RESEARCH ARTICLE

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Role of *CD44* and *CD24* Expression on 2-years Disease Free Survival in Patients with Advanced Epithelial Ovarian Carcinoma

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Abstract

Objective: Ovarian cancer is one of the most common cancers with a high mortality rate worldwide. Despite optimal surgical therapy and chemotherapy, recurrence is still common. Cancer stem cells expressing *CD44* and *CD24* are thought to be contributing factors in recurrence. **Methods:** A cohort retrospective study with survival analysis was carried out on advanced ovarian cancer patients who underwent optimal debulking surgery followed by 6 cycles of chemotherapy at Cipto Mangunkusumo General Hospital and Fatmawati General Hospital from January 2019 to March 2023. Immunohistochemical examination was performed on tumor tissue with *CD44* and *CD24* expression were assessed using the H-Score method then determined the cut off-point expression level using the ROC curve. Furthermore, the relationship between these expression levels with the disease-free survival was assessed using the survival curve. **Results:** There were 48 subjects who were included in the study. There were high expression levels of *CD44* in 47.9% and *CD24* in 50% of cases. High *CD44* expression had mean and median survival of 13.2 ± 1.8 and 11 months (HR 5.05, 95% CI 1.84- 13.85). High *CD24* expression had mean and median survival of 13.5 ± 2.4 and 7 months (HR 7.73, 95% CI 2.58 – 23.15). The combination of the two high expressions had mean and median survival of 10.44 ± 1.88 and 7 months. **Conclusion:** High expression of *CD44* and *CD24* will shorten the disease-free survival of patients with advanced ovarian cancer.

Keywords: Ovarian cancer- cancer stem cells- *CD44*- *CD24*- disease-free survival

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Introduction

Ovarian carcinoma is the third most common gynecological carcinoma worldwide after cervical and uterine carcinoma [1]. In 2020, the World Health Organization (WHO) reported 313,959 cases of ovarian carcinoma in women globally through the Global Burden of Cancer (GLOBOCAN) data register, with a mortality rate of 207,252 [2]. According to the data from the Indonesian Society of Gynecologic Oncology (INASGO) for the period 2019–2021, 1,600 cases of ovarian carcinoma were reported in Indonesia, with serous cystadenocarcinoma and mucinous cystadenocarcinoma dominating the cases [3]. Given the poor diagnosis at the advanced stage and delay in diagnosis, ovarian carcinoma remains the second leading cause of death in gynecological carcinoma. The five-year survival rate of ovarian carcinoma in women worldwide is approximately 30%–40% [4].

Despite cytoreductive surgery and chemotherapy, recurrence is common. Ovarian cancer recurrence rates range from 10% in stage I to 20% in stage II, with advanced stages (III and IV) accounting for up to 62%–85% of cases [5]. One of the prognostic factors for recurrence that is currently being studied is the presence of cancer stem cell (CSC) factors. Recent studies have revealed the emerging role of CSC expression in contributing to poor clinical manifestations; therefore, the presence of CSC surface markers, such as *CD24* and *CD44*, can be used to predict prognosis in ovarian carcinoma [6]. High *CD24* expression corresponds with grading, prognosis, and aggressiveness

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of ovarian cancer cells. In a study of epithelial ovarian cancer patients, *CD24* expression was shown to be significantly correlated (p = 0.012) with overall survival after surgery as the primary treatment. Recurrences occur in approximately 60% of individuals with high *CD24* and *CD44* expression who have undergone primary surgery [7]. Moreover, a meta-analysis study examining the significance of *CD44* as a prognostic factor for ovarian carcinoma concluded that high *CD44* expression was significantly associated with five-year survival rates for ovarian carcinoma [6].

To date, no studies have extensively investigated the association of disease-free survival in ovarian carcinoma and the number of cells expressing CSC surface markers, particularly *CD24* and *CD44*. This study aimed to examine the relationship between the number of cells expressing *CD24* and *CD44* using H-score as a parameter with two-year disease-free survival in epithelial ovarian carcinoma.

Materials and Methods

Study Design and Data Collection

This is a cohort retrospective study, and survival analysis was conducted using the survival curve. Data were obtained from medical records and the paraffin archive in the Dr. Cipto Mangunkusumo National Central General Hospital and the Fatmawati Central General Hospital in Jakarta. This research has received a certificate of passing the ethical reviews of Dr. Cipto Mangunkusumo National Central General Hospital (number KET-707/UN2.F1/ETIK/PPM.00.02/2022) and Fatmawati General Hospital (number LB.02.2/VIII.2/I6295/2022).

Subjects

The subjects were all patients diagnosed with epithelial ovarian carcinoma between January 2019 and March 2023 and who met the following inclusion criteria: advanced epithelial carcinoma (stages IIB–IVA) with or without recurrence within two years after complete treatment (surgery and six cycles of chemotherapy) and optimal debulking (residual tumor <1 cm after surgery). The exclusion criteria included patients with non-epithelial ovarian carcinoma or epithelial type in stages IA–IIA as well as patients with progressive disease while undergoing chemotherapy (platinum refractory), double primary tumors, and incomplete medical records or inconclusive paraffin block samples. Recurrence criteria were obtained from clinical and supporting examinations (imaging and tumor markers), while mortality data were obtained from medical records or family follow-up.

Slide Preparations and Cell Counts

Immunohistochemical staining is a gold standard in observing the expression of *CD24* and *CD44*. In this study, reagent optimization was agreed upon at an optimal dilution ratio of 1:400. *CD44* primary antibody GTX102111 (GeneTex, Inc.) and *CD24* primary antibody GTX37755 (GeneTex, Inc.) were utilized. Reagent staining techniques were performed according to the standardized immunohistochemical staining protocol. Further observation was performed using a light microscope at 100x magnification at five fields of view; cells were then counted up to 100 cells per field of view for a total of 500 cells, using ImageJ software. *CD24*-positive staining was observed on the cytoplasm, and *CD44*-positive staining was observed on the membrane (Figures 1 and 2). A semiquantitative scoring method was used to calculate the *CD44* and *CD24* intensities of expressions: values of 0 (none), 1 (low), 2 (moderate), and 3 (high). The total score of cells in each field of view and the number of cells with each intensity of stain were calculated using the Histochemical-score (H-score) formula given below:

H-score = (% of cells stained in the 0 category × 0) + (% of cells stained in the 1 category × 1) + (% of cells stained in the 2 category × 2) + (% of cells stained in the 3 category × 3).

Statistical Analysis

The data from this study are displayed in the form of text, tables, and/or images. Data were analyzed using SPSS software version 24. The sample demographic data variables are provided in tabular form, including frequency and standard deviation. The parameters used in receiver operating characteristic curve (ROC) are the H-scores of both *CD24* and *CD44* expressions. With the sensitivity and specificity value obtained from the curve, a cut-off point was determined. Then, H-score cut-off points were classified as "high expression (≥ cut-off point)" and "low expression (< cut-off point)" based on these cut-off values. The survival curves indicate the relationship between the combination of *CD44* and *CD24* expression and two-year disease-free survival.

Results

Fifty-four patients were eligible for the study and 48 samples met the inclusion criteria, without applying the exclusion criteria. The mean age of the sample was 53.02 ± 3.78 years; the most frequent histopathological type was high-grade serous (50%), with most FIGO stage being IIIB-IIIC-stage macroscopic abdominal spread (58.3%) (Table 1).

The H-score for the *CD44* expression was 86.63 ± 62.87 (4–242), with a median of 78; the H-score for the *CD24* expression was 88.17 ± 34.85 (24–174), with a median of 95 (Table 2). The AUC value of the *CD44* expression was 0.859 (p = 0.000), while that for the *CD24* expression was 0.795 (p = 0.000) (Figure 3 and Supplementary Table 1). Based on both sensitivity and specificity curves of the *CD44* H-score, the cut-off point was at 87, with a sensitivity value of 77% and specificity of 76.9% (Supplementary Figure 1). The cut-off point of the *CD24* H-score was 97, with a sensitivity value of 77% and specificity of 76.9% (Supplementary figure 2). Data were then grouped into "high expression (≥ 87 for *CD44* and ≥ 97 for *CD24*)" and "low expression (<87 for *CD44* and <97 for *CD24*)" based on these cut-off values.

In high *CD44* expression, the mean disease-free survival was 13.2 ± 1.8 months. This value was significantly shorter compared to that in low *CD44* expression, which

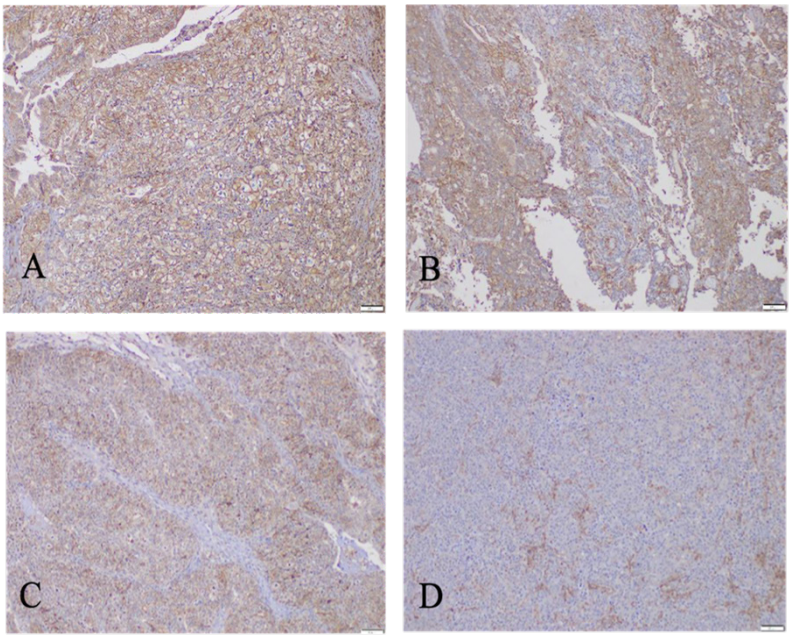


Figure 1. Degree of Immunohistochemical Stain Intensity of *CD44* Expression, Positive in Cell Membrane. (A) High staining, (B) Moderate staining, (C) Low staining, and (D) None.

reached 35.4 ± 3 months (p = 0.000, HR = 5.05; 95% CI, 1.84–13.85) (Figure 4 and Supplementary Table 2). Furthermore, in high *CD24* expression, the mean disease-free survival was 3.5 ± 2.4 months, while in low *CD24* expression, a longer period was also significantly observed with a mean of 37.4 ± 2.5 months (p = 0.000, HR = 7.73; 95% CI, 2.58–23.15) (Figure 5 and Supplementary Table 3). Further, a high expression on both *CD44* and *CD24* markers (*CD44*high /*CD24*low) was found in 15 cases (31.3%). A positive expression only on the *CD44* marker (*CD44*high /*CD24*low) was found in 10 cases (20.8%), and a positive expression only on the *CD24* marker (CD44low/*CD24* high) was found in 7 cases (14.6%) (Supplementary Table 4). Most of the recurrence cases (13 cases, 86.7%) were a combination of *CD44* high and *CD24* high. A high expression on either *CD44* or *CD24* revealed similar percentages—50% on *CD44*high/CD24low and 57.1% on *CD44*low/*CD24*high. In the group of *CD44*low/*CD24*low, no recurrence occurred after two years of complete treatment (Supplementary Table 5). The combination of high *CD44* and *CD24* had the highest two-year recurrence trend compared to the expression of only one CD (mean rank 34.45 vs. 26.21; p = 0.000) (Supplementary Table 6).

Combination of CD44 and CD24 Expression in Relation to Disease-free Survival

In this study, a combination of high expression in

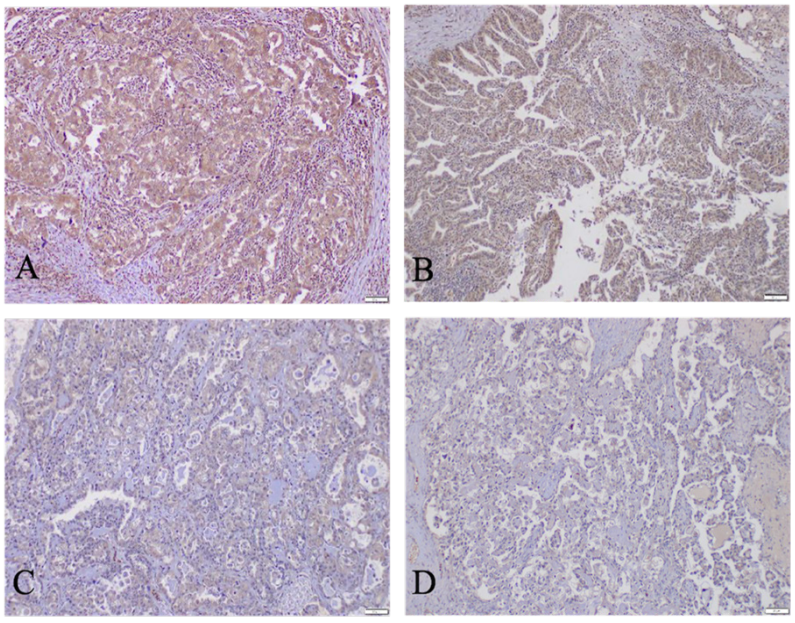


Figure 2. Degree of Immunohistochemical Stain Intensity of *CD24* Expression, Positive in Cell Membrane. (A) High staining, (B) Moderate staining, (C) Low staining, and (D) None.

Table 1. Baseline Characteristics

Variable	Mean ± SD
Age (year)	53.02 ± 3.78
Variable	Frequency N (%)
Histopathological type	
High-grade serous	24 (50%)
Clear cell	13 (27.1%)
Endometrioid	5 (10.4%)
Mucinous	6 (12.5%)
FIGO stage	
IIB - pelvic dissemination	7 (14.6%)
IIIA - abdominal dissemination (microscopic)	10 (20.8%)
IIIB-IIIC - abdominal dissemination (macroscopic)	28 (58.3%)
IVA - lung metastases	3 (6.3%)
Variable	N (min-max) or N (%)
CA-125 levels (U/L)	317 (34 - 5295)
CA-125 ³ 200 U/L	31 (64.6%)
CA-125 < 200 U/L	17 (35.4%)

both markers (*CD44*high/*CD24*high) had the lowest mean survival, with a period of 10.44 ± 1.88 months and a median of seven months. On the other hand, in the *CD44*high/CD24low combination group, the mean survival was 16.94 ± 2.71 months, with a median of 17 months. Moreover, *CD44*low/CD24high expression had a mean survival of 16.81 ± 5.49 months, with a median of 9 months (Table 3). The combination of high *CD44* and *CD24* had a mean disease-free periode of 10.44 ± 1.88 months, with a median of 7 months, while a longer disease-free period was observed in the group of either

Table 2. H-Score Calculation of *CD44* and *CD24* Expressions

Variable	Mean	SD	Median	Min - Max
H-Score *CD44* (500 cells)	86,63	62,87	78	4 - 242
H-Score *CD24* (500 cells)	88,17	34,85	95	24 - 174

Table 3. The Combination of *CD44* and/or *CD24* in Relation to Disease-Free Survival

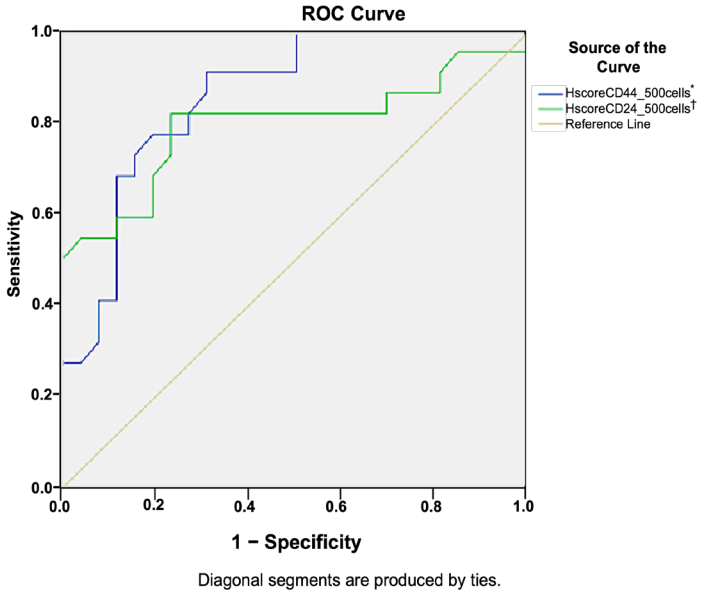
Variable	Mean ± SD (months)	Median (months)
$CD44^{high}/CD24^{high}$	10.44 ± 1.88	7
$CD44^{high}/CD24^{low}$	16.94 ± 2.71	17
$CD44^{low}/CD24^{high}$	16.81 ± 5.49	9

one of the high expression markers (18.94 ± 3.18 and a median of 12 months) (Table 3 and Figure 6).

Discussion

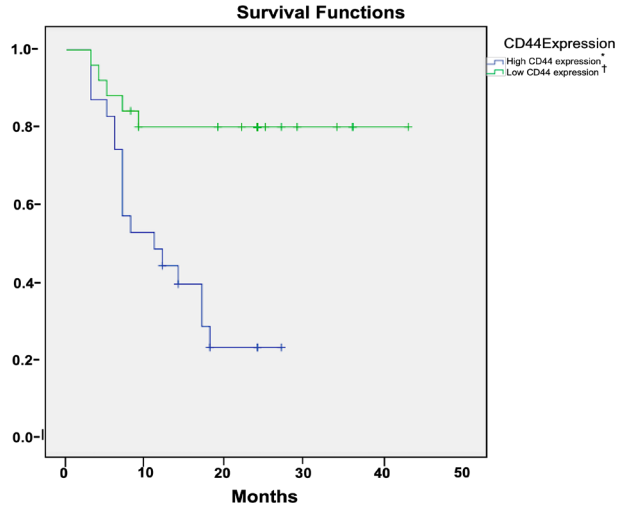
The mean age of the sample was approximately 53.02 ± 3.78 years. Half of the sample in this study included high-grade serous ovarian carcinoma cases (50%). The second most frequent histopathological type was clear cells (27.1%), followed by endometrioid (10.4%) and mucinous (12.5%). According to this study, stages IIIB and IIIC were the most common stages (58.3% of the cases). CA-125 levels were found the most in the range of ≥ 200 U/l (64.6%). This can be thought that the most common histopathological type of the sample was serous carcinoma, and therefore, CA-125 was secreted the most [8].

The H-score calculation for the *CD44* expression was 86.63 ± 62.87 (4–242), with a median of 78, while that for



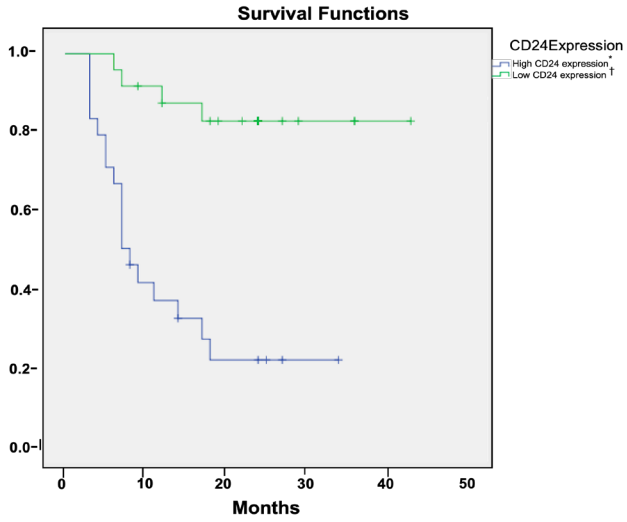
*Blue line indicates H-score for CD44 in 500 cells (AUC = 0.859)
†Green line indicates H-score for CD24 in 500 cells (AUC = 0.795)

Figure 3. ROC Curve of *CD44* and *CD24* H-Score



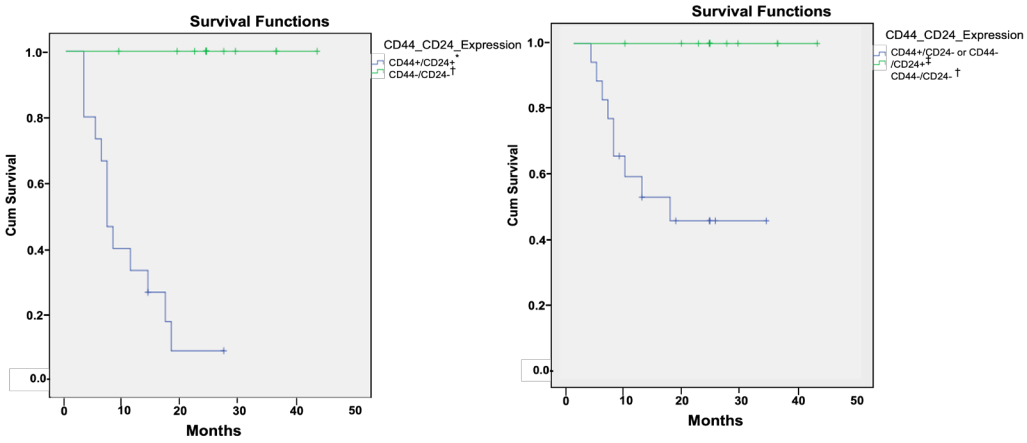
*Blue line indicates high CD44 expression
†Green line indicates low CD44 expression

Figure 4. Kaplan-Meier Curve of *CD44* Expression Against Disease-Free Survival



*Blue line indicates high CD24 expression
†Green line indicates high CD24 expression

Figure 5. Kaplan-Meier Curve of *CD24* Expression Against Disease-Free Survival



Left figure shows survival curve of positive in both expressions* (CD44+/CD24+) compared to both expressions are negative† (CD44-/CD24-)
Right figure shows survival curve of positive in one of the expressions‡ (CD44+/CD24- or CD44-/CD24+) compared to both expressions are negative† (CD44-/CD24-)

Figure 6. Kaplan-Meier Curve of The *CD44* and *CD24* Combination in Relation to Disease-Free Survival

the *CD24* expression was 88.17 ± 34.85 (24–174), with a median of 95. To the best of our knowledge, no previous studies have ever examined the H-score values for *CD44* and *CD24* expression. The mean survival of patients with a high *CD44* expression was 13.2 ± 1.8 months. This range was shorter compared to patients with a low *CD44* expression, which could reach 35.4 ± 3 months (p = 0.000, HR = 5.05; 95% CI, 1.84–13.85). Several studies have stated that *CD24* and *CD44* expressions contribute to poor clinical manifestations in ovarian cancer [6, 9]. A meta-analysis study assessing the role of *CD44* as a prognostic factor for ovarian cancer concluded that high *CD44* expression was significantly associated with five-year survival (RR = 1.42, 95% CI 1.01–2.00, P = 0.05) [6]. This result is also in line with the study by Gao et al. [10], which stated that a strong *CD44* expression reduced the disease-free survival period compared to the weak expression group. Another study conducted in Surabaya concluded that the higher the expression of *CD44*, the faster the recurrence of ovarian carcinoma. Patients with *CD44* expression ≥12.50 are 5.667 times more likely to have a relapse in less than six months compared to patients with *CD44* expression < 12.50 [11]. Overexpression of the *CD44* marker implied a one-year disease free survival rate of 60% (p = 0.036) [10].

In this study, the group with a high *CD24* expression had an average survival of only 13.5 ± 2.4 months, while the group with a low *CD24* expression had a longer survival period of 37.4 ± 2.5 months (p = 0.000, HR = 7.73; 95% CI, 2.58–23.15). A study also concluded that the expression level of *CD24* was found to be significantly associated with progression-free survival (p < 0.01) and overall survival (p < 0.05). Further, the percentage of recurrence-free survival was 57.5% (follow-up range of 29 months) compared to *CD24*-negative ovarian cancer cells (p < 0.01) [12].

Research on epithelial ovarian carcinoma has found that *CD24* expression is significantly correlated (p = 0.012) to the overall survival of patients who received surgery as primary therapy. Furthermore, *CD24* expression was evaluated at surgery after neoadjuvant chemotherapy and a high *CD24* expression was found (p = 0.0025). Nagare et al. [7] showed that approximately 60% of patients with high *CD24* expression after primary surgery experienced recurrences. No recurrences were found within a two-year range in the group with low combined expression on both markers (CD44low/CD24low); this contrasted with high expression on either or both *CD44* and *CD24*, which showed a variety of recurrence events in each combination. Sihombing et al. [13] suggested that the *CD44*+/CD24- combination had a poor prognostic role and chemoresistance in ovarian carcinoma. This difference in results could be due to the different examination methods compared to the study by Sihombing et al. [13], which used the dual-stain *CD44* and *CD24* reagent staining method, while the current study used separate primary antibody reagents for *CD44* and *CD24*, thereby resulting in different expressions for each cell and field of view [13].

In conclusions, high expressions of *CD44* and *CD24* correlate with a shorter mean of disease-free survival. This study also presents the H-Score cut-off value for *CD44* and *CD24* in predicting recurrence within two years, while further internal and external validations are needed to apply these values in clinical practice.

Author Contribution Statement

Yuri Feharsal: Data curation and analysis; Andrijono: Conceptualization; Chamim Shobari Singoprawiro: Conceptualization; Lisnawati: Conceptualization;Trevino Aristarkus Pakasi: Methodology; Andi Darma Putra: Review and supervision of the manuscript; Fitriyadi Kusuma: Review and supervision of the manuscript; Tricia Dewi Anggreni: Review and supervision of the manuscript; Erlina: Supervision and assistance in pathological anatomy laboratory; Sarwanti: Supervision and assistance in pathological anatomy laboratory;Tha'atam Mardhiyah: Writing and administration.

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Approval

This study is part of an approved student thesis.

Conflict of Interest

None of the authors have any conflict of interest associated with this study.

Ethical Declaration

This research has received a certificate of passing the ethical review of Dr. Cipto Mangunkusumo National Central General Hospital with number KET-707/UN2.F1/ETIK/PPM.00.02/2022 and Fatmawati General Hospital with number LB.02.2/VIII.2/I6295/2022

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