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Profile of HER2/neu, estrogen receptor and heat shock protein 27 expression in early and late onset Indonesian breast cancer patients

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Abstract

Introduction Early onset breast cancer (occurring under age 40) tend to exhibit a different, aggressive phenotype, and are usually associated with hereditary BRCA1 or BRCA2 mutations. We investigated the levels of Hsp27, HER2/Neu, and ER positivity, in early onset breast cancer patients. **Materials and Methods** Eighteen paraffin blocks of tissues from patients diagnosed with invasive ductal carcinoma of no specific type (IDC-NST) age below 39 years (mean 34 years) who underwent surgery or surgical biopsy in Dr. Soetomo General Hospital, Surabaya, in the period of January 2014 - December 2014. The control group consisted of 112 patients aged older than 40 who underwent resection with the same diagnosis within the same period. All specimens were examined for Hsp27 expression, ER status, and HER2/Neu amplification. **Results** The younger patient pool typically has higher tumor grade and show less Hsp27 expression. Sixteen (88.9%) of early-onset patients has grade II or III cancer, with 55.55% presenting with grade III cancer. Meanwhile, 79 (70.53%) of late onset patient has grade II or III cancer, with 26.78% presenting with grade III cancer. Seventy-seven out of 112 (68.75%) patients in the older group has positive ER status, compared to 8 out of 18 (45.45%) of early-onset patients. All within the younger patient pool show Hsp27 score less than the median score. HER2/Neu scoring distribution is similar across age groups. Statistical analysis using Chi-square and Spearman rank correlation test show a statistically significant difference in Hsp27 expression between early-onset and late-onset patients, but fail to show statistical significance between ER status or HER2/Neu score and age. **Conclusion** Difference in Hsp27 levels show a statistically significant difference between early-onset and late-onset breast cancer patients. Meanwhile, estrogen receptor expression and HER2/Neu amplification failed to show statistically significant difference between early-onset and late-onset patients.

Key words: *early onset breast cancer, breast cancer, Hsp27, HER2/Neu, estrogen receptor*

Introduction

Breast cancer is the most common cancer among women and remains one of the most common cause of death. According to WHO GLOBOCAN Breast Cancer Factsheet 2012[1], breast cancer is the most common cancer in the world, with an estimated 40.2 cases per 100.000 people (36% of all cancers), and has the second highest mortality rate among all cancers.

Breast cancer is extremely uncommon below the age of 20, where benign breast lesions are much more common[2]; but its occurrence increases according to age and at the age of 90, about 20% of women will have had breast cancer. Because the incidence of early-onset breast cancer is low, and the risk is calculated to be increased up to 100-fold after menopause, breast cancer is thought to be very heavily influenced by hormonal factors. Age at menarche and menopause is also important as it determines the time frame of the patient's exposure towards carcinogenic hormones.[3]

Breast cancer is further histopathologically divided into several types. The first is invasive ductal carcinoma with no specific type (NST), which has no speficic differentiating feature[2]. This classification is an exclusion-based diagnosis, and the most common type of breast cancer. The second type is intraductal carcinoma, which is a potentially invasive precancer lesion in the breast, where abnormal cells are found to line one or more of the breast ducts. Invasive lobular carcinoma comprises 5-10% of all breast cancers.[4] Another method of classification is by the TNM method, which stages cancers based on its primary tumor size, nodal and distant metastasis. According to its receptor status, breast cancers can express endocrine receptors (estrogen and progesterone), HER2 receptor, all of these or none of these. This classification will determine the chemotherapy and endocrine therapy regime.

About 75% of all breast cancers are *ER-Positive*, which means the tumor responds the presence of estrogen, and 65%of these are also *PR-Positive*, which means it also responds to progesterone.[5] ER has proved to be a very successful therapeutic target for ER+ cancers, as proved by anti-estrogenic drugs such as tamoxifen and raloxifen. ER+ cancers is also correlated with better histological differentiation and has a better prognosis.[6]

About 20-25% of all breast cancers make an excess of a protein called HER2/Neu, and these cancers have the tendency to be more aggressive and rapidly growing. The drug Trastuzumab/Herceptin has dramatically reduced the risk of recurrence in such cases.[7] About 10-17% of all breast cancers are triple-negative, showing none of the receptors mentioned above. These cancers tend to be bigger in size and most are high-grade NST invasive ductal carcinoma.[8] The lack of any specific therapeutic target makes the prognosis for these cancers worse, with the lowest point within the first 3-5 years.[9]

Heat shock protein 27 (Hsp27) is a small molecular weight heat-shock protein that acts as a chaperone for protein folding induced by heat or other stress.[10] Induction of this protein by estrogen holds a significant role in the pathogenesis of breast cancers. The ability of estrogen to induce Hsp27 was proven in a study by Porter et al.[11] which showed that the addition of 12-beta-estradiol to MCF-7 breast cancer cell line doubled the levels of Hsp27 mRNA. Hsp27 is also thought to function as an anti-apoptotic agent, interacting with both the mitochondrial-dependent and independent pathways of apoptosis. Due to this function, Hsp27 is thought to play a large role in resistance towards several chemotherapeutic

drugs such as gemcitabine and doxorubicin.[12]

Hsp27 also has the capacity to regulate cell adhesion and migration. Several studies show the activity of Hsp27 in increasing metastatic potential of tumor cells in rats and increase resistance towards therapy.[13] This role in cell migration is important, because the last step of tumor development involve tumor invasion to surrounding tissues and dissemination into metastatic colonies in distant organs.[14] The increase of Hsp27 in tumor cells may be caused by several genes such as p53 loss-of-function or by protooncogenes such as HER and c-Myc.[15] Overexpression of Hsp27 has been associated with shorter *disease-free survival.*

Theoretically, we know that Hsp27 is a downstream target of estrogen receptor signalling and plays a role in cancer cell division, which lends to the theory that the expression of Hsp27 and estrogen receptor should be correlated. 11 However, the connection between Hsp27 and estrogen receptor expression as analysed by prior studies seem to be ambiguous. Several studies, such as those by Hayes and Thor, Love and King, Straume *et al.* and Ciocca and Calderwood[16-19] indicated the presence of a positive correlation between Hsp27 expression and estrogen receptor positivity. On the other hand, several other studies such as those by Asfour *et al.*[20] and Seymour, Bezwoda and Meyer[21] did not find any significant correlation between Hsp27 score and estrogen receptor status. Also, samples analysed in studies that show significant positive correlation often include those who are already undergoing various forms of therapy, effects from which may befuddle the results.

While breast cancer treatment has rapidly evolved over the decades with increased use of adjuvant therapies, mortality from breast cancer in early-onset patients has not decreased in the last 36 years.[22] Tumors in younger patients tend to be more advanced and less curable, and often have familial factors that pose them to be less responsive to therapy, such as BRCA1 or BRCA2 mutations. BRCA-mutated tumors tend to have higher grade and less responsivity to hormone therapy than tumors diagnosed in older women.[23] Young breast cancer patients often present with larger tumor and have metastatic lymph nodes or vascular invasion, and therefore do not follow the trends of breast cancer treatment in general. In this study, we seek to elucidate how hormonal receptors and HER2/Neu expression intersect with the age of presenting patients, which may provide insight as to why early-onset breast cancer has worse prognosis.

Materials and Methods

This study is an analytical observation study designed as a single-sampling cross sectional study. The study samples are paraffin blocks from patients diagnosed with invasive ductal carcinoma of no specific type (IDC-NST) regardless of grade or stage, aged 39 years old or younger (mean: 34 years) who underwent surgery or surgical biopsy in Dr. Soetomo General Hospital, Surabaya, within the period of January 2014-December 2014. The control group consisted of 112 patients diagnosed with IDC-NST aged 40 or older who underwent resection within the same period; all specimen in the control group is tested for ER and HER2/Neu status. The exclusion criteria are patients who already underwent other forms of therapy, such as preoperative chemotherapy or radiotherapy. All samples were treated with immunohistochemistry by staff from Anatomic Pathology Department of Dr. Soetomo General Hospital. Results are cross-tabulated and analyzed with Chi-square test. Furthermore, Spearman ranked correlation test was performed to discover whether there is any statistically significant correlation between the variables.

Results

Early-onset breast cancer, defined as those occurring at or below the age of 39, occurs in a total of 18 (13.84%) of all patients that satisfy the inclusion criteria. The most prominent age range overall is 51-60 years (53.84%) with total *mean* age of 54.03 years old. Within the early-onset group, the mean age is 34.1 years old. The youngest participant is 26 years old and the oldest is 74 years old. Within the early onset group, 8 samples (44.44%) show positive estrogen receptor status whereas 67 samples (58.03%) in the late-onset group show positive estrogen receptor status (Table 1).

All patients under the age of 30, and more than half (53.33%) of those aged 31-40, present with Stage III cancer upon admission. A total of 15 (77.78%) of early-onset patients present with Stage III or IV cancer, while 79 (70.53%) of late onset patients present with stage III or IV cancer. Stage IV cancer is most prominent in those aged 60 or above (30.8%). Sixteen (88.9%) of early-onset patients has grade II or III cancer, with 55.55% presenting with grade III cancer. Meanwhile, 79 (70.53%) of late onset patient has grade II or III cancer, with 26.78% presenting with grade III cancer. Grade III cancer is most prominent in the younger than 30 (66.7%) and 31-40 years old (40%) age group (Table 2).

Cross tabulation between patients' age and estrogen receptor positivity seemed to show higher propensity for negative expression in the younger population (Figure 1). In patients with positive ER status, 77 of 85 (90.58%) is aged 40 or older. 77 out of 112 (68.75%) patients aged 50 or older has positive estrogen receptor status. Meanwhile, in the early-onset group, 10 out of 18 (55.55%) patients have negative estrogen receptor status. Two (66.66%) patients in the below 30 age group has negative estrogen receptor status, while 8 (53.55%) patients in the 30-40 age group has negative estrogen receptor status. In all age groups above 40 years old, 62.5%, 75.7% and 53.8% showing positive ER status in the 40-50 years old, 50-60 years old and >60 years old groups, respectively (Table 3).

Table 1. Data of the characteristics of the research subjects

Category	Range	Amount
Age (years)	<30	3 (2.3%)
	31-40	15 (11.5%)
	41-50	16 (12.3%)
	51-60	70 (53.8%)
	>60	26 (20%)
Estrogen receptor status	Positive	85 (65.4%)
	Negative	45 (34.6%)
Hsp27 expression	0-3	17(13.2%)
	4-6	47 (36.1%)
	7-9	48 (36.9%)
	10-13	18 (13.8%)
HER2/Neu expression	Negative	26 (20%)
	+1	18 (13.8%)
	+2	26 (20%)
	+3	60 (46.2%)

Hsp27, heat shock protein 27; HER2/neu

Cross tabulation between age and HER2/Neu status show that each age group mostly has similar distributions of HER2/Neu intensity, with a total of 46.15% of patients staining strongly positive for HER2/Neu expression (Figure 2). One-fifths of all patients show no HER2/Neu expression. This distribution mostly holds for every age group except for the age group below 30 years old, where one patient (33.3%) showed weak HER2/Neu staining intensity and two patients stained strongly positive (66.6%) for HER2/Neu expression. The oldest age group which included patients above 60 years old showed the least amount of HER2/Neu negative samples, with only 15.4% of samples rendering negative staining (Table 4).

Cross tabulation between age and Hsp27 score shows that all patients aged 50 years and below has Hsp27 score that is less than the median score, suggesting that

Table 2. Cross tabulation between age and grade/stage at presentation

Stage	Age					Total
	<30	31-40	41-50	51-60	>60	
I	0	1 (6.7%)	0	4 (5.7%)	2 (7.6%)	7 (5.4%)
II	0	3 (20%)	4 (25%)	15 (21.4%)	8 (30.8%)	30 (23.1%)
III	3 (100%)	8 (53.3%)	8 (50%)	34 (48.6%)	8 (30.8%)	61 (46.9%)
IV	0	3 (20%)	4 (25%)	17 (24.3%)	8 (30.8%)	32 (24.6%)
Grade	**Age**					**Total**
	<30	**31-40**	**41-50**	**51-60**	**>60**	
I	0	2 (13.3%)	4 (25%)	21 (30%)	8 (30.8%)	35 (26.9%)
II	1 (33.3%)	7 (46.7%)	7 (43.75%)	33 (47.14%)	9 (34.6%)	57 (43.8%)
III	2 (66.7%)	6 (40%)	5 (31.25%)	16 (22.86%)	9 (34.6%)	38 (29.2%)

Table 3. Cross tabulation between age and estrogen receptor status

ER status	Age					Total
	<30	31-40	41-50	51-60	>60	
Positive	1 (33.3%)	7 (46.7%)	10 (62.5%)	53 (75.7%)	14 (53.8%)	85 (65.4%)
Negative	2 (66.7%)	8 (53.3%)	6 (37.5%)	17 (24.3%)	12 (46.2%)	45 (34.6%)
Total	3	15	16	70	26	130 (100%)

ER, estrogen receptor

Table 4. Cross tabulation between age and HER2/Neu status

HER2/Neu score	Age					Total
	<30	31-40	41-50	51-60	>60	
-	0	4 (26.7%)	4 (25%)	14 (20%)	4 (15.4%)	26 (20%)
+	1 (33.3%)	2 (13.3%)	2 (12.5%)	8 (11.4%)	6 (23.1%)	18 (13.84%)
++	0	3 (20%)	3 (18.7%)	16 (22.9%)	4 (15.4%)	26 (20%)
+++	2 (66.6%)	6 (40%)	7 (43.8%)	32 (45.7%)	12 (46.1%)	60 (46.15%)
Total	3	15	16	70	26	130 (100%)

Table 5. Cross tabulation between age and Hsp27 score

Hsp27 score	Age					Total
	<30	31-40	41-50	51-60	>60	
0-3	2 (66.6%)	9 (60%)	0 (0%)	6 (8.6%)	0 (0%)	17 (13.1%)
4-6	1 (33.3%)	6 (40%)	14 (87.5%)	26 (37.1%)	0 (0%)	47 (36.1%)
7-9	0 (0%)	0 (0%)	2 (12.5%)	33 (47.1%)	13 (50%)	48 (36.9%)
10-13	0 (0%)	0 (0%)	0 (0%)	5 (7.1%)	13 (50%)	18 (13.8%)
Total	3	15	16	70	26	130 (100%)

Hsp27, heat shock protein 27

younger patients tend to show less Hsp27 expression (Figure 3). In patients aged 50 or above, 64 out of 96 (66.66%) samples exceed the median score. Eleven out of 17 (64.7%) samples with little to no Hsp27 expression belong to the early-onset age group (Table 5).

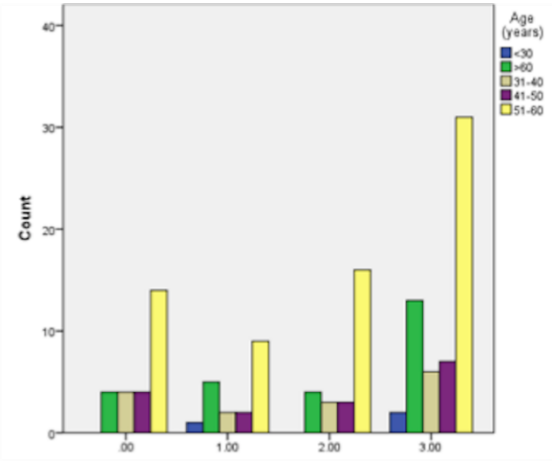

Figure 1. Bar chart illustrating HER2/Neu expression as related to patient age

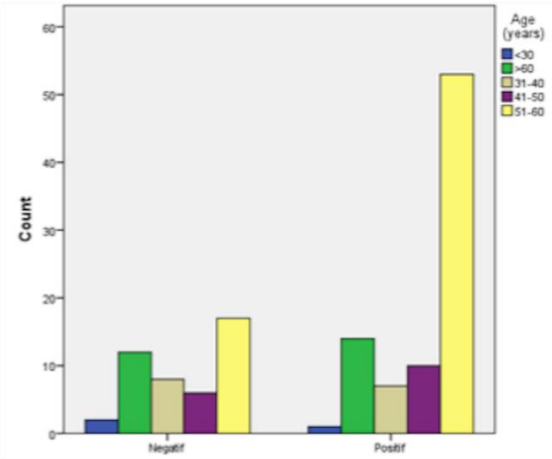

Figure 2. Bar chart illustrating estrogen receptor expression as related to patient age

Chi-square tests were performed correlating Hsp27 score, ER expression and HER2/Neu expression, with the significance threshold set at 0.05. Results for HER2/Neu (N=130, p=0.944) and estrogen receptor expression fail to show a statistically significant correlation. Chi-square results for Hsp27 (N=130, p=<0.001) on the other hand show a strong correlation with patients' age. Similarly, Spearman ranked correlation test showed a statistically significant correlation between Hsp27 expression and patients' age (p=<0.001), but failed to show any significant correlation between age and HER2/Neu expression (p=0.183) or estrogen receptor expression (p=0.281).

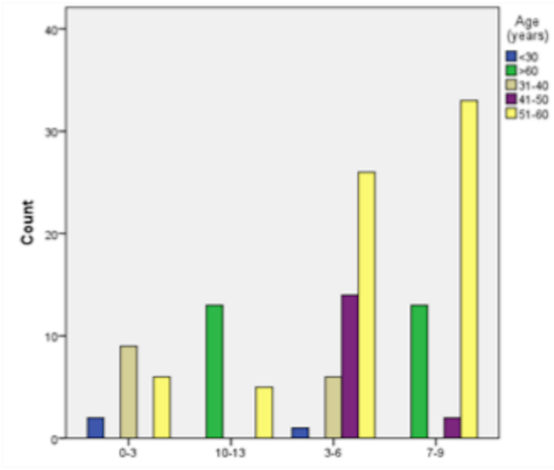

Figure 3. Bar chart illustrating Hsp27 expression as related to age

Discussion

While being relatively rare, early-onset breast cancer show different trends compared to breast cancer in general, often presenting with more advanced tumor stage and grade, and being less responsive to chemotherapy or adjuvant treatment.[22,23] Early-onset breast cancer is also influenced by familial factors such as BRCA1 or BRCA2 mutations, and therefore it is thought to be chemically distinct from breast cancers in general.[24] Study by Sundquist *et al.* indicates that the type of breast cancer associated with lower mortality in older age groups may be a less aggressive disease than the breast cancer developing in younger women.[22] Early-onset breast cancer is also shown to be less responsive to hormonal and adjuvant therapy[16] which renders a population study elucidating the relation between age and hormonal receptor or HER2/Neu status relevant.

This study samples 130 breast cancer patients undergoing treatment in Dr. Soetomo General Hospital, January-December 2014, including 18 early-onset patients aged younger than 40 from various grades and stages of cancer with archived paraffin block specimens of their tumors. All samples are primary tumors and chosen patients are those were never exposed to chemotherapy, hormonal therapy, or immunotherapy. These specimens are processed with immunohistochemistry and staging/grading at presentation, hormonal receptor expression, HER2/Neu amplification and Hsp27 intensity is cross tabulated with data on the patient's age.[9,15]

Cross tabulation between patients' age and grade/stage upon presentation seem to confirm the hypothesis that early-onset tumors tend to have more advanced grade than their late-onset counterparts, with 88.89% early-onset patients presenting with grade II or III tumors, compared to the 70.53 in late-onset patients. The data on staging seem to be equally distributed across all age groups, although we also take into account that most patients tend to present at later stages in the Indonesian society. While existing literature seems to indicate that older patients tend to have positive ER status, while younger patients tend to be ER-negative, Chi-square and Spearman rank correlation test performed on our samples fail to show a statistically significant correlation. This opens up new avenues on investigation regarding ER receptor expression in the Indonesian population – as of now, there is still a lack of data regarding this issue, especially on the prevalence of BRCA1/BRCA2 mutations the Indonesian population, which is connected to early-onset cancer and lack of hormone receptor expression.[25]

Cross tabulation between age and HER2/Neu status also show that each age group mostly has similar distributions of HER2/Neu intensity, with a total of 46.15% of patients staining strongly positive for HER2/Neu expression and one-fifths of all patients show no HER2/Neu expression. This distribution mostly holds for every age group except for the age group below 30 years old. Statistical analysis confirms this result, with both Chi-square and Spearman correlation test failing to show any statistically significant difference.

On the other hand, age and Hsp27 shows significant correlation in our samples. Cross-tabulation between age and Hsp27 score shows that all patients aged 50 years and below has Hsp27 score that's less than the median score, suggesting that younger patients tend to show less Hsp27 expression, while in patients aged 50 or above 66.66% of samples exceed the median score. Both Chi-square ($p = < 0.001$) and Spearman ranked correlation test ($p = < 0.001$) confirms the presence of a strong correlation between Hsp27 and age. Hsp27 is a protein that plays a role in apoptosis and metastasis and has been correlated with resistance towards certain chemotherapeutic drugs. This may be one explanation as to why early-onset cancers tend to be more aggressive and show a worse prognosis [10]

Conclusion

We conclude that early-onset breast cancer does have a connection to more advanced grade and lack of Hsp27 expression. However, our study failed to show statistically significant correlation between age and estrogen receptor or HER2/Neu expression. This shows possible differences in immunohistochemical properties than between early-onset breast cancer and other breast cancers, which could influence future therapeutic approach. Further study is needed to possibly connect these data to BRCA1/BRCA2 mutations, or by performing further patient follow-up or larger multicentre studies.

Ethical Approval

The study was assessed Health Research Ethics Committee, Dr. Soetomo General Hospital, Surabaya, and obtained ethical approval on 15 April 2015 (ethical certificate 261/Panke.KKE/IV/2015)

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Conflict of Interest

The authors declare no conflict of interest

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