Original Article

Role of conventional cytology and cell block methods for diagnosis of malignant pleural effusions

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Background: The study evaluates the role of a combined cytological smear (CS) and cell block (CB) approaches in malignant pleural effusion (MPE), highlighting specificity and sensitivity referred to histology and the incidence of different types of malignancies.

Methods: We retrospectively included 223 patients over a duration of 5 years. Pleural fluid was collected and CS together with CB were made from the fluid. Immunocytochemical (ICC) stains were applied when requested by pathologist. Histology confirmation and medical records were recorded to confirm or not cytological diagnosis.

Results: The combined approach demonstrated a diagnostic accuracy of 95%, 92% sensitivity, 96% specificity, positive predictive value of 92%, negative predictive value of 96%, positive likelihood ratio of 23 and negative likelihood ratio of 0.08. Lung cancer was found to be the commonest (23 cases, 33.8%) malignant neoplasm diagnosed in the pleurae followed by breast cancer (14 cases, 20.6%), gastrointestinal cancer (7 cases, 10.3%) and mesothelioma (6 cases, 8.8%).

Conclusions: In our experience the combined approach definitively has proven to have an excellent diagnostic yield in the MPE, allowing to identify the nature of malignant cells in most of patients.

Keywords: Cell block (CB); cytological smear (CS); immunocytochemical stains (ICC stains); malignant pleural effusion (MPE)

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Introduction

Pleural effusion is a clinical condition characterized by excessive accumulation of fluid inside the pleural cavity. The causes that determine the formation of an effusion can be many, among them, heart failure, pneumonia, tuberculosis but also metastatic tumors or cancer of the pleural cavity. The cytological analysis of the pleural fluid is the first-line diagnostic test together with the biochemical analysis, especially in the suspicion of malignancy (1,2). In fact, thoracentesis is a simple procedure with low complication

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rates (3) and therefore more accessible and cost-effective than potentially higher performance methods such as video assisted thoracoscopy (VAT). The guidelines from the American College of Chest Physicians (ACCP) and the British Thoracic Society (BTS), recommend cytological smear (CS) of two samples of pleural effusions (4,5). If procedures are non-diagnostic, more invasive investigations such as image-guided pleural biopsy or thoracoscopic biopsy are recommended. However, the international literature of the past three decades describes a sensitivity range of 40–87% of pleural fluid cytology for the detection of MPE (4-7). This also varies with the histopathology of the tumor; in fact, sensitivity for adenocarcinoma was reported as 60% in a study versus only 30% for mesothelioma and MPE due to adenocarcinoma is more easily diagnosed than squamous cell carcinoma, sarcoma and lymphoma (5,8). The limits of the CS are in fact linked to poor morphological details often due to the overlapping of cells, the presence of inflammatory cells that can overlap neoplastic cells and the loss of important morphological characteristics in the sample preparation phase (9). The cell block (CB) method overcomes these limits because it allows the study of tissue architecture and a better conservation of the morphological features which allows a better differentiation between malignant and non-malignant cells, but also for the further characterization of cells with special stains and ICC (10). The CB technique is an ancient method for assessing body cavity fluids. Scientific guidelines indicate that the preparation of CB from pleural effusion samples, in addition to CS, allows the "microhistology" of the solid cell portion which can lead to greater diagnostic accuracy. Its main advantage is the preservation of the tissue architecture, obtaining multiple sections for special staining and immunocytochemistry (10-12). However, it is not used in routine daily clinical practice but performed only at the discretion of the pathologist or clinician. However, it is a simple method that requires no special training or tools. It is safe, cost-effective and reproducible even in rural areas with limited resources (13). On the contrary, in our institution all pleural fluids are used to obtain both CS (a conventional smear and a thin layer) and CB. The aim of the study is to evaluate the diagnostic accuracy, sensitivity and specificity of this combined approach, referring to histological confirmation or clinical data. We present the following article/case in accordance with the STROBE reporting checklist (available at http://dx.doi.org/10.21037/jxym-20-66).

Methods

Patients were enrolled after retrospective research using the laboratory information system. The database included cytopathological case number, gender, cytological diagnosis. Pleural effusion samples obtained between January 2015 and December 2019 were included with the aim of assessing sensitivity, specificity, negative and positive predictive value, diagnostic accuracy, negative and positive likelihood ratio of this combined approach (CS+CB) for the detection of MPE referring to the gold standard, i.e., histological confirmation, and clinical data, if histology was not available.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by institutional ethics committee. Informed consent was taken from the patients.

One hundred thirty-two patients were male (59.2%) and 91 were female (40.8%). Fresh PE was centrifuged at 1.750 rpm for 10 min and the supernatant removed. A few drops were drawn from the sediment and one direct slide smear was prepared and submitted for May-Grünwald-Giemsa staining. Another smear was prepared using a ThinPrep method and this slide was fixed and submitted for Papanicolaou staining. To prepare CB, Agar solution was added to the sediment, followed by refrigeration to form a solid clot. The clot was fixed in 10% neutral buffered formalin solution and automatically processed into a paraffin-embedded block. Two histological slides were cut and hematoxylin and eosin (H&E) and PAS staining were performed. All samples were reviewed by two cytopathologists. ICC stains were applied in cases of PE positive or suspicious for malignant cells as described in *Figure 1*. For cytological diagnosis, the conventional diagnosis criteria were divided into three categories as benign, malignant, and suspicious for malignancy. In CB examination, histopathological diagnosis was done in cases with sufficient cell counts. Immunocytochemistry panels on CB sections were applied according to the suspected tumor type based on cytomorphology.

Statistical analysis

Statistical analysis was performed using a statistical software package (SPSS 25.0; SPSS Inc., Chicago, IL, USA) for Windows. Mean ± standard deviation (SD) (normally distributed data), median and range (non-normally distributed data) and percentage frequencies was calculated.

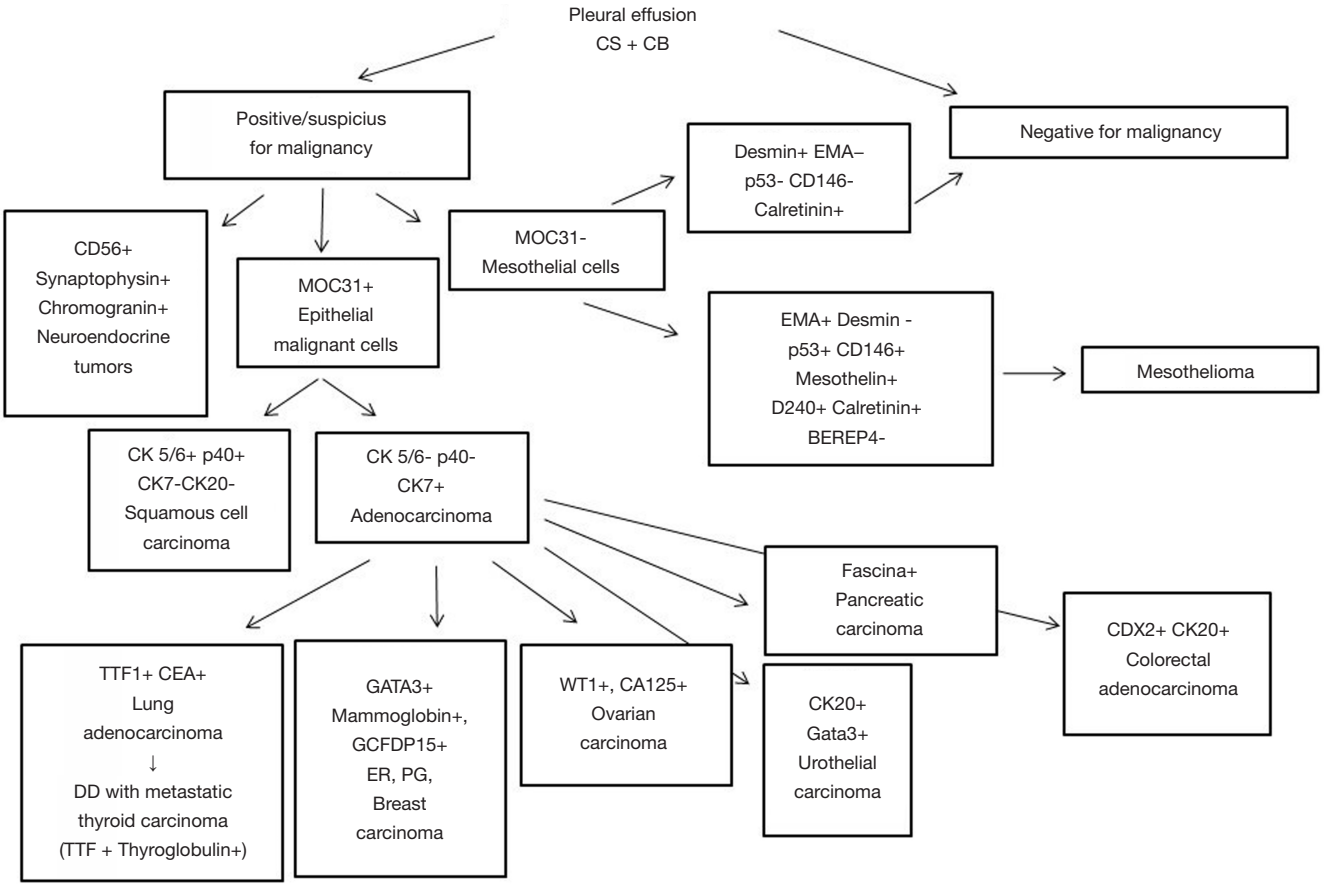

Figure 1 Diagnostic algorithm applied in our Institution. Two smears and a cell block are performed on all samples. In case of morphology suspicious or suggestive for malignancy, immunocytochemistry was carried out, using an average of three antibodies per sample.

Within-patient comparisons were made by paired *t*-test and Fisher's exact test, as appropriate, at significance levels of $P<0.05$.

Results

Out of 223 patients, 68 were positive for malignancy (30.5%), 6 (2.7%) were suspect for malignant disease, 5 (2.2%) are inadequate samples for diagnosis. The number of females with malignant cytology (37 cases, 40.7%) was significantly higher than males (31 cases, 23.5%) (P=0.0074) (*Table 1*). The main cause of MPE was metastatic adenocarcinoma. Using the combined approach and integrating cytomorphology with ICC, like described in *Figure 1*, we have identified site of origin of malignant cells in 50 cases (73.5%). With reference to histological and clinical data (for patients in whom the biopsy was not performed), the combined approach (CS+CB) on pleural effusion showed a sensitivity of 92%, a specificity of 96%, a positive predictive value of 92% and a negative predictive value of 96% with a diagnostic accuracy of 95%. The positive likelihood ratio was 23 and the negative likelihood ratio was 0.08. The most common primary site involved in the MPE for the total cohort was lung (23 patients, 33.8%), followed by breast cancer (14 cases, 20.6%), gastrointestinal cancer (7 patients, 10.3%; in detail 4 colon, 2 pancreas and biliary tract, 1 stomach), mesothelioma (6 patients, 8.8%), ovarian-carcinoma (4 patients, 5.9%), head-neck squamous carcinomas (2 patients, 2.9%), lymphoma (1 patient, 1.5%), endometrial cancer (1 patient, 1.5%) and neuroendocrine tumor (1 patient, 1.5%). For the remaining 9 patients (13.2%), the pathologists were able only to discern malignancy as adenocarcinoma in 6 cases and epithelial origin of MPE in 3 cases, but could not go further because of insufficient materials for further ancillary studies, lack of clinical history of a primary tumor site or nonspecific

Table 1 Distribution and diagnosis of pleural effusion specimens by year and gender

Year	PE positive for malignancy, N (%)	PE negative for malignancy, N (%)	PE suspicious for malignancy, N (%)	PE inadequate for diagnosis, N (%)	Male with MPE, N (%)	Female with MPE, N (%)	Male negative for MPE, N (%)	Female negative for MPE, N (%)
2015	6 (2.7)	39 (17.5)	0 (0)	0 (0)	1 (0.4)	5 (2.2)	28 (12.6)	11 (4.9)
2016	22 (9.9)	44 (19.7)	2 (0.9)	0 (0)	10 (4.5)	12 (5.4)	25 (11.2)	19 (8.5)
2017	19 (8.5)	22 (9.9)	2 (0.9)	4 (1.8)	9 (4.0)	10 (4.5)	16 (7.2)	6 (2.7)
2018	12 (5.4)	20 (9.0)	1 (0.4)	0 (0)	9 (4.0)	3 (1.3)	12 (5.4)	8 (3.6)
2019	9 (4.0)	19 (8.5)	1 (0.4)	1 (0.4)	2 (0.9)	7 (3.1)	13 (5.8)	6 (2.7)
Total	68 (30.5)	144 (64.6)	6 (2.7)	5 (2.2)	31 (13.9)	37 (16.6)	94 (42.2)	50 (22.4)

PE, pleural effusion; MPE, malignant pleural effusion.

morphology and/or immunocytochemical (ICC) staining patterns. Stratifying by gender, the most common etiology for a malignant pleural effusion (MPE) for women was breast cancer (14 patients, 37.8%), followed by lung cancer (9 patients, 24.3%) ovarian cancer (4 patients, 10.8%) and gastrointestinal cancer (3 patients, 8.1%). For men the common etiology was lung cancer (14 patients, 4.2%) followed by gastrointestinal cancer (4 patients, 12.9%) and mesothelioma (4 patients, 12.9%). ICC studies were performed on 74 (33.2%) CB, with a median of 3 (25th to 75th percentiles 1–4) immunostains per case. From a total of 231 immunostainings, TTF-1 (35, i.e., 47.3% of ICC), CK 7 (29, i.e., 39.2% of ICC), CK 20 (24, i.e., 32.4% of ICC), MOC31 (16, i.e., 21.6% of ICC), WT1 (15, i.e., 20.3% of ICC), calretinin (12, i.e., 16.2% of ICC), mammaglobin (10, i.e., 13.5% of ICC), estrogen receptor (10, i.e., 13.5%), D240 (9, i.e. 12.2% of ICC), CDX2 (8, i.e., 10.8% of ICS), EMA (7, i.e., 9.5% of ICS), GATA3 (6, i.e. 8.10% of ICS), CK5/6 (5, i.e., 6.8% of ICC) were the most commonly used.

Discussion

Thoracentesis is a non-invasive technique and widely available in many hospitals. This technique allows to obtain the pleural fluid to perform cytological investigations in the MPE suspicion. The combined CS and CB techniques increases the diagnostic value of CS alone (14-20). CS is the first line method in association with biochemical tests if there is pleural effusion and a suspicion of malignancy. However, literature data suggest a low sensitivity of this method.

The major limitation of this method is related to the low ability to distinguish reactive mesothelial cells from neoplastic mesothelial and epithelial cells. These limits are due to the artifacts related to the preparation and staining techniques, but also to the limit of the absence of a three-dimensionality that causes overcrowding of cells and overlap with poor resolution and difficulty of interpretation. The CB technique allows to overcome many of these limitations

In fact, allowing to obtain a histological piece, it provides more morphological details such as the presence of cellular spheres, papillae, clusters, it also allows to obtain multiple sections and stainings and above all ICC stains (9,13,14,17,21).

Last but not least, the use of CB gives the possibility to conserve the material and to use it for molecular biology tests such as histological samples (11,12,14,22,23). However,

despite the guidelines invite to use CB for the advantages of this well established technique (24), CB is considered time consuming and is performed only in few Institution (5,25). A recent survey among last two-year Spanish residents of Pneumology or Internal Medicine showed that only 16% of 139 responders in their clinical practice actively ordered a CB when confronted with a suspected malignant effusion, whereas the remaining either never did (27%) or left the decision to the pathologist discretion (57%) (26). In our Institute CB was carried out on all pleural fluids received for cytological investigations. In addition to the CB, 2 smears are performed, one thin layer smear and one conventional smear, then two stains are performed (Papanicolaou and MG) and a cytochemical stain with PAS. An ICC panel is also performed on samples showing cells suspected for malignancy or with atypical characteristics, which provides an average of 3 antibodies addressed as described in *Figure 1*. Using this protocol, the sensitivity and specificity data and the diagnostic accuracy are widely increased compared to literature data (27-29). The performance data obtained are also superior to two successive samplings with repeated thoracentesis (30), confirming the usefulness of the combined cytological diagnosis compared to more invasive and less available methods, such as pleural biopsy in VAT. In keeping with our data it should be noted that in specialized centers, mesothelioma can be diagnosed using pleural fluid cytology in up to 73% of the cases (31), highlighting the importance of the cytologist's skills. Currently, experienced cytopathologists promote cytological diagnosis of mesothelioma without the need for biopsy, if pleural fluid cytoarchitectural and ICC features are conclusive. The diagnostic protocol for MPE, used in our center, also made possible to identify the site of origin in 73.5% of cases. The frequency of malignancy shown are in agreement with the literature (32,33), which highlights a prevalence of lung tumors in men and breast tumors in women. In our experience, the frequency of hematological tumors is lower than the literature data but this is probably linked to the presence of a flow cytometry service in another department, constituting a bias in our study. Other limitation is that it is a retrospective study and pathologists were not blinded of clinical data. Furthermore, we have analyzed all pleural fluids received at the Pathology and Histology Unit and not all those performed at our hospital; this could generate a sample selection bias. Finally, in half of pleural effusions the malignancy was not confirmed by histological data but it was based on solid clinical data.

Conclusions

In conclusion, considering the challenging implications due to the presence of malignant cells in pleural effusions, an accurate cytologic evaluation represents a critical and mainstream diagnostic tool being easy, safe and cost-effectiveness, reducing complications of a more aggressive biopsy procedure. Our study confirms that the combined approach (CB+CS) increases the diagnostic yield in MPE. CB method was a simple, inexpensive and did not require any special training or instrument. Bridging the gap between cytology and histology CB should be considered as a useful adjuvant technique along with CS in evaluating pleural fluid cytology, being the preferred substrate for ICC. Our study suggests that pathologists should be use this approach in their clinical practice and clinicians should be encouraged to request CB, along with the conventional routine cytology, in order to increase diagnostics sensitivity and establish a more definitive cytopathological diagnosis.

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appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by institutional ethics committee (No. PA 12 01, CHT 03-2019). Informed consent was taken from the patients.

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