Correlation between CD44 expression on histopathological grading, metastasis, survival overall (SO) and disease free survival (DFS) on women breast cancer

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ABSTRACT

Breast cancer is one of the health problems in the world. In Indonesia, it was highest prevalence of cancer in 2018. CD44, a non-kinase transmembrane glycoprotein, plays an important role in cancer development and progression. It is responsible for mediating of the adhesion between adjacent cells and also between cells and the extracellular matrix. The aim of study was to investigate the relationship between the CD44 expression and histopathological grading, metastasis, overall survival (OS) as well as disease free survival (DFS) in breast cancer patients. This was a retrospective cross sectional study using data on medical records of breast cancer cases at Dr. Wahidin Sudirohusodo General Hospital, Makasar from 2016 to 2018. Examination of CD44 expression were performed from paraffin block samples. Data of the CD44 expression and medical records obtained were then presented in tables and statistically analyzed using chi-squared and Kaplan–Meier survival analysis. No significantly relationship between the CD44 expression and histopathological grading was reported (p>0.05). However, significantly relationship between the CD44 expression and metastasis was reported (p<0.01). Patients with metastasis having high CD44 expression was significantly higher (90%) than those patients without metastasis (60%) (p<0.01). No significantly relationship between the CD44 expression and OS or DFS was reported (p>0.05). In conclusion, there is relationship between CD44 expression and metastasis in the breast cancer patients. However, there is no relationship between CD44 expression and histopathological grading, OS as well as DFS.

ABSTRAK

Kanker payudara merupakan salah satu masalah kesehatan di dunia. Di Indonesia, prevalensi kanker payudara paling tinggi pada 2018. CD44, glikoprotein transmembran non kinase, berperan penting dalam perkembangan dan progresivitas kanker. CD44 berperan dalam proses adisi antara sel yang berdekatan dan juga antara sel dengan matrik ekstra selular. Penelitian ini bertujuan untuk mengkaji hubungan antara ekspresi CD44 dan nilai gambaran histopatologi, metastasis, keseluruhan kelangsungan hidup (OS), kelangsungan hidup bebas penyakit (DFS) pada pasien kanker payudara. Ini adalah penelitian potong lintang retrospektif menggunakan data rekam medis kasus kanker payudara di Rumah Sakit Umum Dr. Wahidin Sudirohusodo, Makasar dari tahun 2016-2018. Pemeriksaan ekspresi CD44 dilakukan dari sampel blok paraffin. Data ekspresi CD44 dan rekam medis yang diperoleh selanjutnya dipresentasikan dalam table dan dianalisis secara statistik menggunakan chi-square dan analisis keseluruhan hidup Kaplan-Meier. Tidak terdapat hubungan nyata antara ekspresi CD44 dan nilai gambaran histopatologi (p>0,05). Namun demikian, terdapat hubungan nyata antara ekspresi CD44 dan metastasis (p<0,01). Pasien metastasis yang mempunyai ekspresi CD44 tinggi (90%) lebih besar secara nyata dibandingkan pasien tanpa metastasis (60%). Tidak ada hubungan nyata antara ekspresi CD44 dan OS atau DFS (p>0,05). Dapat disimpulkan ada hubungan antara ekspresi CD44 dan metastasis pada pasien kanker payudara. Namun demikian tidak ada hubungan antara ekspresi CD44 dan nilai gambaran histopatologi, OS dan DFS.
INTRODUCTION

Breast cancer is one of the health problems in the world. It is the most commonly occurring cancer in women and the second most common cancer overall. According to the Global Cancer Statistics 2018, 18.1 million new cancer cases were reported (17.0 million excluding nonmelanoma skin cancer) and 9.6 million cancer deaths (9.5 million excluding nonmelanoma skin cancer) in 2018. In both sexes combined, lung cancer is the most commonly diagnosed cancer (11.6% of the total cases) and the leading cause of cancer death (18.4% of the total cancer deaths), closely followed by female breast cancer (11.6%). According to the American Cancer Society (ACS) in 2012, breast cancer is the second leading cause of death in women (14%) after lung cancer (26%) in the United States. In Indonesia, based on Basic Health Research (Riset Kesehatan Dasar/Riskesdas) data in 2018, breast cancer is the highest prevalence of cancer in Indonesia that is suffered 58,256 patients or 16.7% from 348,809 cases. Hospital based study conducted in Dr. Wahidin Sudirohusodo General Hospital, Makasar reported during a period 2008-2012 as much as 1,497 patients (299 patients a year) were diagnosed as breast cancer. About 39.4% of them aged between 40-49 years.

In the developed countries, more breast cancer is diagnosed in early stages. However, in the developing countries including Indonesia it is diagnoses in late stages. In Padang, the percentage of breast cancer patients with stage IIIA and IIIB were 43.4 and 14.3%, respectively. In West Jakarta, it was reported the most of frequent of female breast cancer were in 45-49 years old, not other specific location subtopography (81.5%), the histopathology type was ductal and lobular neoplasm (51.8%), in stage III (44%), and with metastases to bone (34%).

Various studies have proven the important role of tumor markers in assessing disease progression, monitoring therapy, recurrence and prognostic factors in malignancies. CD44, a non-kinase transmembrane glycoprotein, plays an important role in cancer development and progression. It is responsible for mediating of the adhesion between adjacent cells and also between cells and the extracellular matrix. The CD44 is associated with prognostic factors in breast cancer. The adhesion cells play an important role to construc the basic form of normal breast histology that are important for maintaining tissue integrity. This disruption of adhesion relationship causes loss of network architecture which is a sign of malignant transformation. This study aimed to evaluate the relationship between CD44 expression of histopathological grading, metastasis, overall survival (OS) and disease free survival (DFS).

MATERIALS AND METHODS

Design and subject

This was a retrospective cross sectional study using data on medical records of breast cancer cases at Dr. Wahidin Sudirohusodo General Hospital, Makasar from 2016 to 2018 who met the inclusion and exclusion criteria. The inclusion criteria were women with breast cancer who have been proven by histopathological examination, breast cancer patients with PA results showing histopathological grading by Nottingham Modification of Bloom Richardson System, patients with metastatic breast cancer evidenced by radiological examination (photo thorax, ultrasound, CT scan or MRI), and patient were willing to be involved in this study. The exclusion criteria were breast cancer patients with biopsy results do not show histopathological grading, and suffer
from other types of cancer (eg ovarian, liver, lung cancer). Subjects were selected using a randomized purposive sampling method taking members of the population. Sixty subjects who met the inclusion and exclusion criteria were involved in this study.

**CD44 expression examination**

The paraffin block sample was examined for CD44 expression using CD44 antibody (DF1485, Novocastra Laboratories Ltd., dilution 1:100) then count the percentage of positive cells and their intensity visually using a comparison light microscope. Immunostained samples were evaluated blind by one pathologist. CD44 was stained in cell membrane, with score 0 if < 10% positive tumour cells; 1 if 10-25%; 2 if 25-50%; and 3 if > 50%. Intensity was scored as 0 if unstained, 1 if weakly stained, 2 if intermediate, and 3 if strong. Interpretation of CD44 staining was determined based on multiplication of the percentage of positive cells and the intensity of staining. CD44 was scored as low if (-until +4) and high (+5 until +9). Data of CD44 expression and medical records were then processed, analyzed with chi square and presented in tables, graphs and narratives. This study was approved by the Joint Ethics Committee of Faculty of Medicine Hasanuddin University, Hasanuddin University Hospital, and Dr. Wahidin Sudirohusodo Hospital (Number 258/UN4.6.5.31/PP36/2020).

**Statistical analysis**

Data were presented as frequency distribution or percentage. Chi Square test was used to evaluate the relationship between histopathological grading or metastasis with CD44 expression. Kaplan–Meiersurvival analysis was applied to evaluate relationship of overall survival (OS) or disease free survival (DFS) with CD44 expression.

**RESULT**

The subjects characteristics of the study are presented in the TABLE 1. The largest number of subject aged between 40-49 years (63.7%) with moderate grading (55.0%), not metastatic (50.0%), multiple metastatic sites (15%), no DFS status (56.7%), and no OS status (60.0%). The subjects with high CD44 levels was 75.0% and low CD44 levels was 25.0%.

| TABLE1. Frequency distribution of research subjects characteristics |
|---------------------------------------------------------------|
| Characteristics       | Number | Percentage (%) |
| Age (year old)        |        |                |
| <40                   | 12     | 20.0           |
| 40-49                 | 38     | 63.7           |
| 50-59                 | 14     | 23.3           |
| 60-69                 | 6      | 10.0           |
| >= 70                 | 0      | 0.0            |
| Hispathological grade |        |                |
| Low                   | 4      | 6.6            |
| Moderate              | 33     | 55.0           |
| High                  | 23     | 23.6           |
| Metastatic amount     |        |                |
| Multiple              | 9      | 15.0           |
TABLE 2 shows the relationship between CD44 expression with histopathological grading of the breast cancer patients. The high CD44 expression of patients with high, moderate, and low histopathological grading were 81.0%, 75.8% and 50.0%, respectively. No significantly relationship between CD44 expression with histopathological grading.

### TABLE 2. Relationship between CD44 expression with histopathological grading

| Histopathological grading [n (%)] | CD44 expression [n(%)] | Total |
|----------------------------------|-----------------------|-------|
|                                  | Low | High     |       |
| High                             | 4 (19.0) | 17 (81.0) | 21 (100.0) |
| Moderate                         | 8 (24.2) | 25 (75.8) | 33 (100.0) |
| Low                              | 3 (50.0) | 3 (50.0)  | 6 (100.0)  |
| Amount                           | 15 (25.0) | 45 (75.0) | 60 (100.0) |

Chi square test (p=0.300)

TABLE 3 shows the relationship between CD44 expression with metastasis in breast cancer patients. Patients with metastasis having high CD44 expression was significantly higher (90%) than those patients without metastasis (60%) (p<0.01). A significantly relationship between the CD44 expression and metastasis war observed (p<0.01).
TABLE 3. Relationship between CD44 expression and metastatic

| Metastatic [n (%)] | CD44 expression [n (%)] | Total |
|-------------------|------------------------|-------|
|                   | Low  | High |       |
| Yes               | 3 (10.0) | 27 (90.0) | 30 (100.0) |
| No                | 12 (40.0) | 18 (60.0) | 30 (100.0) |
| Total             | 15 (25.0) | 45 (75.0) | 60 (100.0) |

Chi square test (p=0.01)

FIGURE 1 shows the OS analysis using median with following up to 3 years for survival that could be divided into two groups. From month 0 to 5\textsuperscript{th} month, the survival rate for OS with low and high CD44 expressions was similar with OS value of 75\%. Decrease in 35\% survival rate of patients with high CD44 expression was observed on the 10\textsuperscript{th} month, where as for patients with low CD44 expression on the 17\textsuperscript{th} month. Furthermore, the patients with high CD44 expression showed a progressive decrease in survival rate from month to month. In the 20\textsuperscript{th} month, the survival rate decreased to be <15\% and then 0-5\% in the 25\textsuperscript{th} month. The decrease in survival rate of patients with low CD44 expression in the 20\textsuperscript{th}, 25\textsuperscript{th}, 30\textsuperscript{th}, and 40\textsuperscript{th} months were 20\%, 15-20\%, 10\% and 5-10\%, respectively. No significantly relationship between CD44 expression and OS was observed (p>0.05).

FIGURE1. Kaplan–Meier curve relationship of OS according to CD44 expression
FIGURE 2 shows the prediction test of patient's survival with low and high CD44 expressions using Kaplan-Meier analysis. The DFS degree of patients with high CD44 expression in the 5th, 10th, 20th were 60%, 20% and 5%, respectively. The longer the patients suffer, the more decreased the patient's ability to respond to the effects of the disease. The DFS degree of patients with low CD44 expression in the 5th, 10th, 20th were 65%, 40% and 20%, respectively. Although the DFS degree in low CD44 expression group was higher than that in high CD44 expression group, it was not significantly different.

FIGURE 2. Kaplan–Meier curve relationship of DFS according to CD44 expression

FIGURE 3 shows the CD44 immunohistochemical staining of breast cancer tissues. Low CD44 expression was considered if less than 50% of the positive cells membrane or cytoplasmic were observed. High CD44 expression was considered if more than 50% of the positive cells membrane or cytoplasmic were observed.

FIGURE 3. Immunohistochemistry CD44 Expression stain (scale 400x). A) negative or low expression; B) positive or high expression.
DISCUSSION

CD44 is involved in multiple physiological and pathological processes, such as angiogenesis, cell adhesion, inflammation, and cancer development. It is involved in tumorigenesis and metastasis in many cancer types such as colon, bladder, and gastric cancers. However, the role of the CD44 in breast cancer remains unclear. Previous studies concerning the role of CD44 in tumorigenesis and metastasis gave different results.

In this study, no significantly relationship between the CD44 expression and histopathological grading was reported. The relationship between CD44 expression and histopathological grading has been reported in the previous studies with different results. Farida et al.12 and Jang et al.13 reported that CD44 expression is not correlated with histopathological grading in breast cancer. Otherwise, Moansoori et al.14 and McFarlane et al.15 reported a significant positive correlation between CD44 expression with histological grade. Breast cancer samples with advance histological grade also had higher CD44 expression. In addition, Qiao et al.16 also reported that CD44v6, a variant isoform of CD44, is associated with histopathological grade.

A positive significantly relationship between the CD44 expression and metastasis was reported in this study. Breast cancer patients with metastasis have high CD44 expression (90%) higher than those patients without metastasis (60%). Studies concerning the role of CD44 in tumorigenesis and metastasis in many cancer types including breast cancers have been conducted. Qiao et al.16 reported that CD44v6 expression is significantly associated with poor prognosis, histological grade, and lymph node metastasis in breast cancer patients, especially among Asian patients. Moreover, McFarlane et al.15 also reported that CD44 expression is associated with metastasis-related phenotype.

Kaplan-Meier analysis revealed that no significantly relationship between CD44 expression and OS as well as DFS were observed in this study (p>0.05). Previous studies have been reported with different results. McFarlane et al.15 reported that high CD44 expression correlated with the reduced DFS in lymph node-positive patients and patients with large tumor size. Furthermore, the correlation between CD44 overexpression and the poor OS rate (pooled OR =1.27, 95% CI: 1.04–1.55, Cochran’s Q test p=0.505, and I2 =0.0%) was reported. However, CD44 expression was not associated with the RFS rate (pooled OR=1.04, 95% CI:0.89-1.23, Cochran’s Q test p=0.417, and I2 =2.4%).

CONCLUSION

In conclusion, there is a significant correlation between high CD44 expression and single or multiple metastatic of breast cancer patients indicating it can be used for metastatic prognostic factor. However, there is no significant correlation between CD44 expression and histopathological grading, OS and DFS.

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