

## Structured abstract

### Hypothesis

Cancer stem cells (CSCs) are subpopulations of cells which could contribute to tumor growth, metastasis formation and chemoresistance. CSCs can be detected by surface markers assessed by immunohistochemistry methods. A typical surface marker for CSCs is CD44 (standard form). We assumed, that CD44(s) could serve as a prognostic factor and marker of chemoresistance in patients with epithelial ovarian cancer.

### The aim of study

1. To recruit group of patients with histologically verified epithelial ovarian carcinoma.
2. To evaluate prognostic significance of known prognostic factors in our series of patients.
3. To assess the expression of CD44 in specimens of primary tumors and specimens of implantation metastasis using immunohistochemistry and analyze their correlation.
4. To evaluate the expression of CD44 in relation to known prognostic factors.

To analyze the significance of CD44 expression evaluation for overall survival, disease-free interval and chemoresistance. To find CD44 positivity cut-off by using statistical methods

### Materials and Methods

A retrospective study was performed on 87 patients with histologically verified EOC. All patients were tested for primary tumor specimens, 48 of them were tested with regard to both specimens of primary tumor and implantation metastasis. The CD44 expression was detected by immunohistochemistry methods. The antibody - antigen reaction was evaluated by a pathologist as a percentage of positive cells per single high-power field. We looked for the CD44 expression positivity limit using the Cox regression model. The results were analyzed by methods of univariate and multivariate analysis.

### Results

We confirmed statistically significant prognostic factors for OS and DFI - stage of the disease (I, II x III, IV  $p = 0.0002$  for OS,  $0.0001$  for DFI), postoperative residual tumor ( $p = 0.0003$  for OS,  $p = 0.0001$  for DFI), and papillary serous histological type ( $p = 0.0079$  for OS,  $0.0013$  for DFI). Other factors were not statistically significant. A positive correlation between expression CD44 in primary tumor and in metastasis was found ( $p = 0.001$ , Spearman correlation coefficient  $0.59973$ ). Then we analyzed CD44 expression in tumor and metastatic cells and acknowledged prognostic factors. A significant correlation between serous papillary carcinoma and CD44 expression in the primary tumor ( $p = 0.0493$ ) and in metastatic tissue ( $p = 0.0049$ ) was detected. The expression of CD44 in primary tumor and in metastasis was tested for OS and DFI. No statistically significant cut-off of the expression CD44 in primary tumor or in metastasis for OS was detected. No statistically significant cut-off of the expression CD44 in primary tumor for DFI was found, but we established 2% as a statistically significant cut-off for CD44 expression in metastatic tissue ( $p = 0.0029$ ). Finally, we performed multivariate analysis for OS and DFI. Stage of the disease ( $p = 0,0018$  for OS,  $p = 0, 0025$  for DFI) and postoperative residual tumor ( $p = 0,0046$  for OS,  $p = 0,0032$  for DFI) were statistically significant for OS and DFI in multivariate analysis. CD44 expression was not statistically significant in multivariate analysis for OS and DFI.

## **Conclusions**

1. We recruited a group of 87 patients with histologically verified epithelial ovarian carcinoma. All patients were tested for primary tumor specimens, 48 of them were tested with regard to both specimens of primary tumor and implantation metastasis.

2. Stage of disease, postoperative residual tumor and histological type were established as statistically significant prognostic factors for overall survival and disease-free interval in our series.

3. A statistically significantly positive correlation between expression CD44 in primary tumors and in metastases was found.

4. We found a significant correlation between serous papillary carcinoma and CD44 expression in the primary tumor and in metastatic tissue. No statistically significant correlation for CD44 expression and other prognostic factors was found.

5. No statistically significant cut-off of the CD44 expression in primary tumors or in metastases for OS was detected. No statistically significant cut-off of the CD44 expression in primary tumor for DFI was found. However, we established 2% as a statistically significant cut-off for CD44 expression in metastatic tissue. Stage of the disease and postoperative residual tumor were statistically significant for OS and DFI in multivariate analysis. CD44 expression was not statistically significant in multivariate analysis for OS and DFI.

According to our results, IHC evaluation of CD44 as a CSCs marker in ovarian cancer is not beneficial in diagnostics and therapy of ovarian cancer. More studies are needed to determine the role of detection CSCs in ovarian cancer.

**Key words:** ovarian cancer, CD44, cancer stem cells