Tumor Grades and Lymph Node Status in Breast Cancer Subtypes

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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; Engstrom 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; Engstrom 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 Grade III is associated with Luminal B, HER2 and TNBC subtypes.

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

RESEARCH ARTICLE

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

Yunita Setyawati1, Yeni Rahmawati1, Irianiwati Widodo2, Ahmad Ghozali2, Dewajani Purnomosari1*

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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1Department of Histology and Cell Biology, 2Department of Anatomic Pathology, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. *For Correspondence: d.purnomosari@ugm.ac.id
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,

| Histological grade | Luminal A n (%) | Luminal B n (%) | HER2 n (%) | TNBC n (%) | Total n (%) |
|--------------------|----------------|----------------|------------|------------|-------------|
| 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         | Positive        |
|------------------|------------------|----------------|
| Cases (%)        | 61 (59.8)        | 41 (40.2)       |
| Cases (%)        | 24 (70.6)        | 10 (29.4)       |
| Cases (%)        | 28 (58.3)        | 20 (41.7)       |
| Cases (%)        | 35 (55.6)        | 28 (44.4)       |
| Cases (%)        | 148 (59.9)       | 99 (40.1)       |

Chi-square, p value<0.05
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, alpha beta 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; Elinger 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; Errahhal 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) (Serro 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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