molecular signatures and therapeutic potentials (Review). *Biomed Rep*, **2**, 41–52.