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EFFUSION FLUIDS

BIOCHEMICAL, MICROBIOLOGICAL AND CYTOLOGICAL ANALYSIS IS ESSENTIAL TO MOVE FORWARD IN THE DIAGNOSTIC WORK-UP

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Abstract

The analysis of human effusions is an essential process for the diagnosis and follow-up of many diseases. This is the case, for example, with cancers, where the detection of malignant cells in an effusion may lead to the diagnosis of metastases, of obvious diagnostic and prognostic importance. As regards the cytological component, effusions are analysed in the medical biology laboratory in order to perform cell counts and a morphological examination of the cells. Biochemistry and bacteriology reliably provide key information for the diagnosis and management of the various diseases involved.

Keywords: effusion; diagnosis; diseases

DEFINITION OF AN EFFUSION FLUID

An effusion is defined by the presence of fluid or gas in a natural cavity that normally contains none. The cavities concerned are generally the peritoneal cavity, the pleura, the pericardium, the joints and the bursae. A classical distinction is made between pleural, peritoneal, pericardial and joint fluids. Effusions are sometimes the first sign of an underlying disease, or reflect the progression of a disease already present in the patient.

1. PATHOPHYSIOLOGY AND THE CONCEPTS OF EXUDATE AND TRANSUDATE

Whatever the origin of the effusion fluid, the pathophysiology is similar. Under normal conditions, the serous membranes are lubricated by the secretion of a small amount of fluid produced by the parietal layer and reabsorbed by the visceral layer. An imbalance between secretion and reabsorption causes an effusion. These effusion fluids are traditionally classified into two

1.1. Transudates

They correspond to a reduction in the resorption of the fluid. The causes are mechanical and differ according to the origin of the fluid. Classically, no excess of protein is found and cellularity is low.

1.1. Exudates

They appear following an increase in the production of the fluid. They are most often secondary to a tumour proliferation or to an inflammation originating from an infection, from acute vascular disorders, or from certain connective tissue diseases. The protein level is high, fibrinogen may be present and numerous cells are found. Moreover, each fluid has different characteristics depending on its origin.

2. ASCITIC FLUID

Ascites is a fluid effusion of the peritoneal cavity. Purulent effusions (peritonitis), bloody effusions (haemoperitoneum) and bilious effusions (choleperitoneum) are generally excluded from this definition. Whether ascites is exudative or transudative depends on the cause of the effusion: the transudate forms as a result of portal hypertension, found in cases of cirrhosis, of cardiac disease, or of hepatic metastases with vascular compression causing a mechanical block. Conversely, portal pressure is normal in the case of an exudate. The cause is then external: peritoneal carcinomatosis, infection, pancreatic disease, intestinal perforation, etc.

2.1. Clinical picture and macroscopic examination

Ultrasound is the most sensitive examination for establishing the diagnosis of ascites. The fluid is sampled at the patient's bedside, at the iliac fossa, at the outer third of the line between the umbilicus and the anterior superior iliac spine. It may be performed under ultrasound guidance. The fluid flows out naturally and is traditionally collected into 2 vials, one of which contains an anticoagulant (EDTA or citrate). A blood culture bottle may be inoculated directly at the patient's bedside for bacterial culture (if not, send the sample to the laboratory for this purpose). The first examination is macroscopic: the colour of the ascitic fluid points to a transudative or exudative origin of the fluid. Transudates correspond to clear, opalescent, chylous or citrine-yellow fluids. Exudates are cloudy or reddish, purulent, haemorrhagic or chylous.

2.2. Cytology and cytological features of ascitic fluids

2.2.1. Mesothelial cells

These are the cells that line the inside of the peritoneal cavity in a single cell layer. They are identical to those found in pleural fluids (in which case they originate from the pleural serosa). The cells desquamate from the visceral layer and are the only ones present in the fluid in the normal state, together with a few macrophages. They measure 5 to 7 µm in diameter within the epithelium and increase in volume once in the ascitic fluid, often measuring 15 to 25 µm in diameter. They are rounded in shape and have sharp edges. The appearance of the mesothelial cells changes according to the cause of the effusion and the time between sampling and

The other cells that may be observed in ascitic fluid all originate from outside the cavity. They pass into the fluid as a result of a benign or malignant process: neutrophils (PN), eosinophils (PEo) and basophils (PBa), lymphocytes, monocytes, plasma cells, mast cells, atypical lymphoid cells, etc. On a fluid smear, these cells have an appearance similar to that found on a blood smear.

2.2.2. Mesothelial cells

One of the ways of characterising fluids is classification by the predominant cell population. A distinction is made between:

- **Ascites with neutrophils:**

The presence of neutrophils in an ascitic fluid is strongly suggestive of infection of the fluid. In a cirrhotic patient, a neutrophil count above 250 cells/mm3 requires empirical antibiotic treatment (often a third-generation cephalosporin) to be started even before the organism is identified. This cytology may also be found after irradiation, reflecting inflammation.

- **Ascites with eosinophils:**

This is an exceptional condition, with a female preponderance. The clinical signs are abdominal pain associated with digestive disorders, and blood hypereosinophilia is frequently associated. The aetiological work-up is often negative. It should also be noted that repeated ascitic taps are often responsible for an increase in eosinophils with successive punctures.

- **Ascites with lymphocytes of banal appearance:**

An ascitic fluid with more than 70% lymphocytes should above all prompt a search for peritoneal tuberculosis. Tuberculosis is responsible for 2 to 6% of cases of ascites. It must be investigated by culture of the ascitic fluid on a medium for mycobacteria.

- **Ascites with atypical lymphoid cells:**

Burkitt lymphoma, as well as other non-Hodgkin lymphomas, in particular diffuse large B-cell lymphoma, may spread into the peritoneal cavity. In this case, the lymphoma cells in the fluid have features close to those of blood lymphoma cells. Numerous mitoses are frequently observed in the fluid.

- **Ascites with mesothelial cells:**

In ascites of transudative origin, the mesothelial cells detach from the peritoneal wall and float in the ascitic fluid. Chronic effusions are accompanied by a vacuolisation of the mesothelial cells, which take on a histioid appearance.

- **Ascites with metastatic malignant cells:**

The presence of malignant cells results from direct involvement of the serosa by the lymphatic route, by the blood route, or by simple propagation. Peritoneal carcinomatosis is the second leading cause of ascites. The cancer most frequently responsible for peritoneal ascites is ovarian adenocarcinoma. Cytology allows the diagnosis of metastasis in only about 2/3 of cases. The sensitivity of the examination may be increased by repeating the analysis on three consecutive days.

2.3. Biochemical and bacteriological analysis

Biochemical analysis of the ascitic fluid is requested systematically: protein and albumin assays. The protein assay makes it possible to classify the fluid as an exudate or a transudate. Calculation of the serum albumin / ascites albumin gradient points to the aetiology. This gradient is greater than 11 in cirrhosis or during portal hypertension. Below 11, it points rather to peritoneal carcinomatosis, peritoneal tuberculosis, pancreatic disease or nephrotic syndrome. Certain analyses may be requested less commonly: the triglyceride assay (above 2.25 × 10-3 mol/L in the case of chylous ascites); the amylase assay (when pancreatic ascites is suspected, the ascites amylase / serum amylase ratio is > 6). The lipase assay currently replaces the amylase assay.

For bacteriology, a direct examination is performed systematically, together with culture on conventional medium or in a blood culture bottle. Other analyses are possible, although not performed routinely, such as the assay of the tumour marker CEA for the diagnosis of peritoneal carcinomatosis. Where neoplastic disease or tumour cells are suspected, the ascitic fluid must undergo a histopathological analysis.

3. PLEURAL FLUID

The pleura is the serous membrane that covers the lungs, the rib cage, the diaphragm and the mediastinum. The two layers are contiguous and the virtual space thus formed between them is the pleural cavity. Normally, the two layers are separated only by a minute, almost virtual amount of fluid that facilitates the sliding movements of the two membranes against each other. Pleural fluid is produced by the parietal layer and is then reabsorbed by the visceral layer. An imbalance between the production and the resorption of the fluid may lead to the accumulation of fluid in the pleural cavity. The most frequent causes of pleural effusion are: congestive heart failure, infectious pneumonia, neoplasia or pulmonary embolism.

3.1. Clinical picture and macroscopic examination

When the pleural effusion is unilateral, it is most often due to a pleural or pulmonary disease, unlike a bilateral effusion, which is rather the sign of systemic involvement. The systematic examinations to be performed in order to explore a pleural effusion include a chest X-ray and a pleural tap for analysis of the fluid obtained.

Macroscopically, the pleural fluid can already provide information on the origin of the effusion. A cloudy fluid suggests an infection. A milky white appearance (chylothorax), a uniformly bloody fluid (haemothorax) or a serosanguineous effusion point to a neoplastic cause or to trauma. In contrast, a clear, citrine-yellow fluid does not prejudge the origin.

3.1. Cytology and cytological features of pleural fluids

The overall cell count is of secondary importance in the analysis of pleural fluids compared with the leucocyte differential. The cells found in a pleural fluid are identical to the cells found in ascitic fluids. Mesothelial cells have the same morphological features. Depending on the percentage of the cells encountered, a distinction is made between:

- **Pleural effusions with neutrophils:**

More than 50% of neutrophils indicates an acute phenomenon. Often, the percentage rises to 90%. These are inflammatory or infectious processes.

- **Pleural effusions with eosinophils:**

Eosinophils are above 10%; this value may reach 90%. They are encountered in cancerous and allergic diseases, in the aftermath of a pulmonary embolism, of a pneumothorax or of chest trauma. As with ascitic fluids, repeated taps appear to be a cause of an increase in eosinophils, but this remains controversial. The origin of these eosinophilic pleural effusions often remains undetermined.

- **Lymphocytic pleural effusions:**

The differential comprises more than 50% of lymphocytes. These effusions strongly suggest tuberculosis or lymphoma. The additional examination of choice is, in the first case, pleural biopsy. Immunophenotyping is recommended in the second. Lymphocytic pleural effusions are also found in viral pleurisy, or in pleural effusions secondary to mycoplasma and chlamydia pneumonia. The possibility of pleural invasion by a lymphoid malignancy must also be considered. Lastly, pleural lymphocytosis may be the sign of a chronic effusion.

- **Pleural effusions with a predominance of mesothelial cells:**

This is mainly the case with transudates or with mesotheliomas. In the case of mesothelioma, it should be noted that the malignant mesothelial cells are mostly dystrophic and often in clusters. The appearance of the cells is sometimes confusing and may make the diagnosis hesitant.

- **Pleural effusions with polymorphous populations:**

This is the name given to effusions with a mixed cytological differential: several cell types coexist without any notable predominance (lymphocytes, neutrophils, mesothelial cells). Two orientations are possible: - Benign effusion: the effusion is in this case the non-specific reflection of an inflammatory process. - Malignant effusion: microscopic examination shows the presence of suspicious cells: typically, they have an irregular, deformed nucleus with one or several nucleoli, and chromatin heterogeneity. They are sometimes cohesive and monomorphic. It is important to know how to detect these malignant cells in order to direct the clinician towards an underlying tumour disease.

3.3. Biochemical and bacteriological analyses

Few biochemical tests are used routinely. Proteins reflect the increase in pleural permeability, whereas LDH rather reflects its inflammation. These two markers, with the help of Light's criteria of 1972, make it possible to distinguish between exudate and transudate. As in ascites, protein or albumin gradients may also be calculated in order to determine the origin of the effusion. As a second-line investigation, a haematocrit may be performed to support a haemothorax, a measurement of pH or of glucose for the infectious nature, or else a cholesterol or triglyceride assay in the case of a chylothorax.

In bacteriology, here again, a direct examination may be performed, and inoculation on suitable media or into anaerobic and aerobic blood culture bottles makes it possible to specify the infectious diagnosis. Where tuberculous infection is suspected, the appropriate cultures must be carried out.

As for ascitic fluids, where malignant involvement is suspected, part of the sample must be sent for histopathological analysis.

4. SYNOVIAL FLUID

Synovial fluid (also called joint fluid) fills the space of the joint cavities in order to lubricate them. It is produced by the synovial cells from a plasma filtrate. Analysis of the synovial fluid is a key examination in the diagnostic work-up. Three analyses are particularly performed: cytology, bacteriology and the search for crystals. Biochemistry is of no interest; however, protein, glucose and uric acid may be assayed.

4.1. Clinical picture and macroscopic examination

The tap is performed by a trained operator, using a fine needle. Collection is into tubes containing an anticoagulant, the most appropriate being sodium heparinate or sodium citrate. Other anticoagulants may create artefacts that can be mistaken for crystals. The ideal is an extemporaneous examination of the fluid, at best within a maximum of 6 hours. The first examination establishes the macroscopic appearance: a normal synovial fluid is transparent, clear, very viscous and non-coagulable. The more inflammatory the fluid, the less viscous it is, viscosity being dependent on the concentration and on the degree of polymerisation of the hyaluronic acid secreted by the synovial cells. The leucocytes present in the joint secrete enzymes that depolymerise hyaluronic acid. The macroscopic appearance changes according to the disease: straw yellow or citrine yellow in the case of degenerative arthropathies, cloudy and purulent in septic arthritis. Haemarthrosis gives bloody, non-coagulable synovial fluids. Small white particles, "like grains of rice", may also be sought in rheumatoid arthritis; they correspond to "aggregates" of fibrin and synovial debris.

4.2. Cytology and cytological features

Normal synovial fluid contains fewer than 200 cells per mm3. These are essentially neutrophils, lymphocytes and cells of the synovial lining, or synovial cells. The latter make up the lining of the synovial membrane. They are released in reaction to a stimulus (the tap alone may be enough to cause synovial cells to appear in the fluid). A distinction is made between:

- **Mechanical synovial effusions:**

They are pale yellow in colour and fairly viscous, and have a cellularity below 2000 cells per mm3. These effusions have a predominance of mononuclear elements; they are encountered in osteoarthritis, aseptic osteonecrosis, osteochondritis dissecans, algodystrophy and synovial osteochondromatosis.

- **Inflammatory synovial effusions:**

The fluid is of a more marked colour, straw yellow. It is sometimes purulent in septic arthritis. Viscosity is low. The cell count is above 2000 cells per mm3. As regards the cell differential, several possibilities are found:

- **Predominance of neutrophils:** a percentage of neutrophils exceeding 95% is strongly suggestive of bacterial or microcrystalline arthritis. It should be noted that neutrophils with inclusions (formerly "ragocytes" of rheumatoid arthritis or "LE cells" of lupus) are not specific.
- **Predominance of lymphocytes:** a differential showing more than 70% of lymphocytes most often reflects rheumatoid arthritis, viral arthritis, systemic lupus erythematosus or even tuberculous arthritis.
- **Predominance of monocytes:** a predominance of monocytes is rather suggestive of viral arthritis, but without specificity. Many inflammatory rheumatic diseases may lead to monocytic effusions: rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis, psoriatic arthritis, etc. Synovial monocytosis is therefore not specific.
- **Predominance of eosinophils:** it is rare to observe an effusion rich in eosinophils (that is to say above 10%), except in parasitic arthritis, arthritis in allergic subjects, Lyme disease, or after an arthrography using a contrast medium. The cause often remains undetermined.

- **Haemorrhagic synovial effusions:**

Haemorrhagic fluids are to be distinguished from fluids with blood contamination caused by a traumatic tap or by perforation of vessels bringing a little blood into the syringe. True haemorrhagic fluids may have multiple causes: post-traumatic effusions, haemostasis disorders (haemophilia in particular, which in principle should not be tapped), septic arthritis, synovial haemangiomas, villonodular synovitis, osteoarthritis with ulceration of the subchondral bone. The appearance of these fluids is obviously bloody.

- **Synovial effusions with the presence of atypical cells:**

The search for neoplastic cells is always indicated. The presence of blasts is sometimes observed, especially in acute leukaemia of childhood with joint involvement. Exceptionally, neoplastic cells originating from solid tumours may be observed. A biopsy is indicated in these cases.

4.3. Biochemical and bacteriological analyses

The search for crystals is very important for the diagnosis of microcrystalline arthropathy. It is carried out under the microscope or by spectrometry. In particular, monosodium urate crystals are sought in gout, and calcium pyrophosphate dihydrate crystals in chondrocalcinosis; apatite crystals and other calcium phosphates are found in particular where the subchondral bone is exposed. Other crystals may be found less frequently (calcium oxalate crystals, cholesterol crystals, microcrystals of cortisone derivatives, etc.). Protein and glucose assays complete the biochemical examination.

Synovial fluid may be inoculated on conventional media or into blood culture bottles. The media will be adapted if necessary for the search for mycobacteria. In addition, fastidious pathogens may be sought using molecular biology techniques; Chlamydia and gonococcus in particular should be considered.

References

1. Buffet C. Conduite à tenir devant une ascite. EMC - Hépatologie. 2012;7(3):1-8.

2. Grancher T, Jeanne G. Biologie des liquides d'épanchement. Biomérieux; 2006.

3. Kjeldsberg. Chapter 5: Pleural et Pericardial Fluid. In: Kjeldsberg's Body Fluid Analysis. ASCP Press. 2015.

4. Lesesve J-F. Cyto-hématologie des liquides d'épanchement. Formation Biologie Prospective; 2016.

5. Pastré J, Roussel S, Israël Biet D, Sanchez O. Pleural effusion: diagnosis and management. Rev Med Interne. 2015;36(4):248-55.