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Expression of CD133 and CD117 in 64 Serous Ovarian Cancer Cases

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ABSTRACT

The cancer stem cells (CSCs) represent a minority of tumor cells that are able to proliferate and self-renew and might be responsible for tumor initiation and maintenance. The CD133 and CD117 are the most commonly used markers for the putative CSCs, especially for the ovarian CSCs, but its clinical significance remains uncertain. The aim of this study was to compare the immunohistochemical expression of CD133 and CD117 in 64 primary ovarian high grade serous carcinoma and peritoneal metastasis, and to examine their potential clinical role. CD133 expression was mainly seen in the apical/endoluminal cell surface of tumor cells and was found in 61% of the carcinoma samples and 41% of the metastasis. The median of CD133 positive cells in tumors was 1 (0.1–7)%, and in metastases was 0.6 (0.1–6) %. CD117 expression appeared as a cytoplasmic and/or membranous stain and was found in 81% of the carcinoma samples and 77% of the metastasis. The median of CD117 positive cells in tumors was 1 (0.1–8)%, and in metastases was 0.1 (0.1–7)%. Multivariate analysis has shown that patients with high CD133 expression in tumor cells have significantly shorter disease free survival and overall survival (p=0.025 and p=0.014, respectively). Patients with high CD117 expression in tumor cells have significantly shorter disease free survival (p=0.031). Cox´s proportional hazards model identified expression of CD133 protein in tumor as an independent prognostic factor. Our study indicates that the immunohistochemical assessment of CD133 and CD117 expression may have potential clinical value in predicting disease progression and prognosis in the high grade serous ovarian cancer. CD133 proved to be an independent prognostic factor in the high grade serous ovarian cancer patients.

Key words: *cancer stem cells, CD117, CD133, immunohistochemistry, epithelial ovarian cancer*

Introduction

Ovarian cancer represents the most lethal of all gynecologic malignancies. It is an aggressive disease associated with rapid progression to peritoneal metastases, and poor prognosis for patients[1,2]. Because of lack of early symptoms as well as effective screening, the majority of patients are diagnosed at an advanced stage. Nearly 75% of all patients at stage III or IV disease will achieve a complete clinical remission with primary surgery followed by platinum/taxane based chemotherapy. But 85–90% of patients at advanced stage will have recurrence and die of their disease[3–5].

The mechanisms underlying chemoresistance in cancer are not clear. One hypothesis is that cancers are driven by a small subset (less than 5%) of highly tumorigenic cells with self-renewal properties, analogous to organ-specific stem cells, and called cancer stem cells (CSCs)[6]. According to this model, only the CSCs, but not the remaining cells in the tumor, can propagate tumorigenesis[7]. If CSCs retain the features of tissue stem cells, being rare and entering the cell cycle infrequently, they could constitute a population that is intrinsically resistant to current therapies designed to kill rapidly dividing cells[3]. CSCs have the capacity to divide and expand the cancer stem cell pool and to differentiate into the heterogeneous non-tumorigenic cancer cell types[8]. If this hypothesis is correct, researchers should be refocused their interest on the minority stem cell population that fuels tumor growth. Effective tumor eradication will require obtaining agents

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that can target cancer stem cells while sparing normal stem cells[9].

Putative CSCs can be isolated based on either a surface marker or an intracellular enzyme activity and then assessed by a sphere-forming assay in non-adherent culture or by their ability to initiate new tumor growth when xenotransplanted into immunocompromised mice[10]. The most commonly used markers for CSCs are: ALDH,Bmi1 ,CD24,CD44,CD90,CD105,CD117,CD133,CD166, EpCAM,SP, but there is no apparent consensus about the „best marker" for any particular cancer[11].

Cancer stem cells were initially isolated from blood cancers[12]. Cells within solid tumors are less accessible and cell surface markers required to isolate such cells are difficult to identify[6]. Over the past decade CSCs have been identified for various epithelial malignancies including cancers of the breast, lung, pancreas, colon and prostate[7,13–17]. Cell surface antigen profile expression is defined by flow cytometry and immunohistochemistry techniques. Some studies investigated the clinical role of the immunohistochemically assessed CSCs; unfavorable prognostic role of high CSCs expression and connection with resistance to radiotherapy and chemotherapy have been recognized[16,18–21]. This assumes that high proportion of CSCs signifies a worse prognosis. A role for CSCs in propagating and maintaining metastasis has been proposed[22]. It is supposed that cell lines in the primary tumors and in the metastasis are derived from the same lineage of cancer stem cells[23].

There is still uncertainty relating to the identification of CSCs in ovarian cancer[7]. The most commonly used markers are: CD44, CD117, CD133[11,24–26]. Bapat et al. first present the evidence that aggressiveness of human ovarian cancer may be a result of transformation and dysfunction of stem cells in the ovary[1]. The ovarian cancer-initiating cells are also isolated from human ovarian primary tumor tissues and showed up-regulation of the CD44 and CD117[24,26,27]. The CD133 positive cells were detected in ovarian cancer cell lines, in primary cancers and from ascitic fluid, also in serous and clear cell tumors[25,28]. Recently, Luo et al. demonstrated that human ovarian cancer cells with the CD117+ phenotype possess the unique properties of CSCs (self-renewal, differentiation, tumorigenic potential and chemoresistance)[29].

CD133 is the human homologue of mouse prominin-1, a penta-membrane glycoprotein and a cell surface protein originally found on neuroepithelial stem cells in mice. It is a 120kD protein with five transmembrane domains and two large glycosylated extracellular loops[30]. The function in cancer stem cells has not been established, but it has been found to interact with cholesterol and may be involved in maintaining membrane topology and membrane lipid composition. Its expression has been shown to be restricted to plasma membrane protrusions in epithelial cells[7]. CD133+ phenotype was first used to identify and isolate brain tumor stem cells in malignant tumors and now it has been used to define the CSCs populations in diverse malignancies (lung, pancreas, liver, prostate, colorectal, gastric[16,20,21,31,32]). The CD133 antigen represents a useful molecule to select and enrich the population of ovarian tumor cells which have a higher clonogenic efficiency and proliferative potential[33]. Two splice variants of CD133, CD133/1(akaAC133) and CD133/2(akaAC141) recognize different glycosylated epitopes and most studies use CD133/1.

CD117 (c-kit proto-oncogene) is a 145 kD transmembrane receptor tyrosine kinase that binds stem cell factor (SCF) and is an important regulator of cell growth. The c-kit/SCF interaction is important for the survival and development of stem cells involved in hematopoiesis, in pancreas development and in melanogenesis, but also promotes tumor growth by promoting proliferation and protecting the tumor cell from death[14]. The c-kit gene product is expressed in a variety of normal human tissue cell types, including breast epithelium, germ cells, melanocytes, immature myeloid cells, and mast cells. In ovarian cancer samples it was noticed that cells with 117+ phenotype possess properties of CSCs and chemoresistance[1,24,29,34]. Expression was common in mast cell disease, testicular germ cell tumors, endometrial carcinomas, thyroid carcinomas, small cell carcinomas, malignant melanomas, and ovarian epithelial carcinomas. C-kit positivity is a defining feature of gastrointestinal stromal tumors[35].

Since CD133 and CD117 are the most commonly used markers for putative CSCs, especially for ovarian CSCs, we are decided to analyze immunohistochemically assessed expression of these two markers on the samples from high grade serous ovarian carcinoma and their peritoneal metastasis. Clinical significance of CD133 and CD117 expression is still uncertain, so we analyzed the possible correlation between these two markers and clinical factors, especially disease free survival and overall survival. To our knowledge, there are no existing studies that have compared the expression of the immunohistochemically assessed markers CD133 and CD117 in primary ovarian carcinomas and their peritoneal metastasis in association with potential clinical role.

Patients and Methods

Patients´ data

The study included 64 patients diagnosed with ovarian high grade serous carcinoma between January 1995 and January 2008 who underwent surgery and treatment in the University Hospital Centre Rijeka, Croatia. The study included a retrospective collection of archival samples and clinical information of the patients. The protocol was approved by Hospital Ethical Commission. All patients had advanced disease and were staged according to FIGO classification (International Federation of Gynecology and Obstetrics) as stage III or IV. All patients underwent primary surgery which included total abdominal hysterectomy with bilateral salpingoophorectomy, omentectomy, pelvic and paraaortic lymphadenectomy and multiple peritoneal biopsies, followed by chemotherapy. Patients´ records were reviewed and clinical characteristics as well as follow-up data were noted. All histopathological samples

are reclassified according to latest two-tier grading system of serous ovarian cancer[4]. The primary ovarian high grade serous cancer samples and their peritoneal metastasis samples were analyzed. Response to treatment was grouped into two categories. Complete and partial response (reduction of the tumor and/or reduction of the CA 125 serum level below 35 U/ml) were grouped together and classifies as »Yes« (responders), cases with stabilization of disease (weak response and satisfactory general condition) or progression were classifies as »No« (non-responders).

Immunohistchemistry

Formalin-fixed, paraffin-embedded specimens from 64 patients with serous high grade ovarian carcinoma were analyzed at the Department of Pathology, School of Medicine University of Rijeka, Croatia. Tissue sections of primary tumors and their peritoneal metastases were imunohistochemically examined for CD133 and CD117 expression.

Immunohistochemical staining for CD133 and CD117 protein was done on 4 μm sections from formalin-fixed, paraffin-embedded tissues placed on coated glass slides. After they were deparaffinised in xylene and rehydrated conventionally, antigen retrieval was performed by incubation in a pressurized heating chamber at 125°C for 30 seconds in TrisEDTA buffer (pH9). For CD133 protein slides were incubated with primary antibody (monoclonal-mouse, clone AC133; Milteny Biotec, Germany) diluted 1:50 and incubated overnight at 4°C. For CD117 protein slides were incubated with primary antibody (polyclonal antibody, C-19, c-Kit; Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:200 and incubated overnight at 4°C. After 30 minutes incubation with secondary antibody (Envision), 10 minutes incubation with DAB+chromogen was performed.

Immunohistochemical stained slides for CD133 and CD117 expression were independently analyzed by two investigators who were blinded to clinical data. The positive stained cells were counted in ten random and non-overlapping fields at high magnification (x200). The results were expressed as the percentage of total number of nuclei counted in the same fields[28,36]. For analysis of progression and survival patients were divided into three groups of CD133 and CD117 status: negative (0% of positive tumor cells), 0.1–0.9% (of positive tumor cells), and >0.9% (of positive tumor cells).

Statistical analysis

Statistical analysis of data was performed using Statistica for Windows, release 11.0 (StatSoft, Inc., Tulsa, OK). To analyze the distribution of all cases according to clinico-pathological characteristics was used $?^2$ test or t-test for proportions. $?^2$ test was used to comparison the difference in distribution of cases CD133 and CD117 expression in tumor and metastasis according to clinico-pathological characteristics. Disease free survival was calculated from the date of treatment to the date of progression (biochemical or clinical relapse or partial response) and Overall survival was calculated from the date of treatment to the date of death or the date of last seen. The normality of the distribution of the disease free survival and overall survival, as the expression of examined markers, were checked by Kolmogorov-Smirnov test. The data were presented by median (5^{th}–95^{th} percentile boundaries). The Kaplan-Meier method was used to analyze survival (and recidive probability) and the long-rank test was used to estimate differences in survival (and recidive probability). Prognostic factors were examined by Cox's proportional-hazards regression. All statistical values were considered significant at the p level of < 0.05.

Results

Patient characteristics

Median age of patients was 58.2 (40.1–72.1) years, range 31.9–79.9. Clinico-pathological characteristics of the patients are summarized in Table 1. Significantly more patients was under 65 years old (p=0.004). Significance was noticed in response to treatment and recurrence of the disease (p<0.001). Nearly 69% of all the patients

TABLE 1
CLINICO-PATHOLOGICAL CHARACTERISTICS OF STUDY SAMPLE

Characteristics	All (N=64) N (%)	Statistics p
Age		
<65	44 (68.8)	
≥65	20 (31.2)	0.004*
Residual tumor		
Absent	18 (28.2)	
<1 cm	23 (35.9)	
≥1 cm	23 (35.9)	0.678
Lymph node status		
Negative	19 (29.7)	
Positive	29 (45.3)	
n.a.	16 (25.0)	0.538
Response to treatment		
Yes	44 (68.8)	
No	15 (23.4)	
n.a.	5 (7.8)	<0.001*
Recurrence of the disease		
Yes	57 (89.1)	
No	7 (10.9)	<0.001*

* indicated statistical significance
n.a.– not available

had a good response to treatment, but recurrence of the disease was noticed in 89% of them.

CD133 expression protein in high grade serous ovarian cancer and peritoneal metastasis

CD133 expression was evaluated in a series of 64 high grade serous ovarian carcinoma and their peritoneal metastasis. Expression was mainly seen in the apical/endoluminal cell surface of tumor cells surrounding a lumen (Figure 1a and c). Cytoplasmic CD133 staining was seen in less than 1% of the solidly arranged tumor cells (but not in all positive cases) (Figure 1b). In some positive cases we noticed strongly stained debris in the lumina of the glands lined by malignant epithelium. Tumor samples were positive for CD133 in thirty nine cases (61%), while metastases were positive in twenty six cases (41%). The median of CD133 positive cells in tumors was 1 (0.1–7)%, range 0.1–9%, and in metastases was 0.6 (0.1–6)%, range 0.1–9%. Although we noticed a more positive cases in tumor samples, the difference was not statistically significant ($p>0.05$). However, this result has shown a certain trend toward significance.

Comparing all clinico-pathological characteristics and the frequency of CD133 positive tumors and CD133 positive metastases, we don't reach statistical significance (all $p>0.05$, Table 2).

CD133 expression in tumor and metastasis was classified into three levels: negative (0%), 0.1–0.9%, and more than 0.9%. Distribution of CD133 status is shown in Figure 2. We have noticed that percentage of negative cells are higher in metastasis than in primary tumors, in the group of 0.1–0.9% distribution is similar, and in group of higher positivity we have noticed more positive cells in tumor samples. The difference is significant ($?^2=10.13$, $p=0.006$).

CD117 expression protein in high grade serous ovarian cancer and peritoneal metastasis

CD117 positive stain appeared as a cytoplasmic and/or membranous stain (Figure 1d). Nonspecific background staining of tumor stroma or adjacent ovarian parenchyma was not encountered. Tumor samples were positive in fifty two (81%) cases, while metastases were positive in forty nine (77%) cases (Table 2). Although we have noticed

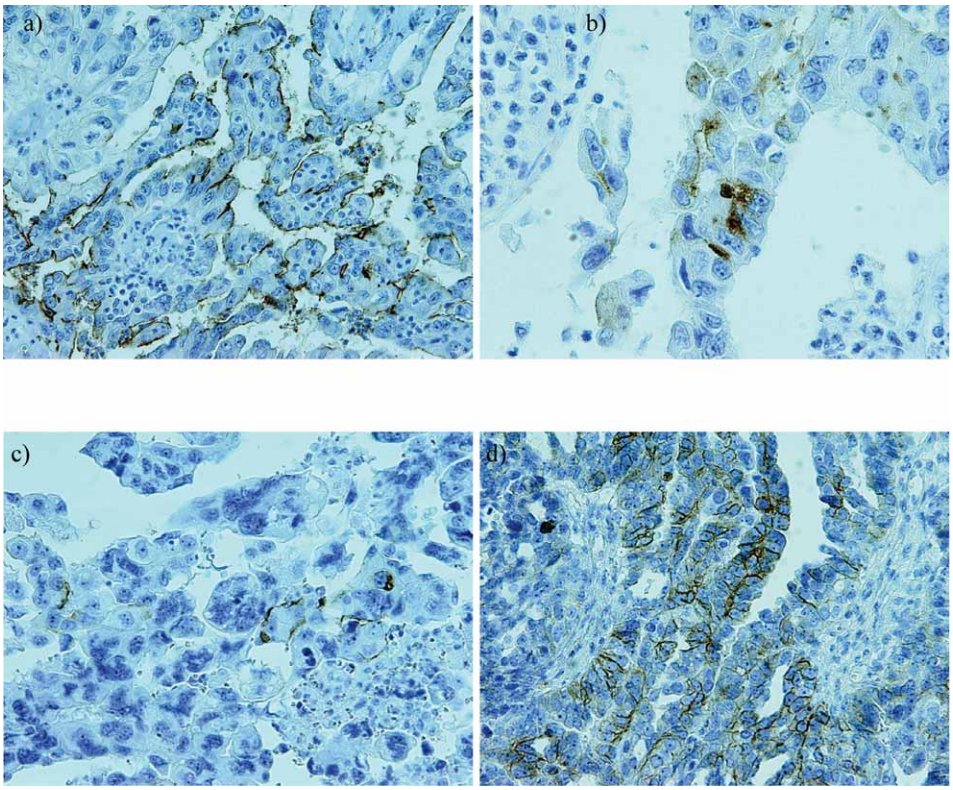

Figure 1. Immunoreaction in the high grade serous ovarian cancer and metastasis. a) High CD133 expression in the high grade serous ovarian cancer (at the apical/endoluminal part of tumor cells). Magnificationx200. b) CD133 positive reaction in the cytoplasm of the single tumor cells. Magnificationx400. c) Low CD133 expression in the peritoneal metastasis (at the apical/endoluminal part of tumor cells) Magnificationx200. d) CD117 positive expression in the primary serous high grade ovarian cancer (cytoplasmic and/or membranous stain). Magnificationx200.

TABLE 2
COMPARISON OF THE CD133 AND CD117 POSITIVITY IN TUMOR AND IN METASTASIS ACCORDING TO CLINICO-PATHOLOGICAL CHARACTERISTICS

Characteristics	Positive CD133			Positive CD117		
	Expression in tumor (n=39) N (%)	Expression in metastasis (n=26) N (%)	c^2 p	Expression in tumor (n=52) N (%)	Expression in metastasis (n=49) N (%)	c^2 p
Age						
<65	26 (66.7)	15 (57.7)	0.22	33 (63.5)	31 (63.3)	0.04
≥65	13 (33.3)	11 (42.3)	0.637	19 (36.5)	18 (36.7)	0.852
Residual tumor						
Absent	10 (25.6)	7 (26.9)		13 (25.0)	11 (22.4)	
<1 cm	13 (33.3)	7 (26.9)	0.31	18 (34.6)	19 (38.8)	0.21
≥1 cm	16 (41.1)	12 (46.2)	0.855	21 (40.4)	19 (38.8)	0.903
Lymph node status						
n.a.	11 (28.2)	8 (30.8)		15 (28.9)	13 (26.5)	
Negative	13 (33.3)	10 (38.4)	0.09	14 (26.9)	11 (22.5)	0.17
Positive	15 (38.5)	8 (30.8)	0.763	23 (44.2)	25 (51.0)	0.683
Response to treatment						
Yes	22 (56.4)	12 (46.1)	0.32	28 (53.8)	26 (53.1)	0.04
No	17 (43.6)	14 (53.9)	0.574	24 (46.2)	23 (46.9)	0.842
Recurrence						
Yes	33 (84.6)	22 (84.6)	0.12	46 (88.5)	43 (87.8)	0.01
No	6 (15.4)	4 (15.4)	0.726	6 (11.5)	6 (12.2)	0.927

* indicated statistical significance

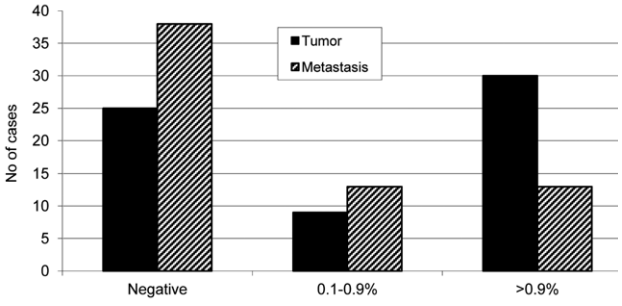

Figure 2. Distribution of the CD133 expression in tumor cells and in metastasis, ?²=10.13, p=0.006.

a more positive cases in tumor samples, the difference was not statistically significant (p>0.05). The median of CD117 positive cells in tumor samples was 1 (0.1–8)%, range 0.1–22%, and in metastases was 0.1 (0.1–7)%, range 0.1–33%.

Comparing all clinico-pathological characteristics and the frequency of CD117 positive tumors and CD117 positive metastases, the result did not reach statistical significance (all p>0.05, Table 2).

CD117 expression in tumor and metastasis was classified in the same way as CD133. Distribution of CD117 status is shown in Figure 3. We have noticed that distribution of cells are similar considering three groups and location of positive cells, the significance was not reached (c^2=1.23, p=0.541).

Prognostic impact of CD133 and CD117 expression

We compare the disease free survival and the overall survival according to three levels of expression of CD133 and CD117 in tumor and in metastasis (Table 3; Figure 4,5,6). The median disease free survival, for all cases, was 11 (1–62) months, range 1–79 months. For CD133 higher positive tumors (>0.9% of positive tumor cells) disease free survival was significantly shorter comparing to CD133 negative tumors and CD133 less positive tumors (0.1–0.9% of positive tumor cells) (p=0.025). The difference in disease free survival between CD133 positive and CD133 negative metastasis was not statstically significant (p=0.514). For CD117 higher positive cases in tumor, disease free survival was significantly shorter comparing to CD117 negative and CD117 less positive tumor cases (p=0.031). The difference in disease free survival between

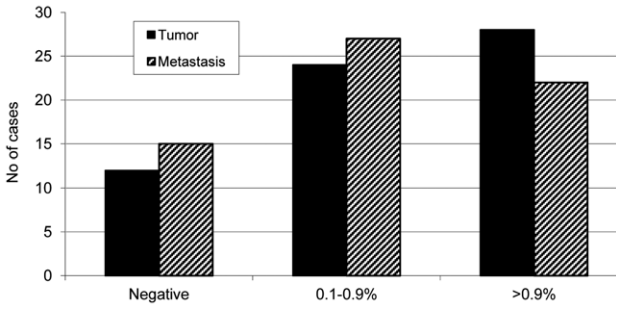

Figure.3. Distribution of the CD117 expression in tumor cells and in metastasis, c^2=1.23, p=0.541.

CD117 positive and CD117 negative metastasis was not statstically significant (p=0.140).

The median overall survival was 26 (1–73) months, range 1–96 months. For CD133 higher positive tumors the median overall survival was significantly shorter (19 months), comparing to CD133 negative and CD133 less positive cases (34 months and 27 months, respectively) (p=0.014). There were no significant differences in overall survival according to CD133 status in metastases; the significance was not reached (P=0.190). Also, there were no significant differences in overall survival according the CD117 status in tumor samples and in metastases (p=0.069 and p=0.055, respectively).

Table 4. shows the results of Cox´s proportional hazards model. Comparing all clinico-pathological characteristics and examined proteins expression, the postoperative survival was significantly related to expression of CD133 in tumor (p=0.024), response to treatment (p< 0.001), and recurrence of the disease (p< 0.001). We can identify expression of CD133 protein in tumor as independent prognostic factor calculated by multivariate analysis.

TABLE 3
COMPARISON OF DISEASE FREE SURVIVAL/OVERALL SURVIVAL ACCORDING TO EXPRESSION OF CD133 AND CD117 IN TUMOR(T) AND IN METASTASIS(M)

		Disease free survival /months	Overall survival / months
	N	Median (5^{th}–95^{th}) percentile	Median (5^{th}–95^{th}) percentile
CD133 (T)			
negative	24	14 (6–73)	34 (10–90)
0.1–0.9%	7	12 (1–25)	27 (1–74)
>0.9%	27	9 (1–25)	19 (1–55)
p		0.025*	0.014*
CD133 (M)			
negative	35	12 (1–73)	30 (1–74)
0.1–0.9%	12	9 (1–30)	16 (1–65)
>0.9%	11	13 (6–63)	23 (9–90)
p		0.514	0.190
CD117 (T)			
negative	11	19 (8–79)	36 (9–96)
0.1–0.9%	21	12 (7–60)	27 (10–73)
>0.9%	26	9 (1–30)	19 (1–65)
p		0.031*	0.069
CD117 (M)			
negative	14	13 (7–79)	32 (9–96)
0.1–0.9%	23	10 (6–27)	23 (10–74)
>0.9%	21	9 (1–36)	18 (1–64)
p		0.140	0.055

*indicated statistical significante difference

Discussion and Conclusion

Our study examined the possible clinical role of the immunohistochemically assessed expression of two potential CSC markers, CD133 and CD117, on samples of high grade serous ovarian cancer and their peritoneal metastases. To our knowledge, there is no data available about similar research.

Ovarian cancers are extremely heterogeneous, and this may suggest that different populations of CSCs can be responsible for tumorigenicity in different histological subtypes, although it is possible that several markers are expressed by the same population of ovarian CSCs[7,37].So, we analyzed only high grade serous ovarian tumors for better correlation of the results. We can assume that same populations of the putative CSCs can express several different markers. Other authors considered that some overlap of the potential stem cell marker can occur[25,28]. CD133 positivity in the tumor and in the metastasis samples was

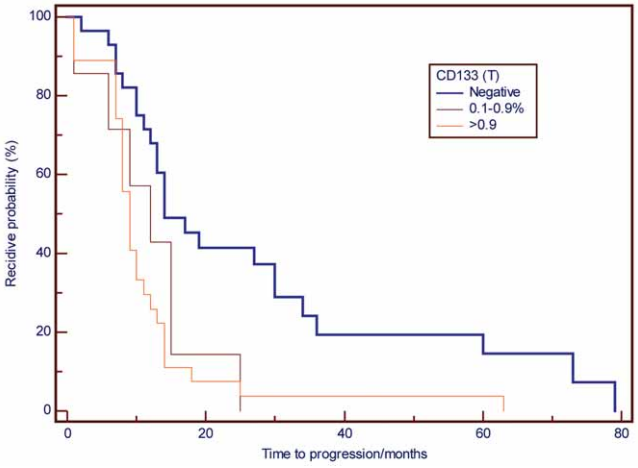

Figure 4. Disease free survival curves according to status of CD133 (T) expression, p=0.025.

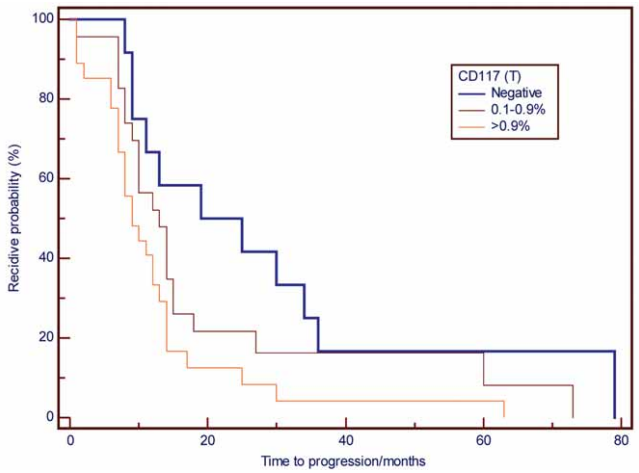

Figure 5. Disease free survival curves according to status of CD117 (T) expression, p=0.031.

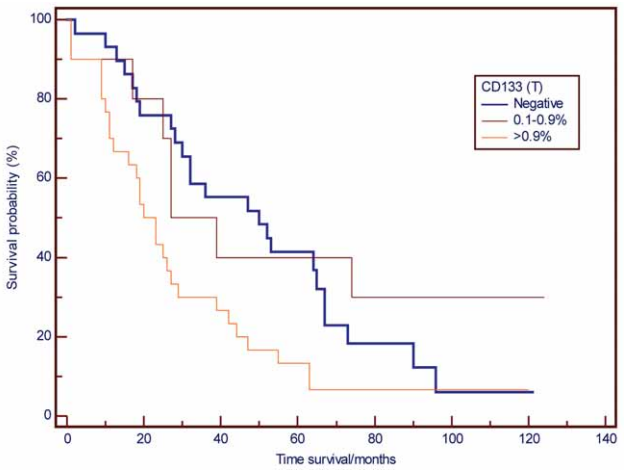

Figure 6. Overall survival curves according to status of CD133 (T) expression, p=0.014.

seen mainly at the apical/endoluminal surface, and less than 1% of the positive malignant cells showed diffuse cytoplasmic pattern. Immunohistochemically determined CD133 positivity expressed at the apical/endoluminal surface was also noticed in samples from pancreatic ductal adenocarcinomas[15], and ovarian carcinoma[36], while Zhang et al. in ovarian cancer samples observed predominantly membrane/cytoplasmic expression[18]. CD117 positivity was noticed as a cytoplasmic and/or membranous stain in tumor and in metastasis samples. Our observation is consistent with data from the literature[27,29].

CD133 positivity was seen in the 61% of tumors. Maeda et al. presents a similar results (60%)[21]. In other studies authors noticed higher (80%)[15,19], or lower number of positive cases (31%)[36,18], so the rank of positivity in the literature is quite wide. CD117 positivity was noticed in our study in high number of tumors, 81%, and our results are similar to those obtained in the literature[14,35]. Some authors noticed lower number of CD117 positive cases (40% and 52%, respectively)[27,29].

The CSCs provide a model of the cancer metastasis in which these cells are able to colonize, expand, and differentiate into heterogeneous tumor phenotypes similar to primary tumors[38]. Both, the primary tumors and the metastases would display similar genetic and expression profiles because both populations are supposedly derived from the same lineage of cancer stem cells[32]. Our results are in the agreement with this hypothesis, because we have found expression of both markers in the samples from tumor and from metastasis. The number of CD133 positive cases was somewhat higher in tumor than in metastasis, but the difference was not statistically significant. The same results we obtanied for CD117. In the literature we did not find the data for the immunohistochemically assessed expression of the CD133 and CD117 for metastasis samples.

TABLE 4
MULTIVARIATE ANALYSIS ASSESING THE SURVIVAL WITH PROGNOSTIC FACTORS
(COX PROPORTIONAL HAZARD REGRESSION)

Variables	b	SE	P	HR	95% CI za HR
CD133 (T)	0.337	0.106	0.024*	1.33	1.03–1.98
CD133 (M)	0.122	0.132	0.346	0.89	0.53–1.32
CD117 (T)	0.039	0.113	0.716	1.04	0.84–1.27
CD117 (M)	0.034	0.053	0.072	1.02	0.98–1.04
Residual tumor	0.118	0.152	0.292	0.92	0.58–1.27
Lymph node status	0.124	0.386	0.840	1.22	0.56–2.32
Reccurence of dis.	–0.055	0.022	<0.001*	0.96	0.92–0.97
Response to treatm.	0.564	0.099	<0.001*	1.82	1.42–2.12

* indicated statistical significance
(b- regression coefficcient, SE- Standard Error, HR- Hazard Ratio)

In our study, the median of CD133 positive cells in tumor specimens was 1 (0.1–7)%, and in metastasis specimens was 0.6 (0.1–6) %. The median of CD117 positive cells in tumors was 1 (0.1–8)%, and in metastasis was 0.1 (0.1–6)%. These results indicate that CD133 and CD117 could represent a possible marker for CSCs, because CSCs are only a very small portion, less than 5%, of the total tumor cell population.

The percentage of CD133 expressing cells was significantly higher in primary ovarian cancer than in peritoneal metastases. Distribution of CD117 positive cells in tumor samples and in metastases is similar to CD133 distribution; we noticed higher percentage of CD117 positive cells in tumor samples, but significance is not reached. This observation may have several reasons. There are studies that suggest that two forms of CSCs exists: stacionary CSCs which are incorporated in tumor and persists in all steps of tumor progression; the other form are mobile CSCs that represent smaller pool of tumor cells. They are located at the tumor-host interface and they are capable to disseminate and form metastatic colonies[39]. Also, we can assume that metastasis in peritoneum cavity has different microenvironment than primary tumor in the ovary and these different conditions may altered expression of potential CSCs marker. Bignotti et al. detected 156 genes that were expressed differentially between ovarian serous papillary carcinomas and omental metastases[40]. Our result is with accordance with one found in the literature[33]. Ferrandina et al. used FACS (fluorescence activated cell sorting) for determining the percentages of CD133-1 and CD133-2 in ovarian carcinoma samples and in omental metastases. Both the percentages of CD133-1 and CD133-2 expressing cells were significantly lower in omental metastases. Although the two different methods are used for assessment of positive cells (FACS versus immunohistochemistry) similar results were obtained in both studies.

Multivariate analysis showed that tumor expression of both markers has statistical significance according to disease free survival. For overall survival only CD133 expression in tumor samples reached the significance. The expression of both markers in metastasis samples didn`t show correlation to prognosis.

Our immunohistochemical results show that expression of CD133 in tumor samples best correlate with prognosis. Expression of both markers in metastasis samples has no prognostic significance. Similar results for tumor expression were obtained by other authors[18,20,21]. Ferrandina et al. found that CD133 expression not provide additional prognostic information for ovarian cancer patients at all[36]. But, in the large study (400 ovarian cancer patients) Zang et al. found an association between CD133 status and prognosis[18]. CD133 is possibly one of the most reliable molecular marker for cancer stem cells in various solid tumors[18], and CD117 has only recently been regarded as a possible CSCs surface marker[29].

Some studies reported that CSCs phenotype was more resistant to platinum-based therapy, which supports the idea that CSCs may be responsible for chemoresistance of ovarian tumors and serve as chemotherapeutic targets for reducing disease recurrence[24,25]. It was hypothesized that CD133 and CD117 are putative marker for CSCs and defining the methods to identify and isolate CD133 and CD117 positive cells in tumor can help adapting the therapy to individual cases or possibly developing targeted therapy in the future.

Conclusion

Our study indicates that the immunohistochemical assessment of CD133 and CD117 expression may have potential clinical value in predicting disease progression or prognosis in high grade serous ovarian cancer patients. According to our results, CD133 proved to be an independent prognostic factor in high grade serous ovarian cancer patients.

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IZRAŽAJ BILJEGA CD133 I CD117 U 64 BOLESNICE SA SEROZNIM RAKOM JAJNIKA

S A Ž E T A K

Tumorske matične stanice (cancer stem cells-CSCs) predstavljaju manji dio stanica u tumoru koje se mogu samoobnavljati i proliferirati, te su vjerojatno odgovorne za početak i održavanje tumorskog rasta. CD133 i CD117 su najčešće korišteni biljezi za potencijalne CSCs, osobito za CSCs jajnika, no njihov klinički značaj još uvijek je nedovoljno istražen. Cilj ovog istraživanja bio je usporediti imunohistokemijski izražaj biljega CD133 i CD117 kod 64 slučaja primarnog seroznog raka jajnika visokog gradusa i pripadajućih peritonealnih metastaza, te ispitati njihovu potencijalnu kliničku ulogu. Izražaj biljega CD133 uočen je u apikalnim/ endoluminalnim dijelovima tumorskih stanica, u 61% tumora i 41% metastaza. Medijan CD133 pozitivnih stanica u tumorima bio je 1 (0,1–7)%, a u metastazama 0,6 (0,1–6)%. Izražaj biljega CD117 uočen je kao citoplazmatsko i/ili membransko bojenje, a pronađeno je u 81% tumora i 77% metastaza. Medijan CD117 pozitivnih stanica u tumorima bio je 1 (0,1–8)%, a u metastazama 0,1 (0,1–6)%. Multivarijatna analiza pokazala je da bolesnice s jačim izražajem biljega CD133 u tumoru imaju statistički značajno kraće vrijeme do pojave recidiva i vrijeme preživljenja (p=0,025 odnosno p=0,014). Bolsnice s jačim izražajem biljega CD117 u tumoru imaju statistički značajno kraće vrijeme do pojave recidiva bolesti (P = 0,031). Cox-ovom regresijskom metodom izražaj biljega CD133 u tumoru uočen je kao nezavisni prognostički čimbenik. Naša studija pokazuje da imunohistokemijski izražaj biljega CD133 i CD117 može imati potencijalnu kliničku vrijednost u predviđanju progresije bolesti i prognoze kod seroznog raka jajnika visokog gradusa. Izražaj biljega CD133 je nezavisni prognostički čimbenik kod pacijenata sa seroznim rakom jajnika visokog gradusa.