

## Diagnostic limitations of biochemical evaluation of peritoneal effusions in differentiating malignant and non malignant pathologies

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**Abstract** Treatment of malignant peritoneal effusions is generally palliative, therefore quality of life issues, as well as the risks and the benefits of the therapeutic options become more critical. Cytomorphological examination alone, provide only limited sensitivity for the detection of metastatic carcinoma cells in many cases of serous effusions. Early diagnosis and management of peritoneal metastases from cancer patients represent new directions of researches. Current study was aimed to differentiate peritoneal liquids encountered in 81 available cases, on various biochemical criteria. The cases were chosen to show both biochemical patterns (benign and malignant) and in this way to achieve a diagnostic value of the biochemical method. A panel of 17 biochemical markers: total proteins (TP), albumin (ALB), lactate dehydrogenase (LDH), total cholesterol (TC), glucose (GL), total lipids (TL), triglycerides (TG), alpha amylase (AA), alkaline phosphatase (ALP), urea (U), total bilirubin (TB), direct bilirubin (DB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), magnesium (Mg), iron (Fe), potassium (K) were determined from the resulted supernatant after centrifugation in blood and peritoneal fluid. It is concluded that a suitably chosen panel, consists of the best specific markers found, can be of great value for initial differentiation and subsequent guidance in the diagnosis.

**Keywords:** biochemistry, peritoneal effusions, benign, malign

### 1. Introduction

The differential diagnosis of ascites often leads to confusion and an inability to exclude its multitude of causes in many patients. In this paper we outline the clinical features and laboratory investigations that usually elucidate the cause of ascites for the clinician in a simple and logical manner. Roughly 80-85% of cases of ascites are related to underlying chronic liver disease, but cardiac failure, tuberculosis, malignancy-related ascites and other less common causes should always be considered. Careful evaluation of the patient, including a clinical history, physical examination and diagnostic paracentesis should routinely be performed to determine the cause of ascites [1].

Both in etiology as the break in the pathogenesis of peritoneal effusions are many issues unclear, solution which would facilitate diagnosis of treatment setting in associated cancer.

Ascites is a term used for pathological accumulation of peritoneal fluid in the peritoneal cavity over the quantities available in normal conditions (less than 50 ml) [2], met in a variety of conditions. Peritoneal fluids are found, in normal conditions, in small amount in the space created between the parietal and visceral peritoneal serous, and the term of peritoneal effusions is used to represent an accumulation in excess of this fluid. Peritoneal cavity normally contains about 100 ml of peritoneal fluid, which is changing into a transudate at a rate of approximately 50% per hour, produced by visceral capillaries and drained by lymphatic vessels.

Conventional cytology technique, as shown by previous studies can not be used as the unique method in determining the disease process responsible for the accumulation of peritoneal fluid in patients diagnosed with cancer. Most people who present themselves to the doctor because of ascites

syndrome are diagnosed with liver cirrhosis (75%), other etiologies are more rarely met: malignancy (10% of cases), heart failure (3%), peritoneal tuberculosis (2%), chronic pancreatitis (1%) etc. [3].

The liquid is generally classified as transudate or exudate (Light's criteria) [4, 5] depending on the amount of albumin, protein content and LDH increased site.

The management of malignant effusions of unknown etiology is a constant evolution. The paracentesis may be useful in reducing symptoms [6]. The first report on cytological examination of peritoneal fluid to detect subclinical metastasis was presented since 1971 [7].

Malignant peritoneal effusions come from direct extension and metastasis process is due to a reabsorption unbalanced. Break of malignant origin is usually recurrent and is often associated with an unfavorable prognosis [8]. Malignant ascites is defined generally as the break of peritoneal containing malignant cells, so in most studies described the efforts of researchers to detect isolated cancer cells in peritoneal fluid, cytopathology method. In 10-20% of cases analyzed, it is difficult to detect primary tumor is often presented as a clinical abnormalities [9].

Numerous studies have shown a sensitivity of conventional cytology diagnosis of 57.3% and a specificity of 89% for detecting malignant cells in peritoneal effusions [10]. There are some a gray area in which the cytopathologist is facing with different problems in determining the nature of mesothelial cells, which can be reactive, atypical or malignant [11]. Therefore, numerous other techniques are needed to assess the peritoneal fluid to increase accuracy of the diagnosis of malignancy.

Because the cytopathology method as the only method sensitivity analysis compared a comprehensive set of analysis, diagnosis becomes difficult to differentiate in the examination of liquids in question [12].

To this end, in the current study were tested other additional methods for diagnosing peritoneal fluid, including biochemistry analysis, immunocytochemistry and cytomorphometry.

Current study was aimed to differentiate peritoneal liquids based on various criteria biochemical and diagnostic accuracy comparing with commonly used biochemical markers (total

protein, albumin gradient serum / ascites and peritoneal fluid LDH's). Identification of biochemical processes that take place in the process of accumulation of peritoneal fluid may improve the identification and application of targeted therapies in malignant cancers. This study aims to highlight the usefulness of biochemical examination of peritoneal fluid and its utility in preventing the risk of cancer complications.

## 2. Experimental

The study was conducted during September 2007 - January 2010 and was based on assessing 81 peritoneal fluids (obtained by abdominal paracentesis) and serum obtained from patients admitted in Constanta County Emergency Hospital (SCJUC). The informations represented by clinical data and histopathologic diagnosis of all patients were relevant for establish the patient's clinical condition.

Biochemical parameters were assessed from the supernatant sample of peritoneal effusions and serum collected in the same day, (previously centrifuged at 3000 rpm for 10 min., with Cytospin centrifuge) by the methods used in biochemistry laboratory for estimating serum biochemical markers.

Biochemical determinations were performed respecting the protocols indicated, on patients with peritoneal effusions associated with at least one type of neoplasia; measurement of TP, LDH, TC, TG, TL, GL, AA, ALP, TB, DB, U, AST, ALT, Fe and Mg were obtained using automatic biochemistry analyzer Hitachi 917 (serial 198,338). Determination of ALB and K were carried out using semi-automatic biochemistry analyzer Cormay Multi (serial 801,260,331).

Biochemical parameters were evaluated both from effusions considered benign and also from malignant peritoneal effusions. Were selected only cases in which microbiological examination was negative, because intra-abdominal infections significantly alter the biochemical composition of peritoneal effusions. Also, the same measurements were evaluated on the serum collected from the same patients, in the same day.

After conducting the cytology method on the 40 peritoneal fluid obtained from patients that had in

clinical history at least one malignancy, only 26 were confirmed as effusions with malignant cytology (65%), the remaining 14 (35%) were only suspected for malignancy, without a certain diagnostic. Were considered suspicious for malignancy, those effusions without obvious characters of malignancy, but associated to cancer, (4 cases associated with liver carcinoma, 4 cases associated with ovarian carcinoma, 2 cases associated with carcinomas of gastrointestinal origin, 2 cases with pulmonary carcinoma and 2 cases associated with breast carcinoma).

The samples were considered to represent the benign conditions if there were no signs of infection, cancer cells or if other parameters values were not changed. Following the results, we also calculate the effusions / serum ratio (PE/S) and the serum-effusion gradient (SAG) (the difference between the recorded value of the parameter studied in serum and the value recorded in peritoneal effusion). When this ratio is greater than 1 unit (activity parameter in effusions being higher than in serum), the ratio was considered positive.

As the values obtained from biochemical determinations were not significantly different between the six types of cancer, we have established three cohorts of patients: group 1 (lot I) - with benign peritoneal cytology, group 2 (lot II) with inconclusive cytology (suggestive for malignancy but without a certain diagnostic) and group 3 (lot III) - with malignant peritoneal cytology.

To estimate the accuracy of laboratory biochemistry, statistical interpretation was made with Office program Excel, the test "two-tails unpaired t-test", determining the values of p. The association was considered significant when  $p < 0.05$ . Test t-test was used to establish the correlation between concentrations of biochemical markers of positive and negative peritoneal effusions.

### 3. Results and discussions

41 cases (from the total of 81 cases who developed peritoneal fluid) were found to be benign effusions (liver cirrhosis), 4 cases were associated with hepatic carcinoma, 4 cases with lung carcinoma, 18 cases with ovarian carcinoma, 3 cases with breast carcinoma, 9 cases with gastrointestinal

carcinoma and 2 cases with peritoneal mesothelioma (Table 1).

**Table 1.** The distribution of peritoneal effusions according to primary disease and cytology diagnostic

Lots	Primary affection	Total number of cases (n=81)	Cytology diagnostic
Lot 1 (n=41)	Cirrhosis	41	Negative peritoneal effusions
Lot 2 (n=14)	Liver cancer	4	Suspicious for malignancy (peritoneal effusions malignancy associated)
	Ovarian cancer	4	
	Gastrointestinal cancer	2	
	Breast cancer	2	
	Pulmonary cancer	2	
	Peritoneal mesothelioma	0	
Lot 3 (n=26)	Liver cancer	0	Positive (malignant peritoneal effusions)
	Ovarian cancer	14	
	Gastrointestinal cancer	1	
	Breast cancer	2	
	Pulmonary cancer	7	
	Peritoneal mesothelioma	2	

\*n – number of cases

The biochemical markers values obtained from peritoneal effusions were found to be much lower than the values obtained from serum patients, except for lactate dehydrogenase (LDH's serum values was greater than values founded in effusions in 3/40, 7.5% cases from group 3) (see Table 2).

The TP values obtained from serum of all patients was included in the reference serum (6.2-8.0 g/dL). TP values obtained from the peritoneal effusions were increased both in cases of Lot 3 (24/26, 92.30%) but also in cases from Lot 2 (11/14, 78.57%), although the cytology showed unclear signs of malignancy. In the control group (represented by Lot 1), mean values of TP were below the limit of 2.5 g / dL (1.66 g/dL), and the values obtained in lot 2 and lot 3 were over this value (2.63, respectively 3.7). Values of SAG and TP in cirrhosis cases and in malignant cases were less than 0.5 (Table 3).

**Table 2.** Mean values of biochemical markers in serum and peritoneal effusions

Biochemical markers		Lot I	Lot II	Lot III	Serum reference values
TP, g/dL	serum	6.3±1.3	7.7±1.4	7.1±0.9	6.2-8.0
	peritoneal effusions	1.66±0.04	2.63±0.12	3.7±0.15	-
ALB, g/L	serum	3.7±0.51	3.78±0.41	4.12±0.85	3.5-5.1
	peritoneal effusions	2.3±0.29	2.8±0.31	3.4±0.44	-
LDH, UI	serum	278±2.41	393±3.34	388±3.15	220-280
	peritoneal effusions	151±1.9	427±2.81	394±2.5	-
ALP, UI	serum	270±2.14	68±1.12	202±2.36	40-140
	peritoneal effusions	154±1.87	41±0.93	113±1.58	-
AST, UI	serum	92±1.29	54±0.80	41±0.73	8-40
	peritoneal effusions	24±0.32	19±0.21	17±0.26	-
ALT, UI	serum	55±0.81	44±0.72	14±0.12	5-30
	peritoneal effusions	21±0.21	13±0.11	9±0.089	-
AA, mg/dL	serum	42±0.94	66±1.01	59±1.05	28-100
	peritoneal effusions	11±0.13	18±0.16	48±1.33	-
DB, mg/dL	serum	0.54±0.07	0.91±0.14	0.68±0.09	<0.2
	peritoneal effusions	0.5±0.06	0.66±0.08	0.42±0.05	-
U, mg/dL	serum	27±0.38	25±0.29	31±0.33	15-40
	peritoneal effusions	25±0.29	24±0.26	29±0.30	-
TC, mg/dL	serum	245±2.13	233±2.09	185±1.97	160-240
	peritoneal effusions	31±0.34	95±1.31	61±1.03	-
TG, mg/dL	serum	227±1.93	166±1.57	158±1.48	55-160
	peritoneal effusions	44±0.98	35±0.82	21±0.77	-
TL, mg/dL	serum	734±3.15	711±2.98	787±3.62	550-750
	peritoneal effusions	241±2.26	278±2.44	693±3.06	-
GL, mg/dL	serum	167±1.56	95±0.98	121±1.03	70-120
	peritoneal effusions	121±1.03	79±0.81	71±0.88	-
TB, mg/dL	serum	1.02±0.11	1.54±0.16	1.66±0.18	0.1-1.2
	peritoneal effusions	0.87±0.14	2.4±0.24	2.9±0.36	-
Fe, µg/100mL	serum	88±1.02	117±1.47	120±1.49	80-160
	peritoneal effusions	18±0.19	11±0.16	9.2±0.09	-
Mg, mg/dL	serum	1.9±0.23	2.5±0.29	2.3±0.27	1.8-2.6
	peritoneal effusions	1.5±0.16	2.22±0.22	2.11±0.21	-
K, mmol/L	serum	3.5±0.50	3.7±0.69	4.1±0.86	3.6-5.5
	peritoneal effusions	1.9±0.23	3.1±0.35	2.9±0.36	-

Thus, the total protein had diagnostic value only by their determination from peritoneal effusions.

Serum ALB values of all patients were within the reference serum (3.5-5.1 g/L). ALB values from benign peritoneal effusions (lot 1) were lower than those recorded in lot 1 and lot 2. SAAG (difference between serum ALB values and effusions ALB

values) was increased in patients with cirrhosis than in patients with carcinoma. Thus, patients with SAAG over than 1.1 g/dL admitted in SCJUC for liver cirrhosis (1.85) g/dL were and hepatic carcinoma (1.1 g/dL), other cases presenting a values lower than a unit (0.98 for group 2, respectively 0.72 for group 3).

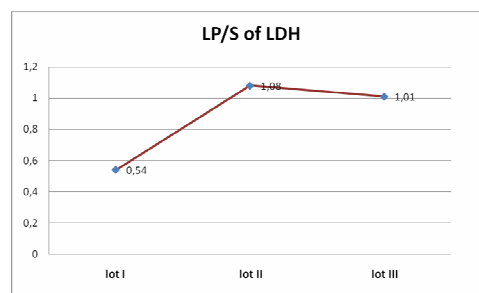
**Table 3.** Values of effusions/serum ratio (PE/S) and serum-effusion gradient (SAG)

Biochemical markers		Lot I	Lot II	Lot III
TP	PE/S	0.26	0.34	0.52
	SAG	4.64	4.9	4.1
ALB	PE/S	0.62	0.74	0.82
	SAG	1.85	0.98	0.72
LDH	PE/S	0.54	1.08	1.01
	SAG	127	561	430
ALP	PE/S	1.75	1.65	1.78
	SAG	124	38	86
AST	PE/S	3.83	2.84	2.41
	SAG	68	35	24
ALT	PE/S	2.61	3.38	1.55
	SAG	34	31	5
AA	PE/S	3.81	3.66	1.22
	SAG	31	48	47
DB	PE/S	0.04	0.25	0.26
	SAG	0.92	0.72	0.61
U	PE/S	1.08	1.04	0.06
	SAG	2	1	2
TC	PE/S	7.9	2.45	3.03
	SAG	214	124	138
TG	PE/S	5.15	4.74	7.52
	SAG	183	131	137
TL	PE/S	3.04	2.55	1.13
	SAG	493	433	94
GL	PE/S	1.38	1.20	1.70
	SAG	46	16	26
TB	PE/S	0.85	1.55	1.74
	SAG	0.17	0.86	1.24
Fe	PE/S	0.20	0.09	0.07
	SAG	70	106	118
Mg	PE/S	0.4	0.3	0.2
	SAG	0.7	0.8	0.9
K	PE/S	1.6	0.6	1.2
	SAG	0.5	0.8	0.7

The LDH values obtained from serum and effusions collected from patients associated with carcinoma were significantly different from those obtained from benign cases ( $p < 0.05$ ). In serum patients, the lactate dehydrogenase values obtained from all patients of lot 1 were included in the reference values (220 - 280g/dL), while the LDH

values from Lot 2 and Lot 3 were 1.4 times higher than average of the lot 1. Serum LDH values were higher than the values encountered in 3 / 40 of effusions (7.5%) from group 3. In 35/41 (85.36%) cases from group 1 the LDH values obtained from effusions were higher than the values obtained from serum. LP/S of LDH registered in benign cases was under 1 unit (0.54), while in cases 2 and 3, the ratio was calculated to be about one unit (1.08, respectively 1.01) (**Fig. 1**).

LDH presented low values in malignant liver diseases without complications, bringing important contributions in differential diagnosis of carcinoma.

**Fig. 1.** Ratio of the value obtained in serum lactate dehydrogenase and the peritoneal effusions

ALP obtained from serum presented an average value (270 IU) in cases with cirrhosis greater than twice than maximum value of serum reference values (40-140 IU); serum ALP values obtained in effusions from group 2 and 3 ranged in the reference serum values (lower than in group 1).

By calculating the difference between ALP obtained from serum and from effusions, the values obtained in the lot 2 and lot 3 were significantly lower than those registered in benign cases (113, respective 86 over those 202).

In all cases, biochemical serum values were higher than those of effusions. Values obtained by determination of ALP in serum and peritoneal effusions could not highlight any differences between benign cases and carcinoma related cases.

AST values recorded in serum patients with liver cirrhosis (group 1) was superior to those obtained in lots 2 and 3. A statistically significant difference was recorded by calculating the SAG (65 for lot 1, compared to 35 for lot 2, and 24 for lot 3).

Although, AST and ALT serum values of liver carcinoma and hepatic cirrhosis had values above reference values and could not establish any correlation between values obtained in the AST and ALT from effusions of the three studied groups.

Values obtained by measuring AA, DB and U were within normal limits, both in serum and peritoneal fluid, being unable to highlight any differences between the two types of peritoneal effusions.

The results obtained by determining serum TC (75/81, 92.59%) were within the range of reference values (160-140 mg/dl) and could not make any correlation between the three studied groups. Instead TC values were significantly high in effusions ( $p < 0.001$ ) from lot 2 (12/14, 85.71%) and from lot 2 (24/26, 9.30% accuracy in differentiating cirrhosis from malignancy), compared to control group. Also, TC values were significantly lower in effusions where reactive cells were encountered, with atypical characteristics (14/14, 100%). SAG of TC, both in group 2 (124) and also in group 3 (138), was lower than the values registered in the control group (214) (**Table 2**).

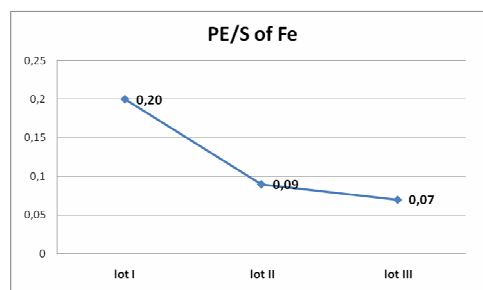
In the control group, serum TG levels have exceeded the reference range in 31/41 cases 75.60%, in lot 2 and lot 3, values falling within normal limits. High TG level was preserved in peritoneal fluid of patients with cirrhosis, compared with levels recorded in group 2 and 3. Between lots 2 and 3, there was no statistically significant difference in terms of values recorded by measuring the TG.

As in the 4 cases (out of 14 cases of effusions suspected for malignancy) associated with liver carcinoma, the TL values of effusions was lower than in other cases, we conclude that TL values are lowering in liver diseases (cirrhosis and liver carcinoma).

Serum GL level was increased in group 1 and had a normal value in lots 2 and 3, compared to the reference range values. Sugar concentrations were low in most cases of cancer (36/40, 90%) and was increased in most cases of liver cirrhosis (32/41, 78.04%). Significantly lower concentration of serum GL levels were recorded in all cases of liver carcinoma (4/4, 100%). GL values lower than normal may be due to the higher level of liver glycogen metabolism of tumor cells.

Serum TB registered in lots 2 and 3 was greater than that determined in group 1. Values registered had a significantly greater increase in case of peritoneal effusions from group 2 and 3 (over 2 or 3 times higher than the value of lot 1).

Levels of Fe registered in effusions of lots 2 and 3 were lower than those registered in group 1 (up to 2 times lower in malignant cases as compared to group 1). Statistically significant differences ( $p < 0.05$ ) were observed by comparing the three groups, by calculating the ratio of Fe (PE / S) (**Fig.2**).



**Fig. 2.**Ratio amounts iron in effusion and serum

Magnesium acts as a cofactor for enzymes with necessary of ATP, thus registering a low activity in cases of cirrhosis (38/41, 92.68%) and an increased activity in malignant effusions (19/26, 73.07%) and in suspected for malignancy effusions (8/14, 57.14%). Serum Mg was framed in reference serum values (1.8 - 2.6 mg/dL).

Potassium, essential for intracellular electrochemical balance, was increased in effusions from lots 2 and 3, with significantly increased values in gastrointestinal cancers (8 / 9, 88.88%). Serum K was framed in reference serum values (3.6 - 5.5 mmol/L) (**Table 2**).

TP concentration of ascites fluid is influenced by serum TP concentration (a directly proportional relationship) and by pressure from the portal vein, (inverse relationship).

A low level of serum TP in cases of cirrhosis can be explained by the low capacity of the liver with hypoalbuminaemia and low levels of coagulation factors. They cause pressure drop phenomenon colloid osmotic and portal hypertension, thereby causing accumulation of ascites and particularly increase the level of TP in

peritoneal fluid. In the malignant process, an increased TP level compared to the one existing in cirrhosis should be explained by the many changes that are taking place in the process of metastases. In cirrhosis, oncotic pressure exerted by plasma proteins (especially albumin) decreases with the decrease of serum TP levels, and leads to further loss of fluid from interstitial area [13].

ALB is a water soluble protein, synthesized by the liver, important for maintaining plasma volume and oncotic pressure. A value of SAAG over 1.1g/dL (portal hypertension) would indicate an accumulation of peritoneal fluid due to higher portal pressure (cases of cirrhosis) [14].

A higher value for differential diagnosis than the concept transudate - exudate, has the SAAG that directly correlates with portal hypertension, and TP determination is not influenced by diuretic therapy or paracentesis. A value of SAAG in 1.1g/dL indicates an obstruction of lymphatic vessels, due to peritoneal fluid accumulation, or an infection of peritoneal serous (malignant cases) [15].

SAAG could distinguish between malignant peritoneal effusions (without liver involvement) (both with a high concentration of TP). However, could not distinguish between the benign effusions, carcinoma and liver cirrhosis; instead, was shown to be a useful marker for classifying the peritoneal effusions associated with these categories: effusions associated with portal hypertension and effusions non-associated with portal hypertension.

LDH activity from peritoneal fluid comes from activity of granulocytes and tumor cells. Thus, in cases of tumors taken to study, concentrations of LDH appear to have elevated low levels of glucose. Influx of blood glucose stimulates insulin secretion and increase pancreatic secretion of glucagon. Insulin facilitates the transport of glucose through cell membranes. Moderate decrease of blood sugar levels in malignant cases could be explained by depletion of liver glycogen amount, due to a high metabolism of tumor cells.

A high level of TC in peritoneal effusion is an indicative of an involvement of cancer process, that affects the peritoneal serosa, but certainly detectable analysis are not specified in these conditions. This elevate TC value may be associated with the disintegration of many cells, a phenomenon that occurs in primary tumors and peritoneal metastases

of serosa. This causes an irritation of peritoneal serosa, an increase of the permeability in affected membrane, and, hereby, cholesterol can penetrate the lymphatic vessels from the peritoneal cavity, thus accumulate in the peritoneal fluid [16].

TB level was higher than the values existing in normally cases, explained by several causes: the decline due to hemolytic colloid osmotic pressure and bleeding due to deficiency of coagulation factors (synthesized in the liver). Transaminase values were moderately elevated, but not represented a specific indicator (value of discrimination between group 1 and group 2 recorded by determining the AST was 45%, 18/40, and ALP of 27.5%, 11/40).

Biochemistry of peritoneal exudates from group 2 differed statistically significantly ( $p < 0,05$ ) from transudates (cases of liver cirrhosis, group 1) only by determining the following parameters: TP (percentage of discrimination 62.5%), SAAG (85%), LDH (85.36%), TC (percentage of discrimination 90%), TG (75.60%), GL (78.04%), TB (60%), K (72,5%) and Mg (67.5%).

Biochemistry serum, collected from studied patients, could not be able to make differences between the three groups, only by determining the LDH and TB. Thus, of all measured parameters, the highest accuracy in the differential diagnosis between malignant and benign cases (cirrhosis) was obtained by measuring peritoneal effusions TC (90%), peritoneal effusions LDH (85.36%) and SAAG (85%). Thus, the combination of these biochemical markers may represent a starting point for discrimination the benign ascites towards from the malignant effusions, especially in the presence of a negative cytology.

#### 4. Conclusions

Biochemical evaluation successfully completed the first stage in diagnostic of carcinoma. As the success of treatment depends on diagnostic accuracy, laboratory chemistry can support the cytological evaluation of peritoneal fluids and can successfully complete the clinical pattern in order to establish an initial diagnosis of malignant transformation, a better evaluation of the progress of neoplasia and can control effective the therapeutic treatment.

The conclusions from this study may open new research direction and may represent valuable information for assessing the function biochemical level of therapy, for maintenance of peritoneal serosa function in normal parameters.

## 5. References

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