Editorial

Additional techniques in serous effusions

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Abstract. Cytological examination is a valuable diagnostic tool in case of a serous effusion. The first manifestation of malignancy may be an effusion of the pleural, pericardial, or peritoneal cavity, especially in carcinoma of the ovary, or lung, and malignant mesothelioma. In other malignancies effusions may occur in the course of the disease. The contribution by Motherby et al. in this issue of ACP focuses on the contribution of image and flow cytometry to establish the presence or absence of malignancy in serous effusions [16]. They point out that the sensitivity of DNA image cytometry in equivocal effusions may be as high as 87.5%, and that for the detection of malignancy, DNA image cytometry is superior to flow cytometry.

1. Introduction

The body cavities have visceral and parietal linings, enabling “free” movement of enclosed organs. These surfaces are covered by mesothelium, a single layer of polygonal or flattened mesothelial cells [13,17]. Serous fluid in the body cavities originates from subserosal vessels, and is removed by lymph vessels. Typically in the absence of pathology little serous fluid is present [25]. A quantity of serous fluid sufficient of tapping, indicates a pathologic process in the body cavities. The amount of serous fluid is influenced by the permeability of vessels, hydrostatic pressure, lymph drainage disturbances, colloid-osmotic pressure, and by secretion of tumors. An increased amount of serous fluid may be either a transudate (low protein content), due to infiltration of blood serum across the physically intact vascular wall, or an exsudate (high protein content), due to active accumulation of fluid apparently associated with damage of walls of capillaries. If the effusion is transudative, therapy is mostly directed toward the underlying cause, e.g., congestive heart failure, or cirrhosis [17]. If the effusion is exsudative, attempts will be made to define the etiology, e.g., inflammatory processes or tumors. The diagnosis of malignancy in serous effusions is mostly established via cytology.

Cells which may be present in serous fluids are mesothelial cells, macrophages, erythrocytes, lymphocytes, neutrophilic and eosinophilic granulocytes, and malignant cells. A proliferation of mesothelial cells can be caused by an inflammatory process of the

serosa, presence of foreign body material, malignancy, and in a longstanding fluid. Reactive mesothelial cells easily desquamate into the serous fluid. The number of proliferating mesothelial cells may be high in chronic inflammation, carcinoma, trauma, lung infarction, processes with necrosis, and mesothelioma. A proliferation of mesothelial cells may be very hard to distinguish from malignant cells by routine cytological examination.

2. Effusions in malignancy

Malignant cells in serous effusions may be found in patients with primary tumors (malignant mesothelioma) or with secondary tumors (carcinomas, sarcomas and lymphomas). Malignant mesothelioma, known for the causal relationship with asbestos, is rare, has a very long latency period of 25–40 yrs, and a bad prognosis with a survival rate after 2 years of 20%. In men metastatic cancers in pleural effusion are mostly carcinomas of the lung, lymphomas/leukemias, and stomach; in ascites it concerns mostly tumors of the stomach, pancreas, and lymphomas/leukemias. In women metastatic cancers in pleural effusion are mostly of the breast, lymphomas/leukemias, ovary, stomach, and lung; in ascites it concerns mostly tumors of the ovary, breast, and stomach [17].

It is of importance to realize that in most cases of malignancy always two types of “atypical” cells are present in the effusion: reactive proliferating mesothe-

lial cells and a population of tumor cells. An exception when only solitary tumor cells are present is lobular adenocarcinoma of the breast and adenocarcinoma of stomach of the linitis plastica type. Many effusions associated with cancer may result from an indirect mechanism. This may be an effusion that develops as a result of venous obstruction caused by a neoplasm, or an effusion developing as a result of inflammatory changes, secondary to the presence of a neoplasm. In such effusions neoplastic cells may not be found.

3. Clinical importance

The major clinical importance of cytological examination of serous effusions is to establish the presence or absence of malignancy. In case of malignancy the type of the malignancy must be established, so that directed treatment can be started. In an effusion of a patient with a known malignancy it should be established whether it concerns a recurrence or a second primary tumor. Increasingly it is further required to be able to monitor the differentiation or changing expression of cell-biological characteristics (like Her2Neu and estrogen and progesterone receptors in breast cancer) of the tumor. Clinicians should be aware of the importance of adequate information to the pathologist and of optimal sampling.

Nowadays it has become feasible to examine small amounts of serous effusions. Previously, a limited amount of cell material and sub optimal fixation of cell samples restricted the number of stains that could be performed. The process of embedding of a part of the cell samples in agarcyto cell blocks has changed that. Agarcyto blocks enable immunocytochemical and cytochemical staining of identical cells [12,17]. It also enables correlation of the morphological and immunocytochemical information of the agarcyto cell blocks with previously removed histological material. Immunocytochemical and cytochemical reactions on agarcyto cell blocks are completely comparable with those of routinely processed tissues. Thus agarcyto cell blocks help to compare morphological, immunocytochemical and cytochemical information and are valuable for optimization of cytological diagnosis of effusions.

4. Additional techniques

The contribution by Motherby et al. in this issue of ACP focuses on the contribution of image and flow cy-

tometry to establish the absence or presence of DNA-aneuploidy in case of primary or secondary malignancy in serous effusions [16]. They point out that the value of routine cytological examination is limited by a relatively low sensitivity of 58%, and that this may be increased to 87.5% by performing DNA image cytometry, while having a specificity of 100%. Even in case of cytological equivocal effusions the sensitivity was still 75%, at the same specificity. In earlier studies by Decker et al. and Kayser et al. even higher sensitivities were found [7,11]. Decker et al. suggested that cytology should be combined with image DNA cytometry, giving in their study a sensitivity and specificity of 98.5% and 100%, respectively, [7].

Motherby et al. further demonstrate in this issue of ACP that for the detection of malignancy, DNA image cytometry is far superior to flow cytometry [16]. A low sensitivity of flow cytometry is in agreement with previous findings in fluids [2,6].

In the study by Kayser et al., which showed the results of a prospective, remote image DNA cytometry analysis using the Euroquant server, it was noted that 40% of effusions did not meet the technical requirements for image DNA cytometry [11]. This is in line with one of the recommendations in the study by Motherby et al. of a strict consideration of the ESACP standards [11,16].

Earlier, DNA image cytometry has been shown to be valuable to discriminate between benign effusions, effusions of patients with a malignant mesothelioma and effusions of patients with a secondary malignancy. Christen et al. [5], Oberholzer et al. [18], and Garcia-Bonafe et al. [9] have shown in this journal that image analysis of nuclear chromatin texture and nuclear geometry may also help to discriminate between malignant mesothelioma cells and reactive mesothelium cells.

Next to DNA cytometry, immunocytochemistry is increasingly important for examination of serous fluids. Motherby et al. [14,15] demonstrated in two nicely executed studies that a combination of DNA-aneuploidy and a positive immunocytochemical reaction with a monoclonal antibody Ber-EP4 may result in an accurate detection and discrimination of metastatic carcinomas and mesotheliomas. Immunocytochemistry has become the most widely used ancillary technique to establish the type of malignancy in serous effusions. Several markers have been described in the literature as important for the discrimination between benign, primary and secondary malignancies in serous effusions. Relevant markers may

be epithelial markers (cytokeratin 7, 8 and 20, EMA), lymphoid (LCA, CD5, CD15, CD20, CD68, kappa and lambda light chains), mesenchymal (vimentin, desmin) and mesothelial markers (calretinin), as well as other specific biomarkers (CEA, B72.3, Oc125, S100, E-Cadherin, Ber-Ep4, HMB-45, HMFG2, Her-2Neu, estrogen and progesterone receptor, chromogranin, thyroglobuline) [1,3,4,8,10,19,20,22,24,26]. Gupta et al. recently described a panel of common immunostains (EMA, CEA, Cytokeratin, B72.3, HMB45, Vimentin, S100, LCA, L26, and kappa and lambda light chains) to be useful in confirming or suggesting the site of the primary tumor [10]. Increasingly it thus has become possible to establish the type of cancer as well as other factors relevant for prognosis. By combining the clinical data, with cytomorphologic and immunocytochemical features it becomes feasible in most cases to discriminate between different malignancies, such as malignant mesothelioma, metastatic adenocarcinoma of the breast, ovary, stomach, colon, kidney, or thyroid, and specific malignancies such as small cell carcinoma, squamous cell carcinoma, sarcoma, Ewing's sarcoma, malignant melanoma, non-Hodgkin lymphoma, and Hodgkin's disease.

Also molecular techniques may offer an additional possibility of enhancing the diagnostic accuracy and may provide information regarding the origin of the primary tumor. This may also be applicable when a very limited number of cells or even no intact cells are present as often is the case in cerebrospinal fluids. In a small study we demonstrated that by PCR a leptomeningeal metastasis of a lung cancer could be demonstrated by the presence of a known tumor specific *K-ras* mutation in the supernatant of a cytological negative CSF [23].

5. Conclusion

Accurate diagnosis of a serous effusion mandates the use of ancillary methods such as immunocytochemistry and, as described in this issue of ACP by Moterby et al., by image DNA analysis [16]. The value of flow cytometry for this task is limited [16]. Molecular techniques may aid in an increasingly demanding patient management. Serous effusions should be prepared in such a way that routinely image DNA analysis, immunocytochemistry and molecular techniques can be carried out. Agarcyto cell blocks may become a standard procedure to study series of biomarkers for even small amounts of effusions. These techniques require a high quality of the cell preparation and staining techniques.

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