Original Article

A comparative study of conventional cytology and cell block method in the diagnosis of pleural effusion

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Background: In a patient with pleural effusion, cytological study (CS) is one of the most useful investigations, especially when malignancy is suspected. Instead of applying only CS, the pleural fluid can be further processed using the cell block (CB) technique, which may augment the diagnostic utility. The aim of this study was to compare the diagnostic yields of CS, CB, and the combination of both, regardless of the etiology of pleural effusion.

Methods: A cross-sectional study was conducted on patients with pleural effusions who underwent thoracentesis from June 2015 to May 2016. All samples were submitted for routine biochemical analysis, CS, and CB histology. The results of cytopathological studies were compared to the final diagnoses.

Results: Out of a total of 353 samples, the final diagnoses included 278 (78.8%) malignancies, 41 (11.6%) infectious diseases, 16 (4.5%) other inflammatory diseases, and 18 (5.1%) transudative pleural effusions. CS and CB provided a similar diagnostic yield (48.7% *vs.* 49.9%, P=0.69), while the combination of both gave a higher yield (57.2%) (P<0.001, compared with CS). Among 278 malignant pleural effusions (MPE), the diagnostic yields of CS and CB were 61.2% and 61.9%, respectively. Combined CS and CB improved the diagnostic yield to 71.2% (P<0.001). However, both CS and CB had low diagnostic yields in infectious pleuritis, other inflammatory diseases, and transudative pleural effusions.

Conclusions: In MPE, CB provides a similar diagnostic performance to CS, while application of both techniques can significantly increase the diagnostic yield. However, in other pleural diseases, CB and CS had limited values in diagnosis.

Keywords: Cell block (CB); cytology; pleural effusion; malignant pleural effusion (MPE)

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Introduction

Thoracentesis is a diagnostic procedure for patients with pleural effusion. Pleural fluid (PF) obtained from the procedure should be submitted for biochemical, microbiological, and cytological study (CS). In cases of suspicion of malignant pleural effusion (MPE), CS is extremely useful as it provides a diagnostic rate of 60%, ranging from 40% to 87% (1-4). CS is important not only in diagnosis but also in staging and further guiding treatment for malignancy. Many widely used guidelines, such as those of the American College of Chest Physicians (ACCP) and the British Thoracic Society (BTS), recommend CS of two samples of pleural effusions (1,4). If the procedures turn out to be non-diagnostic, further invasive investigations such as imaged-guided pleural biopsy or thoracoscopic biopsy are recommended for a definitive diagnosis.

The challenges of obtaining a diagnosis from CS include indistinct morphological details, overlapping or

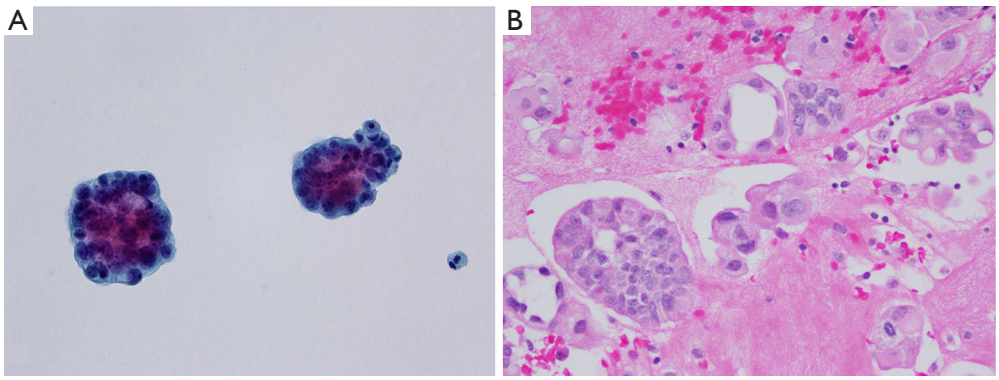

Figure 1 Cytologic and histologic findings in a patient with pleural effusion. (A) Cytology specimen demonstrates metastatic adenocarcinoma with tumor cells arranged in three-dimensional configuration (Papanicolaou staining, ×400); (B) corresponding cell block section of the same patient (hematoxylin and eosin staining, ×400).

overcrowding of cells, abundance of inflammatory cells, paucity of representative cells, and cell losses or changes (5). To overcome these limitations, cell block (CB) method was developed to provide better tissue architecture and morphological features for differentiating between malignant and non-malignant cells and also for further processing via special stains and immunohistochemistry (6). Although CB technique has been known for nearly a century, there have been few reports and a limited number of samples involving the direct comparison of CS and CB on consecutive patients for diagnosis of pleural effusion (5,7-12). In addition, most studies have focused on the diagnostic yield in MPE. The aim of this study was to compare the diagnostic yields of CS, CB technique and the combination of both, regardless of the etiology of pleural effusion.

Methods

Patients

A cross-sectional study was conducted at Ramathibodi Hospital, Mahidol University, Thailand, from June 2015 to May 2016 on patients >15 years of age with pleural effusion, as demonstrated on chest radiographs, who underwent thoracentesis for diagnostic purposes. Written informed consent was obtained from all patients before commencement of the procedure. The study protocol was approved by the Ethics Committee on Human Experimentation of Ramathibodi Hospital, Faculty of Medicine, Mahidol University (ID 05-58-15).

PF was sent for biochemical [protein, glucose, lactate dehydrogenase, and adenosine deaminase (ADA) level] and microbiological [Gram and acid-fast bacilli (AFB) staining and bacterial and mycobacterial culture] analysis, white blood cell count and differential count, and CS and CB for diagnostic evaluation. Other diagnostic investigations of PF were performed according to clinical suspicions.

CS

15 mL of fresh PF was centrifuged at 2,500 rpm for 20 min and the supernatant removed. One direct slide smear was prepared from cell sediment and submitted for Papanicolaou staining. 15 mL of CytoLyt® solution (Hologic, Marlborough, MA, USA) was added to the remaining sediment and centrifuged at 600 rpm for 20 min. The supernatant was poured off and the sediment was placed into a vial of PreservCyt® solution (Hologic) and left to stand for 15 min. The vial was run on an automated ThinPrep® 2000 processor (Hologic) giving one liquid-based slide. The slide was fixed and submitted for Papanicolaou staining. Both conventional and liquid-based slides were sent for cytological evaluation (*Figure 1A*).

CB study

15 mL of fresh PF was centrifuged at 6,000 rpm for 5 min and the supernatant removed. Agar solution was added to the specimen, 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. A histological slide was cut and hematoxylin and eosin (H&E) staining was performed (*Figure 1B*). Microbiological and immunohistochemical

Table 1 Demographic data of 353 patients who underwent diagnostic thoracentesis

Variables	N (%) or mean ± SD
Age, years, mean ± SD	62.1±15.5
Gender, male	161 (45.6)
Side of pleural effusion, right	198 (56.1)
Amount on chest radiograph	
Less than 1/3 hemithorax	24 (6.8)
1/3 to 1/2 hemithorax	101 (28.6)
1/2 to less than whole hemithorax	164 (46.5)
Whole hemithorax	64 (18.1)
Exudative effusion	335 (94.9)
Final diagnosis	
Malignancy	278 (78.8)
Lung cancer	133
Non-small cell lung cancer	132
Small cell lung cancer	1
Non-lung cancer	114
CNS tumor	1
Head and neck cancer	4
Breast cancer	33
Thymic cancer	7
Germ cell tumor	2
Hepatocellular carcinoma	4
Gastrointestinal carcinoma	28
Gynecologic cancer	25
Prostate cancer	2
Soft tissue sarcoma	5
Skin cancer	2
Mesothelioma	1
Hematologic malignancy	31
Leukemia	2
Lymphoma	28
Multiple myeloma	1
Infectious diseases	41 (11.6)
Bacterial infection	16
Tuberculous pleuritis	24
Fungal infection	1
Others	16 (4.5)
Lupus pleuritis	5
Uremic pleuritis	4
Chylothorax	1
Hemothorax	3
Pulmonary embolism	1
Reactive pleuritis	2
Transudative effusion	18 (5.1)

SD, standard deviation; CNS, central nervous system.

(IHC) stains were applied in cases where special staining was indicated and requested.

A diagnosis was established when CB or CS results were defined as malignant disease or specific non-neoplastic disease. CB or CS diagnosis of non-specific inflammation was considered to be non-diagnostic, although the final diagnosis proved to be a benign process. The final diagnosis in non-diagnostic CB or CS results was reached by thoracoscopic pleural biopsy, biochemical results, microbiological results, clinical manifestations, radiographic findings, and improvement or progression on treatment and follow-up.

Statistical analysis

All data were analyzed with a statistical software package (SPSS for Windows version 16.0; SPSS, Chicago, IL, USA). Values were expressed as mean ± standard deviation for continuous variables, and as frequencies and percentages for categorical variables. The diagnostic yields of CS and CB were compared using McNemar's test. All statistical tests were two-sided and $P<0.05$ was considered to be statistically significant.

Results

There were 353 patients, comprising 161 males and 192 females with a mean age of 62.1±15.5 years. The demographics of the study population are shown in *Table 1*. CS and CB provided similar diagnostic yield (48.7% and 49.9%, respectively; P=0.69). However, a combination

Table 2 Final diagnoses and diagnostic methods for 151 non-diagnostic cytological study and cell block histology patients

Final diagnoses	Diagnostic methods	Numbers
Malignancy		
Lung cancer		
Non-small lung cancer	Thoracoscopic pleural biopsy	1
	Response to chemotherapy	25
Non-lung cancer		
	Thoracoscopic pleural biopsy	2
	Response to chemotherapy	38
Hematologic cancer		
Lymphoma	Thoracoscopic pleural biopsy	2
	Response to chemotherapy	12
Infectious diseases		
Bacterial infection	Response to antibiotics	14
Tuberculous pleuritis	Thoracoscopic pleural biopsy	2
	Positive AFB culture + response to anti-tuberculous drugs	1
	High adenosine deaminase level + response to anti-tuberculous drugs	19
Fungal infection	Positive fungus culture	1
Others		
Lupus pleuritis	Response to treatment	5
Uremic pleuritis	Response to hemodialysis	4
Chylothorax	Biochemical analysis	1
Hemothorax	Hematocrit of pleural effusion >50%	3
Pulmonary embolism	Clinical manifestation+ radiographic findings + improvement on follow-up	1
Reactive pleuritis	Clinical manifestation + response to treatment	2
Transudative pleural effusion	Biochemical analysis + response to treatment	18

of CS and CB gave a higher yield (57.2%) than CS alone (P<0.001). The diagnostic methods for the 151 patients with non-diagnostic CS or CB are shown in *Table 2*. For cancer patients for whom definite etiologies of pleural effusions were not achieved by cytopathology, primary cancers were all established and pleural effusions decreased after chemotherapy.

Regarding the etiologies of pleural diseases, CS and CB rendered similar diagnostic yields in MPE (61.2% and 61.9%, respectively; P=0.89). In 108 negative CS MPE patients, CB revealed malignant disease in 28 patients (25.9%) and a combination of CS and CB improved the diagnostic yield significantly compared with CS (P<0.001) (*Table 3*). In infectious pleuritis, CS could diagnose in one case of empyema thoracis and one tuberculous pleuritis. CB provided additional diagnosis in one other case of empyema thoracis and one tuberculous pleuritis. In other inflammatory diseases and transudative pleural effusions, CS and CB had no role in diagnosis.

CB achieved a diagnostic yield similar to that of CS in all subgroup of malignancies. Although not reaching statistical significance, CB tended to have higher diagnostic yield than CS in hematologic malignancy (*Table 4*). Submission of CB improved the diagnostic yield to CS in all subgroup of malignancies.

All CB specimens with confirmation of malignancy

Table 3 Diagnostic yield of cytological study (CS), cell block (CB), and the combination of both techniques (CS + CB)

Final diagnosis	Diagnostic yield				
	CS (n, %)	CB (n, %)	P value	CS + CB (n, %)	P value (compared with CS)
Malignancy (n=278)	170 (61.2)	172 (61.9)	0.89	198 (71.2)	<0.001
Infectious diseases (n=41)	2 (4.9)	4 (9.8)	0.5	4 (9.8)	0.5
Others (n=16)	0 (0.0)	0 (0.0)	N/A	0 (0.0)	N/A
Transudative effusions (n=18)	0 (0.0)	0 (0.0)	N/A	0 (0.0)	N/A
Total (n=353)	172 (48.7)	176 (49.9)	0.69	202 (57.2)	<0.001

N/A, not available.

Table 4 Diagnostic yield of cytological study (CS), cell block (CB), and the combination of both techniques (CS + CB) in subgroups of malignancy

Final diagnosis	Diagnostic yield				
	CS, n (%)	CB, n (%)	P value	CS + CB, n (%)	P value (compared with CS)
Solid malignancy (n=247)	159 (64.4)	156 (63.2)	0.77	181 (73.3)	<0.001
Lung cancer (n=133)	98 (73.7)	91 (68.4)	0.23	107 (80.5)	0.004
Non-lung cancer (n=114)	61 (53.5)	65 (57.0)	0.52	74 (64.9)	<0.001
Hematologic malignancy (n=31)	11 (35.5)	16 (51.6)	0.12	17 (54.8)	0.03

that underwent more specific evaluations as requested by the oncologists were adequate for further examination, including IHC staining in 87 patients (lung cancer, 38; non-lung cancer, 37; and hematologic malignancy, 12) and molecular testing for lung cancer [epidermal growth factor receptor (EGFR) mutations, 63; and anaplastic large-cell lymphoma kinase (ALK) rearrangements, 39].

Discussion

Diagnosis of pleural disease can be made by direct examination of the pleura itself or by indirect evaluation of PF that accumulates in the pleural cavity. As it is a non-invasive technique and available in most hospitals, thoracentesis to retrieve PF for examination is well accepted as an initial investigation. In MPE, CS from PF provides a diagnostic rate of 60%, ranging from 40% to 87% (1-4). During thoracentesis, closed-blind pleural biopsy can be performed simultaneously to obtain pleural tissue for histology. However, its diagnostic yield is less sensitive than CS, as pleural metastases tend to be focal in the parietal pleura (13,14). In addition, pleural biopsy sometimes fails to provide adequate tissue. Furthermore, complications such as pneumothorax, hemothorax, extravasation of PF, and injury to adjacent organs may occur. Therefore, closed-blind pleural biopsy is not routinely recommended in all cases, and has been replaced by image-guided pleural biopsy.

To enhance the diagnosis from cytohistology of PF, the sediment from centrifuged PF can be processed as CB for histology. Although CB technique has been described for nearly a century and is well-known among cytopathologists, it is still underprescribed by clinicians. Most of the published studies were conducted by cytopathologists, and PF samples selected for investigation had already been submitted to the laboratory, conditions which do not resemble daily clinical practice. Therefore, we conducted this study to explore the benefits of CB when integrated as a part of PF examination in routine clinical practice.

There are many fixative substances for binding the sediment cells before embedding into paraffin blocks, including formalin, alcohol-formalin, alcohol-acetic acid-formalin, agar, plasma thrombin clot, CytoLyt-prefixed thrombin clot, HistoGel™, and inverted filter sedimentation (5-8,15). Although some studies have evaluated cell morphology and IHC performance of CB using different fixatives (6,15), there is no consensus guideline in this process, leaving the choice up to each institute based on availability and cost-affordability. In this

study, agar solution was used to form a solid clot. The cost per CB sample was $27 US.

In MPE diagnosis, CB has certain advantages over CS. Improper smear, fixation, and staining techniques in CS can cause cell overlapping or overcrowding, cell loss, artifacts, and poor background staining, while these are less frequent in CB (5-8). Cellularity is higher by CB compared with CS and is concentrated in one small area that can be evaluated at a glance, with all cells lying in the same focal plane of the microscope (11,12). In addition, CB provides better cellular morphological details, such as better nuclear and cytoplasmic preservation, intact cell membrane and crisp chromatin; there is also less difficulty in microscopic observation, in spite of the presence of excess blood in the background (12). Regarding tissue architecture, adenocarcinoma cells, especially from the lung, breast, and gastrointestinal tract, may not clearly exhibit cellular morphology of malignancy; better morphological details and tissue architecture pattern are required for diagnosis (5,11,12). Unfortunately, CS has the limitation of a lack of tissue architecture. Singly scattered cells are predominantly found in CS, whereas architectural patterns such as glands, sheets, three-dimensional cell clusters, and cell balls are commonly demonstrated in CB, resulting in increased sensitivity of diagnosis of MPE by CB method (12). Discrimination of reactive mesothelial cells and malignant cells is a major challenge in CS, as reactive mesothelial cells may express large irregular nucleoli, coarse chromatin, and enlarged nuclei, mimicking malignancy (5,7,8,11,12). In contrast, in CB the nucleoli are not as prominent as in CS and the pseudo-acinar or acinar structures can be better appreciated (11,12). Finally, CB specimens can be stored and multiple sections performed by routine staining, special staining, IHC staining, and also molecular testing as in our study and previous reports (16,17), while storage of CS remains a practical problem. However, CB entails a risk of losing material during preparation, especially in the case of fixation technique (18) that might explain negative CB results but positive CS results in some cases. Similar to our results, previous studies showed an additional diagnostic rate of CB to CS around 10–15% in MPE (5,7,8,12,19).

In tuberculous pleuritis, granulomatous inflammation, which is a key to cytopathological diagnosis, usually occurs throughout the parietal pleura, resulting in a high diagnostic yield of closed-blind pleural biopsy (2,4,20,21). In contrast, this reaction rarely exfoliates into the PF, and consequently there are low diagnostic yields of CS and CB. PF-ADA level of greater than 40 U/L achieves a high diagnostic rate similar to that of closed-blind pleural biopsy, which may render routine closed-blind pleural biopsy unnecessary in the initial thoracentesis (21).

As expected, CS and CB had no role in diagnosis of non-infectious inflammatory pleuritis and transudative effusion. Clinical manifestations and biochemical analysis are required to obtain a diagnosis in these conditions.

There were some limitations in our study. As pleural effusion in cancer patients does not usually resemble MPE (so-called paramalignant effusion), a clinical diagnosis of response to chemotherapy may not be valid for concluding a final diagnosis of MPE in negative CS and CB patients. We did not perform other invasive diagnostic procedures to reach a final diagnosis when there was no any clinical benefit to the patients as suggestion by the oncologists, especially in known advanced malignant diseases. In addition, we did not routinely perform special staining to define all subtypes of malignancies if there was no treatment benefit. However, if required, all CB specimens with confirmation of malignancy were adequate for further examination.

In conclusion, we demonstrated that in MPE, CB method provided a similar diagnostic performance to CS, while submission of both techniques can significantly increase the diagnostic yield. However, in other pleural diseases, CB and CS had limited values in diagnosis, requiring clinical presentation as well as biochemical and microbiological examinations of PF.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study protocol was approved by the Ethics Committee on Human Experimentation of Ramathibodi Hospital, Faculty of Medicine, Mahidol University (ID 05-58-15).

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