Correlation CD24 and CD44 expression against aggressiveness breast cancer

Aida Farida*, Wresnindyatsih, Venni Yuliantini

Department of Anatomical Pathology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

E-mail : aida_farida63@yahoo.com

Abstract. Cancer stem cells (CSC) in breast cancer are the source of cells that are resistant to chemotherapeutic agents that are responsible for recurrence and metastasis. The existence of CSC is a benchmark for success in strategies for prevention and treatment of breast cancer. Cell surface markers known as cluster of differentiation (CD) 24 are expressed in breast cancer. CD44 is a cell surface protein composed of glycoproteins, high enough to be expressed in breast cancer. Sample taken with technique proportional random sampling. The population of this study is a specimen that has been diagnosed molecular subtype as a breast cancer from January 2015 arrived October 2017, consist of 44 sample, 11 luminal A samples, 11 luminal B samples, 11 HER2 samples and 11 triple negative samples. CD24 expression was found most negative (81.8%) and CD44 expression found the most positive at 84.1%. Expression CD24 correlation with molecular subtype found that the results of the chi-square test found a correlation between expression CD24 and molecular subtype (p-value = 0.036). There correlation expression CD24 molecular subtype but there were no correlation CD24 with limfovaskular invasion, grading, and Ki67. There no correlation expression CD44 with limfovaskular invasion, grading, molecular subtype and Ki67.

1. Introduction
Cancer stem cells (Cancer Stem Cell / CSC) is a small population between malignant tumor cells with the ability to regenerate new tumor cells and is also known as "tumor-initiating cells". Like normal stem cells, CSC has the ability to repair itself, can cause different offspring and are able to utilize the signalling pathways, these cells have the ability of tumorigenic activity that allows them to form tumors when transplanted into animals [1].

Breast cancer is the most common malignancies in women and the second cause of death from cancer in women. CSC in breast cancer are the source of cells that are resistant to chemotherapeutic agents that are responsible for recurrence and metastasis. The existence of CSC is a benchmark for success in strategies for prevention and treatment of breast cancer. This current prevention strategy that focuses on CSC so as to reduce the risk of breast cancer progression to bigger attention [2]. In this context, biomarkers that can predict the progress of the disease and the prognosis of cancer are needed.

Cell surface markers known as cluster of differentiation (CD) 24 are expressed in breast cancer [3]. While CD44 is a cell surface protein composed of glycoproteins, high enough to be expressed in breast cancer [4]. The activity of several extracellular proteins that play a role in the regulation of adhesion, proliferation, growth, survival, migration, and angiogenesis associated with CD44 [5]. The findings of Hajj et al., (2003) showed that CD44 + / CD24 - expression was a CSC marker for some breast cancer phenotypes, especially in aggressive types [6].
This study will explore the relationship between the expression of CD24, CD44 on the aggressiveness and progression of breast cancer in Dr. Moh. Hoesin Palembang. So that it is expected to develop diagnostic and prognostic markers for breast cancer.

2. Methods
This research is an observational study with cross sectional design. Sample taken with technique proportional random sampling. Big sample on research this is 44 sample consists of from 11 luminal A samples, 11 luminal B samples, 11 HER2 samples and 11 triple negative samples. The place of sample collection is part of Pathology Faculty of Medicine Sriwijaya Universitas/ Hospital Dr. Moh. Hoesin Palembang. When research was conducted on January 1st 2017 until June 30th 2018. The population of this study is a specimen that has been diagnosed histopathology as a breast cancer from January 2015 arrived October 2017.

After data collection, these variables were processed using SPSS tools. Then univariate analysis was conducted to determine the frequency distribution of each variable studied and bivariate analysis using chi-square test to determine how much the relationship between CD24 and CD44 expression on breast cancer aggressiveness and progressiveness.

The aggressiveness of breast cancer is determined by several conditions including high degree of malignancy (grouped according to histopathological features), molecular subtypes namely triple negative / basal-like and the presence of metastasis. The expression of ER (estrogen receptor), PR (progesterone receptor), HER2, and Ki67 proteins is the basis for determining molecular sub-types breast cancer. Luminal A sub-type is characterized by ER and PR expressions, Ki67 <14%. Luminal B is characterized by ER, PR, HER2 and Ki67> 14%, While the HER2 sub-type is characterized by the expression HER2, ER and PR, Ki67> 14%. And the triple negative sub-type is marked HER2, ER and PR, and Ki67> 14%. HER2 and triple negative subtypes are known as a type of breast cancer with aggressive growth which is rapid spread and high risk for metastasis.

Assessment CD24 and CD44 expression in immunohistochemistry do semi-quantitative, observed with microscope light binoculars Olympus brand type CX 22. Assessment grading examination namely CD24 and CD44 immunohistochemistry based on percentage the area that is reflected and intensity staining on the membrane cytoplasm (grades 0 to 2). Grade 0, no colored, grade 1, colored <50% and grade 2, colored ≥ 50% of tumor cells. Grade this certified to be two group that is negative if grade 0, positive when grade 1 and grade 2.

3. Results
The univariate analysis aims to determine the frequency distribution by characteristic clinicopathologic (age, limfvascular invasion, grading, molecular subtype) see table 1. The results of the analysis showed that the mean age was 49 years with the lowest age of 26 years and a maximum age of 91 years. Age categorized into age <40 years and> 40 years of age are mostly in those aged> 40 years is 77.3%, based on limfvascular invasion distribution obtained largely positive LVI namely 79.5%, 59.1% were grade III with subtypes as many as 11 samples (25%) in luminal A, luminal B, her-2 and triple (-).

Distribution frequency by Ki67 earned mostly positive, namely 61, 4%. In distribution frequency-based expression CD24 and CD44 expression found that CD24 expression was found negative CD24 which is 36 people (81.8%), while the expression of CD24 positive in 8 people (18 2%) see figure 1. Distribution frequency-based expression CD44 was found that the expression of CD44 was found positives that 37 (84.1%), while the negative CD44 expression of 7 people (15.9%). Molecular subtype CD44'/CD24+ much as 75% as triple negative, CD 24+ with HER2+ positive much as 36,4%, see table 2 and figure 2.

The bivariate analysis aims to determine the correlation expression of CD24 and CD44 with clinicopathologic characteristic. This analysis uses a chi-square statistical test. Expression CD24 correlation with limfvascular invasion found that the Fisher Exact Test results are not correlation CD24 expression with limfvascular invasion (p-value = 1.000) p> α. Expression CD24 correlation with grading found that the results of the Fisher exact test did not have a correlation between expression CD24 and grading (p-value = 0.240) p> α. Expression CD24 correlation with molecular...
subtype found that the results of the chi-square test found a correlation between expression CD24 and molecular subtype (p-value = 0.036) p < α see table 3. Correlation of CD24 expression to Ki67 was found that the results of the chi-square test there was no correlation expression CD24 with Ki67 (p-value = 1.000) p > α.

\[ \text{Figure 1. CD24 positive expression in subtype molecular luminal A breast cancer (200x).} \]
\[ \text{Figure 2. CD44 positive expression in subtype molecular luminal B breast cancer (200x).} \]

| Table 1. Frequency distribution based on clinicopathological characteristics. |
|-------------------------------------------------|
| Clinicopathological characteristic | Amount (n) | Percent (%) |
|-------------------------------------|------------|-------------|
| Age                                 |            |             |
| - ≤40 y.o                           | 10         | 22.7        |
| - >40 y.o                           | 34         | 77.3        |
| - Rerata ± SD                       | 49.84 ± 11.93 |             |
| - Minimum                           | 26         |             |
| - Maximum                           | 91         |             |
| LVI                                 |            |             |
| - Negative                          | 9          | 20.5        |
| - Positive                          | 35         | 79.5        |
| Grading                             |            |             |
| - II                                | 18         | 40.9        |
| - III                               | 26         | 59.1        |
| Molecular subtype                   |            |             |
| - Luminal A                         | 11         | 25.0        |
| - Luminal B                         | 11         | 25.0        |
| - Her – 2                           | 11         | 25.0        |
| - Triple (-)                        | 11         | 25.0        |
| KI67                                |            |             |
| - Negative                          | 17         | 38.6        |
| - Positive                          | 27         | 61.4        |

Correlation with the CD44 expression limfsovaskular invasion it was found that the results of the Fisher Exact Test there is no correlation with limfsovaskular invasion CD44 (p-value = 1.000) p > α. The correlation between expression CD44 and grading it was found that the test results of the Fisher Exact Test there is no correlation between expression CD44 and grading (p-value = 1.000) p > α. The correlation between expression CD44 and molecular subtypes it was found that the results of the chi-square test there was no correlation between expression CD44 and molecular subtypes (p-value = 0.917) p > α see table 3. Expression CD44 correlation with Ki67 it was found that the results of the chi-square test did not have a correlation between expression CD44 and Ki67 (p-value = 1.000) p > α.
4. Discussions

Breast cancer is the most common cancer in women. In 2012 according to the International Agency for Research on Cancer (IARC) breast cancer is still a disease number one in the world and in Indonesia, more than 80% of cases are found at an advanced stage [7,8]. The incidence of breast cancer increases with age. The risk of cancer increases two times up to the age of menopause. Risk factors for breast cancer which are irreversible factors are women, increasing age, race and ethnicity, family history of breast cancer, having certain genes derived, namely BRCA 1 or BRCA 2, ATM, TP 53, CHEK2, PTEN, CDH1, STK11, and PALB2, previous history of breast disease, various conditions of benign breast lesions, history of chest radiotherapy at a young age, exposure to diethylstilbestrol during pregnancy, early menarche and late menopause. Risk factors that can be changed related to lifestyle are obesity, alcohol consumption, lack of physical activity, not having children or the first child born after 30 years, not breastfeeding, using oral contraceptives and hormone replacement therapy after menopause [9].

| Expression | Amount (n) | Percent (%) |
|------------|------------|-------------|
| CD24       |            |             |
| - Negative | 36         | 81.8        |
| - Positive | 8          | 18.2        |
| Total      | 44         | 100.0       |
| CD44       |            |             |
| - Negative | 7          | 15.9        |
| - Positive | 37         | 84.1        |
| Total      | 44         | 100.0       |

Table 2. Frequency distribution based on CD24 and CD44 expression.

Table 3. Correlation of CD24 and CD44 expression with molecular subtype.

| Expression | Molecular subtype | Total n(%) | p value |
|------------|-------------------|------------|---------|
| CD24       | Luminal A n (%)   | Luminal B n (%) | Her-2 n (%) | Triple (−) n (%) | 11 (100,0) | 7 (15,9) |
| - Negative | 9 (81.8)          | 10 (90.9)  | 6 (54,5)  | 11 (100,0)       | 36 (81.8)  | 0,036   |
| - Positive | 2 (18,2)          | 1 (9,1)    | 5 (45,5)  | 0 (0)            | 8 (18,2)   |         |
| Total      | 11 (100,0)        | 11 (100,0) | 11        | 11               | 44 (100,0) |         |
| CD44       | Luminal A n (%)   | Luminal B n (%) | Her-2 n (%) | Triple (−) n (%) | 11 (100,0) | 7 (15,9) |
| - Negative | 2 (18,2)          | 2 (18,2)   | 2 (18,2)  | 1 (9,1)          | 37 (84,1)  | 0,917   |
| - Positive | 9 (81,8)          | 9 (81,8)   | 9 (81,8)  | 10 (90,9)        | 44         |         |
| Total      | 11 (100,0)        | 11 (100,0) | 11        | 11               | 44 (100,0) |         |

In this study, the mean age of patients was 49 years with the lowest age of 26 years and a maximum age of 91 years. Age is categorized into ages < 40 years and > 40 years, most of the age is in the age of > 40 years, which is 77.3% more than patients aged <40 years (22.7%). The results of this study are not much different from Marice Sihombing's research in 2011 where there were The research likewise conducted by Silvia Sagita in 2012 where the average age of breast cancer patients was 48 years with an age range of 22 to 85 years more patients aged ≥ 40 years (68.9%) than patients aged <40 years (31.1%). [10]. Arzu et al's study in 2017 found that the average age of breast cancer patients was slightly higher than this study, which was 51.1 ± 6.7 years [11]. In addition, research conducted by FZ Lamiri et al in 2015 showed the average age of patients with advanced breast cancer patients was 45.83 ± 11.05, where there were more patients aged ≥ 45 years (58%) than patients aged <44 years (42%) [12].

This histology grading assessment is assessed based on the Nottingham Combined Histologic Grade system (Elston-Ellis Modification of Scarff and Bloom-Richardson Grading System). This system assesses breast tumors based on three tumor characteristics namely tubular formation, cell...
nucleus pleomorphism, and mitotic count. The most commonly published system for breast cancer stage is the TNM system published by the American Joint Committee on Cancer Staging (AJCC) Union for International Cancer Control (UJCC). This stadium is used to classify breast cancer patients into prognostic groups, make treatment decisions, predict responses to treatment, clinical trials and epidemiological studies [13].

In this study the majority of breast cancer patients with grade III (59.1%). This is in line with the results of a study by Mabula et al. (2012) in Tanzania, where grade III was the most common grade in breast cancer patients, which was 63.8% followed by grade II at 34.1% and grade I at 2.1% [14]. It also supported by the results of research by Leong et al. in India, about 60% of breast cancer patients were found to belong to grade III and has metastasized to the lymph nodes [15]. In addition, the results of research from Ebughe et al. in Nigeria also found that grade III was the most common prevalence of breast cancer, which was around 66.7% [16].

Ki67 showed that breast cancer patients largely positive, namely 61.4%. This result is in line with Noda et al research in 2016 where the results of immunohistochemistry examination on the proliferative index Ki-67 showed that Ki-67 was higher in the high expression category by 86.2%, while the low expression category was 13.8%. This result is also consistent with the results of Chan et al. study in Taiwan that 84% of breast cancers express Ki-67> 10% and only 15% express Ki-67 <10% [17].

The cluster of Differentiation (CD) 24 is a small protein found on the cell surface and composed of glycoproteins found in several cancer cells. CD24 plays a role in maintaining the bonds between cells and cells with the extracellular matrix [18]. The cluster of differentiation (CD) 44 is a cell surface protein composed of glycoproteins, which are specific receptors for hyaluronic acid, increasing migration from cells and high enough to be expressed in some cancer cells [3].

In this study, the majority of patients with breast cancer have expression 81.8% negative CD24 and CD44 expression by 84.1% positive. This result is not in line with the research of Hajj et al., (2003) which showed the expression of CD44+/CD24- is a CSC marker for breast cancer, especially in aggressive types. Likewise, a study conducted by Jang MH et al. in 2016 which stated that breast cancer stem cells express CD44+/CD24- that has high tumorigenesis capacity compared with other cells [19].

In addition, with the bivariate analysis it was found that not correlation expression CD24 with limfovaskular invasion (p-value = 1.000), grading (p-value = 0.240), and Ki67 (p-value = 1.000), but there is a correlation expression CD24 molecular subtype (p-value = 0.036). Whereas, by expression CD44 showed no correlation expression CD44 to limfovaskular invasion (p-value = 1.000), grading (p-value = 1.000), subtype molecular (p-value = 0.917) and Ki67 (p-value = 1.000). This result is in line with Achmad Gozali's research in 2017 where there was no correlation between the presence of CD44+/CD24 stem cells - histological grading of breast carcinoma (p > 0.05) but in 2017 this study found a meaningful correlation between CD44+/CD24-stem cells with proliferation rates (ki67) and high in breast carcinoma and histopathological subtypes (p < 0.05).

5. Conclusions
CD24 expression was found most negative (81.8%) and CD44 expression found the most positive at 84.1%. There correlation expression CD24 molecular subtype but there were no correlation CD24 with limfovaskular invasion, grading, and Ki67. There were no correlation expression CD44 with limfovaskular invasion, grading, molecular subtype and Ki67. CD44+/CD24- more aggressive and metastases far and at the most triple negative. CD44+/CD24- is a stem cell property that has high tumorigenesis capacity compared with other cells.

6. References
[1] Jaggupilli A and Elkord E 2012 Significance of CD44 and CD24 as cancer stem cell markers: an enduring ambiguity Clin. Dev. Immunol. 2012
[2] Lee H J, Choe G, Jheon S, Sung S-W, Lee C-T and Chung J-H 2010 CD24, a novel cancer biomarker, predicting disease-free survival of non-small cell lung carcinomas: a retrospective study of prognostic factor analysis from the viewpoint of forthcoming (seventh
new TNM classification J. Thorac. Oncol. 5 649–57
[3] Tanitchi K, Nishimori I and Hollingsworth M A 2011 Intracellular CD24 inhibits cell invasion by posttranscriptional regulation of BART through interaction with G3BP Cancer Res.
[4] Naor D, Nedvetzki S, Golan I, Melnik L and Faitelson Y 2002 CD44 in cancer Crit. Rev. Clin. Lab. Sci. 39 527–79
[5] Rangaswami H, Bulbule A and Kundu G C 2006 Osteopontin: role in cell signaling and cancer progression Trends Cell Biol. 16 79–87
[6] Ricardo S, Vieira A F, Gerhard R, Leitão D, Pinto R, Cameselle-Teijeiro J F, Milanezi F, Schmitt F and Paredes J 2011 Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype J. Clin. Pathol. 64 937–46
[7] Torre L A, Bray F, Siegel R L, Ferlay J, Lortet-Tieulent J and Jemal A 2015 Global cancer statistics, 2012 CA. Cancer J. Clin. 65 87–108
[8] Hoda S A and Ginter P S 2015 Robbins and Cotran Pathologic Basis of Disease Am. J. Clin. Pathol. 144 172
[9] Simpson R M L, Wells A, Thomas D, Stephens P, Steadman R and Phillips A 2010 Aging fibroblasts resist phenotypic maturation because of impaired hyaluronan-dependent CD44/epidermal growth factor receptor signaling Am. J. Pathol. 176 1215–28
[10] Sihombing M and Sapardin A N 2014 Faktor Risiko Tumor Payudara Pada Perempuan Umur 25-65 Tahun di Lima Kelurahan kecamatan Bogor Tengah J. Kesehat. Reproduksi 5 175–84
[11] Sagita S 2013 Analisis hubungan tingkat pendidikan pasien dengan kanker payudara stadium dini di instalasi rawat inap Rumah Sakit Ciptomangunkusumo Jakarta tahun 2012
[12] Ozsoy A, Barça N, Dolek B A, Aktaş H, Elverici E, Araz L and Ozkaraoğlu O 2017 The Relationship Between Breast Cancer and Risk Factors: A Single-Center Study Eur. J. breast Heal. 13 145
[13] Alizart M, Saunus J, Cummings M and Lakhani S R 2012 Molecular classification of breast carcinoma Diagnostic Histopathol. 18 97–103
[14] Mabula J B, Mchembe M D, Chalya P L, Giiti G, Chandika A B, Rambau P F, Masalu N and Gilyomo J M 2012 Stage at diagnosis, clinicopathological and treatment patterns of breast cancer at Bugando Medical Centre in north-western Tanzania Tanzan. J. Health Res. 14
[15] Leong S P L, Shen Z-Z, Liu T-J, Agarwal G, Tajima T, Paik N-S, Sandelin K, Derossis A, Cody H and Foulkes W D 2010 Is breast cancer the same disease in Asian and Western countries? World J. Surg. 34 2308–24
[16] Ebughe G A, Uigure G U, Nnoli M A, Bassey I-A, Nwagbara V J, Udosen J E, Oyorominya O E, Chukwuebo C C, Ugbeh T I and Omotoso A J 2013 Histological type and tumour grade in Nigerian breast cancer: Relationship to menarche, family history of breast cancer, parity, age at first birth, and age at menopause IOSR J Dent Med Sci 7 58–63
[17] Chan Y-J, Chen B-F, Chang C-L, Yang T-L and Fan C-C 2004 Expression of p53 protein and Ki-67 antigen in phyllodes tumor of the breast JOURNAL-CHINESE Med. Assoc. 67 3–8
[18] Kristiansen G, Winzer K-J, Mayordomo E, Bellach J, Schlüns K, Denkert C, Dahl E, Pilsarsky C, Altevogt P and Guski H 2003 CD24 expression is a new prognostic marker in breast cancer Clin. cancer Res. 9 4906–13
[19] Jang M H, Kang H J, Jang K S, Paik S S and Kim W S 2016 Clinicopathological analysis of CD44 and CD24 expression in invasive breast cancer Oncol. Lett. 12 2728–33