The Yield of Malignancy for Early Fixation versus Routine Fixation of Pleural Fluid Samples

Ria Katrina B. Cortez, RN, MD,[1] Richard Baron B. Yee, RN, MD,[1] and Jessie F. Orcasitas, MD[1]

Abstract

Background: The etiology of pleural effusion remains unclear in nearly 20% of cases. One way to diagnose malignancy is by doing pleural fluid cytology. There are factors that influence the yield of pleural fluid cytology and one of them is appropriate and timely fixation of samples. Currently, there is no local consensus regarding the timing with which the specimen should be fixed.

Objective: The study aims to compare the yield of malignancy between early fixation versus usual fixation of pleural fluid samples, meaning there is no set time for fixation to be done.

Methodology: The study employed a prospective cross-sectional research design. All patients with pleural effusion who fulfilled the criteria set by the study were included. Two sets of pleural fluid samples were collected amounting to 20cc each. First sample was assigned as Bottle #1 and placed immediately with fixative while the second sample was assigned as Bottle #2. Bottle #2 underwent routine fixation which follows no fixed or standard time of fixation. The time difference between the fixation of two sample groups greatly varied with Bottle #1 fixed immediately right after collection while Bottle#2 depends on the time it will be processed by the laboratory personnel. Both samples were submitted for cell block and cell cytology reading.

Results: Characteristics of the 55 patients included in the study showed age group range from 41 to 65 years of age, with 27 male and 28 female patients. Only one third had history of smoking. There were 21.82% who had family history of cancer and with and suspicious mass on chest radiograph. Out of 55 patients, 29 patients had history of previous diagnosis of cancer, 23 had recurrent pleural effusion, and 28 had chest radiograph with suspicious nodules. Based on gross appearance, there were 20 serous and 21 sanguineous pleural fluid noted. Mean cell count was high (1,115.50 ± 741.02) with lymphocytic predominance (82.56 ± 24.46). Elevated protein concentration (5,388.25 ± 8,230.46) and LDH (484.17 ± 248.72) were noted. Glucose (8.78 ± 6.68 mmol/L) was low. There were 21 patients who had high WBC, 24 with high protein and 16 with elevated LDH. There were 3 patients who were positive for AFB and none for KOH. Comparative analysis showed that the pleural fluid samples assigned to the routinely fixed group which were handed to the nurse after thoracentesis, then forwarded to the laboratory through a ward laboratory aide or patient watcher for fixation with with 95% alcohol by the laboratory personnel significantly had a longer duration of 406.62 minutes as compared to immediately fixed at 12.27 minutes (P<0.01). For diagnosis of malignancy, significantly more cases were diagnosed in the immediately fixed group with 36.36% cases versus 18.18% (*p=0.016*).

Conclusion: Among patients with suspected malignant pleural effusions, early fixation of pleural fluid samples resulted in higher histopathology yields as compared to those fixed after going through the routine fixation.

Keywords: Malignant pleural effusion, pleural fluid analysis, lung malignancy

Introduction

Pleural effusion is a frequent complication of advanced malignancy with significant associated morbidity and mortality. Pleural effusions were present in 15% of patients who died of malignancies in one post-mortem series.[1] No local data has been published regarding its incidence or prevalence. However, the United States recorded an estimated incidence of more than 150,000 cases of malignant pleural effusion annually.[2]

The etiology of pleural effusion remains unclear in nearly 20% of cases.[3] One way to diagnose lung malignancy is pleural fluid cytology. An etiological diagnosis can be established in approximately 75% of patients with knowledge of the pleural fluid cytology along with biochemistry and clinical presentation.[4] The accuracy of cytologic examinations of malignant pleural effusion is around 60% (range of 40-87%).[5]

[1] Department of Internal Medicine, Southern Philippines Medical Center, Davao City, Philippines
Correspondence: Ria Katrina B. Cortez, RN, MD
Email: riakatrinabcortez@gmail.com

There are factors that influence the yield of pleural fluid cytology. A minimum of 10ml of specimen is optimal for cytological evaluation.[6] Should other studies be required, small portion of the specimen may be needed. An increase in the volume of fluid submitted for cytologic analysis of more than 50ml does not show a significant increase in the diagnostic yield during malignancy evaluation.[7] Diagnostic tests for pleural fluid include the following: cell count, protein, lactate dehydrogenase, pH, glucose, amylase, gram stain, culture, and cytology. The pleural fluid should be immediately placed in the appropriate specimen tubes and bottles, and then sent to the laboratory for analysis. Another important factor to consider is the time at which fixing is done. Cells become denatured and undergo lysis over time. This can result in incompletely-fixed tissues with poor quality, distorted, and inadequately stained tissues. Thus, pleural fluid should be immediately placed in the appropriate specimen tubes and bottles, and then sent to the laboratory for analysis.[8]

This procedure costs approximately less than a thousand pesos in a local hospital in Davao City. One loses the opportunity of a cheap, fast and minimally invasive diagnostic modality for malignancy if not done properly.[9,10] Currently, there is no local consensus regarding the timing with which the specimen should be fixed. Hence, the study aims to compare the yield of malignancy between early fixation versus routine fixation of pleural fluid samples in which pleural fluid samples assigned to this group were handed to the nurse after thoracentesis, then forwarded to the laboratory through a ward laboratory aide or patient watcher for fixation with 95% alcohol by the laboratory personnel.

Research Question. Among patients with suspected malignant pleural effusions, will early fixation of pleural fluid samples result in higher histopathology yields?

General Objective

This study aimed to compare the yield for malignancy between early/immediate fixation versus usual/routine fixation of pleural fluid samples from patients with suspected malignant pleural effusion.

Specifically, this study had the following goals:

1. To describe the socio-demographic and clinical characteristics of the included patients
2. To describe the laboratory characteristics of the pleural fluid of the patients
3. To compare percentage yield for malignancy for early and routinely fixed slides.

Significance of the Study. Histologic confirmation of malignancy can be done in several ways – pleural fluid cytology after thoracentesis, lymph node biopsy, CT scan guided biopsy, core needle biopsy, or open biopsy. The least expensive, least invasive, and the least risk for patients is pleural fluid cytology. A higher yield would refrain from doing additional invasive test after the initial thoracentesis. The yield of this procedure may be small but if we can increase the yield with early and better preservation of the specimens, this will be of great benefit. The patient will be spared the added cost and trauma of repeated unnecessary thoracentesis and pleural fluid testing with properly prepared pleural fluid specimens. For the physicians and laboratory personnel, they will be guided regarding proper handling of the pleural fluid, specifically timely fixation. Moreover, once reliable results are out, physicians will be able to establish that the cause of pleural effusion is secondary to malignancy thus avoiding work-ups and more invasive procedures to diagnose malignancy. Once results establish that the pleural effusion is malignant, further tests and more invasive procedures will be avoided. Specifically, this study would determine if early fixation of pleural fluid will increase the yield of malignancy compared to the routine fixation as described above. Thus, this will aid the hospital in generating more accurate pleural fluid cytology results in terms of diagnosing malignant pleural effusion.

Methodology

Research design. The study employed a prospective cross-sectional research design.

Participants. This study included patients 18 years and above with radiographic findings of significant pleural effusion with pleural fluid level more than two intercostal spaces or at least 10mm on lateral decubitus chest xray.

Inclusion Criteria: a) Age $\geq$ 18 years old, b) Patients with high suspicion for malignancy (Current or with $\geq$10 pack/year smoking history, Strong Family History of Cancer, Recurrent pleural effusion, Chest Radiograph with suspicious nodules and/or mass, Pleural Fluid characteristic which is suggestive of malignancy, e.g., serosanguinous or bloody pleural fluid (at least one of the above criteria should be fulfilled), and Patients must have informed consent

Exclusion Criteria: a) Patients without consent and b) Patients with transudative pleural effusion with definite diagnosis of non- malignant pleural effusion

Operational Definition of Terms

High Risk patients for Malignancy: These are patients who have high percentage of malignancy both primary lung malignancy as well as metastatic malignancy. Patients who have fulfilled at least 1 criteria of high suspicion for malignancy as stated above are included.

Early fixed samples: These are pleural fluid samples which are immediately fixed with 95% alcohol at bedside after it was obtained through thoracentesis.

Routinely fixed samples: These are pleural fluid samples which are handed to the nurse who then forwards the specimen through a ward laboratory aide or patient watcher to the laboratory to be handled and fixed with 95% alcohol by the laboratory personnel. There is no actual protocol which guides the laboratory personnel as to the ideal time of fixation. No actual mean recorded time was recorded.

Yield of malignancy: These are the results of the cell block and cell cytology study which will determine if atypical or malignant cells are present in the pleural fluid sample.

Sampling procedure. All patients with pleural effusion who have fulfilled the criteria set by the study were included. No potential confounders nor modifiers were identified. No biases were identified. Two sets of pleural fluid samples were collected amounting to 20cc each. First sample was assigned as Bottle #1 and placed immediately with fixative while the second sample was assigned as Bottle #2. Both samples were submitted to the histopathology laboratory for cell block and cell cytology reading.

Intervention and Comparison. Upon the approval of the ethics committee and the department chair, the recruitment of patients has commenced. An informed consent was secured. The following information were collected from the patients: Name, Age, Sex, Hospital registry number, Date acquired

Before thoracentesis, proper information regarding the procedure was well explained. The researchers gave emphasis on the possible risks and complications following the procedure so as to avoid confusion, misinformation, and future problems if ever the patient experiences adverse reactions during or after doing the procedure. Also, explanation regarding the reason the procedure is being performed as well as the suspected diagnosis; the risk, benefits, and alternatives of the procedure; the risks and benefits of the alternative procedure; and the risk and benefits of not undergoing the procedure were stressed out.

Thoracentesis was then conducted. The procedure adhered to strict asepsis technique and followed the standard techniques prescribed by the American College of Chest Physicians/Philippine College of Chest Physicians. Equipment were completely checked before starting the procedure. Patient were well prepared for the procedure which included adequate anesthesia and proper positioning. Standard aseptic technique was used for the remaining steps of the procedure. Two pleural fluid samples were obtained and saved amounting to 20cc each. Samples were then labelled as Bottle #1 for Cell block and cell cytology A and Bottle #2 for Cell block and cell cytology B. Each Sample above was labelled with the Patient's name, Age, Sex, Hospital registry number and Time and date of collection. Bottle #1 was fixed immediately after thoracentesis by the primary researcher while bottle #2 was fixed by the laboratory personnel on duty after it has been submitted to the laboratory.

Both specimens were handed by the researchers to the laboratory personnel for pleural fluid studies. Fixed samples were then smeared into glass slides. Smeared samples were labelled with the patient's name and control number of the same labelled pleural fluid sample. Only the investigator and the technician knew the assignment of the samples. The smeared samples were delivered to the pathology resident in-charge for the study. One pathologist read both the slides from the same patient. Both pathology resident and consultant were blinded to the samples. No standard protocol regarding time of fixation has been established. A master list of the time of fixation of the cell block and cell cytology bottles #1 and #2 was kept by the principal investigator. Collated data were then submitted and analyzed by the statistician.

Independent Variable. The Independent Variable are the following: a) Early fixed slides, and b) Routinely-processed slides

Dependent Variable. The dependent variable is the yield of malignancy.

Sample Size Computation. Sample size for this study was computed using https://select- statistics.co.uk/ calculators/sample-size- calculator-two-proportions with the following assumption: (1) Yield of malignancy to fluid cytology is 50%.[21,22] Computations were made in order to detect 25% difference in yield of malignancy between two groups. In a computation for comparison of two proportions carried out at 95% confidence level, it resulted to a sample size of 55 patients per group (total of 110 pleural fluid samples) with an 80% power of rejecting the null hypothesis if the alternative holds.

Data Handling and Analysis. The principal investigator ensured that all data collected in the study were of utmost confidential nature. Data analysis was performed to ensure quality results. The demographic and clinical profile made use of descriptive statistics such as mean and standard deviation for continuous data and frequency and percent for categorical data. The comparative analysis made use of t-test for two proportion in comparing percentages between two groups and t-test for mean to determine significant difference between the means. The SPSS® version 24 was used to analyze the data.

Ethical Considerations. Participant's consent was obtained prior to enrollment in the study.

Ethics Review. The proponents of the study secured an approval from the Cluster Ethics Research Committee of the Southern Philippines Medical Center prior to doing the research. A similar approval was also secured from the Department of Internal Medicine of the same institution with the approval of a consultant in-charge. The proponents secured an approval from other departments in the same institution, with regards to the department's participation in this study.

Results

Characteristics of the 55 patients who were included in the study are listed in *Table I*. There were no eligible samples that had been excluded in the study. All participants have complete data for each variable of interest. The age group ranges from 41 to 65 years of age, with almost balanced sex distribution. Only 1/3 shows history of smoking with a pack per year between 13.5 to 46.5. Roughly 21% showed history of cancer and suspicious mass with half of the patients suggesting

Table I. Sociodemographic and Clinical Characteristics of the patients

Characteristics	Number (n = 55)
Age in years, mean (±SD)	53.2 ± 12.3
Sex, frequency (%) Male Female	 27 (49.09%) 28 (50.91%)
Smoking history, frequency (%) No Yes	 36 (65.45%) 19 (34.55%)
Mean pack years ± SD	30 ± 16.5
Family history of cancer, frequency (%) No Yes	 43 (78.18%) 12 (21.82%)
Previous diagnosis of cancer, frequency (%)	29 (52.73%)
Recurrent pleural effusion, frequency (%)	23 (41.82%)
Radiograph with suspicious nodules, frequency (%)	28 (50.91%)
Radiograph with suspicious mass, frequency (%)	12 (21.82%)

Table II. Biochemical and Cytological characteristics of Pleural fluid

Characteristic	Values
Pleural Fluid gross appearance (%) Serous Sanguineous Serosanguinous	 20 (36.36%) 21 (38.18%) 14 (25.45%)
Mean cell count ± SD	1,115.50 ± 741.02
Mean Differential count (± SD) Neutrophils Lymphocytes Nil	 17.44 ± 24.49 82.56 ± 24.46 0
Protein (mg/dL, ± SD)	5,388.25 ± 8,230.46
LDH (mg/dL, ± SD)	484.17 ± 248.72
Glucose (mmol/L, ± SD)	8.78 ± 6.68
Positive AFB, frequency (%)	3 (5.45%)
Positive KOH, Frequency (%)	0 (0%)

Table III. Comparison of Yield for Malignancy

Parameter	Early fixed (mins)	Routinely fixed (mins)	p-value
Mean time from extraction to fixation of pleural fluid (± SD, mins)	12.27 ± 9.58	406.62 ± 306.22	< 0.01
With diagnosis of malignancy by cytology, frequency (%)	20 (36.36%)	10 (18.18%)	0.016

history of previous diagnosis of cancer, recurrent pleural effusion and chest radiograph with suspicious nodules.

Table II shows the characteristics of pleural fluid samples. The patients display more of a balanced number of serous and sanguineous pleural fluid gross appearance. Mean cell count was 1,115.50 ± 741.02 with lymphocytic predominance of 82.56 ± 24.46. There was noted elevated protein concentration which ranged from 5,388.25 ± 8,230.46 and elevated LDH with range of 484.17 ± 248.72. Glucose was low with range of 8.78 ± 6.68 mmol/L. There were 3 patients who tested positive for AFB and none for KOH.

Comparative analysis was done between the two groups. *Table III* showed that routine fixation had a significant longer mean time from extraction to fixation of pleural fluid (406.62 minutes) as compared to immediately fixed at 12.27 minutes (P<0.01). There was a significantly higher yield for malignancy in the immediately fixed group versus routinely fixed group, with 36.36% and 18.18% cases respectively, (P=0.016).

Discussion

Our yield for malignancy of pleural fluid study that was fixed immediately was noted to be significantly higher as compared to those fixed after going through the usual routine. In terms of extraction to fixation of pleural fluid, the result showed that routinely fixed group significantly has a longer duration of 406.62 minutes as compared to immediately fixed at 12.27 minutes (*p<0.01*). Significantly more cases were diagnosed using immediately fixed group with 36.36% cases versus 18.18% (*p=0.016*). This contradicted the study that says fixative is not necessary and there is no significant alteration of cell morphology noted if the specimen is processed within 12 hours or kept refrigerated at 40°C up to 72 hr.[19] Currently, there are no studies regarding at which exact hour after collection cell alteration happens. We highly suggest the addition of an equal volume of 50-95% ethanol when longer delay is anticipated.

Our patients' ages ranged from 41 to 65 years, with an almost balanced sex distribution. Among the 20 patients who had higher yield for atypical cells, mean age was 53.7 years also with equal sex distribution. This is similar to the study of Antonangelo, et al. however his population had a female preponderance of 66% which is due to bias of having more breast cancer cases and is commonly seen in females.[21] In contrast, Saha, et al. described that among 166 patients, majority were male (72.89%) with lung cancer (88.97%) as leading cause and followed by Non-Hodgkin's lymphoma (3.67%) and intraductal breast carcinoma (7.35%).[24] This finding was similar to other studies with male preponderance noted.[25-27] In all these studies, majority of the population had lung cancer followed by breast cancer and lymphoma. This suggests that sex may have an association with a specific type of cancer and not directly to malignant pleural effusion. Predominance of lung cancer patients may lead to higher number of male patients while breast cancer may lead to female preponderance.

Only one-third of the 55 patients had history of smoking with pack years between 13.5 to 46.5. In the study of Nair, 46% of patients had history of smoking as well as in a study conducted in Myanmar with reported 82.2% of ever smokers among the study group.[25] It is a known factor that smoking is a risk factor for lung carcinoma.

Smoking triggers mutagenesis, initiation and progression of lung cancer but it is not clearly known if cigarette smoke affects pleural malignancy and malignant pleural effusions. This possibility is likely due to the pro-inflammatory and pro-angiogenic properties of smoke. In support, Sophia Magkouta et al. has stated that cigarette smoke promoted malignant pleural effusion formation by enhancing tumor associated inflammation.[28] Also, in the study of Soe, et al., 60 patients (82.2%) of malignant pleural effusions are heavy smokers or ex heavy smokers.[26] In our study, we noticed that among the 20 patients positive for atypical cells, half of them had smoking history. Since majority of cases were not lung carcinoma (9.09%) but instead breast (27.27%) and ovarian cancer (27.27%), this may be the reason of the slightly lower incidence of smoking.

Among 55 patients in our study, 21% showed history of cancer and suspicious mass, with half of the patients suggesting history of previous diagnosis, recurrent pleural effusion, and radiograph with suspicious nodules. In the study of Wu, et al., there was no difference in history of smoking between groups with positive or negative pleural fluid cytology and 60% had a previous diagnosis of malignancy.[17] Saha noted male (75.2%) and female (11.11%) patients were smokers.[24] History of repetitive pleural fluid aspiration was found in 76 cases (45.78%). Primary cancer was diagnosed in 136 (81.93%) cases, which is predominantly lung cancers (88.97%) cases. On chest x-ray, mass lesion was detected in 85 cases (51.2%).

In the study of Gadewad, et al., computed tomography of thorax had findings suggestive of lung masses, nodules or infiltrates (57%), mediastinal adenopathy (51%), pleural nodularity (43%), lymphangitic carcinomatosa (11%), and chest wall involvement (6%).[27] Among the 20 patients positive for atypical cells in our study, half were previously diagnosed with cancer, with breast and ovarian cancer (27.27% each) as leading diagnosis followed by lymphoma (20%), lung (9.09%), endometrial (9.09%) and nasopharyngeal cancer (9.09%). There were 70% of them who had previous recurrent pleural effusion and 75% had radiographic findings of mass and/or nodules.

There was a balanced number of serous and sanguineous pleural fluid gross appearance in our study. However, in the 20 patients who were positive for atypical cells, majority of fluid were bloody (55%) followed by serosanguinous with 25% and serous 20%. Mean cell count was 1,115.50 ± 741.02 with lymphocytic predominance. Elevated protein concentration and LDH were noted. Glucose was low. There were 3 patients who tested positive for AFB and none for KOH. This is supported in the study of Antonangelo, et al., half of the malignant pleural effusions (50.7%) were described as serohemorrhagic effusions with $p < 0.001$ which was followed by yellow citrine appearance with 47.9%.[21] Most exudates have >1000 nucleated cells/uL but <5000/uL as with malignancy.

When the disease had an insidious onset, as with malignancy, the fluid will be lymphocytic predominant (>80%). In the same study, protein concentrations range was 4.2 $\pm$ 1.0 g/dL (4,200 $\pm$ 1000mg/dL). LDH levels were in the range of 1,177 $\pm$ 675 with noted low glucose concentration. In support, Gadewad, et al, noted 52% of subjects had blood-stained fluid while 48% had straw colored fluid.[27] All of the malignant pleural effusions were exudative. Mean pleural fluid protein level was 4.71(± 0.72) g/dl mean pleural fluid LDH level was 623.98 (± 81.14) U/L. Mean glucose level was 64.98 (± 10.86) mg/dl. Mean total WBC count was 1307 ± 478.89/mm^3. Mean lymphocyte count was 71.12 ± 4.69, neutrophils 18 ± 5.38. Similarly, Saha described macroscopic pleural fluid appearance to be hemorrhagic in 91 cases (54.82%) and straw colored in 75 cases (45.18%).[24] All the cases were exudative with lymphocytes as most predominant cell (mean ± SD of 49.97 ± 8.35%). The mean value of pleural fluid glucose was 38.75 mg/dl. These findings were consistent with the 20 patients who had positive atypical cells with noted high mean cell count (1,424.55) with lymphocytic predominance, low glucose (6.50mmol/L) and high protein (3,696.72mg/dL). However, LDH was lower compared to other studies. All 20 patients were negative for AFB and KOH.

Strengths, Limitations and Recommendation.

Pleural fluid cytology is the simplest definitive method for obtaining a diagnosis of malignant pleural effusion. Thus, timely handling and preparation of samples should be done. There is no local consensus regarding the exact time needed to add the fixative. Our study shows that we can increase the yield of malignancy if we do immediate fixation of the fluid. This process will prevent cells from undergoing lysis and denaturation. We should not allow specimen to stand for 12 hours or more.

The study has potential limitations. The profile of patients should have included the specific type of malignancy – primary or metastatic, work exposure, previous hospital admission if any, and tuberculosis exposure.

Based on the results and identified limitations of the study, the researchers recommended 1) to include the abovementioned variables and 2) to conduct a different study comparing yields of malignancy at different times of fixation less than 12 hours among patients with high suspicion for malignancy in order to determine the most appropriate time for viability of specimens.

Conclusion

Our study showed that among patients with suspected malignant pleural effusions, early fixation of pleural fluid samples resulted to higher histopathology yields as compared to those fixed through the usual route. Patient demographics and pleural fluid characteristics and laboratory results may help in the diagnosis but are not pathognomonic or specific. The results of this study will serve as a guide to help improve current practice of

handling pleural fluid for histopathologic analysis.

Conflicts of Interest

The main proponents and the adviser declared no conflict of interest. Dr. Jessie Orcasitas is an active consultant in the Department of Internal Medicine, Section of Pulmonology, Southern Philippines Medical Center.

Funding

The principal investigators used personal funds for the expenses of this research.

References

1 Rodriguez-Panadero F, Borderas Naranjo F, Lopez-Mejias J. Pleural metastatic tumours and effusions: frequency and pathogenic mechanism in a post-mortem series. Euro Respir Journal 1989; 2:366-369.
2 "Management of Malignant Pleural Effusions". American Journal of Respiratory and Critical Care Medicine, 162(5), pp.1987-2001.
3 Karkhanis VS, Joshi JM. Pleural effusion: diagnosis, treatment, and management. Open Access Emerg Med. 2012; 4:31–52.
4 Collins TR, Sahn SA. Thoracocentesis. Clinical value, complications, technical problems, and patient experience. Chest. 1987;91(6):817-822.
5 Na MJ. Diagnostic Tools of Pleural Effusion. Tuberc Respir Dis (Seoul).2014;76(5): 199–210.
6 Buckley O, Benfayed W, et al. Thoracocentesis for Potential Malignancy: Does Volume Matter? J HK Coll Radiol. 2008; 11:72-75.
7 Abouzgheib W, Bartter T, Dagher H, Pratter M, Klump W. A prospective study of the volume of pleural fluid required for accurate diagnosis of malignant pleural effusion, Chest. 2009;135(4):999.
8 Agarwal SP. Manual for Cytology. India: Directorate General of Health Services Ministry of Health and Family Welfare Government of India. 2005.
9 Valerio E, Nunes W, et al. A 2-year retrospective study on pleural effusions: A Cancer Centre experience. Cytopathology. 2019; 00:1-7.
10 Hooper C, Lee YC, Maskell N. BTS Pleural Guideline Group. Investigation of a unilateral pleural effusion in adults: British Thoracic Society Pleural Disease Guideline 2010. Thorax. 2010; 65 Suppl 2: ii4-ii17.
11 Ong KC, Indumathi V, Poh WT, Ong YY. The diagnostic yield of pleural fluid cytology in malignant pleural effusions. Singapore Med J. 2000;41(1):19-23.
12 Light, RW. Erozan, YS. Ball, WC. Cells in the pleural fluid: their value in differential diagnosis. Arch Intern Med. 1973; 132:854-60.
13 Motherby H, Nadjari B, Friegel P, Kohaus J, Ramp U, Bocking A. Diagnostic accuracy of effusion cytology. Diagn Cytopathol. 1999;20(6):350-357.
14 Metzgeroth G, Kuhn C, Schultheis B, Hehlmann R, Hastka J. Diagnostic accuracy of cytology and immunocytology in carcinomatous effusions. Cytopathology. 2008;19(4):205-211.
15 Porcel JM. Handling Pleural Fluid Samples for Routine Analyses. 2013. p19.
16 Sallach SM, Sallach JA, Vasquez E, Schultz L, Kvale P. Volume of pleural fluid required for diagnosis of pleural malignancy. Chest. 2002;122(6):1913-1917.
17 Wu H, Khosla R, et al. The minimum volume of pleural fluid required to diagnose malignant pleural effusion: A retrospective study. Lung India. 2017;34(1):34–37.
18 Light RW. Pleural Diseases. 6th ed. Philadelphia: Lippincott Williams & Wilkins. 2013.
19 Nguyen GK. M.D. Essentials Of Fluid Cytology. 2009. p10.
20 Michael CW, Davidson B. Pre-analytical issues in effusion cytology. 2016;1(1):45–56.
21 Antonangelo, L., Vargas, FS. et al. Effect of temperature and storage time on cellular analysis of fresh pleural fluid samples. Cytopathology. 2011;23(2),103– 107.
22 Asim M, Saleem N. Diagnostic yield of pleural fluid cytology in malignant pleural effusion. Journal of Islamabad Medical & Dental College (JIMDC); 2012(2):65-68
23 Heffner JE, Klein JS. Recent advances in the diagnosis and management of malignant pleural effusions. Mayo Clin Proc. 2008;83(2):235-250.
24 Saha K, Maikap MK, Maji A, Moitra M, Jash D. Demographic, clinical, biochemical, radiological and etiological characteristics of malignant pleural effusions from Eastern India. Indian J Cancer. 2017;54(1):257-261.
25 Nair RS. Clinical Profile Of Malignant Pleural Effusions In A Tertiary Care Centre. 2018;2(31):3472.
26 Soe Z, Aung Z, Tun KD. A clinical study on malignant pleural effusion. International Journal of Collaborative Research on Internal Medicine & Public Health. 2012;4(5):761-79.
27 Gadewad N, Deokar K, Ghorpade S. Clinical Profile of Patients Presenting with Malignant Pleural Effusion to a Tertiary Health Care Centre. The Journal of the Association of Physicians of India. 2017;65(8):28-31.
28 Magkouta S, Glynos K, Pappas A, Papapetropoulos A, Kalomenidis J. Effect of smoking on experimental malignant pleural effusion. European Respiratory Journal. 2015;46 Suppl 59.