Ascites in Ovarian Carcinoma – Reliability and Limitations of Cytological Analysis

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ABSTRACT

Objective: *The objectives of this study were to examine the validity of ascitic fluid cytology in the detection of pathological findings, to examine the percentage of false positive and false negative results in the cytology of ascitic fluid and to determine the validity of peritoneal cytology in relation to the histopathological type of the ovarian tumour.*

Methods: *This retrospective study included 170 peritoneal cytology findings. The study was conducted from January 2010 to December 2012. The experimental group included 76 cytology findings obtained from patients diagnosed with ovarian carcinoma, whereas the control group was composed of 94 cytology findings of benign ovarian tumours and liver cirrhosis ascites. The patients with ovarian carcinoma had grades III as well as grades I and IIc but only in cases where operative and pathological finding indicated a ruptured or perforated tumour capsule.*

Results: *The sensitivity of peritoneal cytology is 68.92%, specificity is 93.61%, positive predictive value is 89.65%, and negative predictive value is 78.57%. In 30.02% of patients, the peritoneal cytology showed false negative results, while in 6.38%, the results were false positive. The highest percentage of false negative findings was 77%, found in endometrioid carcinoma.*

Conclusion: *Peritoneal cytology of ascitic fluid is highly specific but has relatively low sensitivity, particularly in the case of endometrioid ovarian carcinoma. In order to increase sensitivity, peritoneal cytology should be combined with monoclonal antibodies and other biochemical and immunohistochemical markers.*

Keywords: Ascites, ascitic fluid cytology, ovarian carcinoma

Ascitis en el Carcinoma Ovárico – Confiabilidad y Limitaciones del Análisis Citológico

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RESUMEN

Objetivo: *Los objetivos de este estudio fueron examinar la validez de la citología del líquido ascítico en la detección de hallazgos patológicos, examinar el porcentaje de resultados positivos falsos y negativos falsos en la citología del líquido ascítico, y determinar la validez de la citología peritoneal en relación con el tipo histopatológico del tumor ovárico.*

Métodos: *Este estudio retrospectivo incluyó 170 hallazgos de citología peritoneal. El estudio se llevó a cabo desde enero de 2010 hasta diciembre de 2012. El grupo experimental incluyó 76 resultados de citología obtenidos de pacientes diagnosticados con carcinoma ovárico, mientras que el grupo de control estuvo integrado por 94 hallazgos de citología de tumores ováricos benignos y ascitis de la cirrosis hepática. Los pacientes con carcinoma ovárico tenían grados III, así como grados I y IIc, pero sólo en los casos en los que el hallazgo operativo y patológico indicaba una cápsula de tumor rota o perforada.*

Resultados: *La sensibilidad de la citología peritoneal es 68.92%, la especificidad 93.61%, el valor predictivo positivo 89.65%, y el valor predictivo negativo 78.57%. En 30.02% de los pacientes, la citología peritoneal mostró hallazgos negativos falsos, mientras que en el 6.38%, los hallazgos fueron positivos falsos. El mayor porcentaje de los hallazgos negativos falsos fue 77%, encontrados en el carcinoma endometrioide.*

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Conclusión: *La citología peritoneal del líquido ascítico es altamente específica, pero su sensibilidad es relativamente baja, particularmente en el caso del carcinoma ovárico endometrioide. Con el fin de aumentar la sensibilidad, la citología peritoneal debe combinarse con anticuerpos monoclonales y otros marcadores bioquímicos e immunohistoquímicos.*

Palabras claves: Ascitis, citología del líquido ascítico, carcinoma ovárico

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INTRODUCTION

Ascites is a large amount of fluid accumulated in the abdomen. Under normal conditions, several litres of peritoneal fluid are produced daily and it is not accumulated but effectively absorbed.

Ascites of malignant aetiology appear in only 10% of all ascites cases (1). Non-malignant aetiology ascites are most commonly caused by liver and heart diseases. Malignant ascites most frequently present in gynaecological, gastrointestinal and breast carcinomas. A combination of malignant ascites and carcinomatosis of the peritoneum is present in 15–30% of cases (2).

Ascites can be exudative and transudative. Transudates make up 90% of ascitic fluids and they are caused by conditions of non-malignant aetiology. This fluid is clear, with a small number of cells and low level of albumin. An exudate is usually malignant, cloudy, with a greater number of cells and a higher level of proteins than transudate (3).

This differentiation is enhanced by the serum-ascites-albumin gradient (SAAG). If SAAG is > 1.1, the values point to a transudate caused by portal hypertension, cirrhosis, hepatic congestion, portal vein thrombosis *etc.* If SAAG is < 1.1, the exudate is most likely of malignant aetiology or by an infectious process in the peritoneum, nephrotic syndrome and hypoalbuminaemia from malnourishment (4).

It is believed that the pathogenesis of malignant ascites is multifactorial and that the most important pathogenetic mechanisms include increased vascular permeability, lymphatic drainage obstruction, increased difference in hydraulic pressure and reduced difference in oncotic pressure (5).

A two-way permeability of blood vessels is necessary for normal supply of nutrients and gases and waste removal. The permeability can be basal, acute vascular (a consequence of short exposure to vascular endothelial growth factor – VEGF) and chronic, which is a characteristic of pathological angiogenesis.

Vascular endothelial growth factor causes the process of neovascularization and angiogenesis and the result is hyperpermeability and increased porosity of the endothelial membrane, which is followed by migration and proliferation of endothelial cells and creation of new capillaries. Besides VEGF, neovascularization is also influenced by fibroblast growth factor (bFGF), angiogenin, transforming growth factor (TGF α and β) and interleukin 8.

Ascites is the most common complaint of patients with ovarian carcinoma. In 54% of patients with peritoneal carcinomatosis, ascites was the first detectable sign of malignancy (6).

More than two-thirds of patients that report to the doctor have grades III and IV of the disease. Survival rate in advanced stages (III and IV) is 5–20% (7).

The presence of malignant ascites in malignancies of the secondary localization is a worse prognostic marker compared to ovarian carcinoma, and the survival period from the moment of detection is 7–13 weeks (8).

The purpose of this study was to test the validity of ascitic fluid cytology in the detection of pathological cytology results, to test the percentage of false positive and false negative results of ascitic fluid cytology and to determine the validity of peritoneal cytology in relation to the histopathological type of ovarian tumour.

SUBJECTS AND METHODS

A retrospective analysis was used for the research which included 170 peritoneal cytology results during the period from January 2010 to December 2012. The experimental group was composed of 76 cytological findings obtained from patients diagnosed with ovarian carcinoma, while the control group included 94 cytological findings of benign ovarian tumours (fibroma, dermoid cysts, endometrioses, serous and mucinous cysts) and ascites in liver cirrhosis. The patients with ovarian carcinoma were in stages III as well as in stages I and IIc but only in cases where operative and pathological findings indicated a ruptured or perforated tumour capsule. Cytological findings of ascitic fluid and peritoneal cavity effusion were sampled at the Gynecology and Obstetrics Clinic in Niš and scanned at the Institute of Pathology at the Clinical Center Niš. All results were statistically processed by a formula for measuring validity, χ^2 test and presented in tables and graphs.

RESULTS

The histopathological distribution of findings from the experimental group is presented in Fig. 1. The highest percentage of patients had serous type of carcinoma (71%), which is described as the histopathologically most frequent type of ovarian carcinoma in the literature.

Cytological findings of ascitic fluid obtained from the mentioned 76 patients with ovarian carcinoma who were classified into the experimental group (stage III and stage I and IIc with perforated capsule) was negative-false negative in 23 (30.2%) patients (Table 1).

Table 1: Distribution of false negative cytological findings of ascitic fluid with respect to the histological type of tumour

Histological type	Total number of histological type	Number of negative cytological findings	Percentage of false negative findings
Serous	54	15	27.77%
Mucinous	6	2	33.33%
Endometrioid	4	3	77%
Clear cell	6	2	33.33%
Anaplastic	4	1	25%
Granulo-cellular	2	0	0%
Total	**76**	**23**	**30.2%**

Table 2: Distribution of false positive peritoneal cytology findings with respect to the cause and histological type of tumour

Histological type	Total number of histological type	Number of positive cytological findings	Percentage of false positive findings
Fibroma	9	0	0%
Dermoid	11	0	0%
Endometrioma	13	2	15.38%
Serous	39	2	5.12%
Mucinous	20	2	10%
Liver cirrhosis	2	0	0%
Total	**94**	**6**	**6.38%**

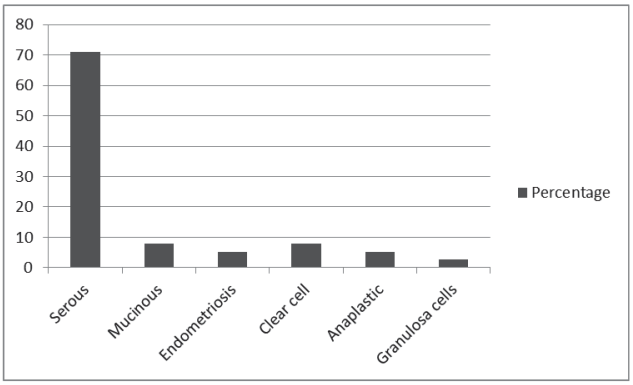

Fig. 1: Distribution of histopathological findings from the experimental group.

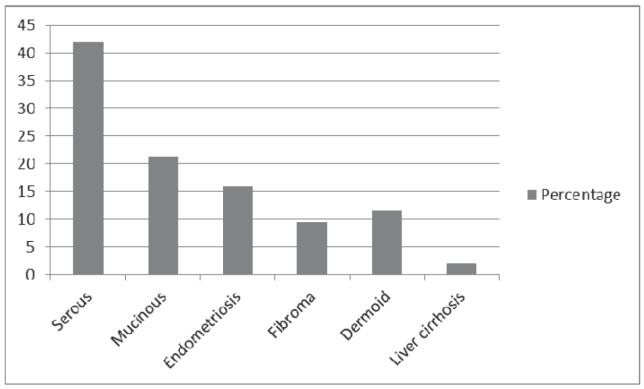

Fig. 2: Distribution of histopathological findings from the control group.

Histopathological distribution of findings from the control group is shown in Fig. 2 and it also shows the highest frequency of benign tumours to be of the serous type (41.98%). The findings of peritoneal cytology obtained from the control group (94 patients) with benign histopathological ovarian tumour or ascites caused by liver cirrhosis was positive-false positive in six (6.38%) patients (Table 2).

Figure 3 shows that the percentage of false negative results in patients with ovarian carcinoma in the experimental group was 30.2%, whereas the percentage of false positive results in patients with benign tumour was 6.38%.

Using the formula for measuring sensitivity and specificity, the data for peritoneal cytology validity were obtained. The measured sensitivity of peritoneal cytology was 68.92%, specificity was 93.61%, positive predictive value (PPV) was 89.65%, negative predictive value (NPV) was 78.57% and overall validity was 82.35% (Fig. 4)

Table 1 shows the number of false negative results with respect to histological type of malignant tumour. It can be seen that, with respect to the histological type, the highest percentage (77%) of false negative results was in endometrioid ovarian carcinoma (out of four endometrioid carcinomas, three

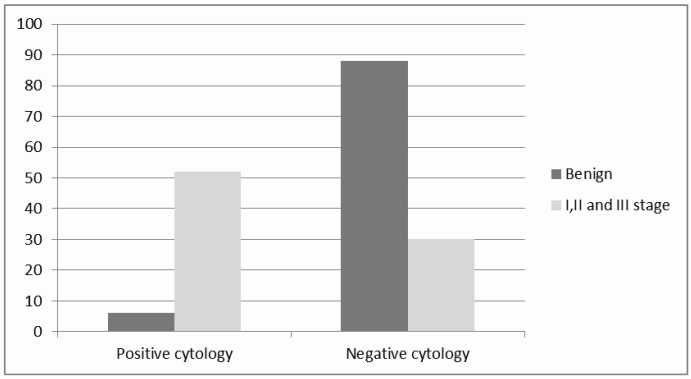

Fig. 3: Combined representation of the distribution of peritoneal cytology findings from experimental (pathological findings – stages I, II and III) and control (benign findings) groups.

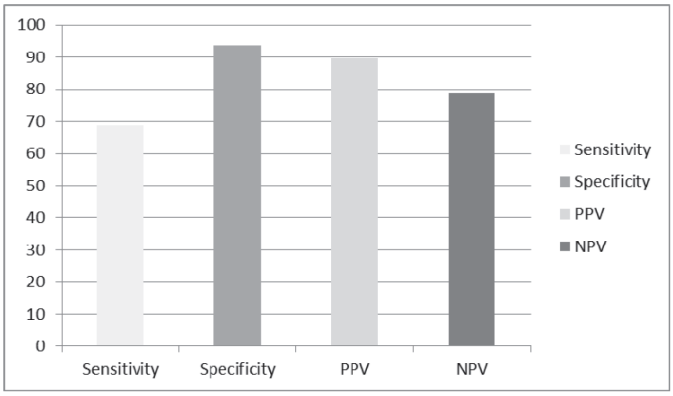

Fig 4: Graphic representation of validity parameters of peritoneal cytology. PPV: positive predictive value; NPV: negative predictive value

were false negative results), while the lowest percentage was with the granulo-cellular type. Although the percentages show that serous carcinoma was the most frequent (71% of all findings), 27.77% were false negative. The difference in frequency of false negative cytological findings with respect to the histological type is statistically significant because χ^2 e = 34.75 > $\chi^2_{0.01}$ = 9.21.

Table 2 shows the number of false positive results with respect to the histological type of tumour. The highest percentage of false positive results in the control group was with endometrioid ovarian cysts – 15.38% (out of 13 endometriomas, two were false positive). The difference was not statistically significant because χ^2 e = 5.34 < $\chi^2_{0.05}$ = 5.99.

DISCUSSION

The main characteristics of malignant ascites are increased concentration of asctic fluid proteins, increased lactate dehydrogenase, a large number of leukocytes and positive cytology for the presence of malignant cells.

A positive cytological finding is important in subclassification of stages I and II of the disease and it represents an important predictive factor in prognosis and recurrence. However, an increasing number of studies shows that morphological examination of cytological samples is not a highly sensitive diagnostic tool.

The reason for false positive cytological results is inadequate interpretation of reactively altered mesothelial cells. These cells are enlarged and they have a dense cytoplasm, a big nucleus with a nucleolus and may contain vacuoles. Endosalpigniosis can also present a problem in differential diagnosis and it should be carefully determined that it is a well-organized group of uniform cells with scarce basophil cytoplasm and a nucleus with a well-defined membrane, fine chromatin and a small nucleolus.

Endometriosis represented a big problem in differential diagnosis. In endometriosis, there are round cells organized into three-dimensional groups and layers, with round and bean-shaped nucleus which has fine chromatin and scarce vacuolated cytoplasm. The most sensitive findings for endometriosis are macrophages with haemosiderin.

These are the reasons why literature data state that peritoneal cytology can be false positive in 4.5% of cases (in our research it was 6.38%). Some recent studies also describe a relatively high percentage of false negative findings which exceeds 20% (in our research it was 30.2%). The reasons for such a high percentage of false negative cytological results of ascitic fluid may be in the bad distribution of cells in the sampled ascitic fluid, bad preparation, or insufficient cell exfoliation, and since cytology is a subjective method, errors may be due to inadequate interpretation of findings (9).

The highest percentage of false negative results in our research was in the group of patients with endometrial carcinoma. In addition to the stated parameters which point to the benign nature of endometrioid carcinoma, cytological elements which point to the endometrioid carcinoma should also be considered: the appearance of three-dimensional groups of cells with large pleomorphic eccentric nuclei with rough chromatin, emphasized nucleolus and abundant cytoplasm. Cytological finding which points to serous carcinoma shows cells which are separate or in irregular incohesive groups, with large pleomorphic nucleus and emphasized nucleolus.

The sensitivity of peritoneal cytology stated in other studies ranges from only 50 to 60% (in our research it was 68.92%), up to 97% depending on the study, disease stage and peritoneal inclusion (10).

In patients with stage Ic, cytology was positive in 75% and when the peritoneum was included, it was 94% (11). The sensitivity of cytology when peritoneum was included was 82.9% and specificity was 98.1% [in our research it was 93.61%] (12). The examination of total validity of cytology in some publications showed somewhat lower sensitivity which was 60% and high specificity of almost 100% (13).

The result of primary cytology of ascitic fluid is an important parameter in the diagnosis, therapeutic approach and disease prognosis. The result of secondary cytology after the treatment is also an important independent prognostic marker which is highly correlated with the optimal effect of surgical treatment, recurrence and overall survival rate. In positive

secondary cytology, survival is 13 to 32 months, while in negative cytology, it is > 48 months (14).

Considering all aspects of validity of ascitic fluid cytology, particularly with certain histological types (endometrioid ovarian carcinoma), it is believed that further research is necessary as well as the use of specific additional immunohistochemical markers in order to reduce as much as possible the percentage of cytological errors which cause wrong grading and inappropriate treatment.

In addition to measuring the concentration of alkaline phosphatase, lactate dehydrogenase, fibronectin, as well as tumour markers CA-125, CEA, p53, β HCG, there is also a specific group of panel antibodies, primarily MOC-31 and Ber-EP4. These antibodies are important for differentiation of mesothelial and cancerous cells and it may also contribute to the differentiation of antibodies into adherent cells and non-adherent carcinogenic (15).

From the remaining biomarkers, telomerase has lately been mostly tested. Telomerase is an enzyme necessary for normal replication of chromosomes and constant growth of cancer cells. Telomerase activity is absent in the majority of somatic cells. On the other hand, telomerase expression has been confirmed in almost 100% of ovarian carcinoma cases. Contrary to 24–54% of cases, where, despite the fact that residual disease was diagnosed, cytology and second-look surgery were negative, telomerase was almost 100% positive (16).

In our study, we concluded that peritoneal cytology of ascitic fluid is highly specific (93.61%) but it has a relatively low sensitivity (68.92%). In 30.02%, peritoneal cytology had false negative results and in 6.38%, it showed false positive results. Such distribution of cytological findings may cause inadequate grading of the disease and inadequate therapeutic approach (not applying the necessary chemotherapy or application of unnecessary chemotherapy). The highest percentage of false negative results was with endometrioid ovarian carcinoma (77%), so it is necessary to be very careful when cytological scanning is used with this type. In order to increase sensitivity, peritoneal cytology should be combined with other available biochemical and immunohistochemical markers.

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