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STUDY ON THE CYTOLOGY OF PLEURAL FLUID IN DIFFERENT PLEURITIS

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Key words: *pleural fluid, pleurisy, cellular elements*

INTRODUCTION

Normally, there is a small volume of fluid in the pleura, which fulfills two important functions: it ensures easy sliding of the pleura during respiratory movements, significantly reducing the friction force and allows keeping the lungs "glued" to the chest wall, allowing optimal expansion. of lung tissue. The volume of liquid is between 0.1-0.3 ml / kg, and the total protein concentration is below 1.5 g%, providing a colloid-osmotic pressure of 3-5 cm of water. The thickness of the fluid layer varies between 5-30 micrometers, being higher in the lower part of the pleural cavity. The liquid contains cells, between 1500 and 4500 cells / mm3, proteins and polysaccharides.

The cells that can be found in the pleural fluid are mesothelial cells, macrophages, lymphocytes, polymorphonuclear, neutrophils, eosinophils, mast cells and basophils. It is estimated that the filtration rates in the parietal pleura are physiologically 100 ml / h and the resorption capacity is 300 ml / h in the visceral pleura. Lymphatic drainage of proteins and fluids with their reabsorption from the pleural cavity involves an active pumping phenomenon. Proteins, cells, and particles are absorbed exclusively by the lymphatics of the parietal pleura through pores with a diameter of 2-4 microns larger in the intercostal pleura, mediastinum, and diaphragm and which put the pleural cavity in direct contact with the lymphatic plexuses. This allows a lymphatic drainage capacity of up to 600 ml / 24h. Thus, the production of pleural fluid overflow can occur through the following mechanisms: increase in hydrostatic pressure in the pulmonary capillaries (eg in left ventricular failure); decreased oncotic pressure (nephrotic syndrome); increase in negative pleural pressure (atelectasis); increasing capillary permeability through mediators of inflammation leading to increased protein and fluid secretion; alteration of lymphatic disturbance; passage of fluid from the peritoneal cavity. In practice these mechanisms do not intervene in isolation and especially in exudate they combine.

Transudative pleurisy occurs in two situations: when the pressure in the capillaries increases, or when the plasma oncotic pressure decreases to such an extent as to exceed the reabsorption capacities of the visceral and lymphatic pleura. The vast majority of transudative fluids have a level of pleural proteins below 25 g / l. It should be borne in mind that once the initial cause, which led to the formation of the liquid, has been removed, the reabsorption of this type of liquid can be done very quickly (up to 200 ml / h). In these situations, the amount of protein in the liquid is concentrated and these transudates can become false exudates.

The formation of exudative pleurisy is done by directly affecting the pleura. The rate of resorption of exudative fluids is much slower than that of transudates because it depends on the removal of proteins from the liquid (which can only be done by lymphatic route). Also, the pleural repair process is slow. Another common cause of exudative pleurisy is decreased lymphatic disturbance in the parietal lymphatics. This type of mechanism is found in lymphatic blockages secondary to neoplastic invasion, is without a direct touch of the pleura, or in obstructions of lymphatic disturbance in the brachiocephalic confluences. A third cause of exudative pleurisy is a significant increase in negative intrapleural pressures. This mechanism can be found in atelectasis, or obstruction of the main airways (sleep apnea), which generates very high negative intrapleural pressures.

MATERIALS AND METHODS

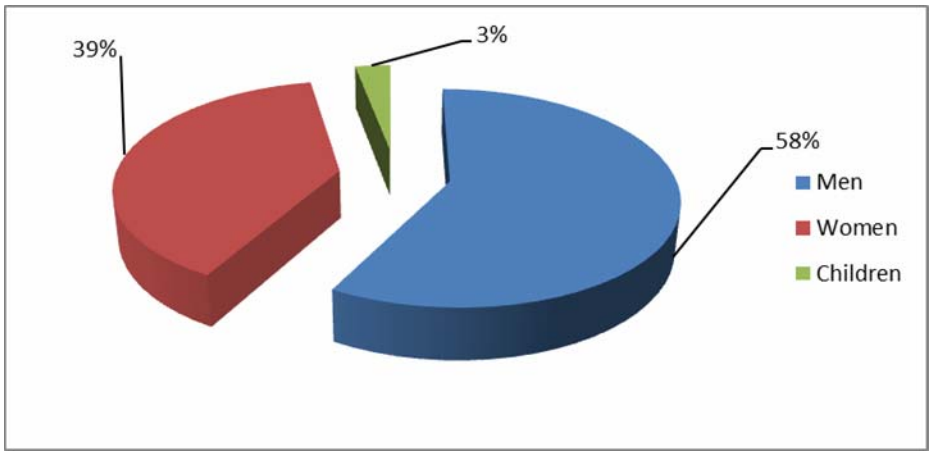
The biological material used in the investigation was peripheral blood and bone marrow collected from 100 patients admitted to the Bacau County Emergency Hospital. The working method used in the analysis of pleural fluid was the execution of sediment smears obtained by centrifuging the pleural fluid, fixation, rapid drying of the smears by stirring, Gram staining, methylene blue and May-Grunwald-Giemsa staining, respectively.

RESULTS AND DISCUSSIONS

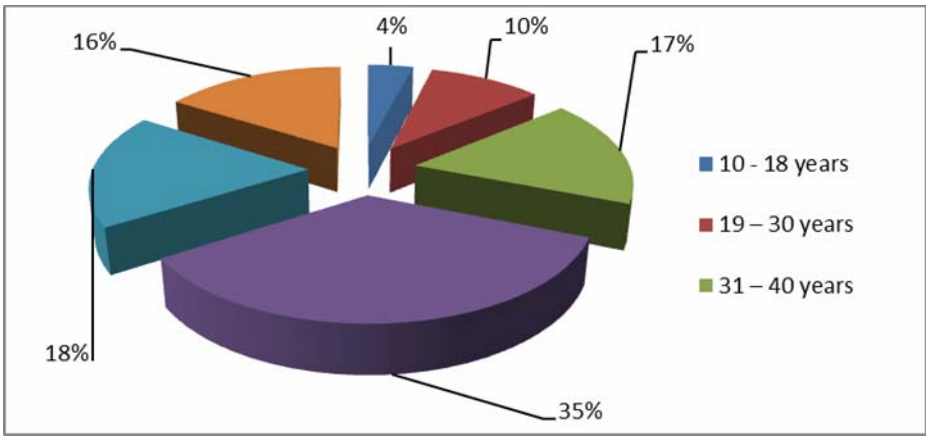
The types of pleurisy identified together with the parameters analyzed and statistically processed are represented in graphs, as follows.

Of the total number of patients studied, the percentage (graph 1) shows that the most affected are men (58%), women have such diseases in a smaller number (39%) and children very rarely (3%). Although lung disease can occur at a very young age, it affects the body in adulthood, usually over 30 years

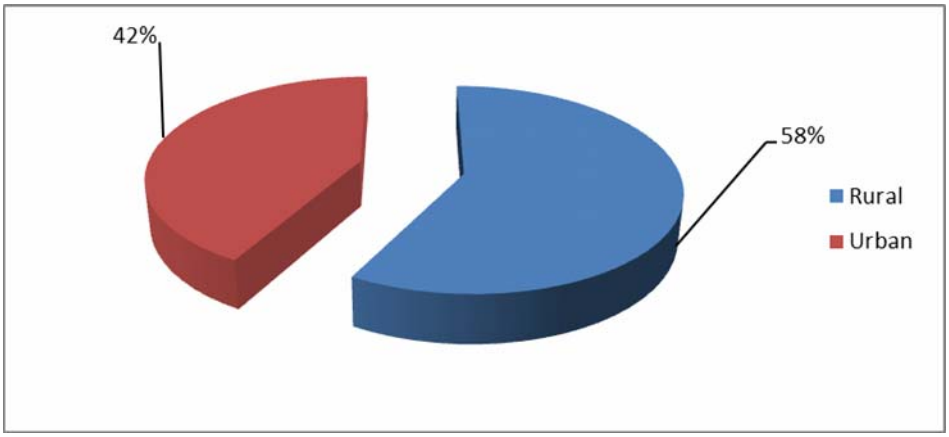
(Figure 2). Patients with pleurisy from rural areas have a slightly higher frequency (58%), compared to those from urban areas (42%), an aspect represented in graph 3. The frequency of lung diseases identified in the group of patients studied is presented as a percentage in graph 4. It is thus found from this graph that the most common pleurisy are serofibrinous (47%) followed by those of T.B. (25%) then neoplastic pleurisy (16%) and less often purulent (empyema) and hemorrhagic pleurisy (6%).



Graph 1. Percentage distribution by sex of the investigated patients



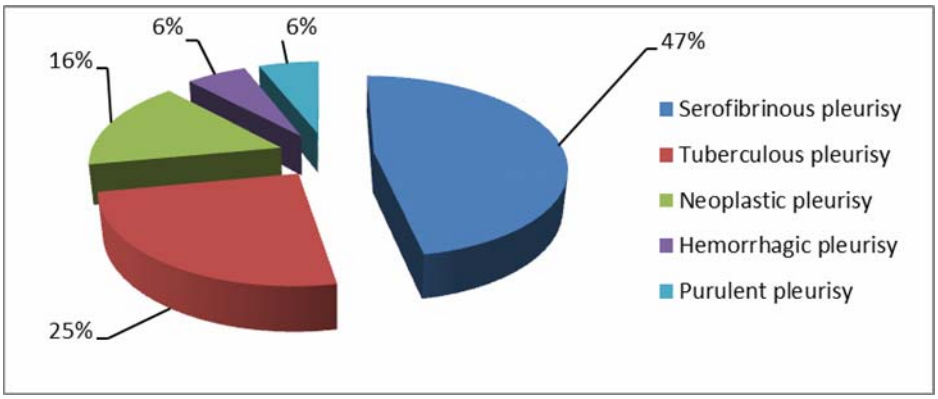
Graph 2. Percentage distribution by age groups of the investigated patients



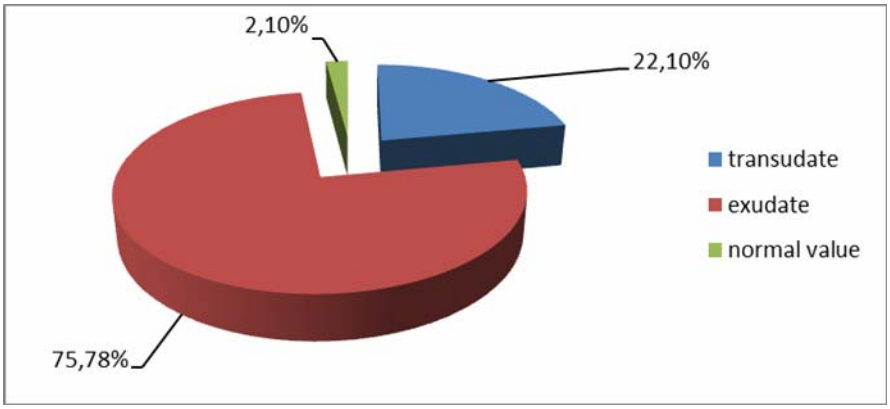
Graph 3. Percentage distribution by origin of domicile of the patients investigated

Cellular elements present in serofibrinous pleurisy and their interpretation

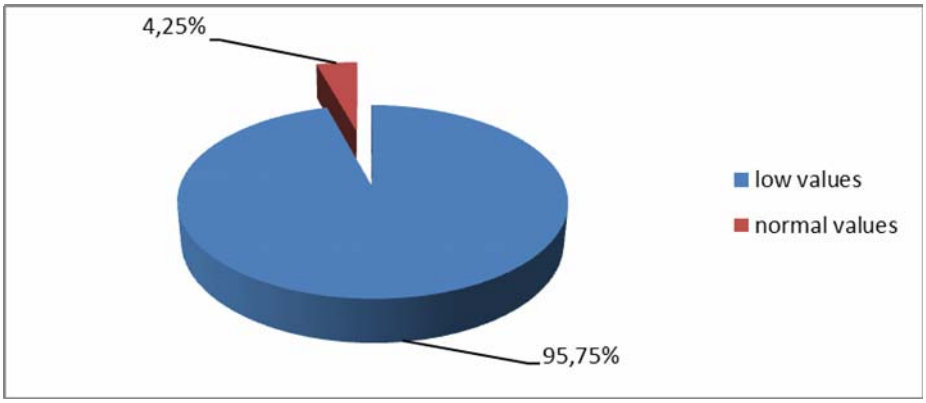
In the pleural fluid in patients with serofibrinous pleurisy is found the regular presence of the following cellular elements: frequent red blood cells and frequent lymphocytes, sometimes relatively common histocytes, granulocytes or mesothelial cells (Figure 1). The identification of blood elements such as erythrocytes in the pleural fluid implies the existence of hemorrhagic phenomena, because of lung lesions; the association of the presence of erythrocytes with lymphocytes and granulocytes, denotes the existence of infectious and inflammatory processes of T.B. type, respectively.



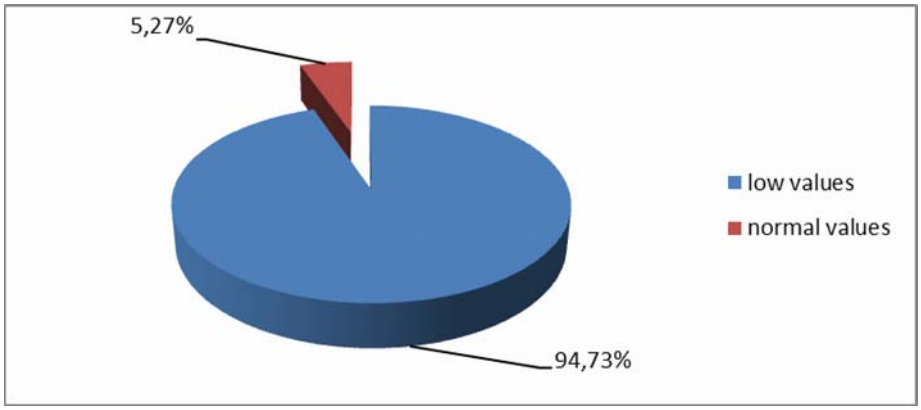
Graph 4. Frequency of pleurisy types in the group of patients investigated



Graph 5. Differentiation of pleural fluid according to the values of total pleural proteins, between transudate and exudate



Graph 6. Percentage distribution of patients investigated by glycopleuria values



Graph 7. Percentage distribution of patients investigated by pleural LDH values

The identification of reticulohistocytic cells (histiocytes) denotes the need for intense phagocytic processes, while the presence of mesothelial cells, even rare denotes phenomena of intense irritation, inflammatory or allergic processes, but also neoplastic (Figure 3). Although rare, atypical cells have been identified in pleural fluid because they are small and isolated, unorganized in placards and have no active division, they may be reticulohistocytic elements that can sometimes mimic tumor cells and lead to misdiagnosis. Of the 47 cases, the majority are male (27) aged between 30 - 70 years, most of them being 40 years old and a 17 - year - old boy, all from rural areas.

Cellular elements present in pleurisy with etiology T.B. and their interpretation

In patients with pleurisy T.B. certainly, the predominance of the following cellular elements is found in the pleural fluid: erythrocytes and very frequent lymphocytes, granulocytes, mesothelial cells and frequent histocytes and, very rarely, the presence of the Koch bacillus (Figure 2). The cellular elements identified in T.B. pleurisy. are very similar to those identified in serofibrinous pleurisy, since most serofibrinous pleurisy evolves into forms of T.B. Of the 25 cases, 16 are men of various ages (26 - 78) predominating those aged 40 and a 15-year-old boy, all from rural areas.

Cellular elements present in hemorrhagic pleurisy and their interpretation

In the case of hemorrhagic pleurisy, erythrocyte cellular elements predominate in the pleural fluid, in very large numbers, but also accompanied by other blood elements, namely lymphocytes, granulocytes, mesothelial cells and common histocytes, because hemorrhagic phenomena occur due to pathological processes extending to vessels (caseous and cavitary tuberculosis) or neoplastic erosion that occurs following the invasion of the vascular wall by malignant tumors. The presence of lymphocytes, granulocytes, histocytes and mesothelial cells indicates intense irritative and infectious inflammatory phenomena.

Of the 6 cases of hemorrhagic pleurisy, 3 are women and 3 men aged between 20 and 50, most of them from rural areas.

The cellular elements present in purulent pleurisy and their interpretation

In purulent pleurisy, the characteristic presence of a high number of granulocytes and lymphocytes is found in the pleural fluid, while erythrocytes are relatively common. The high number of granulocytes especially GSN (neutrophils) and lymphocytes indicates a marked infectious and inflammatory process. Of the 6 cases, the majority were female, 2 women aged 30 and 60, respectively, 2 girls aged 12 and 13, respectively, from rural areas.

Cellular elements present in neoplastic pleurisy and their interpretation

On all examined pleural fluid smears, the presence of atypical cellular elements arranged in plates with active division is found together with blood elements (erythrocytes, lymphocytes), reticulohistocytic (histiocytes) or mesothelial elements. Any cell type can be neoplasia, becoming atypical. Cells are called atypical because they are characterized by: large size (increased nucleo-cytoplasmic ratio) nuclear deformities, nucleus hyperchromasia (due to increased amount of DNA), chromatid abnormalities (loss of fineness, granular, irregular densifications), nucleotide abnormalities (nucleoli hypertrophied, uneven, deformed) atypical mitoses, cytoplasm with many nuclei, chromosomal aberrations (uneven distribution of chromosomes, delays in the migration of chromosomes in anaphase).

The identification of atypical cells, which are therefore neoplastic cells, arranged in placards and in active division, is the sure diagnosis of neoplastic pleurisy, even if the malignant cells are small (Figure 4). Neoplastic cells can sometimes be mimicked by some bell cells, such as histiocytes. When small, atypical, isolated cells without active division and

organized in placards are identified in the pleural fluid, they are not malignant cells. Most types of pleurisy with neoplastic etiology are found in men (10) aged between 30 and 70 years, predominantly the age of 30 years, coming from both rural and urban areas. From the obtained results and their interpretation, it results that the different types of pleurisy can be identified, confirmed, or refuted based on the examination of the cellular elements present in the pleural fluid, these being characteristic of different pleurisy.

It should be noted that in the case of neoplastic pleurisy, the presence of atypical malignant cells even in very small numbers is sufficient to confirm the disease in its onset form. Literally the number of atypical cells increases, and other cells characteristic of neoplasms appear in the pleural fluid (red blood cells, mesothelial cells, lymphoreticular blastoid elements), because of massive obstructions in the vessels, tissues or organs. All the diseases studied are accompanied by the inflammatory effect, as a defense reaction of the body against the aggressive action of microbial agents (in our case the Koch bacillus, generator of pulmonary TB), but also against various mechanical factors that appeared after the microbial attack (production of cavitary lesions, vascular obstructions, tissue obstructions, cell invasions). In this case, the organism tends to "locate" the action of the harmful agent and to restrict the area of action of the agent, respectively its area of penetration. As a result, in all smears of the pleural fluid, regardless of the type of disease (pleurisy), the presence of leukocyte cells is found, as the main cells participating in the inflammatory process.

The action of recognition, capture, destruction, and purification of the agents provoking inflammation, as well as the removal of the damaged material, implies the obligatory participation of some cellular systems. The lysosomal systems have the complete lysis capacity of the substances, containing a harmful biological information. However, organisms also have extremely complex modes of action through cellular mediation, whose ultimate role is to expose the material recognized as heterologous (heterologous) before they are exposed to the lytic action of lysosomal enzymes. Thus, in the pleural fluid in the studied patients, the following categories of cells were identified, because of the inflammatory effect (reaction):

- **phagocytic cells**: granulocytes and histiocytes, which intervene in the removal of biological agents of inflammation and altered substances, using lysosomal lytic enzymes;
- **lymphoid cells**: lymphocytes, which mainly provide the immune defense function of the body, a function that is performed in cooperation with macrophages;
- **accessory cells**: mesothelial cells, which frequently show phagocytic properties, can become malignant or simulate malignant aspects, making it difficult to differentiate the cytological aspect;
- **foreign cells**: tumor cells, identified on the basis of malignancy criteria (nuclear changes, grouping of cells in aqueous, papillary or muliform formations) to which are added red blood cells (erythrocytes).

The presence of erythrocyte cells in pleural fluids, demonstrates the vascular lesions that always accompany the inflammatory process, their number increases greatly when extensive lesions are involved in larger vessels (hemorrhagic pleurisy).

In our study, microbiological examination did not allow the development in culture of pathogens, although the Koch bacillus was present on some specific stained smears, Ziehl-Neelsen. However, their inflammatory effects have been identified in all cases, smear, or radiology. Direct examination and culture of pleural fluid are rarely positive even in the case of TB pleurisy, but radiologically the bacillary impregnation syndrome + tuberculous AHC is present.

Pleural effusion is an accumulation of fluid in the virtual space between the pleural, visceral, and parietal sheets. The causes can be pulmonary or extrapulmonary.

Pleural effusion can be classified according to the mechanism of occurrence in:

- **transudate** - the result of an imbalance of hydrostatic pressure - characterized by a low protein content and occurs in heart failure or nephrotic syndrome.
- **exudate** - inflammatory, pleural causes, which lead to active fluid secretion with a high protein content. Transudates occur due to a mechanical circulatory barrier, while exudates are the consequence of various inflammatory or neoplastic processes.

The pathogenic mechanisms involved in the development of transudates are represented by the disturbance of the balance between the hydrostatic pressure and the colloid osmotic pressure. Inflammatory exudates, for which the differential diagnosis with malignant processes must be considered, occur in the context of a tuberculosis, bacterial infection or in connection with a pulmonary embolism.

For the differential diagnosis between exudate and transudate, in practice the determination of the number of total proteins in the pleural fluid is used, considering that a value of total proteins below 3 g / dL indicates a transudate, and over 3 g / dL indicates an exudate. The classification of spillage as exudate or transudate is the first step in establishing its etiology. In the experimental group of patients 22.10% have total pleural proteins below 3 g / dL (transudate), 75.78% over 3 g / dL (exudate) and only 2.10% have normal value (graph 5).

Decompensated heart failure is the most common cause of transudation, and over 80% of pleural exudates occur parapneumonically, from malignant causes (breast, lung, ovarian, gastrointestinal tract, kidney, lymphoma, leukemia, mesothelioma) or because of pulmonary embolism. Other inflammatory causes include systemic lupus erythematosus, rheumatoid arthritis, asbestosis, tuberculosis. Transudates may be associated with hypoalbuminemia (in liver cirrhosis or nephrotic syndrome), atelectasis, lung cancer, portal hypertension, peritoneal dialysis.

Confirmation of the diagnosis. Other biochemical parameters investigated in pleurisy of different etiologies are glycopleuria and pleural LDH.

Glycopleuria is normally greater than 50% of serum blood glucose; decreases significantly in TB pleurisy, purulent pleurisy, and neoplastic pleurisy. In our group of patients, it is found that only 4.21% of them have normal values of glycopleuria, the remaining 94.73% have modified value, well below the normal value (chart 6).

Pleural LDH (lactate dehydrogenase) is considered normal at a value of 2/3 of the maximum serum value, considered 200 IU / l. In the group of investigated patients, 5.27% have normal values, while 94.73% have much higher values (graph 7). The combined determination of total protein and LDH concentrations, as well as the ratios between the pleural fluid concentration and their serum concentration allows the differentiation of exudate transudate, especially in malignant effusions, which appear as transudates if only proteins are determined, as well as in heart failure, when they can be erroneously classified as exudates.

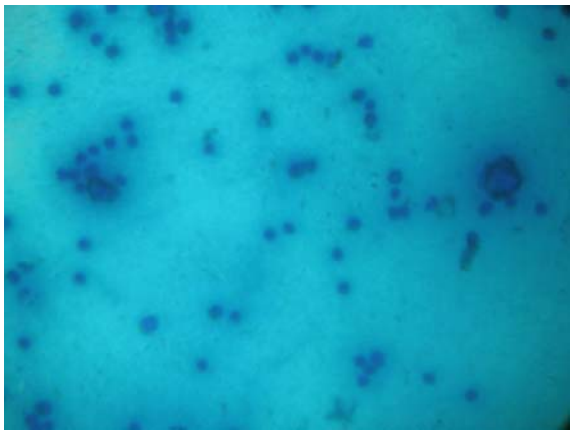


Figure 1. Neutrophils and mesothelial cells in the pleural fluid

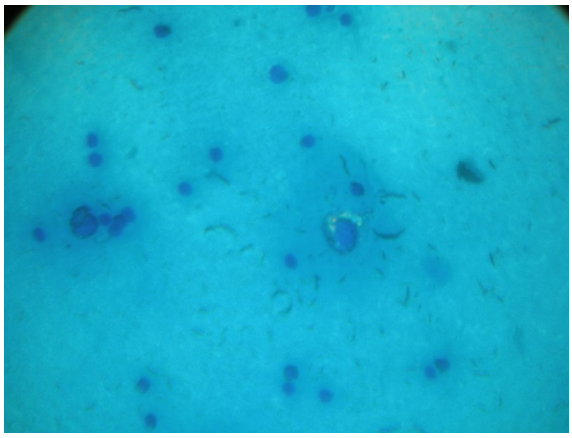


Figure 2. Ziehl-Neelsen stained smear from pleural fluid (TB pleurisy), the presence of Koch bacillus is observed

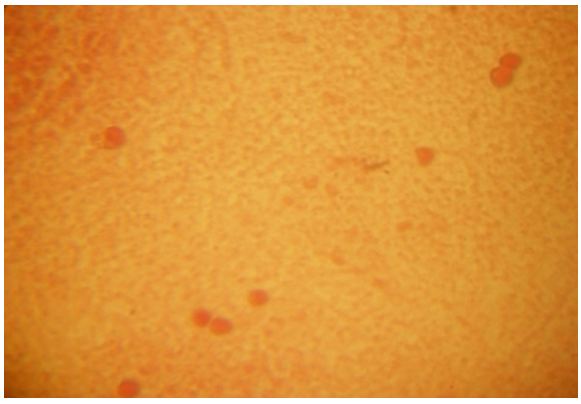


Figure 3. Mesothelial cells in the pleural fluid.

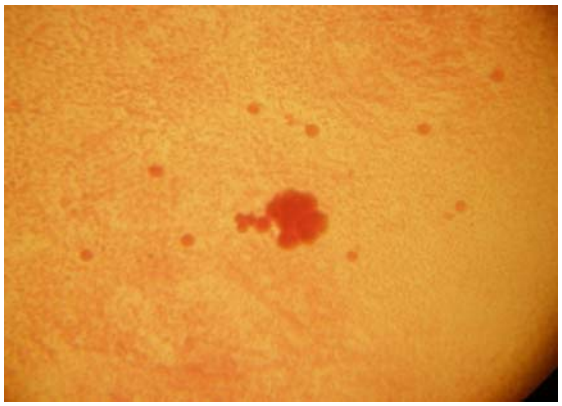


Figure 4. Neoplastic cells in the pleural puncture fluid

CONCLUSIONS

- Cytological examination of pleural fluid is one of the most important means of investigation for the etiological diagnosis of pleural effusions. The efficiency of this examination has increased due to the improvement of the techniques of harvesting, processing, and examination of the products.
- The use as a working technique of the smear made from the sediment obtained by centrifuging the pleural fluid and the May-Grunwald-Giemsa staining, gives very good results in identifying cellular elements, especially malignant ones.
- In the studied patients, the following categories of cells were identified in the pleural fluid: reticulohistocytic elements (histocytes), blood elements (granulocytes, lymphocytes and erythrocytes), mesothelial cells and tumor cells.
- Cell categories can be cytological formulas characteristic of different diseases based on they can be identified and confirmed.
- However, there are also possibilities of error in identifying a disease by this method, the main cause being the weight of delimiting the normal mesothelial elements, degenerated or atypical, from malignant cells. For this reason, it is necessary to know in detail all the cellular elements and their evolution.
- While in *serofibrinous pleurisy* the picture of cellular elements does not presuppose the predominant existence of certain characteristic cell types, in *T.B. pleurisy*, numerous lymphocytes and enterocytes are characteristic, in *hemorrhagic pleurisy*, the large number of erythrocytes, in *purulent pleurisy*, the large number of granulocytes and lymphocytes, and in *neoplastic forms*, malignant cells.
- In the case of *neoplastic pleurisy*, the presence of atypical malignant cells, even in very small numbers, is sufficient to confirm the disease in its onset form. Subsequently, the number of atypical cells increases, and other cells characteristic of neoplasms appear in the pleural fluid (red blood cells, mesothelial cells, blastoid elements, lymphoreticular elements, etc.).
- All the studied diseases are accompanied by the inflammatory effect - as a defense reaction of the organism against the aggressive action of microbial agents * in this case Koch's bacillus) but also against various mechanical factors that appeared after the microbial attack (vascular, tissue destruction, cell invasions). As a result, in all the studied pleural fluid smears, regardless of the type of pleurisy, the presence of leukocyte cells (granulocytes, lymphocytes) is found as the main cells participating in the inflammatory process.
- The presence of erythrocytes in the pleural fluid demonstrates the vascular lesions that accompany the inflammatory process, their number increases greatly when large vessel lesions are involved (hemorrhagic pleurisy).
- **Microbiological examination** did not allow the development in culture of pathogens, although they were the Koch bacillus was present on some specific stained smears, Ziehl-Nielsen. However, their inflammatory effects have been identified in all cases, smear, or radiology.
- **The biochemical investigation** shows that in the experimental group of patients 22.10% have transudate as a mechanism of occurrence, 75.78% exudate and only 2.10% have the normal total protein value.
- **Glycopleuria** has a modified value, well below normal, in 94.73% of the investigated patients, while **pleural LDH** has much higher values in 94.73% of patients.
- Out of the total number of patients studied, it is found that this type of disease mainly affects men (58%), less women (39%) and occasionally children (4%).
- Diseases can affect very different ages between 12 and 78 years but are more common in the 41-50 age segment.
- Lung diseases affect both urban and rural populations. However, a slightly higher percentage is observed in the case of the rural population, possibly due to a poorer diet and physical work in poorer hygienic conditions.

ABSTRACT

100 patients with different types of pleurisy, hospitalized in the Bacau County Emergency Hospital, were investigated. The study was performed on pleural fluid from which pellets obtained from centrifugation, Gram stains, methylene blue and May-Grunwald-Giemsa staining, respectively, were performed.

The following categories of cells were identified in the pleural fluid: reticulohistocytic elements (histocytes), blood elements (granulocytes, lymphocytes, and erythrocytes), mesothelial cells and tumor cells. Cell categories can be cytological formulas characteristic of different diseases based on they can be identified and confirmed.

Thus, in *serofibrinous pleurisy* the picture of cellular elements does not presuppose the predominant existence of certain characteristic cell types, *in T.B. pleurisy*, numerous lymphocytes and enterocytes are characteristic, in *hemorrhagic pleurisy*, the large number of erythrocytes, in *purulent pleurisy*, the large number of granulocytes and lymphocytes, and in neoplastic forms, malignant cells.

REFERENCES

1. ANASTASATU C., 1972 — *„Fiziologie* Editura Didactică şi Pedagogică, Bucureşti. (Physiology Didactic and Pedagogical Publishing House, Bucharest).
2. ANASTASATU C., 1975 — *„Fiziologie Clinică",* Editura Didactică şi Pedagogică, Bucureşti (Clinical Physiology, Didactic and Pedagogical Publishing House, Bucharest).
3. ANASTASATU C., 1980 — *„Pneumoftiziologia Clinică",* Editura Medicală, Bucureşti (Clinical Pneumoftiziology, Medical Publishing House, Bucharest).
4. ANGELESCU V., 1963 — *„Elemente de Embriologie",* Editura Medicală, Bucureşti (Elements of Embryology, Medical Publishing House, Bucharest).
5. BUNGETZIANU GH., Olga Moldovan, 1987 — *„Investigaţia Microbiologică în Tuberculoză Microbacterioze"* Editura Academiei, Bucureşti (Microbiological Investigation in Tuberculosis Microbacteriosis" Academy Publishing House, Bucharest).
6. CLAICIU I., 1979 — „ *Practica Luptei Anti-Tuberculoase",* Editura Medicală, Bucureşti (Practice of Anti-Tuberculosis Fighting", Medical Publishing House, Bucharest).
7. COMAN G. CONSTANTIN, 1972 — *„Pleurezii purulente",* Editura Medicală, Bucureşti (Purulent Pleurisy", Medical Publishing House, Bucharest).
8. GHERASIM L., 1995 — *„Medicină Internă",* Editura Medicală, Bucureşti (Internal Medicine", Medical Publishing House, Bucharest).
9. IONESCU C., 1977 — „*Patologia Generală a Tuberculozei*", Editura Dacia, Cluj-Napoca (General Pathology of Tuberculosis", Dacia Publishing House).
10. POPA G., 1971 — „ *Citodiagnosticul prin puncţie în practica medicală* ", Editura Medicală, Bucureşti (Cytodiagnosis of Malignant Tumors", Medical Publishing House, Bucharest).
11. PRISECARU MARIA, CRISTEA TINA OANA, STOICA IONUȚ, 2011 – *Histologie animală*, Editura Alma Mater Bacău (Animal Histology, "Alma Mater" Publishing House, Bacău), ISBN: 978-606-527-115-9
12. PRISECAR MARIA, CRISTEA TINA OANA, VOICU ROXANA, 2011 – *Biologie celulară şi moleculară*, Editura Alma Mater Bacău (Cellular and molecular biology, "Alma Mater" Publishing House Bacau), ISBN: 978-606-527-116-6.
13. PRISECARU MARIA, IONUȚ STOICA, 2017 – *Imunologie generală şi clinică*, Editura Alma Mater Bacău (General and clinical immunology, "Alma Mater" Publishing House Bacău), ISBN 978-606-527-572-0
14. PRISECARU MARIA, IONUȚ STOICA, DANIELA TIȚĂ, FLORIAN PRISECARU, 2021 - *Ghid de educaţie pentru sănătate şi de patologie umană, vol. III – Bolile diferitelor organe şi sisteme*, Editura Alma Mater Bacău (GUIDE TO EDUCATION FOR HEALTH AND HUMAN PATHOLOGY, Volume III - *Diseases of different organs and systems*, Alma Mater Bacău Publishing House), ISBN 978-606-527-65; 978-606-527-663-5

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