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Pleurisies – the experience of "Tudor Vladimirescu" Hospital of Pneumology II: morphological study

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Abstract

Pleural effusions are still representing a challenge in daily practice. *Materials and Methods*. This retrospective study on 221 patients with pleurisies hospitalized in our unit is focused on the contribution of different types of pleural fluid morphological evaluation in setting a correct etiological diagnosis. The algorithm of investigation included: gross aspects assessment on X-ray records and by direct observation of pleural liquid obtained by thoracentesis and microscopic assessment on cytology slides of pleural fluid and on histopathological samples obtained by pleural needle biopsies. *Results*. Mycobacterial etiology was the most frequent, with 72% of all cases, followed by tumoral etiology. Cytologic examination of pleural fluid was useful in establishing the final diagnosis in 66.1% of cases, histopathological assessment being imposed for the rest of cases. *Discussion*. Imagistic investigation offered appropriate information concerning the site and extention of pleural effusions and guided, in certain cases, the needle biopsy. Gross aspect of pleural fluid oriented quite well the suspicion diagnosis. The use of a set of cytological "formulas" was useful in filtering subsequently the suspicion diagnosis. Histopatholgical examination of pleural tissue samples established the final diagnosis in cases where etiology was still uncertain after laboratory and cytological examination and subtyped further the pathologic processes within each main category of etiology. *Conclusions*. A correct diagnosis of pleural effusions could be achieved only by going through a precise algorithm of investigation where, besides thorough clinical examination and laboratory tests especially of pleural liquid, morphological assessment and in particular cytologic examination of pleural liquid and histopatological examination of pleural tissue samples are essential.

Keywords: pleural effusion, pleurisy, cytology, histopathology.

Introduction

Pleural effusions are pathologic processes that are still putting problems to pneumologist and not only in the daily practice. One of them is, as we mentioned before, the wide range of etiological factors, which generate not rarely a more or less overlapping of clinical pictures or paraclinical investigations results. Another important issue is the disabling potential of pleural effusions knowing that an inefficient therapeutical strategy may result in an important cut of the respiratory function [1]. Therefore, the sooner the positive and especially etiologic diagnosis is established, the better is for patient's outcome.

The paraclinical investigation arsenal is so diversified that only the medical culture of the one which is establishing the diagnosis and the available paraclinical investigations he has are crucial. Besides the laboratory tests of pleural fluid, cytological examination of pleural fluid and histopathological examination of pleural tissue samples are ones of the tools of great value in setting out a correct etiological diagnosis.

Standard cytological examination cannot precise diagnosis in all cases. For instance, it does not differentiate between pleural adenocarcinoma and mesotheliomas or between lymphomas and reactive lymphocytosis without special studies [2] even its sensitivity concerning malignant pleural effusions is anyway around 65–71%, depending mainly on repeated thoracenteses but not on larger volumes of pleural fluid [3–9].

Biopsy of parietal pleura, described for the first time in 1955, has become over the years, the most sensitive diagnostic tool, being used when a first cytology is negative in effusions of unknown origin and especially where histology is crucial, such as in tuberculous pleurisy and malignancy [10–14]. The improvements of sampling techniques, which started with closed ("blind") percutaneous needle biopsy continued with

image(CT)-guided biopsy and finished with thoracoscopic biopsy influenced the diagnostic rate which became around 87% for malignancies and 90.1% for tuberculosis in case of thoracoscopic biopsies [2, 15–17].

However, we have to keep in mind that percutaneous needle biopsy can be easily performed on an outpatient basis, while thoracoscopy is more complex and requires hospitalization of the patient, that biopsy needles provide a better yield in tuberculosis than in malignancy, probably due to the different degree of diffuse involvement of the parietal pleura and, finally, that closed pleural biopsy is less sensitive than cytology in malignant effusions, even when both are repeated [18–20].

Therefore, morphological assessment of pleural fluid and pleural tissue, preferable after thoracoscopy for the latter, together with a thorough clinical examination and a complete biochemical investigation of pleural fluid, could offer to the clinician the satisfaction of establishing the right diagnosis for each pleural effusion.

This study represents the second part of an extensive survey of 443 cases with diagnosis of pleural effusion hospitalized in the Pneumology Department of "Tudor Vladimirescu" Hospital of Pneumology, in Gorj county and whose first part, including analysis and discussion of clinical and laboratory parameters, was published above [1].

Materials and Methods

Materials

Our study base consisted of 221 patients selected from the larger group of 443 cases with diagnosis of pleural effusion mentioned above.

Cases were selected based on four valuation criteria of which the first three were mandatory:

- radiologic examination;
- pleural puncture (thoracentesis);
- cytologic examination;
- histopatohologic examination.

The study materials included: X-ray records, pleural puncture records, cytology records and histopathology records.

The selected group was subsequently divided in three series, as we mentioned also in our previous paper [1], according to the pleurisy etiology as follows:

- **Group I**: tuberculous (TB) pleurisy;
- **Group II**: malignant (M) pleurisy;
- **Group III**: infectious (INF) non-TB pleurisy;

Methods

The study was retrospective and was structured in two main stages:

- Gross morphological assessment;
- Microscopic study, with two steps:
  - Cytological assessment;
  - Histopathological assessment.

For cytological examination, smears were prepared from the sediment obtained by pleural fluid centrifugation after supernatant removal and stained with May–Grünwald–Giemsa (MGG) solution.

For histopatological examination, pleural tissue fragments were fixed in 10% neutral buffered formalin solution, embedded in paraffin and the 4 μm sections were stained with Hematoxylin–Eosin (HE) and trichrome van Gieson.

Histopathological examination was accomplished only when the algorithm of examination (clinical paraclinical and morphological) could not precise the diagnosis (Table 1).

Table 1 – ***Cases distribution depending on etiology and type of morphological investigation***

Group	Algorithm investigation			
	TOTAL	Gross	Cytology	HP
Group I – TB	159	159	159	54
Group II – B	41	41	41	14
Group III – INF	21	21	21	7
Total	**221**	**221**	**221**	**75**

The following parameters were assessed:

- Site of pleural effusion which included:
  - location in one of pleural cavities;
  - topography inside the pleural cavity.
- Quantity of pleural liquid;
- Macroscopic aspect of pleural effusion;
- Morphological aspect of cytology specimens;
- Morphological aspect of histopathological specimens.

Data from every patient were introduced in a computer Access database and processed with the module Microsoft Excel, both parts of Microsoft Office 2003 Professional package. Graphical charts were done with the module Microsoft Excel.

The cytopathological and histopathological aspects were selected using an Olympus CX31 microscope with the ×4 eyepiece and the ×4, ×10, ×20 and ×40 Plan Apo objectives. The most significant images were acquired using an Olympus DP12 digital camera and the AnalySIS Pro 3.2 software.

Analysis of some of the parameters required a primary data filtering, consisting in definition of sets of specific categories.

- For **site** assessment – left cavity and right cavity;
- For **topography within pleural cavity** – great cavity, interlobar fissure, diaphragmatic space;
- For **quantity** of fluid: reduced (<250 mL); moderate (250–750 mL); massive (>750 mL);
- For **gross appearance**: serous; citrine; hemorrhagic (serous-hemorrhagic and franc hemorrhagic); purulent (serous-purulent and franc purulent);
- For TB lesions smears:
  - Phase I smears (≅50% lymphocytes – L – + (≅35% neutrophils – PMN – + mesothelial cells);
  - Phase II smears – lymphocytosis between 75% and 95%;
  - Phase IIa smears – purulent fluid (bacillary aggression extremely active).

- For TB lesions on histopathological samples the staging of Sugiyama M and Horiguchi T [17] was used (even the authors designed it mainly for macroscopic appearance of pleural lesions observed during thoracoscopy):
  - Stage I (redness and swelling stage) – the parietal pleura is reddened, swollen, and with tiny white nodules;
  - Stage II (nodule dissemination stage) – the parietal pleura is extensively reddened and swollen, with military white nodules extending diffusely and coalescing together;
  - Stage III (fibrin deposition stage) – white fibrin deposits extend over the pleura in a cord-like and a membrane-like fashion;
  - Stage IV (pleural thickening stage) – the fibrin deposits become fibrous, representing a chronic stage.
- For cytological and histopathological aspects of mesotheliomas, the morphologic criteria and the correspondence between them we used are listed in Table 2.

Table 2 – ***Morphological criteria of classification for mesotheliomas***

	Examination		
	Cytology	***Histopathology (WHO) [21]***	
Morphology	Differentiated	Monophasic	Epithelioid
			Sarcomatoid
		Biphasic	
	Undifferentiated	Undifferentiated	

Results

Gross morphological assessment

Site of pleural effusion

The overall distribution of cases depending on site of pleural effusion showed that right pleural cavity was more frequently affected that the left cavity (Table 3).

Table 3 – ***Site distribution***

Site	No. of cases	%	R/L ratio
Left cavity	92	41.6	
Right cavity	***129***	***58.4***	**1.4**
Total	**221**	**100**	

Within the same pleural cavity, pleural effusions were mostly located in the great cavity (almost 80% of patients) (Table 4).

Table 4 – ***Topography within the same pleural cavity***

Site	No. of cases	%
Great pleural cavity	***176***	***79.6***
Interlobar fissure	39	17.6
Diaphragmatic	6	2.7
Total	**221**	**100**

Sequestered collections in one of the interlobar fissures or in diaphragmatic space of pleural cavity were observed less frequently.

Pleural fluid quantity

Another evaluated parameter was the quantity of liquid accumulated in the pleural cavity. The volume of liquid was estimated through radiological examination and directly measured after thoracentesis.

Moderate accumulations of fluid, between 500 mL and 750 mL, were found in almost 80% of patients. (Table 5).

Table 5 – ***Distribution depending on fluid quantity***

Quantity	No. of cases	%
Reduced	12	5.5
Moderate	***174***	***78.7***
Massive	35	15.8
Total	**221**	**100**

Massive collections, exceeding 1000 mL were present in a significant number of patients whereas pleurisies with reduced quantity of fluid were rare.

Gross appearance of the effusion fluid

The most valuable gross parameter, which could really offer a key for orienting the diagnosis, was, however, the gross aspect of the liquid pulled out through thoracentesis.

In almost half of the patients, pleural fluid had a citrine appearance. The next aspect, in order of frequency, was the hemorrhagic appearance, observed in 62 patients, meaning almost 30% of cases; it should be noted that more than half of these patients had a clear-cut hemorrhagic fluid (Table 6).

Table 6 – ***Distribution depending on pleural fluid gross aspect***

Type of gross aspect		No. of cases		%	
Serous		*18*		*8.1*	
Citrine		***102***		***46.2***	
Hemorrhagic	Serous-H	27	*62*	12.2	*28.1*
	Hemorrhagic	35		15.9	
Purulent	Serous-P	18	*39*	8.1	*17.6*
	Purulent	21		9.5	
Total		**221**		**100**	

The purulent appearance was observed in only almost 18% of all cases, i.e. 39 patients, the franc purulent pattern being present in more than half of these cases.

The rarest gross aspect was the serous one, encountered in less than 10% of all cases.

Microscopic assessment

Pleurisies with serous pleural fluid

Cytologic assessment

The smears of the 18 cases with serous appearance of the pleural fluid showed a polymorphous cellular population scattered in small groups all over the slide, including some lymphocytes, some neutrophils (PMN) and even rare mesothelial cells (Figure 1).

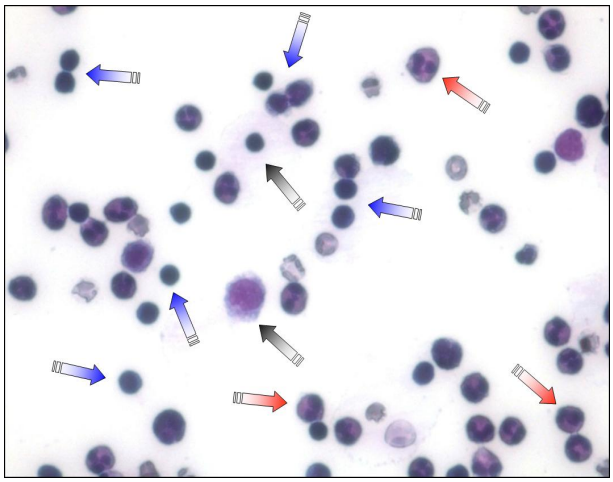

Figure 1 – ***Pleural fluid smear: inconclusive – lymphocytes (blue arrows); neutrophils (red arrows); mesothelial cells (black arrows) (MGG stain, ob. ×40).***

This inconclusive cytologic picture imposed further a pleural biopsy to elucidate the diagnosis.

Histopathological assessment

Histopathological samples revealed in 15 of these cases isolated granulomatous lesions with central incipient caseous necrosis, either of epithelioid (Figure 2, blue arrow) or giant Langhans cells (Figure 2, red arrow) types.

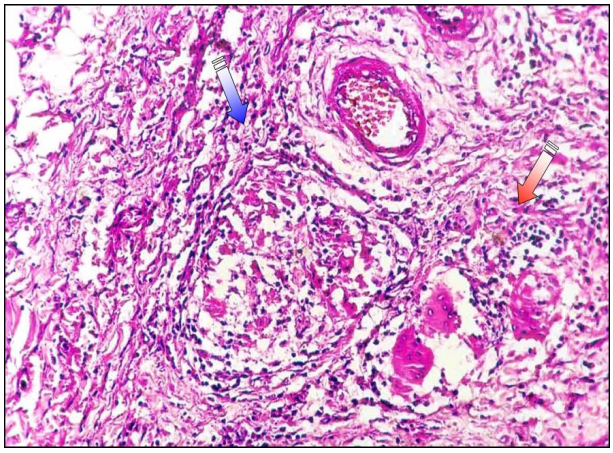

Figure 2 – ***Pleural tuberculosis (HE stain, ob. ×20).***

This morphological picture allowed us to establish the diagnosis of pleural tuberculosis in the first phase of evolution and Sugiyama's Stage I.

In the remaining three cases, histopathological examination revealed a chronic, nonspecific inflammatory process in different stages of evolution.

The inflammatory process was observed in different stages of evolution, from granulation tissue which was organizing the submesothelial structures and the exudate present in pleural cavity (Figure 3a) to fibrosis more or less extended even with vascular hyalinisation sometimes (Figure 3b). In one case, xantogranulomatos cells could be observed within the granulation tissue (Figure 3c).

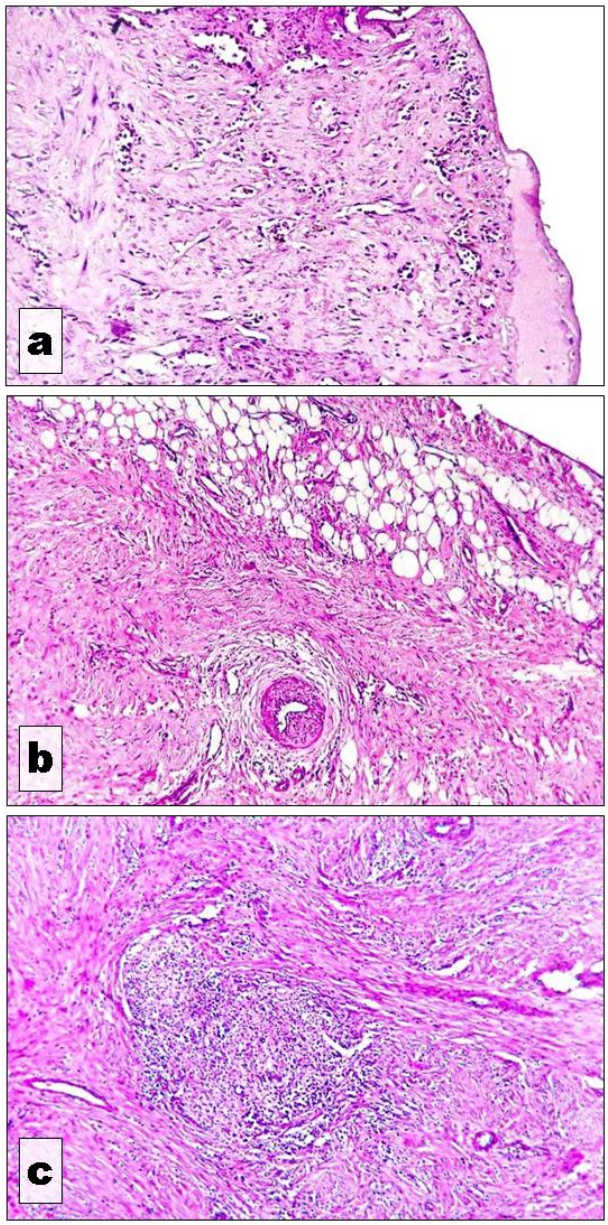

Figure 3 – ***Chronic inflammation of parietal pleura (HE stain, "a" – ob. ×4; "b" and "c" – ob. ×10).***

Pleurisies with citrine pleural fluid

Cytologic assessment

Of the 102 patients whose pleural fluid had citrine appearance, 87 had a smear fully dominated by lymphocytes, strongly suggesting a TB evolving already in the second phase and Sugiyama's Stage II (Figure 4).

Diagnosis was sustained also by clinical and paraclinical investigations.

In other three cases, the cytologic picture was made mainly of mesothelial cells with malignant features and undifferentiated aspect, arranged in irregular, large groups, suggesting a mesothelioma (Figure 5).

Other four cases with citrine pleural fluid had a cytological picture that was confirming the viral etiology established by clinical and paraclinical investigations. The acute viral inflammation was suggested by the significant presence of lymphocytes (Figure 6 – blue arrows), but also of neutrophils

(Figure 6 – orange arrows) and, very important, of some eosinophils (red arrows).

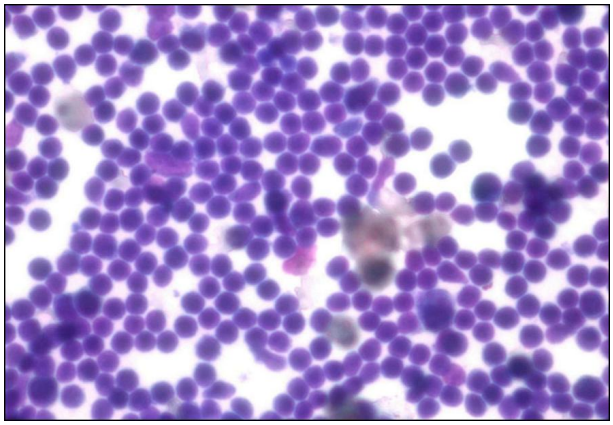

Figure 4 – ***Pleural fluid smear: lymphocytes over 90% (MGG stain, ob. ×40).***

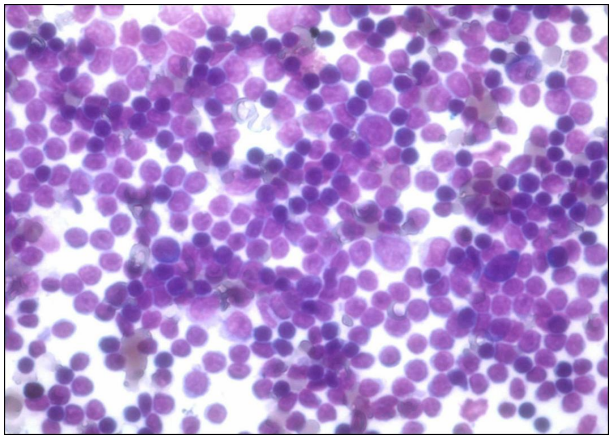

Figure 5 – ***Pleural fluid smear: malignant mesothelial cells + lymphocytes (MGG stain, ob. ×40).***

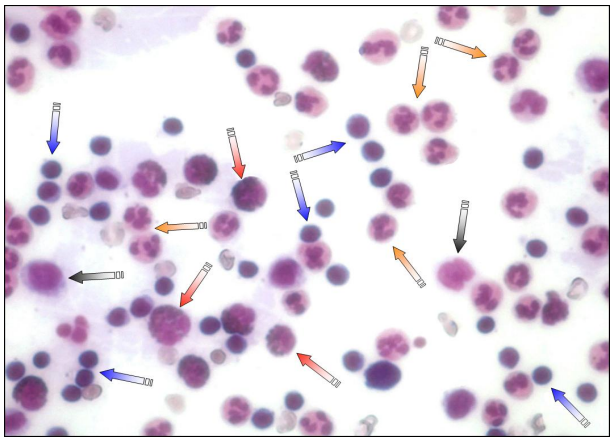

Figure 6 – ***Pleural fluid cytology: lymphocytes + some neutrophils + some eosinophils (MGG stain, ob. ×40).***

Histopathological assessment

In eight patients with citrine appearance of the pleural fluid, the cytological smear was doubtful, imposing a pleural needle biopsy.

Histopathological examination of pleural tissue samples showed the presence of an inflammatory process in two of these patients. In one of these cases, the morphological pattern of the inflammation was acute, with serous-hemorrhagic exudate covering the pleural surface (Figure 7a – blue arrow) and mild hemorrhagic infiltrate in submesothelial space, but also deeply in parietal pleura (Figure 7, a and b – red arrows).

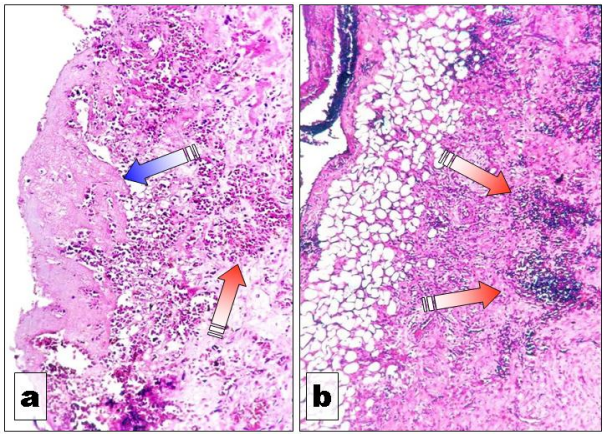

Figure 7 – ***Hemorrhagic inflammation of pleura (HE stain, "a" – ob. ×10; "b" – ob. ×20).***

In the other case, the inflammatory reaction just moved to proliferative stage.

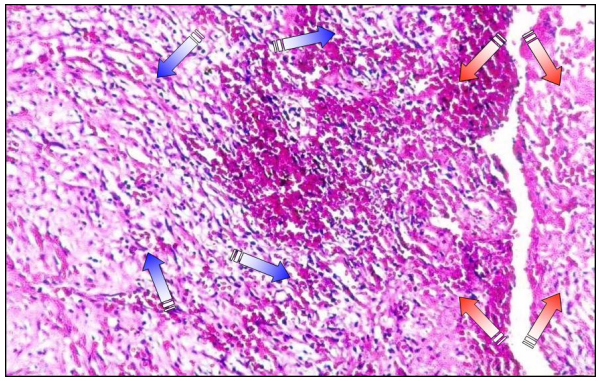

Figure 8 – ***Chronic inflammation of pleura (HE stain, ob. ×20).***

The granulation tissue recently formed in the submesothelial space (Figure 8 – blue arrows) is in process to organize the fibrinous deposits laying on the pelural surface (Figure 8 – red arrows).

In the remaining six cases, tumoral proliferations of biphasic type, with epithelial component organized in tubules, papillae or nests and stromal component mainly of fibrous type, were observed, confirming the clinical suspicion of mesothelioma (Figure 9).

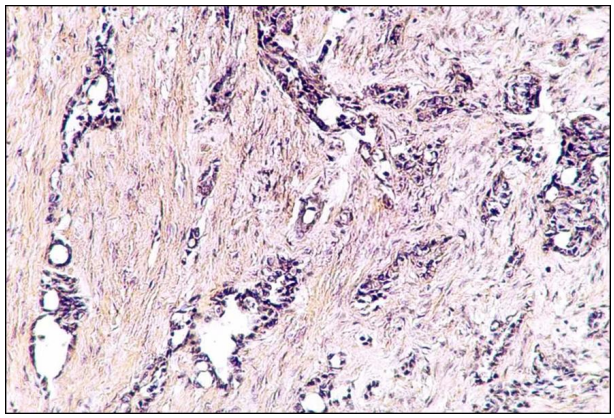

Figure 9 – ***Biphasic mesothelioma with epithelial compound of tubular-papillary pattern (van Gieson's stain, ob. ×10).***

Pleurisies with hemorrhagic pleural fluid

Cytologic assessment

Of the 62 patients whose pleural fluid had less or more franc hemorrhagic appearance, 21 had a suggestive picture for a malignancy.

In one case, malignant mesothelial cells of differentiated type forming extended but well-defined strands on a background composed mostly of erythrocytes and few scattered lymphocytes were observed, confirming the clinical suspicion of a mesothelioma (Figure 10).

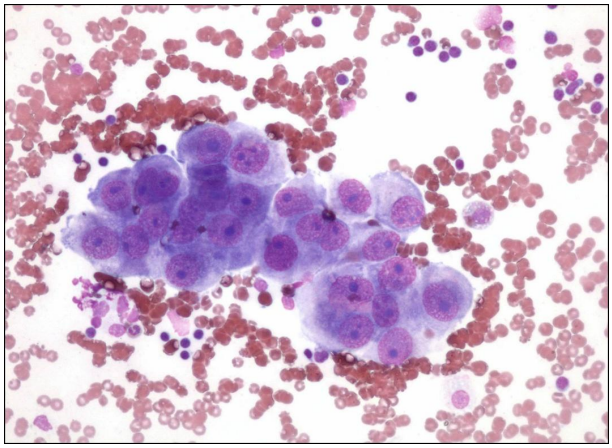

Figure 10 – ***Pleural fluid cytology: differentiated mesothelioma; hemorrhagic pleural fluid (MGG stain, ob. ×20).***

In the remainder 20 cases, the cytologic picture confirmed the pleural involvement in the pulmonary malignancies discovered at clinical and imagistic examination. The cytologic argument was the presence of either isolated or clustered cells with clear morphological characteristics of malignancy (Figure 11).

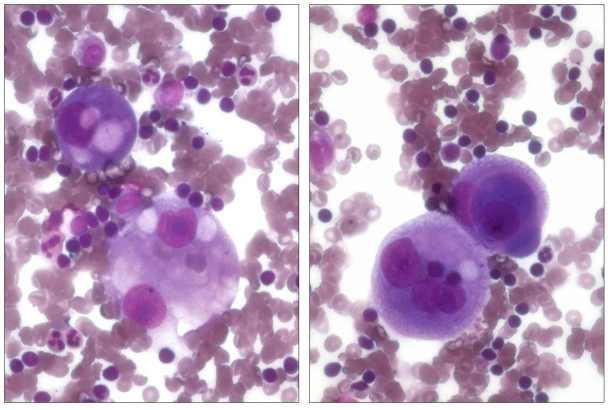

Figure 11 – ***Pleural fluid cytology: monstrous malignant cells with large nuclei, irregular in shape, sometimes multiple, with prominent nucleoli and intra-nuclear vacuoles (MGG stain, ob. ×40).***

Histopathological assessment

The remainder 41 patients whose pleural fluid had a gross hemorrhagic appearance presented inconclusive microscopic features on cytologic smears, either only erythrocytes (Figure 12a) or erythrocytes as main cellular population, accompanied by a smaller number of lymphocytes (Figure 12b).

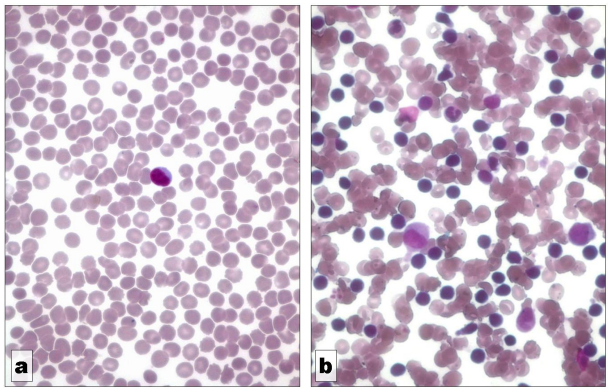

Figure 12 – ***Pleural fluid cytology: predominant hemorrhagic pattern (MGG stain, ob. ×40).***

As in previous situations with doubtful results of cytologic examination, pleural needle biopsy was accomplished.

In 33 of these patients, the histopathological examination revealed pathognomonic lesions for TB.

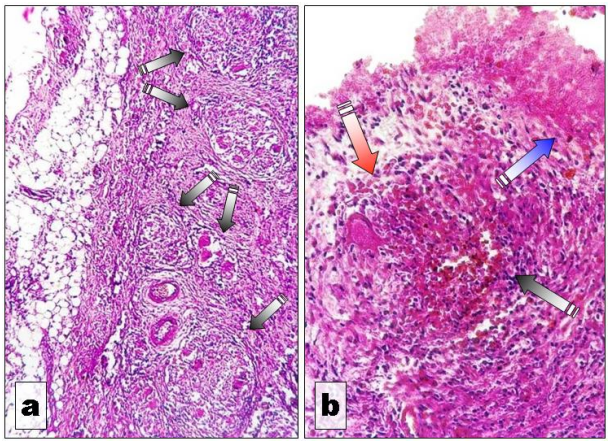

Figure 13 – ***Pleural tuberculosis (HE stain, "a" – ob. ×4; "b" – ob. ×20).***

The entire range of granulomatous features could be observed, i.e. epithelioid granulomas, giant cell granulomas (Figure 13a and Figure 13b – red arrow) with or without central foci of caseous necrosis (Figure 13b – black arrow). Granulomatous lesions were numerous, with a confluence trend (Figure 13a – black arrows) which pleads for classification of these patients in second phase of evolution.

Moreover, in 21 cases deposits of fibrin were covering the mesothelial surface (Figure 13b – blue arrow), meaning that these patients could be classified as Sugiyama's Stage III. The remainder 12 cases with no fibrin deposits but only specific granulomas in submesothelial space wee classified as Sugiyama's Stage II.

In other five patients, the histopathological picture showed the typical morphological aspects of a mesothelioma, but also the argument for the cytological appearance of the pleural fluid, i.e. hemorrhagic foci within tumoral proliferation (Figure 14).

Finally, the last three patients of 41 with hemorrhagic fluid had histopathological pictures of pulmonary carcinomas that invaded the pleura. In two cases, the metastatic tumor was an adenocarcinoma of solid type, with tight cellular proliferations, which were defining

very narrow lumens (Figure 15a). In the third case, the metastatic tumor was a poorly differentiated squamous carcinoma, with malignant cells tightly packed and mild dyskeratosis (Figure 15b).

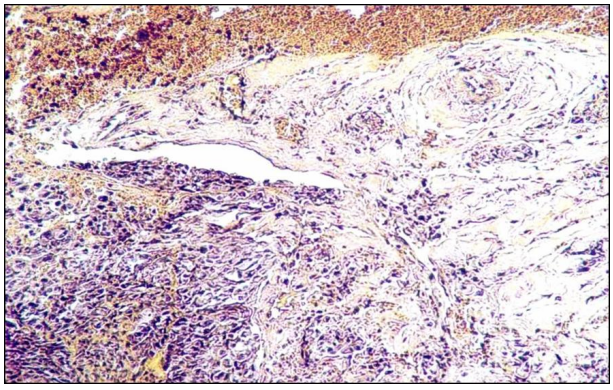

Figure 14 – ***Mesothelioma (van Gieson's stain, ob. ×10).***

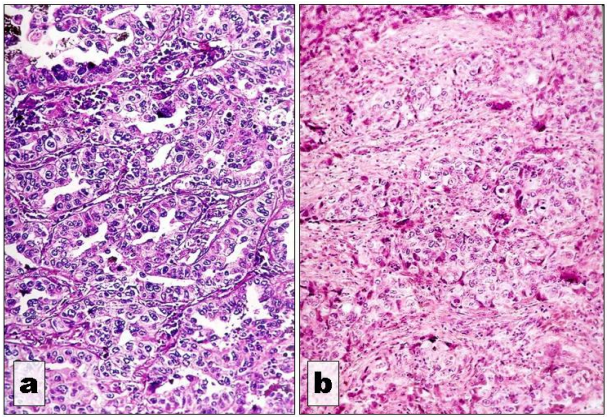

Figure 15 – ***Metastatic tumors: "a" – Pulmonary adenocarcinoma, solid type; "b" – Pulmonary squamous carcinoma, poorly differentiated (HE stain, ob. ×10).***

Pleurisies with purulent pleural fluid

Of the 39 patients whose pleural fluid had a less or more franc purulent appearance, 31 had a cytological picture that, together with clinical, laboratory and imagistic date was sufficient to establish the diagnosis.

Cytologic assessment

In 15 of these 31 cases, the pleural fluid had a serous – purulent aspect. The cytological smear revealed a cellular population with two main types: lymphocytes and neutrophils, the latter without morphological alterations (Figure 16). The clinical and paraclinical data were pleading for a tuberculous pleurisy in the first phase of evolution and the cytological aspect was in accordance to and confirming the diagnosis.

In other three cases, the clinical and paraclinical data were pleading for a tuberculous etiology but patients' condition was not good.

The results of cytological examination revealed a cellular population dominated by neutrophils, some of them being altered, and only few lymphocytes (Figure 17) which raised the suspicion of a bacterial infection overlapping the tuberculous one, suspicion confirmed by the subsequent microbiologic determinations of the pleural fluid.

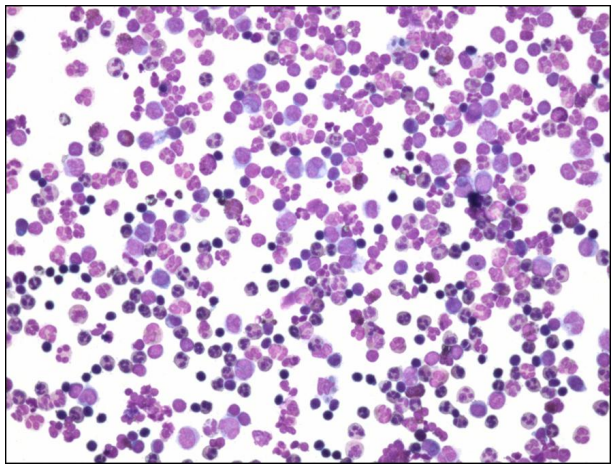

Figure 16 – ***Pleural fluid cytology: lymphocytes + intact neutrophils (MGG stain, ob. ×20).***

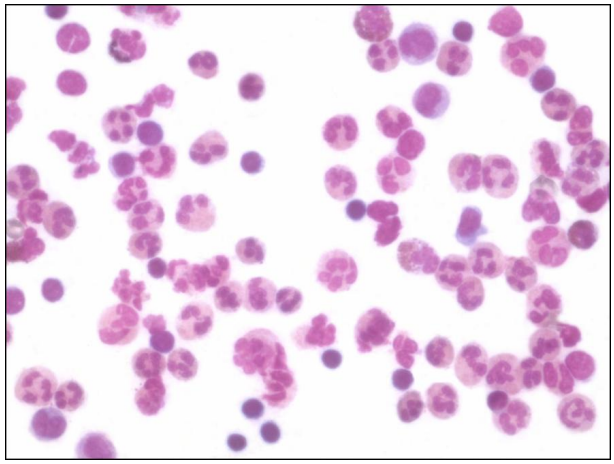

Figure 17 – ***Pleural fluid cytology: intact neutrophils + lymphocytes (MGG stain, ob. ×40).***

Other 10 cases had a pleural fluid with a franc purulent appearance. Clinical data were suggestive for an acute infectious condition.

The cytological picture was characteristic for a bacterial pleurisy, with almost only neutrophils in the microscopic field, many of them being morphologically altered (Figure 18).

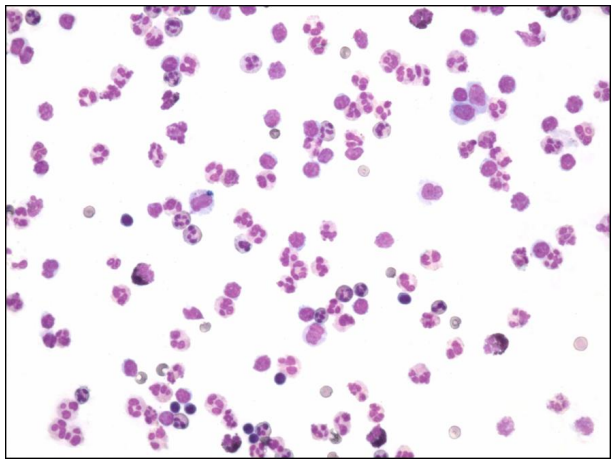

Figure 18 – ***Pleural fluid cytology: neutrophils, many of them altered (MGG stain, ob. ×20).***

Finally, the last three patients with purulent gross aspect of the pleural fluid, known with pulmonary

tumor with pleural invasion, were hospitalized for a signs and symptoms of both an infectious condition and pleural effusion. The cytological examination of the pleural fluid revealed a polymorphous cellular population, with numerous neutrophils, but also lymphocytes, mesothelial cells and malignant cells (Figure 19), pleading for adding of a bacterial infection over a preexisting metastatic pleural effusion.

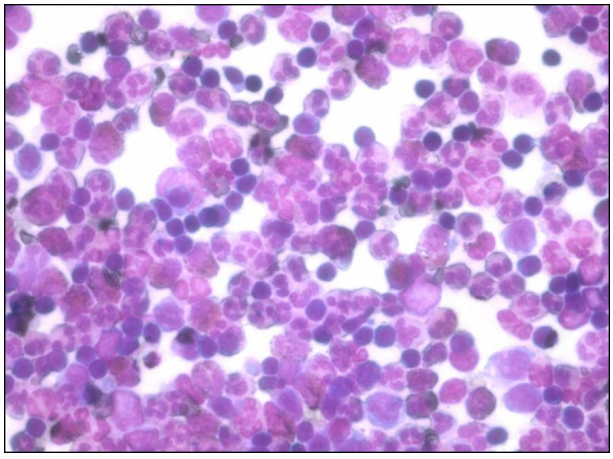

Figure 19 – ***Pleural fluid cytology: lymphocytes + neutrophils + malignant cells (MGG stain, ob. ×40).***

Histopathological assessment

In eight of the 39 patients with purulent gross aspect of the pleural fluid, clinical, paraclinical and cytological data were not sufficient to establish the etiology of pleural effusion, a needle biopsy being therefore required.

In six of these eight cases, the histopathological examination revealed the presence of granulomatous caseous lesions in submesothelial space (Figure 20) and deposits of fibrin and neutrophils over the mesothelial surface. Thus, the diagnosis of tuberculous pleurisy complicated with bacterial infection could be established and the therapeutical strategy could be modified and adapted.

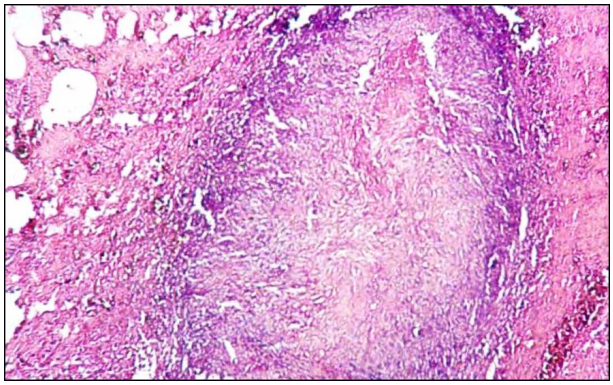

Figure 20 – ***Tuberculosis caseous nodule in the parietal pleura (HE stain, ob. ×4).***

For the last two patients with purulent pleural fluid, the needle biopsy was made just in order to exclude the bacillary etiology of the pleural infection. The histopathological examination revealed the picture of an acute inflammatory reaction with purulent exudate (Figure 21).

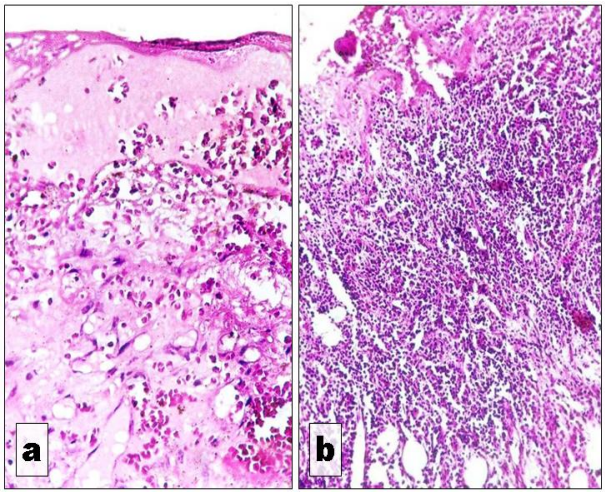

Figure 21 – ***Bacterial purulent pleurisy: "a" – Serous-fibrinous exudate covering mesothelial surface (HE stain, ob. ×20); "b" – Inflammatory infiltrate with neutrophils in subserous space and fibrinous deposits (HE stain, ob. ×10).***

Discussion

The considered series of patients was somehow peculiar, peculiarity given, most likely by the specificity of our medical institution, which was designed 70 years ago as sanatorium for tuberculosis and kept this main profile until today when it is functioning as a hospital for all respiratory diseases.

Therefore, the largest group in this studied series is that of patients with TB pleural effusion which represents almost 3/4 (i.e. 72%) of all studied patients. Besides this group, the other two groups include patients with pleural effusions determined by two other important pathological conditions: malignancy and infection other than TB.

However, even if the patients' distribution depending on etiology is influenced by the above-mentioned particularity of the medical unit, our data are consistent with those of literature, TB being still one of the main causes of pleural effusion, along with malignant disease and heart failure [22].

Gross aspects of pleural effusions

Site of pleural effusion

The analysis of a possible correlation between the location of the pleural effusions and the etiology showed that while inflammatory pleurisies (TB and non-TB) had just a slight predilection for right cavity – 57% of cases in both inflammatory groups (Figures 22 and 23), malignant effusions determined either by mesotheliomas or by metastatic conditions showed a more clear predilection for the right cavity, left cavity being affected in slightly more than 1/3 of cases (i.e. 36.5% – Figure 22).

There was no case in our series, even in the TB group, with bilateral involvement of pleural cavities, results that are concordant with those of other recent studies [23].

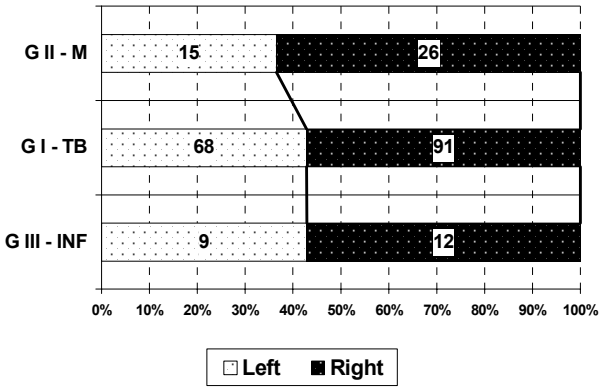

Figure 22 – ***Weight of sites in each group.***

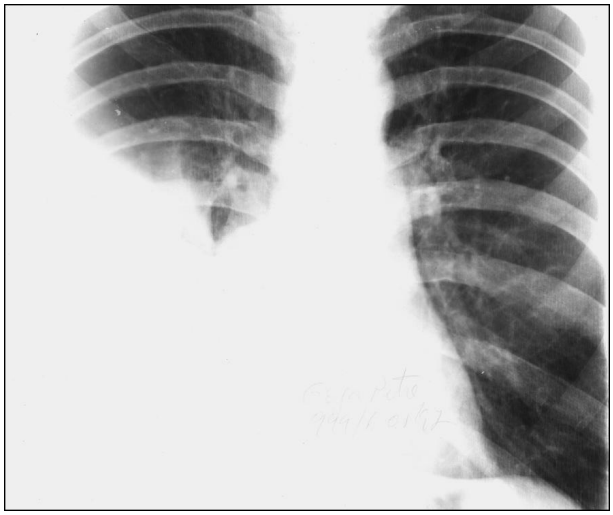

Figure 23 – ***Tuberculous pleurisy of right cavity.***

Pleural fluid quantity

The correlation between the quantity of pleural fluid and the etiology revealed, in contrast with effusion location, differences between the main types of etiological pleurisies. Thus, most of the inflammatory effusions, both TB and non-TB (86.8% and 85.7% respectively) evolved with a moderate amount of fluid accumulation in the pleural cavity, usually occupying less than two thirds of it (Figure 24). This observation is comparable with what other authors have reported in the literature [23–25].

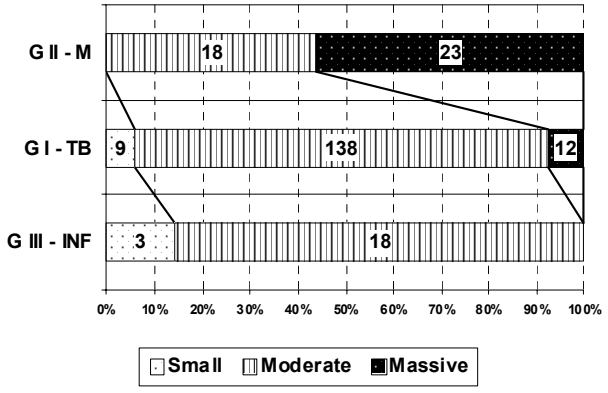

Figure 24 – ***Weight of different types of effusion depending on fluid quantity in each group.***

On the other hand, more than half of the pleural effusions associated with pleural mesothelioma or secondary to a neoplasia with other localization, evolved with large quantities of fluid (>1000 mL), the rest being with moderate quantities (Figure 25).

This is because it is well known that malignant effusions develop usually rapidly and have a great capacity of regeneration after evacuation [26, 27].

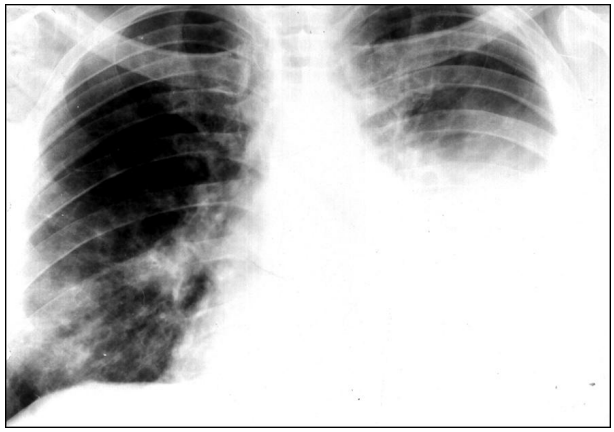

Figure 25 – ***Pleurisy of left cavity of malignant etiology.***

Gross aspect of the effusion fluid

The gross aspect of the pleural fluid is an important parameter because it could offer a fair enough orientation of the diagnosis towards a certain etiology. It also has a very special role in staging the disease evolution, especially in TB cases.

There are two approaches of the fluid gross appearance analysis.

The first is the comparison between the weights of different types of gross aspect in the studied groups.

Thus, malignant effusions were almost all of hemorrhagic type, in more than half of cases, the aspect being franc hemorrhagic (Figure 26).

Only few cases had a serous-purulent appearance, suggesting an overlapping infection.

At the opposite, in non-TB infections the prevailing aspect was the purulent one – more than half of cases, suggesting an acute bacterial infection. In the remaining cases, the aspect was either citrine or serous (Figure 26), suggesting firstly a viral etiology.

TB pleurisies had a wide range of gross aspects.

This variety of gross appearances could be connected with the evolution of TB inflammation.

Thus, the citrine aspect, observed in more than half of cases, suggested that the disease was in the second phase of evolution, while serous and sero-purulent aspects suggested that the disease was in the first phase of evolution. Only few cases had a purulent appearance, suggesting a particularly active bacillary process (Figure 26).

Our observations are consistent with those of literature [23] even we found also some cases with hemorrhagic aspect.

The second approach is which is the etiology suggested by the different gross aspects. Thus, as we can see in Figure 27, the purulent aspect was present in our cases only in infectious conditions, more frequently in acute bacterial infections of pleura but also in TB pleurisies.

The serous, citrine, and serous-purulent aspects were mainly present and therefore they could suggest, as a first etiology, TB.

The hemorrhagic aspect points out before all the malignancy and then TM whereas the serous-hemorrhagic aspect signaled first of all TB and then the malignancy.

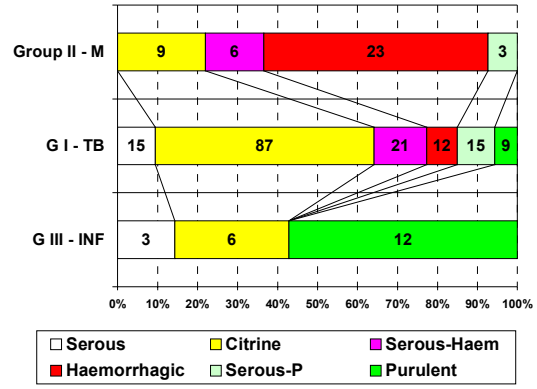

Figure 26 – ***Weight of different types of pathologic condition (studied group) for each type of gross aspect.***

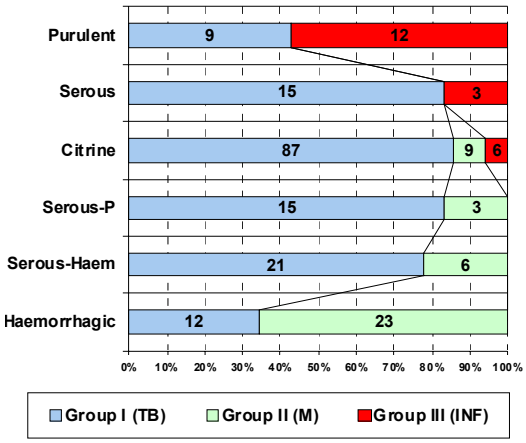

Figure 27 – ***Weight of different types of pathologic condition (studied group) for each type of gross aspect.***

Microscopic aspects of pleural effusions

Cytological aspect of the effusion fluid

In Table 7 are resumed all the cellular patterns observed on cytological smears of all patients included in our study.

Table 7 – ***Cell population in smears of different types of pleural pathologic conditions***

Type of cells on the smear		Group I TB	Group II Neopl.	Group III Others
L	*Predominant*	87		
	+ E (L>E)	21		6
	+ N (L>N)	15		3
E	*Predominant*	12	23	
N	*Predominant*	9		12
	+ L (N>L)	15		
MC	*+ N (N>MC*		3	
	+ L and less E		18	9
	+ E and less L		6	

TB = tuberculosis; Neopl. = neoplasia; L = lymphocyte(s); E = erythrocyte; N = neutrophil; MC = malignant cell(s).

The synopsis defines a quite accurate picture of the relationship between a certain category of etiologic factors and the composition of cellular population present in the pleural fluid.

Thus, the first group of smears was those with only **one type of cell**, fully dominating or dominating the cytological picture:

- A cellular population fully dominated by **lymphocytes** was seen only in TB (dark blue boxes in the Table 7);
- A cellular population fully dominated by **erythrocytes** was observed primarily in malignant effusions but also, in our cases, in TB (red boxes in the Table 7);
- A cellular population fully dominated by **neutrophils** was observed primarily in acute bacterial pleurisies but also in some TB cases, signaling a very aggressive action of bacillary population (orange boxes in the Table 7);
- A cellular population dominated by **malignant cells** was present only in malignant effusions (green boxes in the Table 7).

Then, there were smears presenting the association of at least two main cellular populations on the cytological picture:

- The **erythrocytes** were present in an significant number, and, therefore, shifting the color of gross appearance towards red range, in several situations (pink boxes in the Table 7):
  - together with lymphocytes, but less than them, primarily in TB but also in acute viral infections;
  - together with malignant cells, but not predominating, in malignant effusions.
- The **neutrophils** were present in an significant number, and, therefore, shifting the color of gross appearance towards yellowish range, in several situations (tan boxes in the Table 7):
  - together with lymphocytes but less then them in initial phase of TB;
  - together with lymphocytes but more then them also in TB, but in cases with aggressive bacillary population;
  - together with malignant cells but more than them in overlapping of a bacterial infection to a pre-existing malignant effusion.

Taking into consideration these observations, we could say, together with many other authors, that, even in the pleural effusion, unlike blood, the cellular compound varies from one individual to another depending on the pathologic condition which generated the pleural effusion, the disease gravity and other factors, some **indicative** but not pathognomonic **cytological** "**formulas**" could be designed [27–30].

These indicative "formulas" could be "cell oriented" or even "disease oriented".

A "cell oriented" set of cytologic formulas could be the following:

- **Predominantly lymphocytic smear** with >85% lymphocytes suggests [2, 27]:
  - In the first instance *tuberculosis* in the second phase;

and then:
- *Chronic inflammation* (sarcoidosis, chronic rheumatoid pleurisy;
- *Lymphoma;*
- *Viral infection* (but together with some neutrophils and sometimes eosinophils);

but also:
- Yellow-nail syndrome or chylothorax.

- **Predominantly hemorrhagic smear**, suggests [2, 27]:
  - In the first instance *neoplastic effusion, either primary or metastatic.* In these cases hemothorax can be ruled out by performing a pleural fluid hematocrit level whose value greater than that of peripheral blood with 50% suggests a hemothorax [31];

  and then:
  - *Benign asbestos pleurisy, post-cardiac injury syndrome, trauma and pulmonary infarction;*

  and also:
  - Viral infection and even TB.
- **Predominantly neutrophil smear**, suggests [27]:
  - In the first instance *bacterial piogenic infection* (over 100 000 neutrophils/mm^3 but with degenerative changes as nuclear pyknosis and cytoplasmic vacuoles);
  - Secondly, *the overlap of a bacterial infection on a pre-existing effusion*;
  - But also first phase of TB (but the neutrophils have no degenerative changes).
- **Malignant smear**. Cytological examination can provide confirmation of a malignant pleural effusion but has a diagnostic yield of only 65% in general categories of patients with malignant pleural effusions [4-8]. The presence of malignant cells in pleural fluid has a wide range (between 50% and 90%), being influenced by cancer histotype, number of examined samples, and experience of pathologist. For instance, neoplasms like adenocarcinoma show a great tendency to exfoliation which on the contrary, is reduced in others like Hodgkin's lymphoma [31];
- **Presence of eosinophils** (>10% eosinophils) **in the pleural smear** could have several meanings [32]:
  - Conditions associated with the presence of blood or air in the pleural space;
  - Infections;
  - Malignancy – between 12% and 24% of eosinophilic effusions are malignant in etiology [33–35];
  - But also: drug-induced pleural effusions, pleural effusions accompanying pulmonary embolism, and benign asbestos pleural effusions;
  - It should also be noted that pleural fluid eosinophilia considerably reduces the probability of TB unless the patient has a concomitant pneumothorax or a previous traumatic thoracentesis that has resulted in pleural space hemorrhage [36];
  - **Smear with lymphocytes and erythrocytes**, without any other accompanying cellular element is suggesting either TB or malignancy. More than 50% of malignant pleural effusions have an amount of lymphocytes varying between 50% and 70% of nucleated cells but always associated with a significant amount of erythrocytes [2, 27];

On the other hand, a "disease-oriented" set of cytologic formulas could be the following:
- **Bacterial pleurisies** – cytological picture dominated by *neutrophils*, usually with degenerative changes and acompanied by a reduced number of lymphocytes and mesothelial cells;
- **Chronic inflammatory pleurisies** – cytological picture dominated by *lymphocytes* accompanied by a reduced number of mesothelial cells;
- **Viral pleurisies** – cytological picture dominated by *lymphocytes* accompanied by neutrophils and sometimes eosinophils;
- **TB pleurisies** have a particular behavior, with a dynamic changing cytological picture [27, 37, 38]:
  - In the initial stage of illness (up to first two weeks), the cytologic formula is: lymphocytes – 50%, neutrophils – 35% and mesothelial cells – 15%. Sometimes, neutrophils can predominate;
  - Second stage show a shift toward lymphocyte predominance (between 75% and 95%);
  - It should be reminded however that for the older authors >5% mesothelial cells in pleural fluid were rarely compatible with TB pleural effusions [39]. However, there have been case reports of TB pleural effusions with numerous mesothelial cells [40] analogous to reports in HIV-infected individuals [41];
- **Malignant pleurisies** which rise two aspects:
  - Pleurisies accompanying mesotelioma;
  - Pleurisies accompanying metastatic conditions of the pleura;

Even there are some characteristic cytological features which can evoke both mesothelioma and metastases in pleura it is better for the definitive diagnosis of malignancy not to be made on pleural fluid cytology alone and pleural biopsy for tissue diagnosis is therefore recommended [21].

To conclude, even if cytology is considered to have only indicative value in pleural effusions and especially in the malignant ones, sampling of the fluid for cytological examination is the first step in confirming the diagnosis [21, 42–46].

Histopathological aspect of the effusion fluid

In more than 30% of cases in each of the three groups, clinical paraclinical and cytological data were not sufficient to fully elucidate the diagnosis, requiring a pleural needle biopsy (Figure 28).

There were no problems of histopathological interpretation for the 61 patients with TB or non-TB inflammatory processes, which needed pleural biopsy.

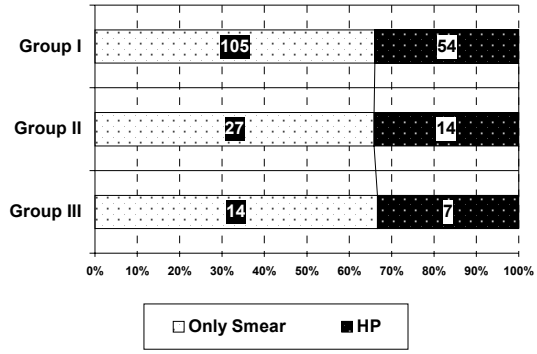

Figure 28 – ***Weight of histopathological examination in each studied group.***

Concerning the tumoral conditions which could generate pleural effusions, it is well known that lung cancer, especially the non-small-cell lung adenocarcinoma histotype, and breast cancers account for about 50–60% of all malignant pleural effusions, smaller but proved contribution being given by mesothelioma (about 15% of malignant pleuritis), kidney cancers and by lymphomas [31].

In our series, of the 41 patients with malignant pleurisies, 15 had pleural mesothelioma and 26 had metastatic pleural effusions, which were, all of them, pulmonary malignant tumors invading the pleura.

Only 14 of the patients with pleural malignancies required a pleural biopsy, which, as we mentioned above, revealed histopathological features characteristic for mesothelioma in 11 cases and for pulmonary malignant metastases in three cases.

Two of them were adenocarcinomas. One of these cases raised some difficulties in interpretation because the morphological picture was reminding somehow of some types of epithelial type mesothelioma with tubular well-packed pattern (Figure 29), situation also mentioned in the literature [47].

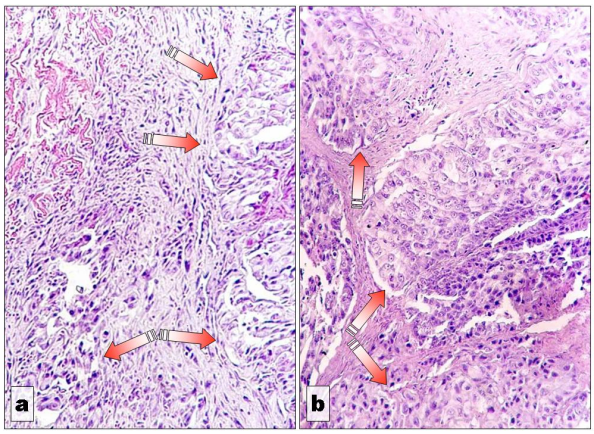

Figure 29 – ***(a) Biphasic mesothelioma (HE stain, ob. ×40); (b) Pulmonary adenocarcinoma with pleural invasion (HE stain, ob. ×40).***

The third case was a poorly differentiated squamous cell carcinoma, which was, somehow, a surprise for us because it is well known that pleural carcinomatosis from squamous cell carcinoma occurs only rarely [47].

Conclusions

Morphological examination of pleural fluid and pleura is of great value in the management of pleural effusions, only if all its steps are accomplished, starting with gross aspect, continuing with cytological examination and, when these are inconclusive, finishing with histological examination of pleural tissue samples.

Cytological examination is a very useful and also more available tool and, even, unlike blood, is far to be stable, sets of "cytologic formulas" with a good enough indicative value could be applied in order to clear up the diagnosis and to avoid the more invasive biopsy.

So, a correct diagnosis of pleural effusions and an adequate therapeutical strategy could be achieved only by going through a precise algorithm of investigation where, besides a thorough clinical examination and a complete set of laboratory tests especially of pleural fluid, morphological assessment and in particular cytological examination of pleural fluid and histopathological examination of pleural tissue samples are essential.

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