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Comparison of Examination Results of Cytocentrifuge Cytology and Cell Block Methods
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Comparison of Examination Results of Cytocentrifuge Cytology and Cell Block Methods on Pleural Fluid Confirmed with Calretinin Immunohistochemistry as Mesothelial Cell Marker

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ABSTRACT

Background
Pleural effusion is an abnormal accumulation of fluid in the pleural cavity, which occurs as a result of disturbance in the balance between production and reabsorption. Cytologycal examination is important as diagnosis, determining the stage and guiding further treatment for malignancy. Effusion fluid is often processed using cytocentrifuge cytology or cell block methods which have their advantages and disadvantages. Calretinin as mesothelial cell marker valuable for establishing a more definitive cytopatholgical diagnosis.

Methods
The disain of this study was analytic observational study with a cross-sectional approach of 49 samples cytologically diagnosed pleural fluid specimens at private hospital in Medan. Examination method cytocentrifuge cytology and cell block followed calretinin immunohistochemistry was done. Data analysis was tested by Chi-square test.

Results
The result of pleural effusion examination were confirmed by calretinin immunohistochemistry, which showed positive mesothelial cell in 45 cases (91,8%), while those who did not show or negative mesothelial cell were 4 cases (8,2%).

Conclusion
There was a significant different between the result of the cytocentrifuge cytology and cell block methods with a p-value < 0,001.

Keywords: Pleural effusion, cytocentrifuge cytology, cell block, calretinin

INTRODUCTION

The pleural cavity is a potential space between the two pleural layers filled with pleural fluid which functions as a lubricant to allow the two pleural layers to touch properly during respiration. Pleural effusion is an abnormal accumulation of fluid in the pleural cavity, which occurs as a result of a disturbance in the balance between production and reabsorption, which may indicate pulmonary, pleural, or extrapulmonary disease.[1,2] This condition is a common clinical and radiological finding, with a wide variety of causes, ranging from quite innocuous effusions accompanying viral pleurisy to those that are prognostically very poor relevant because of congestive heart failure or cancer.[2,3]

According to the World Health Organization (WHO) in 2017 the prevalence of pleural effusion in the world was reported to be 320 out of 100,000 cases in industrialized countries. Cases of pleural effusion in the United States are around 1.3 million cases per year. The estimated causes are congestive heart failure, malignancy and pulmonary embolism, including Kariadi Hospital Semarang with pleural effusion sufferers for women 66.7% and men 33.3%(kalimatnya diperbaiki). A study at H. Adam Malik General Hospital in Medan showed that 136 cases of pleural effusion consisted of 34.6% women and 65.4% men.[4] The prevalence of malignant pleural effusion (MPE) was reported as 15% of all cancer cases.[2]

Prompt diagnosis of the existence and nature of pleural effusion is essential to evaluate the best therapeutic options (diuretics, invasive procedures, etc.). Pleural effusion, and diagnosing the cause of this effusion requires a combination of clinical, radiological and laboratory examinations.[5] Evaluation of patients with pleural effusion can be a challenge for clinicians.[8,9] In patients with suspected symptoms of lung cancer and pleural effusion, the first process to be performed is thoracocentesis,[9-11] which is a diagnostic procedure for patients with pleural effusion. Pleural fluid obtained by this procedure must be submitted for biochemical, microbiological, and cytological studies. Pulmonary or metastatic involvement of the visceral or parietal pleura may come from extrapulmonary malignancy.[10] Many pleural effusions have no clear etiology, so interpretation of pleural fluid results, in addition to clinical and radiological information, is essential in making the diagnosis.[5]

Cytological examination is important not only in diagnosis but also in determining staging, guiding further treatment for malignancy,[1,3,12,13] as well as for evaluating the prognosis of the disease.[1,13] Effusion fluid is often processed using conventional cytology techniques and/or fluid-based cytology.[14] However, cytocentrifuge cytology examination can be a diagnostic problem, because the differentiation between benign and malignant cellular changes may be very difficult to understand, especially in differentiating reactive mesothelial cells from malignant cells,[1,11] given their lower diagnostic yield.[1] Cytodiagnosis by cytocentrifuge cytology smear has a sensitivity of 40-70%, a specificity of 89%, a PPV of 89.3% and an NPV of 69.4%. Cell density, cell loss, and different laboratory processing methods are frequent diagnostic problems among pathologists.[15]

To overcome the limitations of the cytocentifuge cytology method, the cell block method was developed to provide better tissue architecture and cell morphological features to differentiate between malignant and non-malignant cells, and can be examined as further confirmation through special staining and immunohistochemistry and biomolecular. The difficulty in differentiating between mesothelial lesions and adenocarcinoma requires special tests to differentiate between the two, namely calretinin immuno-histochemistry which will be expressed in the nucleus and cytoplasm of mesothelial cells for prognostic, and predictive purposes.[11,16,17]

Pleural effusion cases, especially MPE, are common, requiring a fast and accurate diagnosis associated with further management of the patient. Cytocentrifuge cytology and cell block methods can be used, but it is necessary to know the advantages and disadvantages of each. The cell block examination method has become a regular procedure in other Anatomical Pathology centers in Indonesia, even abroad, but not so in the city of Medan. This examination method has not become a routine procedure in evaluating pleural fluid, so the researchers wanted to invite the Anatomical Pathology center in Medan to apply the cell block method. The cytocentrifuge cytology and cell block methods are also expected to help pathologists work, especially in areas that do not have biopsy and surgical facilities.

METHODS

The design of this research is an analytic observational study with a cross-sectional approach. The study was conducted at the Anatomic Pathology Laboratory of

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Columbia Asia hospital in Medan. The sample in this study was a cytologically diagnosed pleural fluid specimen at 2021-2022. Sample calculation was carried out, obtained a total sample of 49 samples, including inclusion criteria complete sample data, specimen less than 24 hours with a volume of more than 50 ml. The exclusion criteria when the received pleural fluid lysis. Then examined by Kubota cytocentrifuge cytology and cell block methods. Interpretation with The International System (TIS) for reporting serous effusion cytopathology: non diagnostic (ND), negative for malignancy (NFM), atypia of undertemined significance (AUS), suspicious for malignancy (SFM) and malignant (MAL).). Futhermore, the cell block samples was confirmed by immunohistochemistry examination.

Immunohistochemical performance of calretinin by finetest is polyclonal antibody,1: 200 μl dilution is marked with brown color in the nucleus and cytoplasm of mesothelial cells, categorized as follows: positive, when brown appears in the nucleus and cytoplasm of the mesothelial cells and negative, if no brown color appears in the nucleus and cytoplasm mesothelial cells.

RESULTS

Forty-five samples of cytology pleural effusion were obtained in this research. Table 1 shows the distribution of sample characteristics based on age, cytocentrifuge diagnose, and cell block diagnose.

According to Table 1, the median age of the patients was 57,16 ± 14,76 years (range 27-83 years, men predominantly (57,1%). Mostly diagnosis are cytocentrifuge cytology negative for malignancy (57,1%) and cell block (53,1%). Table 2 show the distribution of sample characteristics based on cell block diagnose with calretinin confirmation.

Table 1. Characteristics of 49 patients with pleural effusion.

Charateristics	Amount (n)	Percent (%)
Age average ± SD (years old)	Age avarage 57,16 ± SD 14.76 (min-max: 27-83 years old)	
Sex		
Male	28	57.1
Female	21	42.9
Cytocentrifuge cytology diagnosis		
Nondiagnostic	0	0
Negative for malignancy	28	57.1
Atypia of undertemined significance	1	2.0
Suspicious for malignancy	8	16.3
Malignant	12	24.5
Cell block diagnosis		
Nondiagnostic	0	0
Negative for malignancy	26	53.1
Atypia of undertemined significance	1	2.0
Suspicious for malignancy	10	20.4
Malignant	12	24.5

Table 2. Characteristics of cell block diagnose with calretinin confirmation.

Result examination of calretinin	Amount (n)	Percent (%)
Positive mesothelial cell	45	91,8
Negative mesothelial cell	4	8,2

According to Table 2, calretinin immunohistochemical examination, which showed positive mesothelial cells in 45 cases (91.8%), while those who did not show or negative mesothelial cells were 4 cases (8.2%).

Table 3 show comparison of pleural effusion examination by cytocentrifuge cytology and cell block methods.

Table 3. The comparison between cytocentrifuge cytology and cell block.

Cytocentrifuge cytology	Cell block						p-value
	Not diagnostic	Negative for malignancy	Atypia of undetermined significance	Suspicious for malignancy	Malignant	Total	
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
Not diagnostic	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (100.0)	
Negative for malignancy	0 (0.0)	26 (92.9)	0 (0.0)	1 (3.6)	1 (3.6)	28 (100.0)	
Atypia of undetermined significance	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	<0.001
Suspicious for malignancy	0 (0.0)	0 (0.0)	0 (0.0)	8 (100.0)	0 (0.0)	8 (100.0)	
Malignant	0 (0.0)	0 (0.0)	0 (0.0)	1 (8.3)	11 (91.9)	12 (100.0)	

*Chi square test

There was a significant different between the result of the cytocentrifuge cytology and cell block methods according to Chi-square test with a p-value < 0,001. Comparison test analyses summarized in Table 3 displayed that there was a significant different between the result of the cytocentrifuge cytology and cell block methods with a p-value<0,001.
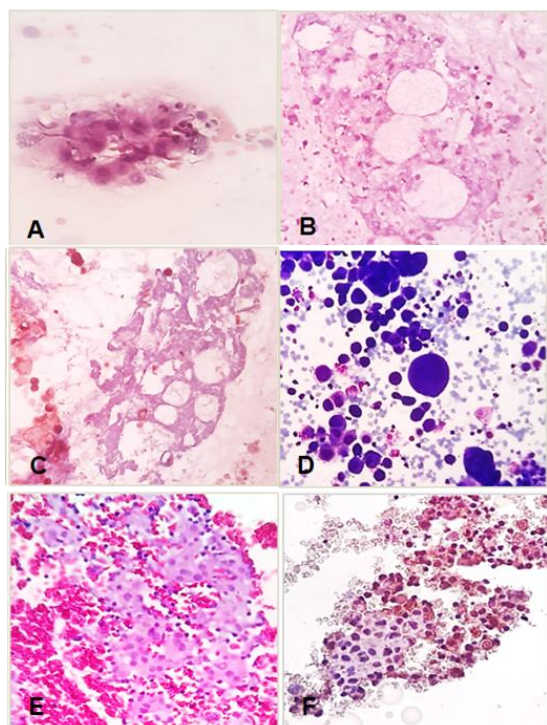

Figure 1. A. Cytocentifuge cytology: malignant (400 times). B. Cell block: malignant (400 times). C. Calretinin: negative (400 times). D. Cytocentrifuge cytology: malignant (400 times). E. Cell block: malignant (400 times). F. Calretinin: negative (400 times).
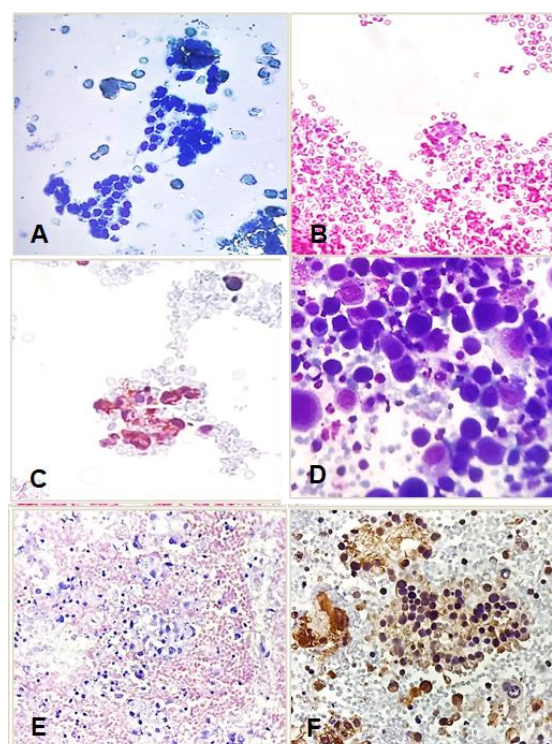

Figure 2. A. Cytocentifuge cytology: Negative for malignancy (400 times). B. Cell block: Suspicious for malignancy (400 times). C. Calretinin: Negative (400 times). D. Cytocentifuge cytology: Negative for malignancy (400 times). E. Cell block: Suspicious for malignancy (400 times). F. Calretinin: negative (400 times).

DISSCUSSION

The mean age of pleural effusion patients in this study was found to be 57.1 ± SD 14.76 years with the youngest being 27 years and the oldest being 83 years. Research conducted by Yosefani et al in 2022 at dr. Mohammad Hosein Palembang, found 56 cases (58.3%) in the age group 40-59 years.[18]

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Research conducted by Dewi Anggita et al in 2016 stated that sufferers of pleural effusion are known to be in the age range of 40-59 years.[19] Another study by Indra Yofi et al at Arifin Achmad Hospital Pekanbaru in 2017 found that patients with pleural effusion were found in the age group of 45-64 years (39.2%).[20] Study conducted by Stephen Walker in 2017 et al showed the incidence of pleural effusion at the age of 60 years.[21] Effusion Pleurisy is often found at a productive age because the function and physiology of the lungs has decreased and there are risk factors for the underlying disease. Increasing age is often associated with a decrease in cell capacity and frequent exposure to pollution and carcinogenic substances.[20]

According to the study of Putriani et al in 2019, it was stated that the averageage of pleural effusion patients is 45-49 years due to inactivation of the methylene tetrahydrofolatereductase (MTHFR) gene which is a trigger for tumor development.[21] Pleural effusion occurs due to an abnormal accumulation of fluid in the pleural space caused by benign or malignant conditions and this occurs frequently in adulthood.[8,23] Another study conducted at Husseini Hospital, Tripoli, Lebanon, for three years found 165 patients aged between 21 and 80 years with pleural effusion. Patients with tuberculous pleural effusion were significantly younger than the others (p < 0.05). Study by Ibrahim et al in 2021 effusions were more common in the first five decades of life (48 of 72 = 66.7% of cases) and were the most common type of pleural effusion, accounting for 48 of 70 (68.6%) of patients aged less than 50 years.[22] This is also in accordance with a large-scale epidemiological study conducted by Zhang et al showing that most young people aged 15 to 24 years have tuberculous pleural effusion.[23] The majority age of patients with empyema (81.8%) and parapneumonic effusion (77.8%) was more than 50 years old.[22]

Malignant pleural effusion is more common in the age group of more than 50 years (73.6%). This study is also in line with the study reported by Zhou et al that the morbidity of most malignant tumors increases with age. Most of the malignant tumors reported from 2011 to 2013 occurred in patients aged 60 years or over in Poland and increased with age, and the highest incidence was between the ages of 80 and 90 years. The incidence of malignant pleural effusion in the UK occurs in 50% of lung carcinoma patients aged over 70 years.[24]

Most of the pleural effusions suffered by men were 28 people (57.1%) in this study, while the other 21 people (42.9%) suffered by women. These results are in line with the study of Ibrahim et al in 2021 during the exudative pleural effusion study period, namely 114 people (69.1%) were men and 51 (30.9%) were women. The female patient was significantly older (p = 0.05), and the male to female ratio was 2.3:1 with the greatest predilection for right-sided effusion.[22]

In this study, all cell block specimens were subjected to further examination with immunohistochemical examination to ensure the presence of mesothelial or non-mesothelial cells, so that a pathologist's doubts could be answered. The results of this study obtained positive mesothelial cells in 45 (91.8%) samples and negative mesothelial cells in 4 (8.2%) samples. These results are consistent with the study of Dey et al which stated that part of the sample from the cell block can be taken, stored for special staining, immunohistochemistry there by increasing specificity.[25] The sensitivity and specificity of calretinin immunostaining as a marker of mesothelial cells is 100% when the staining patterns of various cell types are considered.[20]

In this study, 2 samples were found using the cytocentrifuge cytology method with negative results for malignancy, then followed by the cell block method giving a suspicious picture of malignancy because there were groups of cells which, although showing a clearer morphology, could not yet prove a malignancy. For this reason, a confirmatory examination was carried out using calretinin immunohistochemistry as a mesothelial cell marker. As a result, no brown color was found in the cell nucleus and cytoplasm, this means that these groups of cells are not mesothelial cells, possibly these cells are a group of adenocarcinoma cells, although further immunohistochemical examination is necessary to prove this. The patient who owned the specimen was a man aged 83 years and a woman aged 46 years, clinically supporting a malignant process, namely with the main complaint of shortness of breath and reddish pleural effusion fluid.

In this study, there were 12 samples with malignant results using the cytocentrifuge cytology method, and the results were the same as the cell block method. After confirmation with calretinin immunohistochemistry, 10 samples showed positive results and 2 samples were negative. A sample with a positive result means that these cells are mesothelial cells, while those that are not stained with calretinin are not mesothelial cells, but cells that give the

impression of an adenocarcinoma. They require other immunohistochemical tests, such as thyroid transcription factor-1 (TTF-1) or napsin A in diagnosing an adenocarcinoma.[26]

CONCLUSION

There is a significant different between the results of cytocentrifuge cytology and cell block method with a p-value <0,001. (dihilangkan). Immunohistochemical performance of calretinin as a marker of mesothelial cells in cell block preparations obtained 45 positive and 4 negative stains.

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