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Risk factors of repetitive thoracentesis of malignant pleural effusion in naive non-small cell lung carcinoma

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Abstract---Almost 40% of Non Small Cell Lung (NSCLC) patients develop Malignant Pleural Effusion (MPE). Nowadays, clinicians prefer to perform a repetitive thoracentesis rather than a definitive procedure, which causes pleural loculations and inflammation. This study aimed to identify risk factors of repeated thoracentesis MPE in NSCLC. This was an observational analytic study with a retrospective cohort design held at Prof.Dr. I.G.N.G Ngoerah General Hospital from January 2018 to June 2022. There were 95 subjects who participated in this study. The median time of repetitive thoracentesis MPE in NSCLC is 3 days (95% CI 1,9-4). The difference in location tumor in the peripheral was 3 days (2.25-3.74) while in the central was 7 days (4.24-9.75), $p=0.21$. The difference in histology type, Adenocarcinoma was 3 days (1.86-4.13) while squamous cell carcinoma was 4 days (2.53-5.46), $p=0.69$. Pleural fluid cytology positive was 6 days (2.78-9.22) while negative was 3 days (1.83-4.16), $p=0.51$. The EGFR mutation positive was 4 days (2.48-5.52) while negative 3 days (1.68-4.31), $p=0.78$. LDH levels ≥ 821 IU/L was 3 days (1.49-4.50) while < 821 IU/L was 4 days (2.32-5.67), $p=0.81$. The size of pleural effusion massive was 3 days (1.88-4.11) while non-massive was 4 days (0.93-7.06), $p=0.49$. Conclusion: This study showed that the median time of repetitive thoracentesis MPE in NSCLC is 3 days. Tumor location, histology of lung carcinoma, pleural fluid cytology, EGFR mutation, LDH level of pleural fluid, and effusion size were not the risk factor of repetitive thoracentesis MPE in NSCLC.

Keywords---Repetitive thoracentesis, Malignant Pleural Effusion, NSCLC.

Introduction

Lung cancer is the most common cause of MPE in men and breast cancer in women (Ferlay et al., 2020). MPE indicates that cancer has metastasized far and is a complicating factor in managing lung cancer. Research conducted at the Jakarta Persahabatan Hospital found 120 cases of MPE (52.4%) of 229 cases of

pleural effusion, RS. Dr. Soetomo, 27.23% of MPE cases, and a study at Darmas Cancer Hospital showed 1,731 MPE patients in 2015 out of 2,502 (Fariha et al., 2016). Based on data from the medical records of Prof.Dr. IGNG Ngoerah General Hospital, it is known that from January 2018 to June 2022, there were 138 cases (51.68%) of the 267 cases of NSCLC who had complaints of pleural effusion, and 95 patients (68.8%) of the 138 cases had complaints of reaccumulation pleural effusion requiring repetitive thoracentesis.

Pleural effusion is generally a complication of advanced lung cancer; this condition significantly inhibits respiration and circulation, which affects the patient's quality of life. Approximately 15% of lung cancer patients have pleural effusion at the initial diagnosis, and 50% develop it during the disease (Cheng et al., 2012). Pleural effusion can occur in patients with lung carcinoma of all types of histology, but a preponderance of around 40% of all cases of pleural effusion occurs in adenocarcinoma types (Fariha et al., 2016).

Adenocarcinoma is the most common histological type of NSCLC; this type grows more frequently in the peripheral parts of the lung, making it easier to invade the pleura. (Fariha, et al 2016). Studies showed that patients with MPE are typical patients with lung cancer of the NSCLC type accompanied by Epidermal Growth Factor Receptor (EGFR) mutations (Schwalk et al., 2020).

A definitive pleural procedure should be recommended in patients with recurrent MPE symptoms and who have had an initial thoracentesis. Audra et al in 2020 stated that repeated thoracentesis therapy could increase pleural loculation, complicate future management, and prolong MPE symptoms.

The goals of MPE treatment are to relieve symptoms, minimize unnecessary pleural procedures, reduce the incidence of hospitalization, and improve the quality of life of sufferers (Schwalk et al., 2020). There are several options for the management of MPE. The consideration depends on several factors, including symptoms, patient performance status, type of primary cancer, and the response of cancer to systemic therapy that has been given. Current recommendations for managing pleural effusions in patients with malignancy suggest that therapeutic thoracentesis be performed first to evaluate the drainage effect in patients with shortness of breath and determine the type of effusion. Treatment in the form of thoracentesis or pleural puncture only provides a temporary effect, definitive therapy is the installation of a thoracostomy Indwelling Pleural Catheter (IPC), installation of Water Sealed Drainage (WSD), and pleurodesis (Verma et al., 2016; Schwalk et al., 2020).

Patients who underwent standard pleural procedures experienced fewer additional pleural procedures, fewer procedures in the emergency department, and fewer complications than those who underwent repeated thoracentesis. This explains the importance of timely definitive pleural intervention in managing recurrent MPE. However, a retrospective study of 23,431 patients with MPE showed that only 24% underwent standard pleural procedures versus repeated thoracentesis after reaccumulation of pleural fluid (Ost et al., 2018; Schwalk et al., 2020).

Even though there's no therapeutic management of MPE that can extend survival, poor management will increase patient complaints and reduce mortality in patients' quality of life. This showed the importance of treating MPE patients with an experienced and competent team from various multidisciplinary disciplines with existing therapeutic modalities.

This study aimed to determine repetitive thoracentesis and identify risk factors associated with symptomatic MPE requiring further thoracentesis after the initial thoracentesis. Knowing the risk factors associated with repetitive thoracentesis of MPE is expected to provide additional information for clinicians to identify patients likely to experience repetitive thoracentesis so that more appropriate management can be carried out.

Method

This study was an observational analytic study using a retrospective cohort design of NSCLC patients data between January 2022 to June 2022 in Prof. Dr. IGNG Ngoerah Hospital Denpasar. Research ethics permit was from Udayana University. All patients aged over 18 years who were diagnosed with MPE with NSCLC and treated at Prof. Dr. IGNG Ngoerah Denpasar Bali were included. Exclusion criteria were incomplete data medical records.

The variables in this study are:

The dependent variable is MPE repetitive thoracentesis in NSCLC patients. Repetitive thoracentesis is the period of repeated MPE, calculated from the initial thoracentesis carried out until further thoracentesis before definitive management of NSCLC. Independent Variables: Location of Primary Lung Tumor, Lung Cancer Histology, Pleural Fluid Cytology, EGFR Mutation, Pleural Fluid LDH Level, Size of Pleural Effusion on Chest X-ray. The location of primary tumor is the location of the tumor in the lung lobes consisting of peripheral or central (Roberto et al. 2016). Lung cancer histology results from a lung cancer biopsy show adenocarcinoma, squamous cell carcinoma, and non-small cell carcinoma. Pleural fluid cytology results from an examination to find malignant cells that appear in pleural fluid. The LDH level is the pleural fluid lactate dehydrogenase level obtained from the pleural fluid analysis. EGFR mutation is the result of an examination that shows whether there is a mutation examination in a lung biopsy through a report. The size of the pleural effusion is the number of effusions assessed based on chest radiographs in the two weeks before the initial thoracentesis. Massive pleural effusion, the accumulation of excess fluid on radiological images as high as areas 3 and 4 in the pleural cavity, is above 50% (Grosu et al., 2018). Comorbid diseases include pericardial disease, heart failure, renal failure, and liver failure.

Result and Discussion

Demographic characteristics

The demographic characteristics of the 95 research subjects can be seen in table1. The mean age is 60 years with a minimum age of 38 years and a maximum of 89 years with a predominance of the female sex (51.6%), without

comorbid diseases (62.1%), adenocarcinoma (90.5%), a central tumor location (52.6%), negative pleural fluid cytology examination results (63.2%), negative EGFR examination results (58.9%), massive effusion (76.8%), median LDH of 821 (127 and 28.260 U/L) as seen in Table 1. In this study, the median repetitive thoracentesis MPE in NSCLC patients was three days (95% CI 1.9-4 days) as seen in Figure 1.

Table 1 Characteristic distribution of NSCLC patients with MPE and undergoing repeated thoracentesis at Prof.Dr. I.G.N.G Ngoerah General Hospital

Characteristics	Amount (N = 95)	Percentage (%)
Gender, n (%)		
Man	46	48.4
Woman	49	51.6
Age, mean (SD)		
Age (years)	60.4, p = 0.20	
Comorbid Diseases, n (%)		
With comorbid disease	36	37.9
No Comorbid Diseases	59	62.1
Type of Histology NSCLC, n (%)		
Adenocarcinoma	86	90.5
Squamous Cell Carcinoma	9	9.5
Tumor Location, n (%)		
Peripheral	72	75.8
Central	23	24.2
Cytology Examination, n (%)		
Positive	35	36.8
Negative	60	63.2
EGFR Mutation Examination, n (%)		
Positive	30	31.6
Negative	56	58.9
No inspection	9	9.5
LDH, median (minimum–maximum)		
LDH (U/L)	821 (127-28.260), p = 0.00	
Total Effusion, n (%)		
massive	73	76.8
Not Massive	22	23.2

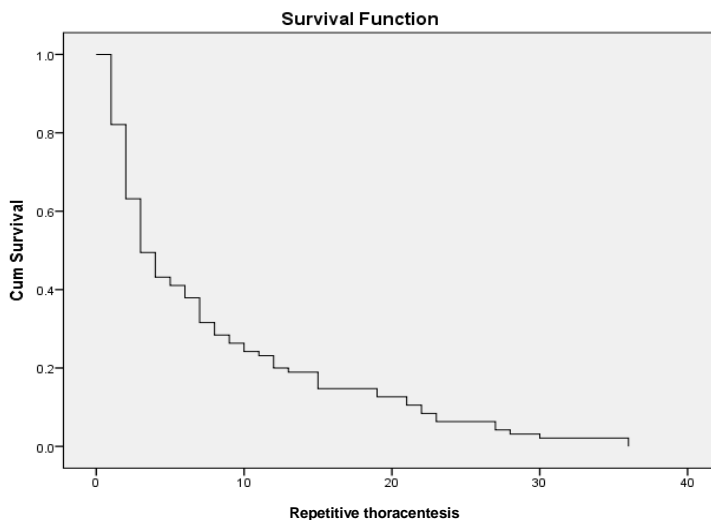


Figure 1 Survival of repetitive thoracentesis of NSCLC patients at Prof.Dr. I.G.N.G Ngoerah General Hospital in January 2018 - June 2022

Fariha et al. in 2016 reported the number of lung cancer patients in Persahabatan hospital with pleural effusion was 106 (63.5%) male patients and 61 women patients (36.5%) with the age range of male patients 29-81 years, while in women the age range is 18-76 years. Deniz et al. 2019 stated that pleural effusion associated with malignancy was found in patients over 50. The mechanisms involved are aging, genomic instability, epigenetic changes, loss of proteostasis, nutritional deregulation, metabolic disturbances, and cellular senescence.

Fifty-nine subjects (62.1%) without comorbid diseases and 36 subjects (37.9%) with comorbid and dominated by Congestive Heart Failure (CHF) 11 subjects 30.5%. Grosu et al., 2019 reported patients with CHF around 85 (8.6%) and there was no significant effect of recurrent MPE within 100 days of observation $p = 0.74$. Schwalk et al. in 2020 reported that CHF was one of the risk factors associated with recurrent MPE, but this was not statistically significant $p=0.47$.

The histological type of lung cancer found in this study was 86 patients (90.5%) with adenocarcinoma, and 9 patients (9.5%) had squamous cell carcinoma. This is in line with research by Syahrudin et al. in 2010 at Persahabatan Hospital, which reported the highest types of lung cancer cell morphology, were adenocarcinoma (90.4%) and squamous cell carcinoma (6.6%). Xu et al. in 2014 also reported a similar thing, that adenocarcinoma was found 85.8%.

Based on the location of the tumor, 23 subjects (24.4%) had tumors in the central area and 72 subjects (75.8%) in the peripheral area. Thirty-five patients (36.8%) had positive cytology on pleural fluid, while 60 patients (63.2%) had negative cytology on pleural fluid. Similar results were shown in the study by Jany et al. in 2019, which reported the results of a review of studies involving 620 pleural cytology specimens, only 61 subjects (9.8%) had positive cytology on pleural

effusion. Among the positive cytology, lung adenocarcinoma was the most common histology account for 25 patients (41%).

EGFR mutations were detected in 30 patients (31.57%), 56 patients (58.94%) had no EGFR mutations detected, and nine patients (9.47%) were not tested. A study by Verma et al. 2016 reported that EGFR mutations are more commonly found in cases of adenocarcinoma accompanied by MPE around 70% compared to lung adenocarcinoma patients with a single mass or nodule presentation. Chang et al. 2016 also reported that 68.4% of subjects with EGFR mutations were found in adenocarcinomas with MPE, which predominantly occur in women, nonsmokers, and predominantly Asian populations.

The results of the pleural fluid analysis showed that the lowest LDH value was 127 U/L and the highest was 28,260 U/L, the median value was 821 U/L. Researcher Audra et al. in 2020 reported that the higher amount of pleural fluid drained, the higher pleural fluid LDH level, associated with a higher risk of symptomatic pleural fluid recurrence ($p < 0.001$).

The majority of NSCLC patients with MPE had massive pleural effusions, 73 patients (76.8%). Audra et al. in 2020 reported larger pleural effusion sizes on chest X-rays were associated with a higher risk of symptomatic pleural fluid recurrence ($p < 0.001$).

Median repetitive thoracentesis MPE in NSCLC patients

In this study, the median repetitive thoracentesis MPE in NSCLC patients was three days (95% CI 1.9-4 days). This showed in **Figure 1**, that 50% of these patients have shown symptoms and signs the need of repetitive thoracentesis MPE on the third day after the initial thoracentesis. The results of this study also showed that NSCLC patients with MPE at Prof. Dr. IGNG Ngoerah General Hospital progressed during their illness, with cumulative percentages reaching the first, second, and third days of 17.9%, 36.8%, and 50.5%. Other studies provided mixed results.

Schwalk et al. in 2020 reported that more than 50% of patients with MPE experienced a recurrence within 100 days of the diagnosis of MPE being made and receiving IPC instillation therapy. Types of histology in the study included lung cancer types of adenocarcinoma, squamous cell carcinoma, unspecified EGFR, and ALK mutations, and definitive therapy of IPC installation, which can cause differences in repetitive thoracentesis. Differences in the number of samples, race, type of mutation, and therapy are the causes of the differences in the obtained repetitive thoracentesis. Ost et al. in 2018 reported that 12,967 patients with lung cancer, lymphoma, and other cancers with MPE in white, black, and Hispanic races with an age range of 66-90 years also showed a median recurrent effusion of 9 days (IQR 25% -75% 3-32 days). The choice of further thoracentesis was carried out only in patients with a survival rate of less than 30 days. In a multi-center study by Grosu et al. in 2019, 988 MPE lung cancer patients who had received lung cancer therapy showed a progressive cumulative incidence of 30% experiencing recurrent MPE recurrence on day 15 and as many as 45% experiencing recurrent pleural effusion on the 60th day. Further

thoracentesis was determined based on complaints of shortness of breath with the Borg Score criteria and radiological images indicating the addition of pleural fluid. In multivariate analysis, it was reported that an increase in the amount of pleural fluid, high levels of LDH, protein, and cholesterol levels of pleural fluid were associated with the danger of recurrence of accumulation of pleural fluid. Lung cancer therapy, such as chemotherapy, radiation, and surgery, can affect the differences in repetitive thoracentesis.

MPE is a complication of advanced NSCLC, significantly affecting the patient's quality of life. MPE originates from direct malignant cell spread, invasion, and pulmonary vasculature by embolizing tumor cells to the visceral pleura or distant hematogenous metastases from the tumor to the parietal pleura. Several factors are thought to affect repetitive thoracentesis, including the location of the tumor periphery through local extension to the pleura, which can obstruct the distal lung parenchyma followed by atelectasis which allows negative pressure in the pleural space resulting in increased accumulation of pleural fluid. The peripheral location of the tumor allows direct invasion into the pleural space. Tumor cells can close the stomata on the surface of the parietal pleura and the lymphatic system, which play a role in the cascade of decreased absorption of pleural fluid. Adenocarcinoma type is the histological type with the highest EGFR mutation rate, which causes uncontrolled signaling associated with proliferation, invasion, angiogenesis, progressive cell metastasis, and cell resistance to apoptosis. Positive cytology indicates the tumors that have metastasized to the pleura resulting in increased permeability of the pleural surface so that the volume of fluid that enters the pleural cavity is more than the volume of fluid due to decreased lymphatic drainage. LDH is a cellular enzyme that can increase its levels non-specifically as a marker of inflammation, indicating cell damage, muscle membranes, and tissue damage. The higher the LDH level of the pleural fluid, the higher the level of inflammation on the visceral and parietal pleural surfaces, which will affect vascular hyperpermeability through the VEGF mechanism.

In principle, MPE management is palliative. The initial thoracentesis intervention as a therapeutic thoracentesis aims to expel pleural fluid. This action can assess the response to shortness of breath after thoracentesis. Complaints of shortness of breath in MPE are multifactorial, but symptoms of shortness of breath are often used as a reference for intervention in MPE patients (Thomas et al., 2014). Rai, 2009 in his writing stated that the causes of shortness of breath in MPE patients by various factors that cause complaints of shortness of breath in patients with lung cancer, such as pleura; apart from MPE, the presence of pneumonia infections, heart failure, and pulmonary embolism could cause complaints of shortness. In the lung parenchyma, there is extensive fibrosis and tumor mass with pulmonary restriction; in the airways, there is obstruction by tumors; in the cardiac and pericardial areas, there is restrictive cardiomyopathy due to tumor infiltration, pericardial effusion, and the presence of chronic heart failure. In blood vessels, pulmonary thromboembolism and tumor embolism occur, as well as other conditions such as pain due to disease progression, invasion of tumor cells into the chest wall, and myopathy, which can cause shortness of breath in NSCLC patients.

Overexpression of vascular endothelial growth factor (VEGF) has been found in most human tumors, including NSCLC, and is associated with increased tumor recurrence, metastasis, and death. Most VEGF effects are mediated through the binding of VEGF R-2, which leads to microvascular permeability, invasion, migration, and survival. Liang J et al. in 2009 reported that increased VEGF could affect tumor angiogenesis, contributing to genetic variation for lung cancer among individuals. Yang F et al. in 2018 reported that the VEGF +460T/C polymorphism was found to be significantly associated with susceptibility to lung cancer only in Asian populations. Chen Y et al. in 2018 explained that through its role in regulating vascular permeability and angiogenesis, VEGF plays a central role in the accumulation of pleural effusion in tumor patients. Banka et al. in 2020 reported that the mechanism of pleural fluid formation in MPE is influenced by many factors, including direct tumor invasion of adjacent structures, hematogenous spread to the pleura, infiltration of the lymphatic drainage system, and upregulation of angiogenic growth factor (VEGF) leading to further fluid formation. Musso V et al. in 2021 explained that an increase in VEGF polymorphisms occurs in the serum of patients with lung cancer accompanied by pleural effusion, therefore VEGF is thought to be a critical pathological factor in the occurrence and development of MPE in patients with NSCLC.

In this study, repetitive thoracentesis MPE in NSCLC patients is thought to be influenced by management factors of advanced-stage patients with clinical shortness of breath which are assumed to be a reference for repetitive thoracentesis intervention, the role of the primary mediator of VEGF in Asian races, which plays a role in blood vessel leakages, lung cancer therapy, such as chemotherapy, radiation, and surgery, can cause differences in repetitive thoracentesis, in addition to various other risk factors that are thought to be associated with pleural fluid reaccumulation when compared to other studies.

Effect of risk factors for repetitive thoracentesis MPE in NSCLC

Identification of MPE repetitive thoracentesis in NSCLC patients requiring further thoracentesis after initial thoracentesis is thought to be influenced by risk factors, including tumor location, histological type of lung cancer, pleural fluid cytology results, EGFR mutations, pleural fluid LDH levels, and pleural fluid effusion size.

Tumor location as a risk factor affecting repetitive thoracentesis MPE in NSCLC

This study showed that the difference in repetitive thoracentesis in the peripheral tumor was 3 (95% CI 2.25-3.74) days, while the central location was 7 days (95% CI 4.24-9.75). However, the difference was not statistically significant ($p = 0.21$) as seen in Table 2 and Figure 2.

Morgensztern et al., in 2012, explained that the pleural fluid's process of entry and exit plays a role in maintaining a constant volume in the pleural cavity. Both fluid and protein leave the pleural cavity through the stomata of the parietal pleura, which lie between the mesothelial cells and communicate directly with lymphatic lacunae, which coalesce and drain into lymphatic channels along the ribs and then to the mediastinal lymph nodes. MPE in lung carcinoma mainly occurs due to impaired lymphatic drainage, embolization of tumor cells to the

visceral pleura, and tumor occlusion of the stomata of the parietal pleura to enlarged lymph nodes in the mediastinum.

Table 2 Median repetitive thoracentesis MPE of NSCLC patients

Variable	Median repetitive thoracentesis (Days)	p
Tumor Location		
• Peripheral	3 (2.25-3.74)	0.21
• Central	7 (4.24-9.75)	
Histology Type		
• Adenocarcinoma	3(1.86-4.13)	0,69
• Squamous cell carcinoma	4 (2.53-5.46)	
Pleural fluid cytology		
• Positive	6 (2.78-9.22)	0.51
• Negative	3 (1.83-4.16)	
EGFR Mutation		
• Positive	4 (2.48-5.52)	0.78
• Negative	3 (1.68-4.31)	
LDH levels		
• LDH \geq 821 IU/L	3 (1.49-4.50)	0.81
• LDH < 821 IU/L	4 (2.32-5.67)	
Pleural effusion size		
• massive	3 (1.88-4.11)	0,49
• Not Massive	4 (0.93-7.06)	

NSCLC that metastasizes to the pleura will increase the permeability of the pleural surface so that the volume of fluid that enters the pleural cavity is more than the volume of fluid that can be removed. In comparison, there is a decrease in lymphatic drainage caused by two different mechanisms. The first mechanism is metastases in the parietal pleura resulting in a blockage of fluid which will leave the pleural cavity to the lymph vessel area so that the channel blocked by the metastases causes a decrease in the ability to remove fluid from the pleural cavity and finally accumulation of pleural fluid occurs. The second mechanism is because the lymph vessels and parietal pleura flow to the mediastinal lymph nodes; when a malignancy attacks the mediastinal area, either primary or metastatic will cause blockage of this channel so that the drainage ability of pleural fluid is reduced. Another mechanism is the occurrence of bronchial obstruction. The lung parenchyma distal to the site of obstruction will have atelectasis when the neoplasm obstructs the main bronchus or the bronchi of one of the lobes. Lung atelectasis will cause negative pressure in the pleural cavity so that fluid will accumulate in the pleura cavity (Stathopoulos et al., 2012; Murthy et al., 2019).

The ability of NSCLC cells to develop in both the central and peripheral areas, followed by their progressive expansion to reach the pleura, explains that the location of the tumor, both central and peripheral, does not affect time of repetitive thoracentesis MPE.

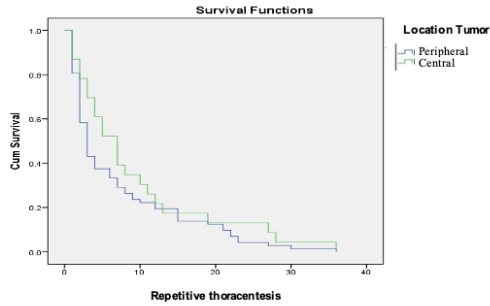
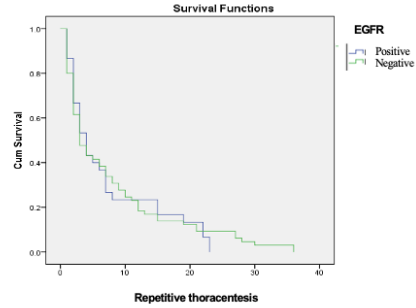
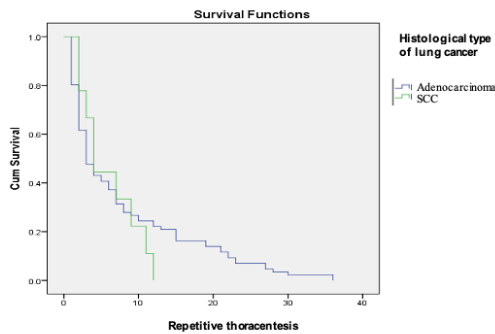
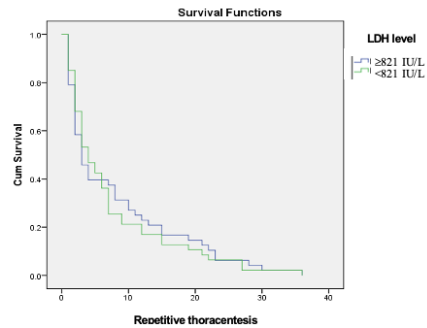
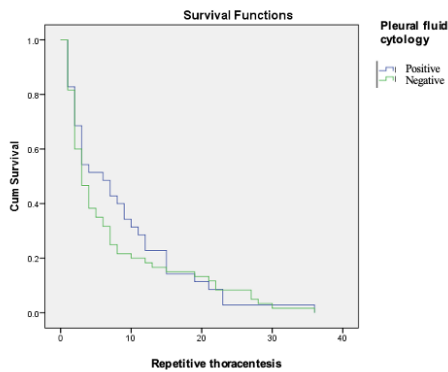
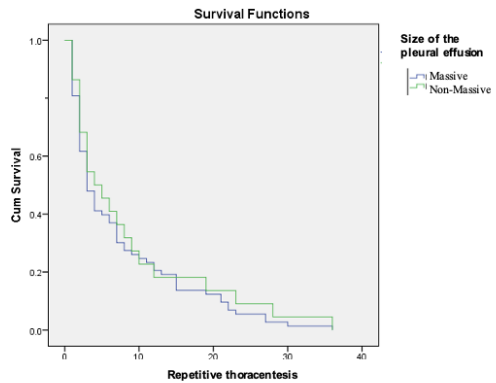
(a) Tumor Location**(d) EGFR Mutations****(b) Lung cancer histology****(e) LDH levels****(c) Pleural fluid cytology****(f) Size of pleural effusion**

Figure 2 Survival of repetitive thoracentesis MPE of NSCLC patients based on (a) tumor location, (b) histological type of lung cancer, (c) pleural fluid cytology, (d) EGFR mutation, (e) LDH level, and (f) the size of the pleural effusion.

The histological type of lung cancer as a risk factor affecting repetitive thoracentesis MPE in NSCLC

This study showed that the difference in repetitive thoracentesis in the Adenocarcinoma group was 3 days (95% CI 1.86-4.13), while squamous cell carcinoma, was 4 days (95% CI 2.53-5.46). However, the difference was not statistically significant ($p = 0.69$). The study's results explained that the differences in the types of cell histology between Adenocarcinoma and Squamous Cell Carcinoma did not affect the occurrence of repetitive thoracentesis MPE after the initial thoracentesis. Other studies regarding the different types of histology affecting recurrent MPE have been limited.

Schwalk et al. in 2020 reported 396 NSCLC patients; more than 50% of patients experienced MPE recurrence within 100 days of the diagnosis of MPE being made. The histological type in the study was predominantly Adenocarcinoma (61.36%), followed by squamous cell carcinoma (49%) and unspecific (22%). In the multivariate analysis, the histological type did not significantly affect repetitive thoracentesis MPE in NSCLC patients who had received lung cancer therapy.

Morgensztern et al. in 2012, reported an increasing association between Adenocarcinoma and the incidence of MPE with the location of the tumor in the periphery, which experienced a higher local extension into the pleura; in addition, this study reported involvement in the central area of the mediastinal lymph nodes, especially the type of lung adenocarcinoma. Syahrudin et al. (2010). Fariha et al. (2016). Elsaka et al. 2018; explained that lung adenocarcinoma more often grows in the peripheral parts of the lung from the alveolar surface epithelium or bronchial mucous glands, which quickly experience local expansion into the pleura, which often causes symptoms of coughing, tightness, recurrent pleural effusion, and chest pain due to infiltration of the parietal pleura and chest wall. This is supported by Murthy et al. in 2019, who stated the same thing: Adenocarcinoma can mobilize mast cells into the pleural space during MPE development through the elaboration of CCL12 chemokines, which leads to the formation of repeated MPE.

In addition to the role of Adenocarcinoma in causing the progression of spread through peripheral and central tumor sites. Rivera et al. in 2013 reported that lung lesions located in other central locations of the lung, such as squamous cell carcinoma, could manifest as endobronchial masses with submucosal spread to reach the pleural area which can cause MPE.

The mechanism for the ability of each NSCLC cell to spread to the pleural cavity has explained that the different types of cell histology between Adenocarcinoma and Squamous cell carcinoma do not affect repetitive thoracentesis MPE

Pleural fluid cytology as a risk factor affecting repetitive thoracentesis MPE in NSCLC

This study showed that repetitive thoracentesis in the positive cytology of pleural fluid compared to the negative cytology of pleural fluid was 6 days (95% CI 2.78-9.22) versus 3 days (95% CI 1.83-4.16). However, the differences were not

statistically significant ($p = 0.51$) as seen in table 2 and figure 2. This explained that differences in pleural fluid cytology results between positive and negative did not affect the occurrence of repetitive thoracentesis MPE after initial thoracentesis. Other studies regarding differences in pleural fluid cytology results affecting recurrent MPE were limited.

Audra et al. 2020 stated that positive cytology ($p=0.008$) was associated with an increased risk of pleural effusion recurrence. While Grosu et al. in 2019 stated that negative cytology (HR: 0.52, 95% CI: 0.43-0.64, $p < 0.0001$) was associated with a reduced risk of pleural effusion recurrence. The location of the primary tumor strongly influences the presence of malignant cells in the pleural fluid. At peripheral tumor sites, cells are found due to direct infiltration. In contrast, at a central location, cancer cells are suspected of spreading to the pleura due to metastatic processes through the vascular or lymphatic systems and are mechanically retained in capillaries or lymph nodes, which then become sites of tumor development (Syahrudin et al., 2010; Fariha et al., 2016; Elsaka et al., 2018). Cytological examination of pleural fluid is a fast, efficient, and minimally invasive method for diagnosing cancer. Fariha et al. 2016 stated that the sensitivity varies between 30-60%, and although the presence of cancer cells in pleural effusion is a diagnostic marker of MPE, the probability of finding cells is deficient. Syahrudin et al. 2010; Brunet al. 2018 also reported that the sensitivity of cytological diagnosis of pleural fluid is determined mainly by sample quality, subjective expertise of the cytopathologist, and sampling skills. Even if no malignant cells are found in the pleural fluid or pleural tissue, the pleural puncture or biopsy results may be false negative. Hariyanto et al. 2020 reported that the specificity of the cytological examination of pleural fluid was high. However, the reported sensitivity varied from 30% to 90%, so it was not clinically practical at certain times. This is in line with Arif et al. in 2020 which obtained positive cytology examination results in 52 cases (15%) of the total number of samples (347) diagnosed with malignancy.

The statistical analysis results that were not significant in this study may be due to sampling quality, subjective expertise of the cytopathologists, and sampling skills which determine the variation in the sensitivity of pleural fluid cytology examination. The possibility of variation in the sensitivity of pleural fluid cytology examination explains that differences in pleural fluid cytology results between positive and negative do not affect the occurrence of repetitive thoracentesis MPE.

EGFR mutation as a risk factor affecting MPE repetitive thoracentesis in NSCLC

This study showed differences in repetitive thoracentesis MPE in patients with favorable EGFR mutations and negative EGFR mutations, namely 4 days (95% CI 2.48-5.52) versus 3 days (95% CI 2.08-3.91) as seen in table 2 and figure 2. However, differences in the two groups were not statistically significant $p = 0.78$. The insignificant difference between the 2 groups has a high chance of experiencing repetitive thoracentesis. Other studies on the differential presence of EGFR mutations affecting recurrent MPE were limited.

Lin et al. 2017 conducted a prospective study evaluating 76 MPE patients with EGFR mutations treated with oral gefitinib. However, most patients experienced an initial decrease in MPE size on radiographs; 48 patients later developed the disease, with almost 70% experiencing recurrent MPE and requiring intervention within 30 days. This confirmed that patients with or without EGFR mutations experience repetitive thoracentesis.

Audra et al. in 2020 reported that based on the results of multivariate analysis, patients with or without EGFR mutations did not significantly affect the occurrence of recurrent pleural effusions in the first 100 days after initial thoracentesis $p = 0.083$. Several other studies have suggested that a positive EGFR potential is more dominant in experiencing recurrent pleural effusions after received lung cancer therapy. Smits et al. in 2012 reported the frequency of favorable EGFR mutations occurring extensively in Asian populations, especially East Asia, with a prevalence ranging from 36.4% to 66.3% in patients, especially adenocarcinoma. Researchers compared the status of EGFR mutations in the primary tumor with the incidence of MPE, showing a significant difference in which patients with MPE had a predominance of EGFR mutations.

Many genetic and epigenetic deviations were found in the development of the NSCLC type. Oncogenic driver mutations are the leading cause of the activation of chemical signaling pathways that lead to uncontrolled cell growth and proliferation. Several mutation drivers that have been identified in the NSCLC case include EGFR. The EGFR pathway plays a vital role in cell growth and proliferation. It often deregulates epithelial cancers in humans, including NSCLC, through increasing protein expression, gene coding, and activating mutations that occur in angiogenesis, tumorigenesis, and inhibition of apoptosis. EGFR overexpression occurs in 60% of NSCLCs, with a higher frequency in adenocarcinoma than squamous cell carcinoma (Brambilla et al., 2014). Immunological analysis and genomic tumor biomarkers have now become routine examinations for NSCLC patients, especially the adenocarcinoma type. Adenocarcinoma is said to be associated with EGFR mutations because uncontrolled signaling is found, which is associated with progressive proliferation, invasion, angiogenesis, cell metastasis, and cell resistance to apoptosis (Brambilla et al., 2014; Karachaliou et al., 2018).

This suggests that EGFR mutations facilitate the migration of cancer cells into the pleural space. Guo et al., in 2011, explained that abnormal activation, amplification, and overexpression of the EGFR gene were found in lung carcinomas and metastases in lung carcinomas that reach the pleura. This is very closely related to the presence of EGFR mutations. Has now become a routine examination for patients with NSCLC, especially the type of adenocarcinoma. Adenocarcinoma is said to be associated with EGFR mutations because uncontrolled signaling is found, which is associated with progressive proliferation, invasion, angiogenesis, cell metastasis, and cell resistance to apoptosis (Brambilla et al., 2014; Karachaliou et al., 2018). This suggests that EGFR mutations facilitate the migration of cancer cells into the pleural space. EGFR mutations cause cell dysregulation, resulting in increased protein expression, gene coding, activated mutations in angiogenesis, tumorigenesis, and inhibition of apoptosis. Vasoactive substance VEGF triggers angiogenesis (indirect effect) caused by cell

proliferation and migration. VEGF is reported to be a vascular endothelial proliferative factor that plays a role in the entire process of tumor growth through its ability to activate host vascular endothelial cells, migration, and increase the proliferation of malignant cells through increasing essential oxygen and nutrients for tumor metastasis. MPE is influenced by many factors, including direct tumor invasion of adjacent structures, hematogenous spread to the pleura, tumor embolism, and infiltration of the lymphatic drainage system, and is strongly supported by the upregulation of the angiogenic growth factor.

Anny et al., 2021 stated that activation of the EGFR pathway is associated with several important mechanisms in tumor development, including proliferation, transformation, cell survival and metastasis, migration, adhesion, motility, and differentiation. That EGFR expression led to the upregulation of VEGF signaling. In line with these researchers, Taberero et al., 2007 stated that EGFR modulates angiogenesis by regulating VEGF or other critical mediators in the angiogenic process. Another study by Theodora et al., 2015 reported that the presence of EGFR mutations in patients with MPE was significantly higher than EGFR without modifications, thus indicating that EGFR mutations facilitate the migration of cancer cells to the pleural space. Le et al., 2020 stated that NSCLC with positive EGFR could encourage an increase in VEGF expression through the upregulation of HIF-1 α in a hypoxia-independent manner. VEGF is a vascular endothelial proliferative factor that plays a role in the entire process of tumor growth through its ability to activate host vascular endothelial cells, migration, and increase the proliferation of malignant cells so that it will affect pleural fluid production and decrease the rate of pleural fluid absorption. NSCLC with positive EGFR had higher levels of VEGF than tumor cells with negative EGFR (Le et al., 2020).

The statistical analysis results that were not significant in this study may be due to the role of VEGF in both NSCLC with positive and negative EGFR. Differences in the number of samples, race, and definitive MPE therapy given in several other studies, as well as differences in definitive cancer therapy, such as chemotherapy and initial radiation, are the reasons for the difference in repetitive thoracentesis.

Pleural fluid LDH level as a risk factor affecting MPE repetitive thoracentesis in NSCLC

This study showed repetitive thoracentesis in the LDH group ≥ 821 IU/L and LDH < 821 IU/L, namely 3 days (95% CI 1.49-4.50) versus 4 days (95% CI 2.32-5.67), as seen in table 2 and figure 2. However, the difference in both groups was not statistically significant ($p = 0.80$). The study's results explained that LDH levels did not affect MPE repetitive thoracentesis after the initial thoracentesis.

LDH is an enzyme needed to catalyze the conversion of pyruvic acid to lactic acid under conditions of anaerobic glycolysis. The results showed that an increase in LDH levels indicates inflammatory markers indicating damage to cells, muscle membranes, and tissue damage (Oda et al., 2006; Prestes et al., 2007). Increased LDH describes an inflammatory condition caused by increased capillary membrane permeability. Upregulation of the LDH enzyme enables the process of glycolysis, which is beneficial for the growth of cancer cells because it can

produce adenosine triphosphate (ATP) much more quickly than oxidative phosphorylation (Magy et al., 2012; Verma et al., 2016). According to Wande et al. 2016, the increase in pleural fluid LDH levels was higher than serum LDH level, indicating malignant cells presence in the pleural fluid. This was not only indicating the presence of malignant cells in the fluid but may also indicate an inflammatory process in the pleura.

Audra et al. in 2020 stated that there was no significant difference between the patient's mean LDH level of 1319.1 U/L and the median LDH level of 693 U/L (104-3972), $p = 0.67$. Based on the results of multivariate analysis, patients with LDH > 693 U/L had HR 1.006 for every 100U/L increase in LDH levels (95% CI 1.52-8.65) $p = 0.004$ for recurrent pleural effusion when compared with LDH <693 U /L in the first 100 days from initial thoracentesis after lung cancer therapy.

Cheng et al. 1999 reported a significant association in patients with MPE with pleural fluid VEGF and LDH levels ($p < 0.005$). Increased expression of the VEGF gene in lung fibroblasts and pulmonary vascular smooth muscle cells is induced by proinflammatory mediators, so researchers believe the VEGF and LDH in the pleural fluid are correlated because both are markers of inflammatory response. Wande et al. 2016, state that the increase in pleural fluid LDH levels was higher than serum LDH indicating that the presence of malignant cells in the pleural fluid causes an increase in capillary permeability, thereby triggering an increase in pleural fluid production. The increase in LDH in this study may have occurred in an inflammatory condition associated with an increase in capillary membrane permeability, which increases pleural fluid production.

The size of the pleural effusion on radiographic images as a risk factor that affects the repetitive thoracentesis MPE of NSCLC

This study showed that the median repetitive thoracentesis of massive pleural effusions was 3 days (95% CI 1.88-4.11) while non-massive pleural effusions were 4 days (95% CI 0.93-7.06), although not significantly different statistically ($p = 0.50$). The study's results explained that the difference in the massive and not massive effusion size did not affect repetitive thoracentesis MPE.

Grosu et al. in 2019 identified 988 MPE patients at NSCLC. Based on the results of multivariate analysis, patients with massive effusions had an HR of 2.2 (95% CI 1.42-3.45) $p = 0.0004$ for recurrent pleural effusions compared with non-massive effusions. Research by Audra et al. in 2020 stated that patients with massive effusions had an HR of 3.6 (95% CI 1.52-8.65) $p = 0.004$ for recurrent pleural effusions, compared with non-massive effusions.

The size of pleural effusions is thought to affect repetitive thoracentesis MPE through obstruction of the lymphatic system, which plays a role in the cascade of decreased absorption of pleural fluid. It was associated with clinical symptoms, such as shortness of breath, a feeling of heaviness in the chest, pain that can arise due to a large amount of effusion in the form of localized pleuritic pain or dull pain, dry cough may occur in some patients. Symptoms of shortness of breath are often used as a reference for intervention in MPE patients. Shortness

of breath on MPE is multifactorial, so the amount of effusion alone does not correlate with symptoms of shortness of breath on MPE (Thomas et al., 2014). The indication for further thoracentesis related to definitive therapy is one of the causes of differences in the period of repeated thoracentesis in NSCLC patient

Strengths and weaknesses of research

This study was the first to describe repetitive thoracentesis MPE of NSCLC patients in Bali, especially at Prof.Dr. I.G.N.G Ngoerah General Hospital. In this study, there was no documented and standardized reason for further thoracentesis in the patients.

Conclusion

The median of repetitive thoracentesis MPE in NSCLC patients was 3 days after the initial thoracentesis. Tumor location, histological type, pleural fluid cytology, EGFR mutations, LDH levels, and size of the pleural effusion on the radiological appearance were not the risk factor of faster repetitive thoracentesis in NSCLC patients.

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