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The role of cytology in the diagnosis of fluid collection syndromes associated with liver cirrhosis. Clinical, epidemiological, cytological and biochemical study of pleural effusion

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Abstract

Cirrhosis, end-stage of many liver diseases, presents many complications during its evolution. One of these is the presence of pleural fluid collection syndrome. This may be a direct consequence of liver disease (hepatic hydrothorax) or may be a random association. A formidable complication due to its consequences is spontaneous pleural empyema. The present study aimed to assess the incidence, the factors that influence its occurrence, and the frequency of the infectious complication.

Keywords: cirrhosis, pleural effusion, spontaneous pleural empyema.

Introduction

Hepatic hydrothorax is a transudate-type fluid accumulation in patients with advanced cirrhosis. The term was introduced by Morrow CS *et al.* (1958) in order to differentiate pleural fluid secondary to cirrhosis from other cases leading to fluid accumulation syndrome in patients with cirrhosis [1–2].

Incidence is assessed differently from one author to another. McKay DG *et al.* (1947) appreciates it as 1% of cases, Albin RJ and Johnston GS (1989) as 6%, Lieberman FL *et al.* (1966) as 5.4%, Cardenas A *et al.* (2004) as 5 to 10%, while Chen TA *et al.* (2003) as in 15% of all cases [3–7].

Pleural effusions are a common pneumologic diagnosis problem, often interdisciplinary, because of many benign or malignant diseases which can cause. In this respect, it is necessary to assess the type of cells in pleural aspirate which can be useful for diagnosis.

The current study aimed to highlight the role of pleural fluid cytology correlation with clinical and laboratory data in patients with cirrhosis associated with pleural effusion.

Materials and Methods

Between 2004 and 2011, we studied a number of 849 patients diagnosed with liver cirrhosis. The batch of 849 patients consisted of 423 (49.82%) females and 426 (50.18%) males with the following distribution depending on the etiology: 253 patients with alcoholic cirrhosis, 137 patients with HBV cirrhosis, 105 patients with HCV cirrhosis, 110 patients with B+C viral hepatitis, 149 patients with viral infection and alcohol consumption, 34 cases with immune cirrhosis, 12 cases with primary biliary cirrhosis, and 49 cases with cryptogenic cirrhosis.

The patients were divided into functional classes according to the Child–Pugh criteria [8].

The diagnostic criteria for hepatic hydrothorax are [1, 2, 9]:

- PMN <250/mm^3;
- proteins <2.5 g/dL;
- effusion proteins/serum proteins <0.5;
- pleural fluid LDH/serum LDH >0.6;
- pleural fluid albumin/serum albumin >1.1;
- pleural fluid bilirubin/serum bilirubin <0.6;
- pH >7.4;
- pleural fluid glucose = serum glucose.

Clinical examination was performed in all patients, and thoracentesis was performed in patients with pleural effusion with biochemical and cytological examination of pleural fluid. Pleural fluid extracted by thoracentesis was centrifuged for 10 minutes and the supernatant was spread on the slide, fixed with methanol and stained with May–Grünwald–Giemsa solution (Merck). In addition, for pleurisy cases suspected as having tuberculous etiology smears were fixed with methanol and stained using the Ziehl–Neelsen technique (Merck).

Results

Of the 849 patients, 270 had pleural effusion with the following etiology: TB – 102 cases, HIV – four cases, HIV+TB – four cases, parapneumonic – 58 cases, hydrothorax – 48 cases, lung carcinoma – 32 cases, mesothelioma – five cases, myocardial infarction/ pulmonary embolism – 17 cases, with the following distribution in relation to functional class (Table 1).

Table 1 – ***Distribution of cases in relation to functional class***

	CHILD A	CHILD B	CHILD C	Total
Tuberculosis	15	72	15	102
HIV	1	1	2	4
HIV + Tuberculosis	1	2	1	4
Parapneumonic	7	38	13	58
Hydrothorax	0	8	40	48
Lung carcinoma	5	23	4	32
Mesothelioma	3	2	0	5
Myocardial infarction / pulmonary embolism	3	8	6	17
Total	35	154	81	270

The diagnosis of hepatic hydrothorax is suspected in cirrhotic patients in advanced stages of disease presenting pleural effusion, most often unilateral and on the right side.

Of the 270 patients in the subgroup with pleural effusion, we established a diagnosis of hepatic hydrothorax in a number of 48 patients.

Patients with hepatic hydrothorax were aged between 42 years and 66 years, corresponding to the distribution of the maximum incidence of cirrhosis in our country (Table 2).

Table 2 – ***Age of patients with hepatic hydrothorax***

	Mean	Standard deviation	Standard error	No.	Min.	Max.
Total	52.521	6.51	0.94	49	42	66
Alcohol	56.444	6.67	2.224	9	48	65
HBV	50.1	3.071	0.971	10	47	53
HCV	53.75	6.519	2.305	8	47	65
HBV + Alcohol	52.2	7.855	3.513	5	44	64
HCV + Alcohol	51.5	6.979	2.849	6	47	65
HBV + HCV	55.5	11.832	5.916	4	42	66
Immune	49	3.464	2	3	47	53
Cryptogenic	50	2.646	1.528	4	48	53

Basically, there were no significant differences in relation to age, etiology and gender of patients, however hepatic hydrothorax was present only in Child B functional class (eight cases, 16.66%) and Child C (40 patients, 83.34%), demonstrating a statistically significant correlation with the functional class.

The cytological study in these patients revealed different aspects depending on the etiology of effusion. Cytological examination of the hydrothorax fluid revealed smears showing low cellularity, consisting of rare inflammatory and mesothelial exfoliated cells (Figure 1).

Smears from pleurisies associated with bacillary etiology smears showed low to moderate cellularity, composed mostly of lymphocytes, as well as rare exfoliated mesothelial cells, often isolated or grouped as spheres or knobs (Figure 2).

Mesothelial cells were spherical or oval, well-defined cytoplasm and centrally located large nuclei with a uniform overall appearance. Direct examination of pleural fluid using the Ziehl–Neelsen staining revealing the Koch bacilli had diagnostic utility (Figure 3, a and b).

The eight cases of AIDS accompanied by sero-fibrinous pleurisy also associated pulmonary tuberculosis in four cases and *Pneumocystis carinii* pneumonia in the other four cases. In these cases, besides lymphocytes and exfoliated mesothelial cells there were exfoliate there were also frequent polymorphonuclear cells. Reactive mesothelial cells were isolated, reduced in number, large and often binucleated (Figure 4).

Similar aspects were present in parapneumonic pleural effusions together with the presence of large numbers of polymorphonuclear cells, rare exfoliated mesothelial cells with reactive changes similar to those of those from other sero-fibrinous pleural effusions (Figure 5).

In a number of 37 cases, sero-fibrinous pleural effusions were associated with malignant neoplasms, which, in five cases, corresponded to epithelial mesothelioma, and in 32 cases to lung carcinoma with metastasis in the pleura. In the five cases diagnosed as mesothelioma smears were to hypercellular and were characterized by the presence of mesothelial-type cells, isolated or compact cell groups with smooth edges or papillary structures without fibro-vascular axis (Figure 6, a and b).

Neoplastic mesothelial cells showed a very broad morphologic spectrum, which ranged from cells with normal size to abnormally large cells with one or multiple large nuclei and a clearly visible pleomorphism (Figure 7).

In 13 cases smear appearance corresponded to lung adenocarcinoma with pleural metastases which was later confirmed histologically and immunohistochemically. We observed hypercellular smears with necrotic background, composed of tumor cells arranged in groups, sometimes with acinar arrangements. They had cuboidal, columnar or polygonal shape, variable dimensions, scant or moderate cyanophilic cytoplasm, and large round or oval nuclei, often eccentric, with granular chromatin and prominent nucleoli (Figure 8, a and b).

In 19 cases, the appearance of the smear allowed the suspicion of well and moderately differentiated squamous lung carcinomas, with highly cellular smears containing individual cells or arranged in three-dimensional cohesive groups within a necrotic background. Neoplastic cells were round, oval or elongated, spindle-shaped, with clear boundaries and dense eosinophilic cytoplasm, large hyperchromatic nuclei and without clearly visible nucleoli (Figure 9, a and b).

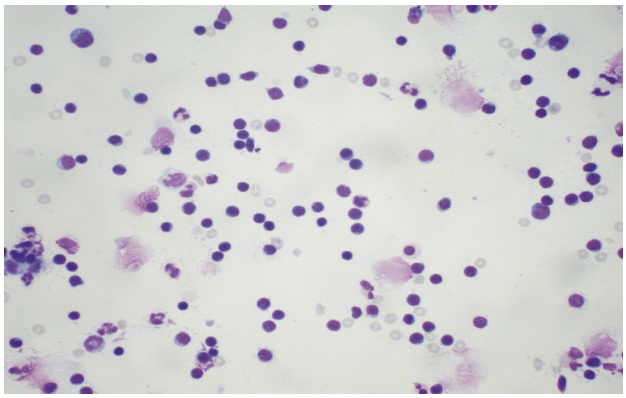


Figure 1 – *Smear from hydrothorax pleural effusion, low cellularity, consisting of rare inflammatory and mesothelial exfoliated cells (Giemsa stain, ×100).*

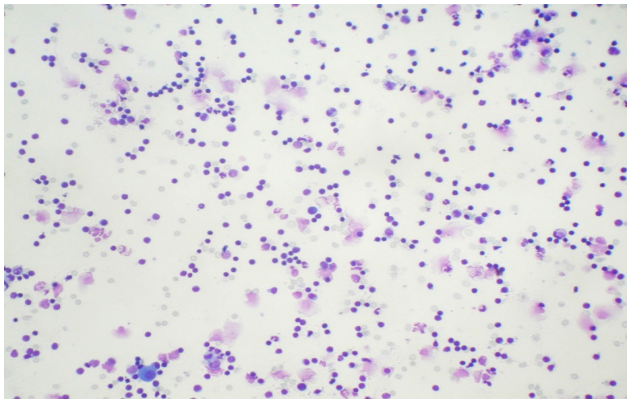
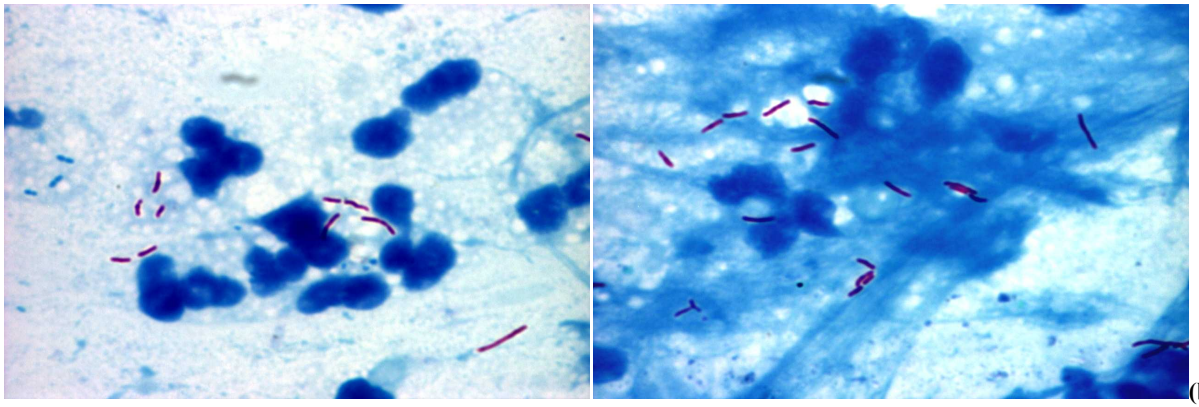


Figure 2 – *Smear from bacillary pleural effusion with moderate cellularity, numerous lymphocytes and rare reactive mesothelial cells (Giemsa stain, ×40).*

(a)



(b)

Figure 3 – *(a) and (b) Smear from bacillary pleural effusion – details: Mycobacterium tuberculosis (Ziehl–Neelsen stain, ×1000).*

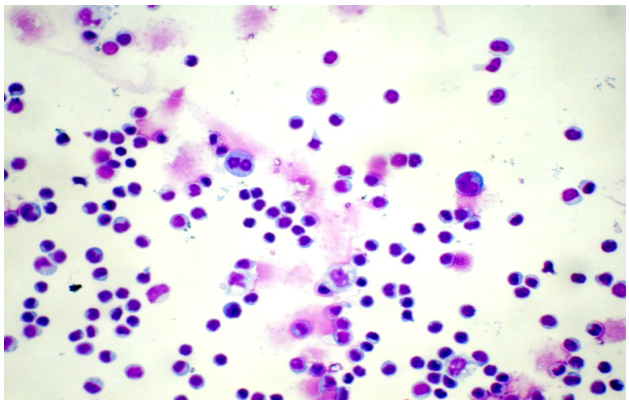


Figure 4 – *Smear from SIDA sero-fibrinous pleural effusion, polymorphonuclear cells and isolated reactive mesothelial cells (Giemsa stain, ×100).*

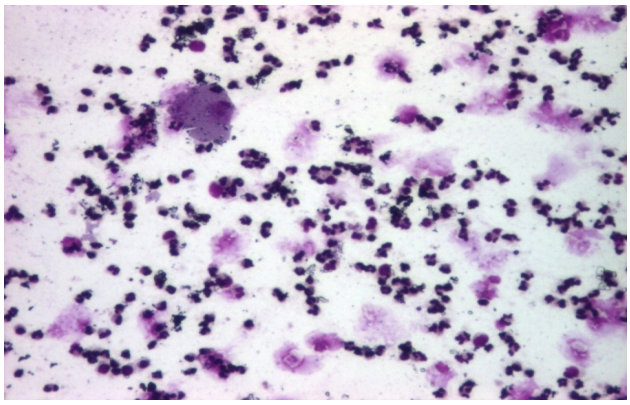
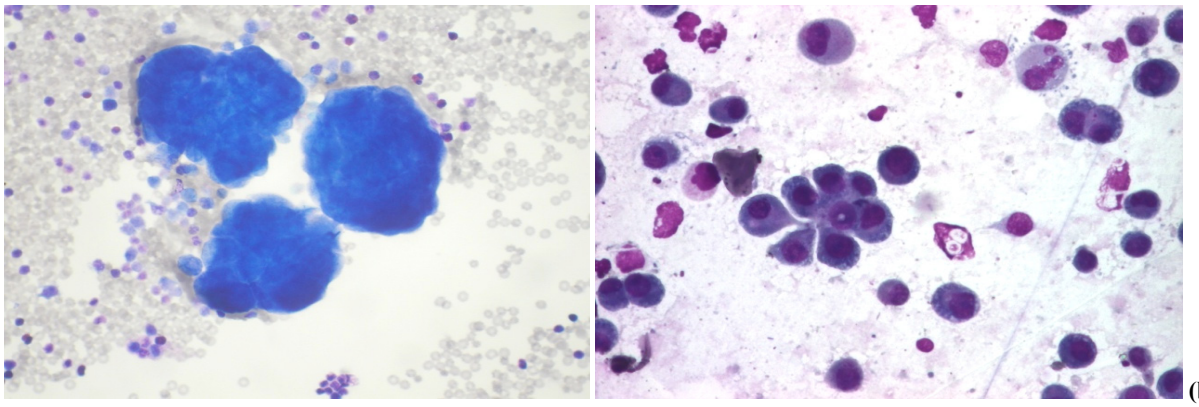


Figure 5 – *Smear from parapneumonic pleural effusion, frequent polymorphonuclear cells, rare reactive mesothelial cells (Giemsa stain, ×100).*

(a)



(b)

Figure 6 – – *Smear from epithelioid mesothelioma pleural effusion: (a) "Ball-like" pattern (Giemsa stain, ×100); (b) Papillary pattern (Giemsa stain, ×200).*

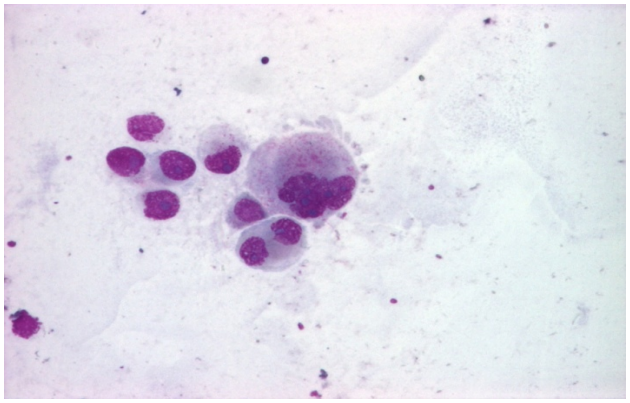
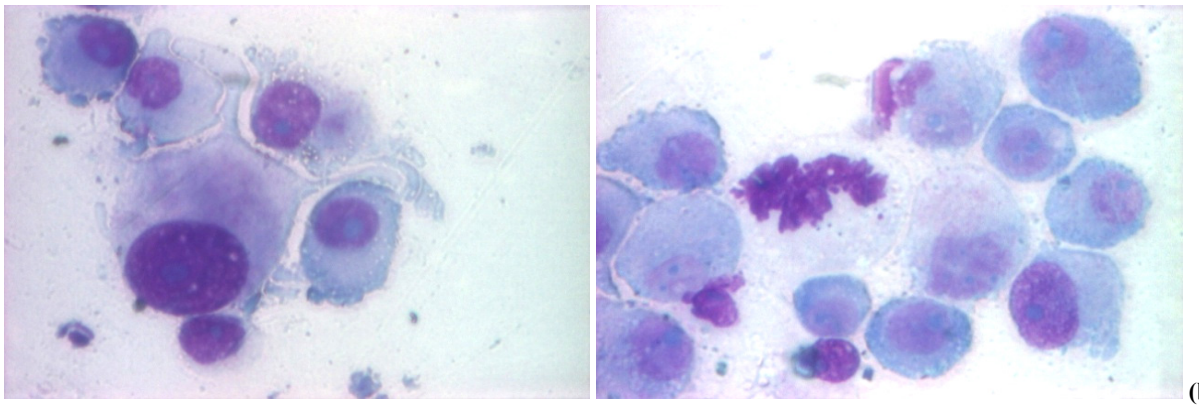
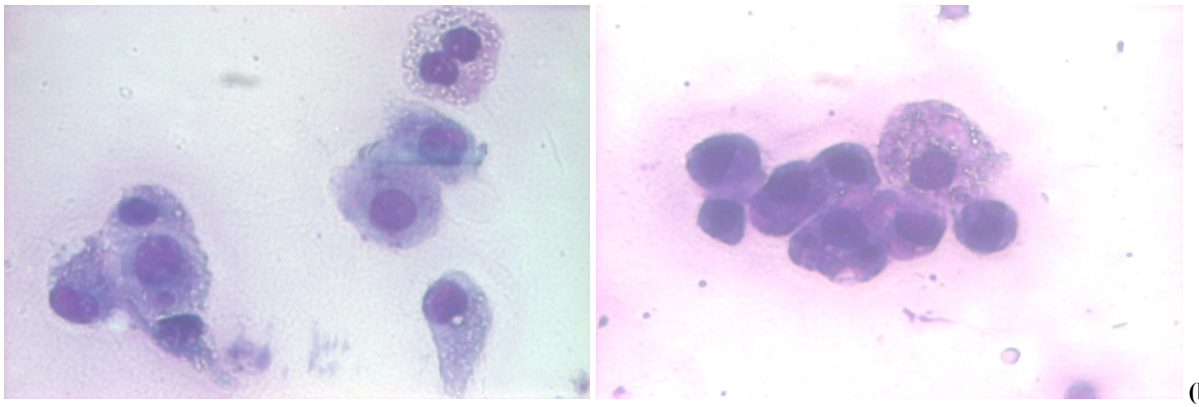


Figure 7 – ***Smear from epithelioid mesothelioma pleural effusion with atypical cells with pleomorphic nuclei and clearly visible nucleoli (Giemsa stain, ×200).***



(a) **(b)**

Figure 8 – ***Smear from lung adenocarcinoma pleural effusion: (a) Large nuclei with prominent nucleoli (Giemsa stain, ×400); (b) Atypical mitosis (Giemsa stain, ×400).***



(a) **(b)**

Figure 9 – ***Smear from moderately differentiated squamous carcinoma pleural effusion: (a) Cohesive groups of cells, isolated spindle cells (Giemsa stain, ×200); (b) Groups of cohesive cells (Giemsa stain, ×200).***

Discussion

The right side hepatic hydrothorax was present in 38 (79.16%) patients, the left side hepatic hydrothorax in six (12.5%) patients, while the bilateral one was encountered in four (8.34%) patients.

The data we obtained is consistent with other studies in the literature. Cardenas A *et al.* (2004) mention the presence of hepatic hydrothorax on the right side in 85% of cases with a bilateral involvement in 15 to 30% of the cases [6].

Reichen J (2007) communicates the presence of hepatic hydrothorax in the right hemithorax in 65% of the cases, 15% in the left hemithorax, with 15–30% of the cases having bilateral location [10].

Questions were raised as to why this type of distribution of hepatic hydrothorax. The causes leading to the development of hydrothorax are incompletely understood. A mechanism for hydrothorax development is supposed to be the existence of communications between the abdominal cavity and chest cavity, through which ascites fluid enters the pleura [11, 12].

Both Cardenas A *et al.* (2004) and Chen TA *et al.* (2003) as well as others have demonstrated the presence of small trans-diaphragmatic communications that allow the unidirectional flow of the fluid from the abdominal cavity towards the pleural cavity where the pressure is negative. These defects were highlighted by computer tomography or thoracoscopy, but these are high-risk methods in patients with an already altered state of health. Cardenas A *et al.* injected intraperitoneally 99mTc–colloidal sulfur or 99mTc–serum albumin and found the presence of the radiotracer in the pleural cavity even provided that the patient has no simultaneous ascites [6, 7].

Another speculative hypothesis is the increased pressure in the azygos vein and the passage of the fluid from the abdomen into the pleura through the trans-diaphragmatic spaces of lymphatics [12, 14].

Benet A *et al.* (1992) have studied the presence of hydro-thorax in cirrhotic patients and using the radiotracer of 99mTc–human albumin or 99mTc–colloidal sulfur, concluded that there is a unidirectional communication of fluid from the abdominal cavity to the pleura [13].

Another speculative hypothesis is the increased pressure in the azygos vein and the passage of the fluid from the abdomen into the pleura through the trans-diaphragmatic spaces of lymphatics [12, 14].

In our study group, hepatic hydrothorax was present in 27 women and 21 men (48 cases in total, 17.7%), a percentage that is consistent with the results obtained by other authors. Of the 48 patients diagnosed with hepatic hydrothorax, a total of 43 (89.58%) patients had simultaneous ascites and five (10.41%) patients had no ascites.

Apart from the presence of these anatomical trans-diaphragmatic defects there were other questions as to which other factors are involved in the development of hepatic hydrothorax.

Eid AA *et al.* (1999) suggested a change in the balance of Starling forces due to hypoalbuminemia that characterizes most patients with liver cirrhosis in advanced stages of the disease. They studied a total of 594 patients with pleural effusion of which 296 patients had hypoalbuminemia [15]. Patients with hypo-albuminemia were divided into three subgroups: albumin >3.5 g/dL, albumin between 2.1 and 3.5 g/dL, and the third group of with albumin <2 g/dL. Following the analysis of the three subgroups of patients, they concluded that the presence of pleural effusion did not differ significantly among them, so they concluded that decreased albumin alone does not lead to pleural effusion, with other possible associated pathogenic conditions influencing this phenomenon [16–18].

It can be said that there are multiple and confounding mechanisms that contribute to the development of hepatic hydrothorax. Thus, some studies have indicated more pathogenic links. Similar to what happens in the case of cirrhotic ascites, it is considered that in the case of hydrothorax, the increase in hydrostatic pressure within the azygos vein together with the hypertension in portal-pulmonary anastomoses between paraesophageal and pulmonary veins are involved [19, 20].

Another mechanism involves the lymphatic drainage. Thus, in cirrhotics, the ascites fluid absorbed by lymphatics is directed through the subdiaphragmatic lymphatic plexi towards the subpleural plexus.

The third mechanism for hepatic hydrothorax development is the direct trans-diaphragmatic crossing of abdominal fluid through the anatomical defects. Anatomical defects were seen in 20% of necropsies. They are usually less than 1 cm in size and are located in the tendinous portion of the diaphragm. Negative pleural pressure favors the passage of the fluid from the abdominal cavity to the pleural one even in the case of a small amount of liquid [11, 12].

Hypoalbuminemia, even if not a determinant factor per se, together with other mechanisms, could explain the presence of hepatic hydrothorax [15].

We found a high statistical correlation between the presence of hydrothorax and the functional class. There were no cases in Child A class, eight cases Child B class (16.66% of cases with hydrothorax), and 40 cases (83.34% of cases with hydrothorax) in Child C functional class.

The presence of hydrothorax was also significantly correlated with the serum albumin level, a fact noted by other similar studies (Table 1).

A total of 12 patients were diagnosed with spontaneous pleural empyema (by similarity with spontaneous bacterial peritonitis). Of the 12 patients with spontaneous pleural empyema, eight patients had positive cultures (five with *Escherichia coli*, one with *Klebsiella pneumoniae*, one patient with *Staphylococcus*, and one with *Pseudomonas*). In the other patients, cultures did not grow on the media used [21, 22].

Nine patients were enrolled in Child C class and three patients in Child B class, correlation that was statistically significant.

Also, serum albumin showed values below 3 g/dL in all patients with spontaneous pleural empyema.

Chen TA *et al.* (2003) found a number of 862 cases with liver cirrhosis, 132 cases with hepatic hydrothorax, of which 17 cases (13%) showing spontaneous pleural empyema [7]. In their study, they found statistically significant correlations with the functional class, low albumin level and prolonged prothrombin time.

Various studies suggest that spontaneous empyema occurs by two mechanisms: spontaneous bacteremia as in spontaneous bacterial peritonitis or by the passage of infected ascites *via* diaphragmatic defects. Normally bacteria are filtered by the liver, but in the context of the existence of a portosystemic shunt blood bypasses the liver and thus bacteremia is more frequent and prolonged. In the case of spontaneous bacteremia blood and pleural fluid cultures are positive for the same organisms, and in case of infections via diaphragmatic defects pleural fluid and ascites cultures are positive for the same microorganism [22, 23].

The macroscopic appearance of spontaneous pleural empyema is generally transparent, yellow-citrine, and it does not clot. Generally, in parapneumonic empyema there are over 250 000 PMN/mm^3 and the fluid is turbid.

Also, all published studies confirm that the main causes of spontaneous pleural empyema are decreased complement levels and opsonic activity [24–27].

The importance of diagnosing spontaneous pleural empyema lies in the high mortality, which it determines (in some studies even up to 38% of the cases) [22].

In the case of febrile patients with advanced cirrhosis and pleural effusion more frequently on the right side, one should bear in mind this diagnosis and antibiotic therapy according to the antibiogram or broad spectrum antibiotic therapy are required from the start.

Pleural effusion is a common diagnostic problem for the pneumologist, often interdisciplinary, due to the numerous benign or malignant conditions, which can cause them. At present, thoracentesis and pleural fluid cytology are usually the first step in the diagnosis.

Assessing pleural aspirate cell type may be useful for the differential diagnosis [28]. Polymorphonuclear predominance reflects an acute process, which is most likely a parapneumonic effusion. However, polymorphonuclear cells are also observed in effusion due to pulmonary embolism, tuberculosis and asbestosis [29]. The presence of pleural lymphocytes is associated most often with tuberculosis or malignancy. However, up to 10% of bacillary effusions are predominantly polymorphic exudates [30], while on the other hand many lymphocytes may also be present in sarcoidosis, rheumatoid pleuritis and chylothorax [28]. Most authors communicate similar issues, reporting tuberculous pleural exudates that are usually characterized by a predominance of lymphocytes and a few exfoliated mesothelial cells [31, 32].

The sensitivity of conventional cytology for the identification of neoplastic cells in pleural effusion is approximately 60%, and the rate of an uncertain diagnosis is 6% [33–35]. Differentiating benign from malignant mesothelial proliferations or of the secondary or primitive origin of neoplasia represents a major problem in the pathology of serous membranes. The study of smear appearance allowed the estimation of malignant etiology in all cases. However, the tumor type, later confirmed histologically and immunohistochemically, was identified only in 72% of the cases. The cytological diagnosis of mesothelioma consists in identifying malignant cells followed by the identification of mesothelial characteristics of neoplastic cells [36–38]. The correct cytological diagnosis of an epithelioid mesothelioma can be established in the presence of numerous groups or large aggregates of epithelioid cells, together with the presence of individual and small groups of epithelioid cells [37, 39].

Conclusions

Hepatic hydrothorax is a relatively rare complication of liver cirrhosis. Hepatic hydrothorax is significantly correlated with the Child functional class. Decreased serum albumin is a contributing factor, but not sufficient for the development of hepatic hydrothorax.

The development of spontaneous pleural empyema is a formidable complication in patients with hepatic hydrothorax, which requires targeted or broad-spectrum antibiotics as early as the time of diagnosis.

The study of pleural fluid cytology in patients with liver cirrhosis associated pleurisy allowed the orientation of the etiologic diagnosis.

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