1. Introduction

Ovarian cancer is one of the most fatal female reproductive system cancers [1]. Based on data provided by the WHO, 225,500 cases of ovarian cancer are diagnosed worldwide each year and 140,200 deaths are reported. Among the female population, according to world statistics, ovarian cancer ranks 7th in the structure of morbidity and 8th in the structure of mortality [2, 3]. According to the Ukraine National Cancer Registry (2019), ovarian cancer occupies the 6th position both in the morbidity and mortality structure of women population [4].

Epithelial malignant neoplasms of the ovaries account for 40 % of all oncopathology [5], with SOC being the leading histotype and covering 68–71 % of epithelial neoplasms [6]. There are 2 types of SOC, which differ in origin, biological behavior and prognosis for patients. Type I is represented by serous carcinoma of low malignancy (LGSC), and type II – serous carcinoma of high malignancy (HGSOC). [7] Although LGSC accounts for only 5 % of all SOC, and the leading role is played by HGSC [8], the study of molecular genetic and biological features of LGSC is extremely important for the ability to predict tumor behavior and selection of targeted therapy.

In recent years, much attention has been paid to the study of CSCS population and their role in the cancer progression. Numerous studies have shown that although CSCS account for only a few percent of tumor mass, they are one of the leading predictors of chemoresistance, recurrence and metastasis of SOC [9, 10]. The population of CSCS in ovarian tumor tissue is determined by a number of surfaces IHC-markers (CD44, CD117, CD133) and intracellular markers (Nanog, Oct4 and Sox2) [11, 12]. The origin of CSCS is also debatable; according to some data, normal stem cells can become a source of the CSCS pool, according to other data, stem tumor cells are transformed from carcinoma cells. In addition, many studies have focused on the plasticity properties of CSCS and their relationship to EMT. According to some researchers, carcinoma phenotype changes dynamically from epithelial to mesenchymal depending on disease stage and presence of metastases, and it correlates with increased levels of CSCS expression and worsening prognosis [13].

The aim of our research was studying of expression surface CSCS markers CD44 and CD117 in LGSC with subsequent recurrence and without it, as well as the relationship between the expression of CSCS and EMT markers to identify possible predictors of recurrence in patients.

2. Materials and methods

The material for our research were case histories, paraffin blocks and slides of 43 patients with LGSC who underwent surgery in the amount of bilateral salpingooophorectomy and hysterectomy with resection or extirpation of the large omentum with subsequent adjuvant chemotherapy and previous neoadjuvant chemotherapy in cases of advanced stages. Surgical interventions were performed at the Institute of Medical Radiology and Oncology. S. P. Grigoriev, Regional Clinical Oncology Center (Kharkiv) in the period from 2013 to 2018. The prevalence of the tumor process was assessed according to the FIGO classification. The study included LGSC with disease stage I–IV: 30 cancers without recurrence over the next 24 months (main group) and 13 cancers with recurrence (comparison group) in the specified time interval. The age of the patients varied from 28 to 56 years (average age 42.7±0.91). Histological type of tumors was determined during examination of slides stained with hematoxylin-eosin. From each clinical case, 1 paraffin block was selected for IHC-study.

All patients signed informed consent for the using their information from case histories, paraffin blocks and slides in our study.

For the IHC study, the material was fixed with 10 % neutral formalin for 24 h, embedded in paraffin, prepared 4 μm thick sections, which were applied to highly adhesive Super Frost slides and dried at 37 °C for 18 hours.

Unmasking heat treatment was performed by boiling the sections in citrate buffer (pH 6.0). UltraVision Quanto Detection Systems HRP Polymer (Thermo scientific) was used to visualize primary antibodies. DAB (diaminobenzidine) was used as the chromogen.

Primary monoclonal antibodies (MCAT) from DAKO (Denmark), TermoScientific (Germany) and Diagnostic BioSystems (USA) were used. The expression of the following markers was studied: E-cadherin (EP7004, Termo Scientific), Vimentin (Diagnostic BioSystems), CD117 (Diagnostic BioSystems), CD44 (Clone: 156-3C11, Dako Cytomation).

To assess the intensity of IHC mark used a semi-quantitative scale 0–3 +: 0 – no expression, + – weak, ++ – moderate, +++ – a pronounced reaction. To quantify the IHC mark, E-cadherin...
and Vimentin took into account the percentage of staining: 0 – no staining, <10 % of nuclei – weak, 10–50 % – moderate, 51–100 % – high expression. To assess the immunohistochemical label CD117 and CD44, the level of staining >10 % was considered high, ≤10 % – low, 0 – no staining. Only cells with moderate (+) and high (+++) color intensity were taken into account.

Statistical analysis was performed using the Mann-Whitney test to assess differences between two groups on the level of trait. Spearman’s rank correlation method was used to assess the correlation. Pearson’s Yets-corrected test was used to assess differences between the study groups. The level of significance≤0.05 was considered significant.

3. Results

Analysis of general clinical characteristics showed that age of patients in the main group ranged from 35 to 51 years (median=42.97±0.81 years), and in the comparison group age fluctuations ranged from 28 to 56 years (median=42.08±2.43 years). Age differences of the compared groups are statistically insignificant (Uemp=191 at Ucrit=132). Menopause occurred in 23.08 % (3/13) of patients from the group of recurrent LGSCs, in the group of LGSCs without recurrence menopause was recorded in 13.33 % (4/30) cases. For LGSCs without recurrence are typical initial (I–II) stages of the disease with no terminal (IV) stage, while recurrent LGSCs are characterized by disease stage III–IV with no cases diagnosed in stage I (χ²=20.95, p=0.0051) and a significant decreasing of cases diagnosed in stage II (χ²=14.37, p=0.0039) (Table 1).

The study did not show association between the patients’ age and disease recurrence in the control group (r=0.402, p=0.0049) and the study (r=0.159, p=0.07). At the same time Fig. 1 shows a direct strong correlation between FIGO stage and development of recurrence of SOC (r=0.69, p=0.00039).

Analyzing the expression of EMT markers (Table 2), it was found that both carcinoma groups are characterized by Vimentin expression at ≥10 %. The control group is typical for a moderate expression level (median: 35.27 %±1.93) with increasing number of cases with high marker expression in the control group and the absence of such cases in recurrence LGSC. There is an inverse relationship between E-cadherin expression and FIGO stage (r=0.32, p=0.034) and development of recurrence (r=0.62, p=0.0003) (Fig. 2).

Considering the expression of surface CSCS markers (Table 3), it was found that CD44 + status had 51.16 % (22/43) of all tumors: in the comparison group CD44 + tumors were observed in 69.23 % (9/13) cases, and in the control group in 43.33 % (16/30) of the studied tumors. In LGSC with recurrence, the level of CD44 expression ranged from 8 % to 14 % with a median of 4.13 %±0.91. No statistically significant differences between marker expression levels in the study groups were found (χ²=2.74, p=0.098).

The dependence of marker expression on age (r=0.128, p=0.41) and the presence of recurrence (r=0.29, p=0.057) was not detected, while the dependence of CD44 expression on the FIGO stage was found (r=0.483, p=0.001) (Fig. 3).

Regarding the CD117 expression, it should be noted that 39.55 % (17/43) of tumors had CD117-negative status: 7.69 % (1/13) belonged to the control group and 33.33 % (16/30) to the main group. The control group was characterized by marker expression level (median: 30.15 %±2.78), and in the comparison group the values ranged from 11 % to 47 % (median=46.57±17.77 %). Interestingly, despite the lack of differences in the levels of E-cadherin expression, in tumors with recurrence there is a trend to decrease the level of IHC-mark (Uemp=43 at Ucrit=132). It was found that LGSC III–IV stages are characterized by a moderate level of E-cadherin expression (χ²=4.03, p=0.04) with a decrease in the number of cases of high marker expression in the control group and the absence of such cases in recurrence LGSC.

### Table 1

<table>
<thead>
<tr>
<th>FIGO stage</th>
<th>Main group n=30 (%)</th>
<th>Comparison group n=13 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6 (20)*</td>
<td>0*</td>
</tr>
<tr>
<td>II</td>
<td>20 (66,67)**</td>
<td>1 (7,69)</td>
</tr>
<tr>
<td>III</td>
<td>4 (13,33)****</td>
<td>10 (76,92)</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>2 (15,38)</td>
</tr>
</tbody>
</table>

Note: * – insignificant difference between I and II stages (χ²=0.46, p=0.5), III and IV stages (χ²=0, p=1); ** – significant difference between II and III stages (χ²=4.37, p=0.0002); *** – significant difference between I–II and III–IV stages of the disease (χ²=20.95, p=0.0001).

### Table 2

<table>
<thead>
<tr>
<th>Expression level</th>
<th>Main group n=30</th>
<th>Comparison group n=13</th>
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<tbody>
<tr>
<td>0 %</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>&lt;10 %</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10–50 %</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>51–100 %</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>FIGO stage</th>
<th>Expression level</th>
<th>Main group n=30</th>
<th>Comparison group n=13</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>II</td>
<td>19</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>–</td>
<td>–</td>
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**Fig. 1.** Graphical representation of the relationship between FIGO stage and LGSC recurrence.
expression at the level of 0–10% with a median of 5.37±1.19 and a range of values from 6% to 19% with a statistically significant ($\chi^2=14.81$, $p=0.00043$) increase in the number of cases of high expression level in comparison group (median 13.85±1.55) and the increase of expression level in the group of recurrent LGSC ($U_{emp}=62$ at $U_{crit}=132$). For stage III–IV there is a trend to increase number of cases with expression > 10%, while for the initial stages more typical expression at the level of 0–10% ($\chi^2=26.68$, $p=0.00067$). A strong correlation was found between the CD117 expression and stage of the disease ($r=0.84$, $p=0.00033$). At the same time, there is no relationship between CD117+ tumor status and patient’s age ($r=0.183$, $p=0.24$) (Fig. 4).

Coexpression of markers, studied in our study, revealed a direct correlation between CD117 and CD44 expression ($r=0.73$, $p=0.001$), as well as between Vimentin expression ($r=0.64$, $p=0.0006$), the inverse correlation between E-cadherin and Vimentin expression ($r=-0.32$, $p=0.038$). Coexpression of CD44 and E-cadherin shows no dependence ($r=0.08$, $p=0.615$). Results are displayed in Fig. 5.

<table>
<thead>
<tr>
<th>Expression level</th>
<th>Main group $n=30$</th>
<th>Comparison group $n=13$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10%</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>CD117</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤10%</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

The results are displayed in Table 3.
4. Discussion

Clinical data of our study show that recurrence of LGSC occur in patients with stage III–IV disease by FIGO \((p=0.0051)\) and were not detected in stage I, although according to the literature LGSC have an indolent course, less often diagnosed on advanced stages and have more favourable prognosis \([7, 14]\). The relationship is confirmed by a direct correlation between disease stage and reoccur \((p=0.00039)\). The presence of large number of patients with stage III–IV in the study, in our view, is due to untimely treatment of patients, because in the early stages ovarian tumors are mostly asymptomatic. An additional adverse factor for early detection of SOC is the low consciousness of women, who neglect preventive gynecological examinations. Detection of recur in the neglected stages is due to the prevalence of the tumor process, which is less susceptible to cytoreduction and treatment. The age of onset of LGSCs in two comparable groups didn’t differ: sick patients aged 42 years, which coincides with treatment. The age of onset of LGSCs in two comparable groups didn’t differ: sick patients aged 42 years, which coincides with treatment. The age of onset of LGSCs in two comparable groups didn’t differ: sick patients aged 42 years, which coincides with treatment.

The obtained data do not contradict the literature about the key role of EMT in tumor metastasis and progression \([16]\).

CD44 expression was observed in 51.16 % of all tumors, and level of expression in the study groups did not change significantly \((p=0.098)\). CD44 +– status didn’t depend on age \((p=0.41)\) and recurrence \((p=0.057)\), but there was a direct correlation with the FIGO stage \((p=0.001)\). Similar data were obtained in other studies \([17, 18]\), at the same time in the work of Sillanpää S. et al. there is controversy about a direct correlation between CD44 expression and highly differentiated tumors detected in the early stages and long-term recurrence-free survival of patients \([19]\). CD117 + – status had 60.47 % of all tumors, recurrent LGSCs were characterized by an expression level>10 % \((p=0.00067)\) with increasing values of the IHC mark. The frequency of expression according to the literature reaches 40 % \([9]\), and the difference with our data can be explained by the characteristics of a particular sample. For the initial stages of the disease according to FIGO, the typical level of CD117 expression was 0–10 % \((p=0.00043)\) with an increase of cases number of high expression in stages III–IV. As for CD44, the CD117 marker did not show a dependence on the age of patients \((p=0.24)\), but there is a strong correlation between the FIGO stage \((p=0.0002)\) and tumor recurrence \((p=0.00033)\), which supported by data from another study \([9]\). At the same time, it was found that the level of CD44 expression increases with increasing expression level of CD117, which confirms the phenotype of ovarian CSCS, because to verify this pool itself, the coexpression of these two markers is always studied \([9]\). Coexpression of CD44 and E-cadherin was not found in our study \((p=0.615)\),

\[y = 0.980 + 0.524 \times \]
\[n = 43\]
\[r = 0.73; P < 0.001\]

\[y = 6.549 -0.0339 \times \]
\[n = 43\]
\[r = 0.08; P = 0.615\]
which is somewhat unexpected, because CD44 is a transmembrane glycoprotein, which, like E-cadherin, is involved in intercellular adhesion. The heterogeneity of the expression of the two markers can be explained by the complexity of the mechanisms of intercellular adhesion and the peculiarities of the microenvironment of tumor cells, because CD44 has many functions and can function as a signalling transmitter, which is also involved in invasion and migration of tumor cells [20].

**Study limitations.** The study analyzed 43 carcinomas, which is a sufficient reference sample. However, it should be borne in mind that obtained results are specific only for LGSC and may lose their relevance and informativeness when applied to high-grade serous ovarian carcinomas due to the different biological origins of two groups of tumors.

**Prospects for further research.** Given the relevance and prospects of studying the role of CSCS in tumor progression, metastasis and recurrence, it is interesting to study the relationship of other CSCS markers (Sox2, CD133, Oct4) with the onset of recurrence of SOC, as well as their relationship with EMT.

6. **Conclusions**

Based on the data of our study, in the risk group for recurrence of LGSC are women with stage III–IV disease according to FIGO, and the likelihood of recurrence increases with increasing stage. Recurrent carcinomas are also characterized by EMT phenomenon with expression level of Vimentin 51–100 % and expression of E-cadherin at level of ≤50 %. In our study, we did not find a prognostic role of the CD44 marker as a predictor of recurrence or worsening of the SOC stage, it should be used, in our opinion, as an additional marker that identifies pool of ovarian CSCS. A prognostic role in the deterioration of the stage and appearance of recurrence was found for CD117, for recurrent LGSC typical expression level: ≤30 %. The direct correlation between CD117 expression and Vimentin proves the common role of EMT process and CSCS in recur and progression of LGSC.

**Conflict of interests**

The author declares no conflict of interests.

**References**


