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Relationship of Tumor Necrosis Factor Alpha, Monocyte-Lymphocyte Ratio, and Neutrophil-Lymphocyte Ratio of Bronchial Washing Fluid with Histopathology, Stage, and Bronchoscopic Lesions of Non-Small Cell Carcinoma Lung Cancer

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ABSTRACT

Background: Chronic inflammation has been shown to be closely associated with immunosuppressive conditions that support the microenvironment for tumorigenesis, tumor progression, and metastasis. The aim of this study was to assess the relationship of inflammatory cytokine levels TNF-α , neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR) with histopathology, stage, and bronchoscopic features of non-small cell carcinoma lung cancer (NSCLC). ***Methods:*** This study was an analytical observational study with a cross-sectional design at the Prof. Dr. I.G.N.G Ngoerah Central General Hospital in January 2023 - September 2023. Data analysis was performed using the Mann-Whitney, Kruskal-Wallis and 2 independent sample t test. ***Results:*** There were 23 samples that met the inclusion criteria. The mean NLR, MLR, and TNF-α in this study were 8.939, 0.095, and 129.46 respectively. NLR was associated with KPKBSK histopathology (p = 0.026), KPKBSK stage (p = 0.003), tumour size classification (T) (p = 0.001) and metastasis (M) (p = 0009). MLR was associated with KPKBSK histopathology (p = 0.035). Smoking status had an influence on the relationship between MLR and KPKBSK histopathology (p = 0.032). TNF-α value was not associated with KPKBSK histopathology, bronchoscopic lesions, and stage. Smoking status had an influence on the bronchoscopic lesions of KPKBSK (p = 0.04). ***Conclusion:*** There are associations between MLR and NLR with histopathology of non-small cell carcinoma lung cancer. There is a relationship between NLR and the stage of non-small cell carcinoma lung cancer.

Keywords: non-small cell carcinoma lung cancer; NLR; MLR; TNF-α

INTRODUCTION

The incidence of cancer cases worldwide continues to rise and mortality rates reflect an increase in cancer risk factors linked to socioeconomic development. By 2020, GLOBOCAN estimates worldwide cancer statistics at 19.3 million new cases and 10 million cancer deaths annually. Efforts have been made to detect cancer early, but most cases are still detected at an advanced stage [1].

The five-year survival rate for lung cancer that has metastasized is still low, even shorter when compared to prostate, breast and colon cancer. Most lung cancer patients are diagnosed when symptoms appear and have an advanced stage of disease where curative treatment options are no longer possible.

There are several screening options for lung cancer including sputum cytology, chest radiography and computed tomography (CT) scan, all with the aim of reducing lung cancer mortality. One of the recommended lung cancer screening tests is a low-dose CT scan (LDCT) performed on high-risk individuals. To further improve the outcomes of lung cancer patients, research into the role of biomarkers in lung cancer diagnosis continues. The lower the stage of lung cancer, the better the survival. As an illustration, the percentage of stage I lung cancer survival can reach up to 80%, and drops significantly to only about 5% in patients with stage IV lung cancer. Patients who actually have lung cancer and are asymptomatic are not diagnosed in a timely manner.

Because most lung cancer diagnoses are obtained when there has been metastasis both locally and to other organs. From the things that have been described, one possible solution that is prioritized to improve the outcomes and survival of lung cancer patients is the existence of an early marker indicator [2].

Inflammation has been shown to be closely associated with all stages of development and malignant progression of almost all cancers. Chronic inflammation is also involved in immunosuppression that supports the microenvironment for tumorigenesis, tumor progression, and metastasis [3]. The immune system consists of inflammatory cells (lymphocytes, macrophages, dendritic cells, natural killer/NK cells) that interact through direct contact or mediators (cytokines and chemokines). The innate immune response is activated first (macrophages, neutrophils, NK cells) and followed by the adaptive immune response consisting of T and B lymphocytes. Inflammatory cytokines will be produced by immune cells. Cancer-associated macrophage cells such as monocytes, NK cells, and the innate immune system will keep the carcinogenesis process going, while Tumor Necrosis Factor (TNF) mediated by interleukins plays a role in the epithelial to mesenchymal transition that will play a role in neovascularization and metastasis of cancer cells [2].

One diagnostic procedure for lung cancer that is minimally invasive and has few complications is bronchoscopy. Tumor cells can be found in the fluid from bronchial washing. Epidermal growth factor receptor (EGFR) mutations have also been detected in the BAL fluid of patients with advanced CKD. Of the lung cancer cases, not all endobronchial lesions are visualized, especially if the cancer is still at an early stage. Detection of circulating tumor DNA (ctDNA) has potential, but is also undetectable in most early-stage lung cancers [4].

Bronchoalveolar lavage (BAL) as a diagnostic modality was first performed in 1974. The application of BAL fluid analysis in pulmonary malignancies has been reported since the late 1980s. Cellular and non-cellular components of the lung organs can be identified with the help of bronchoscopy, such as inflammatory cells of macrophages, lymphocytes, neutrophils, eosinophils, to abnormal cells associated with lung pathology, including microbes, fungi, and malignant cells. Currently, the methods of diagnosis of peripheral lung tumor lesions are more modern with the advent of various techniques such as transthoracic needle aspiration, endobronchial ultrasonography, cryobiopsy. Despite a number of modern diagnostic methods, BAL has a role to narrow down the differential diagnosis of peripheral lesions when other methods are not available. Analysis of BAL fluid in lung cancer can elucidate the characteristics of the tumor microenvironment, the characteristics of the local immune status of the lung and can assist in the diagnosis of immunotherapy-related side effects. LAB fluid requires a series of processes for both qualitative (recognition of fluid constituents) and quantitative (total cell count and inflammatory cell type count) analysis. The main principles of LAB fluid analysis are as follows: liquid volumes of less than 100 mL are referred to as mini LAB and are therefore limited for microbiological examination. Macroscopic evaluation of BAL fluid should be clear, bronchial epithelial cells can be found, if the number of neutrophils increases, it indicates an infectious process. Normal BAL fluid cellular analysis is defined as the presence of 10 million cells with a proportion of > 80% macrophages, < 20% lymphocytes, < 5% neutrophils, and < 0.5% eosinophils. The CD4+/CD8+ ratio is 1.5 in the non-smoker population. Normal BAL fluid is usually sterile. BAL fluid cell type counts are routinely performed with preparations stained with the May-Grunwald-Giemsa (MGG) method to identify non-epithelial cells and lymphoid cells, although malignant cells can also be observed but Papanicolau, Diff-Quick or hematoxylin-eosin staining is preferred. In addition to microscopic examination, flow cytometry applications have been applied in BAL fluid analysis for the identification of lymphoid cells [5].

Various research efforts have been made to detect lung tumor markers in BAL fluid, including elevated CYFRA 21-1, carcinoembryotic antigen (CEA), neuron-specific enolase. BAL fluid is currently also useful as a liquid biopsy material to detect EGFR and T790M mutations of tumor cell DNA in circulation, especially when tumor tissue is considered insufficient for analysis. The part of the lung where the BAL fluid material is collected is very important. When the tumor site can be identified by computed tomography (CT) examination, the BAL fluid collection procedure is directed according to the radiological examination results. If the lesion is disseminated, the medius lobe of the right lung or the lingula of the left lung are the best lung sites for examination. Centrally located lung tumors cannot be diagnosed using BAL material. BAL fluid can be examined using cell smear, fluid cytology and cell block methods which are superior as more cellular material can be retained for molecular and immunohistochemical examination [5].

The lungs are the target of metastases from almost every other organ system malignancy. Various manifestations of metastasis in the lung include lepidic pattern as alveolar nodules, pneumonia-like metastasis, desquamative interstitial pneumonia (DIP)-like, lymphangitic carcinomatosis, disseminated nodules, intravascular lesions, disseminated lesions in the interstitial area of the lung resembling interstitial lung disease, alveolar bleeding or infection. Many specific conditions impact the BAL sampling procedure and pattern, such as airway obstruction and smoking. In individuals with chronic obstructive pulmonary disease (COPD), there is chronic airway inflammation leading to increased numbers of neutrophils in the airway. Smoking alters the cellular pattern of BAL fluid with an increase in the number of total cells, proportion of macrophages, and reverses the CD4+/CD8+ ratio below 1. Various pulmonary changes due to imprinting or inflammatory processes in the area surrounding the tumor including hemorrhage, thromboembolic lesions, eosinophilic infiltration, and infection can be

seen from the BAL fluid, possibly leading to a false positive diagnosis. Alveolar macrophages are the main cellular component in BAL fluid [5].

Proinflammatory activity is triggered by TNF-α , this cytokine is produced by T lymphocytes, NK cells, monocytes, macrophages, neutrophils, and some endothelial cells. In addition to carcinogenesis, TNF-α also plays a role in the systemic inflammatory process that occurs in patients with lung cancer, causing impaired albumin synthesis, as well as triggering hematopoietic stem cells to differentiate into myeloid cells. In relation to inflammatory cytokine production, the ratio between monocytes (a component of the innate immune response) and lymphocytes (a component of the adaptive immune response) is one of the predictors of outcome and prognosis of cancer patients [6].

Analysis of proinflammatory cytokine levels such as TNF-α cannot only be done by BAL examination which requires a large volume of fluid. Bronchial lavage fluid is the fluid obtained after rinsing/instillation of the lung lesion site with physiological saline solution during bronchoscopy. Bronchial lavage fluid specimens have a better direct link to tumor lesions compared to blood samples, and also better reflect intertumoral heterogeneity [7].

Bronchial lavage fluid examination is superior when intrabronchial biopsy procedures cannot be performed, and it can reach more distal areas than bronchial lavage especially in peripherally located lung masses [8]. Cytologic examination of bronchial lavage has a low accuracy, but is minimally invasive, simple, can be performed repeatedly, and rarely causes complications. Not only cellular material, bronchial lavage can now be used to detect nucleic acids from tumor cells, including epidermal growth factor receptor (EGFR) mutations from the supernatant of bronchial lavage fluid . (Ryu et al., 2019). Research by Kartini in 2018 used 3 mL of bronchial washing fluid as a laboratory sample [4]. There were differences in TNF-α levels based on histopathology type and location of lung cancer lesions. Between histopathology types, there was no statistically significant difference between Non-Small Cell Carcinoma Lung Cancer (NSCLC) (8.28pg/mL) and Small Cell Carcinoma Lung Cancer (SCLC) (7.24pg/mL) with a p value> 0.05; and between the central cancer lesion location (8.21pg/mL) and peripheral lesions (7.38pg/mL) obtained statistically significant differences [9].

Based on previous studies, we wanted to evaluate the relationship of inflammatory cytokine levels TNF-α , monocyte to lymphocyte ratio, and neutrophil to lymphocyte ratio with histopathology, stage, and bronchoscopic features of non-small cell carcinoma lung cancer to describe the progressivity of lung cancer.

METHOD

This study was an analytic observational study with a cross-sectional design. The study starts from patients with a diagnosis of lung tumor who undergo a diagnostic bronchoscopy procedure, bronchial lavage is performed in the bronchial segment suspected to be the location of the tumor, then the bronchial lavage fluid is analyzed. The study will be conducted at Prof. Dr. I.G.N.G Ngoerah Central General Hospital from January 2023 - September 2023. The study sample was all patients with a diagnosis of lung tumor in the pulmonary polyclinic room and inpatient room of the Prof. Dr. I.G.N.G Ngoerah Central General Hospital. The inclusion criteria for this study were: 1) Patients aged more than 18 years; 2) Patients with a diagnosis of lung tumor who underwent a flexible bronchoscopy procedure, and obtained a definite diagnosis of non-small cell carcinoma lung cancer through the results of anatomical pathology examination of good material of bronchial washings / bronchial forceps biopsy / bronchial elbow, and / or transthoracic needle aspiration biopsy / malignant pleural effusion / open biopsy at Prof. Dr. I.G.N.G Ngoerah Central General Hospital, who have never received chemotherapy, targeted therapy, or monoclonal antibodies for lung cancer. The exclusion criteria for this study were: 1) Patients with a diagnosis of malignancy in an organ system other than the lung; 2) Patients with a diagnosis of pulmonary tuberculosis when the diagnosis of lung cancer was established; 3) Patients who refused to participate in the study. The analysis was assisted by SPSS version 26 including descriptive data analysis, independent t test analysis, Spearman correlation test, Fischer's Exact and Mann Whitney tests.

RESULT

The number of subjects with a diagnosis of lung tumor who underwent bronchoscopic examination in this study was 23 people. The basic characteristics of the study subjects are shown in Table 1.

TABLE 1: Characteristics of research subjects.

Variables	N = 23 n (%)
Age	
Mean ± SD (years)	61,43 ± 11,317
Gender	
Male	18 (78,3)
Female	5 (21,7)
Bronchoscopic lesions	
Yes	9 (39,1)
No	14 (60,9)
Type of cancer	
Non-squamous NSCLC	19 (82,6)
Squamous NSCLC	4 (17,4)
Cancer stage	
IIIC	2 (8,7)
IVA	16 (69,6)
IVB	5 (21,7)
Tumors	
T1	3 (13)
T2	2 (8,7)
T3	3 (13)
T4	15 (65,2)
Lymphnode	
N0	1 (4,3)
N2	3 (13)
N3	19 (82,6)
Metastasis	
M0	2 (8,7)
M1a	9 (39,1)
M1b	7 (30,4)
M1c	5 (21,7)
Smoking Status	
No smoking	9 (39,1)
Light Smokers	3 (13)
Moderate Smokers	3 (13)
Heavy Smokers	8 (34,8)
Comorbid	
Asthma	1 (4,3)
Hypertension	3 (13)
NLR	
Mean ± SD	8,939 ± 9,644
MLR	
Mean ± SD	0,095 ± 0,138
TNF-α	
Mean ± SD (ng/L)	129,46 ± 37,07

The results of the Shapiro-Wilk test showed that the NLR data in the non-squamous NSCLC group and the absence of bronchoscopic lesions were not normally distributed ($p < 0.05$).

Differences in NLR between histopathology, stage and bronchoscopic lesions were analyzed using the Mann Whitney test (Table 2).

TABLE 2: Testing the normal distribution of NLR data based on histopathology, stage and bronchoscopic lesions of NSCLC.

	n	P-value
Histopathology		
Squamous NSCLC	4	0,966
Non-squamous NSCLC	19	0,001
Bronchoscopic lesions		
Yes	9	0,092
No	14	0,009

The Mann Whitney test results showed a significant difference in NLR between squamous and non-squamous histopathology ($p < 0.05$).

There was no difference in NLR based on NSCLC stage and bronchoscopic lesions ($p > 0.05$), as shown in Table 3.

TABLE 3: Relationship between NLR and histopathology, bronchoscopic lesions, and stage of NSCLC.

	n	Median (min - max)	P-value
Histopathology			
Squamous	4	19,67 (6,08 - 32)	0,026*
Non-squamous	19	3,76 (0,0 - 24)	
Bronchoscopic lesions			
Yes	9	6,08 (0,0 - 24)	0,636
No	14	4,41 (0,0 - 32)	

* = statistically significant.

The results of Spearman correlation showed that there was a significant relationship between NLR and NSCLC stage and classification of tumor stage and metastasis ($p < 0.05$). Table 4 shows that the relationship between NLR and stage and metastasis classification (M) has moderate strength and the relationship between NLR and tumor classification (T) has strong strength. The mean NLR by histopathologic division, bronchoscopic lesion, and stage of NSCLC is shown in Table 5.

TABLE 4: Relationship between NLR and NSCLC stage.

NLR relationship with	n	Spearman correlation coefficient (r)s	P-value
Stadium (IIIC-IVB)	23	0,597	0,003*
Classification of Stage T	23	0,641	0,001*
N stage classification	23	0,150	0,494
M stage classification	23	0,530	0,009*

* = statistically significant.

TABLE 5: Mean NLR by histopathology, bronchoscopic lesion, and stage of NSCLC.

	NLR (Mean ± SD)
Histopathology	
Non-squamous NSCLC	6,746 ± 7,984
Squamous NSCLC	19,352 ± 11,164
Bronchoscopic lesions	
Intralumenal lesions visible	9,54 ± 9,42
No visible intralumenal lesions	8,55 ± 10,11

	NLR (Mean ± SD)
Stadium	
IIIC	0,17 ± 0,24
IVA	7,74 ± 9,33
IVB	16,27 ± 8,55
Tumors	
T1	1,156 ± 1,496
T2	1,189 ± 1,682
T3	2,464 ± 2,274
T4	12,823 ± 9,909
Lymphnode	
N0	15,333 ± 0
N2	2,48 ± 2,53
N3	9,622 ± 10,161
Metastasis	
M0	0,17 ± 0,24
M1a	6,684 ± 8,031
M1b	9,103 ± 11,322
M1c	16,273 ± 8,553

The normality test of MLR data with the study variables is shown in Table 6. The results of the Shapiro-Wilk test showed that MLR data in the squamous NSCLC group were normally distributed (p> 0.05), while in non-squamous and MLR data based on bronchoscopic lesions were not normally distributed (p < 0.05). Differences in MLR based on histopathology and bronchoscopic lesions were analyzed using the Mann Whitney test.

TABLE 6: Testing the normal distribution of MLR data based on histopathology, stage and bronchoscopic lesions of NSCLC.

	n	P-value
Histopathology		
Squamous	4	0,259
Non-squamous	19	< 0,001
Bronchoscopic lesions		
Yes	9	0,004
No	14	0,001

The results of the Mann Whitney test showed that there was a significant difference in MLR between the histopathology of squamous and non-squamous NSCLC (p < 0.05). Mann Whitney test results showed no difference in MLR based on bronchoscopic lesions (p > 0.05), shown in Table 7.

TABLE 7: Relationship between MLR and Histopathology, Stage, and Bronchoscopic Lesions of NSCLC.

	n	Median (min - max)	P-value
Histopathology			
Squamous	4	0,333 (0,00 - 0,462)	0,032*
Non-squamous	19	0,0 (0,0 - 0,250)	
Bronchoscopic lesions			
Yes	9	0,0 (0,0 - 0,462)	1,000
No	14	0,01 (0,0 - 0,333)	

* = statistically significant.

Table 8 shows from the correlation test that there is no significant relationship between MLR and stage or stage classification of NSCLC (p > 0.05). The mean MLR based on histopathology division, bronchoscopic lesions, and stage of NSCLC is shown in Table 9.

TABLE 8: MLR relationship with NSCLC stage classification

MLR relationship with	n	Spearman correlation coefficient $(r)_s$	P-value
Stadium (IIIC - IVB)	23	0,048	0,830
Classification of Stage T	23	0,195	0,372
N stage classification	23	-0,244	0,261
M stage classification	23	0,140	0,525

TABLE 9: Mean MLR based on histopathology, bronchoscopic lesions, and stage of NSCLC.

	MLR (Mean ± SD)
Histopathology	
Non-squamous NSCLC	0,057 ± 0,864
Squamous NSCLC	0,282 ± 0,197
Bronchoscopic lesions	
Intralumenal lesions visible	0,107 ± 0,163
No visible intralumenal lesions	0,088 ± 0,124
Stadium	
IIIC	0
IVA	0,119 ± 0,153
IVB	0,06 ± 0,089
Tumors	
T1	0,005 ± 0,009
T2	0,034 ± 0,048
T3	0,081 ± 0,096
T4	0,125 ± 0,158
Lymphnode	
N0	0,333 ± 0
N2	0,085 ± 0,094
N3	0,085 ± 0,137
Metastasis	
M0	0
M1a	0,065 ± 0,094
M1b	0,187 ± 0,192
M1c	0,06 ± 0,089

The Mann Whitney test results showed no significant difference in TNF-α based on histopathology ($p > 0.05$). The results of the 2 independent samples t test also showed no significant difference in TNF-α based on bronchoscopic lesions ($p > 0.05$).

This also means that there is no significant relationship between TNF-α with histopathology, stage and bronchoscopic lesions of NSCLC. The relationship between TNF-α levels with histopathology, bronchoscopic lesions, and stage in NSCLC is shown in Table 11.

TABLE 11: Relationship between TNF-α levels with histopathology, bronchoscopic lesions, and stage of NSCLC.

	n	Mean± Standard deviation Median (min - max)	P-value
Histopathology			
Squamous	4	130,3 (104,85 - 156,05)	0,570
Non-squamous	19	116,76 (72,06 - 228,67)	
Bronchoscopic lesions			
Yes	9	147.45± 44.164	0,098
No	14	117.90± 27,520	

Table 12 shows that there is no significant association of TNF-α with stage or classification of T, N, and M ($p > 0.05$).

The mean TNF-α based on histopathology division, bronchoscopic lesions, and stage of NSCLC is shown in Table 13.

TABLE 12: Relationship between TNF-α and NSCLC stage classification.

TNF-α relationship with	n	Spearman correlation coefficient $(r)_s$	P-value
Stadium (IIIC - IVB)	23	0,145	0,510
Classification of Stage T	23	0,399	0,059
N stage classification	23	0,163	0,456
M stage classification	23	0,103	0,640

TABLE 13: Mean TNF-α by histopathology, bronchoscopic lesion, and stage of NSCLC.

	TNF-α (Mean ± SD)
Histopathology	
Non-squamous NSCLC	129,26 ± 40,08
NSCLC squamous	130,37 ± 20,91
Bronchoscopic lesions	
Intralumenal lesions visible	147,44 ± 44,16
No visible intralumenal lesions	117,89 ± 27,51
Stadium	
IIIC	144,47 ± 62,26
IVB	126,68 ± 40,15
IVA	132,33 ± 19,68
Tumors	
T1	113,03 ± 42,73
T2	105,29 ± 8,11
T3	112,66 ± 12,53
T4	139,32 ± 39,56
Lymphnode	
N0	156,05 ± 0
N2	116,62 ± 6,52
N3	131,66 ± 39,28
Metastasis	
M0	144,47 ± 62,26
M1a	126,17 ± 45,70
M1b	127,34 ± 35,29
M1c	132,33 ± 19,68

Table 14 illustrates the association of smoking status with histopathology, stage, and bronchoscopic lesions of NSCLC. Fisher's Exact test results showed no association of smoking status with NSCLC histopathology ($p > 0.05$). The Mann Whitney test results showed no significant relationship between smoking status and the stage of JPIC ($p > 0.05$).

There was a significant relationship between smoking and bronchoscopic lesions of NSCLC, but from table 5.15 it appears that the degree of smoking based on the Brinkman index has no relationship to bronchoscopic lesions ($p < 0.05$). It was not possible to analyze the relationship between comorbid asthma and hypertension with the study variables because the number of blank data was too large.

TABLE 14: Relationship between smoking and histopathology, stage and bronchoscopic lesions of NSCLC.

	Smoking		P-value
	Yes (n=14)	No (n=9)	
Histopathology			
Squamous	1 (7,1%)	3 (33,3%)	0,260
Non-squamous	13 (92,9%)	6 (66,7%)	
Stadium			
III C	1 (7,1%)	1 (11,1%)	0,350
IV A	9 (64,3%)	7 (77,8%)	
IV B	4 (28,6%)	1 (11,1%)	
Bronchoscopic lesions			
Yes	8 (57,1%)	1 (11,1%)	0,040
No	6 (42,9%)	8 (88,9%)	

TABLE 15: Differences in smoker status based on NSCLC bronchoscopic lesions.

Smoking Status	Bronchoscopic lesions		P-value
	Yes (n = 9)	No (n = 14)	
Non-Smokers	1 (11,1%)	8 (57,1%)	0,096
Light Smokers	2 (22,2%)	1 (7,1%)	
Moderate Smokers	2 (22,2%)	1 (7,1%)	
Heavy Smokers	4 (44,4%)	4 (28,5%)	

DISCUSSION

Historically, lung cancer has been more prevalent in men compared to women. However, currently the incidence of lung cancer in the female population is increasing [10] There were no female subjects with a history of smoking in this study. There was one female with adenosquamous carcinoma lung cancer and one female with squamous cell carcinoma lung cancer, both with advanced stage (VIA) at diagnosis. Adenosquamous carcinoma lung cancer is a subtype of NSCLC when at least 10% each of adenocarcinoma and squamous cell carcinoma components are found through anatomical pathology examination. Adenosquamous carcinoma of the lung has a low incidence, only about 0.4 - 4% of all lung cancer cases, is more common in men, and is closely associated with a history of smoking. The theory of adenosquamous carcinoma carcinogenesis is that this cancer arises from undifferentiated cells that are bipotential, so they are able to differentiate into adenocarcinoma and squamous cell carcinoma[11].

Squamous cell lung cancer is rarely found in individuals who do not smoke, only about 5% of all cases of JPIC [12]. It is undeniable that SCLC in non-smoking individuals can be caused by environmental tobacco smoke / secondary cigarette smoke. Large case control studies in the United States and Europe resulted in no effect of secondary cigarette smoke on the difference in lung cancer incidence in terms of histopathology type between adenocarcinoma and squamous cell carcinoma of the lung. Most types of lung cancer in women are lung adenocarcinoma [13].

Some factors that increase the risk of lung cancer in non-smoking women include radon gas exposure, occupational exposure, indoor air pollution, human papilloma virus (HPV) infection, and genetic susceptibility [12]. Of the 19 men with SCLC, 14 had a history of smoking, with 3 light smokers, 3 moderate smokers, and 8 heavy smokers. Two men had squamous cell carcinoma lung cancer, while the rest had lung adenocarcinoma. All NSCLC patients were diagnosed at an advanced stage. A number of studies with populations of CKD patients in Japan, China and the US illustrate that most CKD patients with a history of smoking or not smoking present with stages III to IV, but there are differences in survival between the two groups. The absence of a smoking history was a positive independent predictor of CKD survival based on multivariate analysis [13].

TNF-α levels were elevated in all samples, but there was no difference in mean TNF-α between histopathology, bronchoscopic lesions, and stage of NSCLC. Although it is suspected that direct contact with endobronchial lesions can describe the intratumoral situation, in this study, TNF-α levels were increased even though bronchial washings were not performed directly on endobronchial lesions. The results of this analysis are similar to research conducted in Makassar, there is no difference in the average TNF-α levels between central and peripheral tumor lesions. (Kartini, 2018).

Examination of biologically active substances directly produced by tumors is one of the roles of examination with BAL fluid material through bronchoscopy. A study in Poland more than two decades ago showed that the average TNF-α levels in patients with squamous cell carcinoma lung cancer were much higher than in non-malignant lung diseases such as sarcoidosis and COPD (1192 pg/mL, 5.3 pg/mL, and 0.5 pg/mL), and the highest TNF-α concentrations were found in stage IIIb squamous cell carcinoma lung cancer [3].

The findings of the Chinese study reinforced these results, measuring the levels of biomarkers such as VEGF and TGF-β in patients with lung nodules less than 8mm in size. Through the analysis of BAL samples taken from bronchial segments corresponding to the location of lung nodules, it was found that the levels of biomarker cytokines were higher in patients diagnosed with lung cancer, even exceeding the average levels of the same markers in the blood [14]This illustrates that even though lung cancer nodules are small and endobronchial abnormalities are not visualized, the levels of cancer-related inflammatory cytokines detected through BAL fluid are able to represent the microenvironment of lung cancer tumors. TNF-α levels in this study were not shown to be associated with the histopathologic division of squamous and non-squamous lung cancer. The presence of TNF-α causes up-regulation of the enzyme manganese superoxide dismutase (SOD-2) through the NF-κ B pathway which is involved in epithelial to mesenchymal transition (EMT) and associated with tumorigenesis. In vitro studies show SOD-2 levels are elevated in lung adenocarcinoma. Infiltration of TNF-α secreting macrophages in lung adenocarcinoma tissue was associated with SOD-2 expression, supporting the notion that macrophage-associated chronic inflammation contributes to the development of lung adenocarcinoma. Increased SOD-2 expression in lung adenocarcinoma is also associated with metastasis to Lymphnode and TNM stage [15].

Increased levels of the cytokine TNF-α in the tumor microenvironment will stimulate the activity of its receptors, namely tumor necrosis factor 1 and 2 (TNFR1 and TNFR2). Squamous cell carcinoma originates from the basal epithelial cells of the lung. Immunohistochemical examination of squamous cell carcinoma lung cancer tissue illustrates increased expression of TNFR1. In vivo animal studies have shown that TNFR1 overexpression plays a role in the initiation of tumorigenesis and metastasis of lung squamous cell carcinoma. Overexpression of TNFR1 is also found in lung adenocarcinoma, but TNFR2 has a different role. Stimulation of TNFR2 by TNF-α produced by macrophages converts CD4 T lymphocytes into Treg cells that play a role in the progression of lung adenocarcinoma [16].

This study showed that TNF-α levels were not associated with the stage of NSCLC and the TNM classification of NSCLC.

One of the possible reasons why TNF-α cytokine levels in this study were not associated with the stage of NSCLC is that all subjects in this study were diagnosed at an advanced stage of NSCLC (IIIC-IVB). TNF-α cytokine has an important role in the initiation of tumorigenesis and increases tumor cell growth [17]. Therefore, TNF-α levels are expected to continue to increase as the stage of lung cancer increases. TNF-α levels in lung cancer patients with metastases are much higher than those in lung cancer patients without metastases. TNF-α cytokine will increase the expression of CXCL-1 chemokine which plays a role in carcinoma proliferation and resistance to chemotherapy. CXCL-1 levels were found to increase in NSCLC that had metastasized, higher than in early stage NSCLC (IA-IIB) [2].

The NLR value in this study was related to the histopathological division of NSCLC. The NLR value in the squamous NSCLC group was higher than that in the non-squamous NSCLC group. The process of histological changes in squamous cell lung cancer can be observed through sampling from bronchoscopic examination, in contrast to lung adenocarcinoma which often requires sampling from transcutaneous biopsy results or surgical resection. Genetic mutations will occur in normal lung epithelial cells exposed to carcinogenic substances, causing changes ranging from cell hyperplasia, squamous metaplasia, dysplasia (mild, moderate, severe) to carcinoma in situ (Keith and Miller, 2013). (Keith and Miller, 2013). Tumor-associated neutrophils (TANs), which have protumor and immunosuppressive functions in the tumor microenvironment, are more abundant in squamous cell carcinoma lung cancer than in lung adenocarcinoma. In contrast, TAM tended to be more abundant in the tumor microenvironment of lung adenocarcinoma. TAN cell population increases ROS activity, increases the expression of genes that block the activity of T lymphocyte cells [18].

The results of the analysis showed that NLR was associated with tumor classification (T) and metastasis (M) of NSCLC, but was not shown to have an association with NSCLC stage. A retrospective study with NSCLC patient subjects showed that NLR was an independent predictor of poor prognosis, after statistical adjustment for gender, lung cancer histology, stage, smoking history, display status, and chemotherapy. The lack of statistically significant association between NLR and stage of CKD may be due to the fact that all CKD patients in this study were at an advanced stage. A Spanish study examined NLR and its association with lung cancer. Increased NLR value was an independent predictor of poor survival of stage IV CKD patients. The higher T and N stages were associated with an increase in NLR. NSCLC patients with T4 and N3 had significantly higher NLR compared to T1 and N0. The overall survival (OS) of CKD patients with NLR $\geq$ 5 is shorter when compared to patients with NLR < 5 (4.3 months and 8.8 months, respectively, p = 0.01) [19]

The results obtained from this study are in accordance with the findings of a study in South Korea that analyzed BAL samples of NSCLC patients. The results of NLR in BAL fluid have a relationship with NLR in blood. There was no significant difference in NLR between the classifications of Lymphnode (N) [4]. This can be explained because neutrophil infiltration in the tumor microenvironment is an innate immune defense that appears in the early phase in response to the presence of lung cancer cells. Increased expression of the chemokine CXCL-1 supports the recruitment of neutrophils into the lung tumor environment, and CXCL-1 expression increases with the progression of lung cancer stage [2].

The MLR value in this study was related to the histopathological division of NSCLC, and MLR tended to be higher in squamous NSCLC compared to non-squamous NSCLC. Tumor-associated macrophages (TAMs) are the most abundant immune cells in the tumor microenvironment, involved in enhancing tumor invasive properties, angiogenesis, metastasis, and immunosuppression. Alveolar macrophages are far more abundant than interstitial macrophages (Loyher et al., 2018).. The difference in MLR between squamous and non-squamous NSCLC may be due to differences in cellular immune responses during the carcinogenesis process of the two types of lung cancer. A Japanese study showed an increased number of alveolar macrophages located close to the tumor cell collection (peritumoral) was an independent risk factor of poor prognosis for stage I squamous cell carcinoma lung cancer, illustrating the role of macrophages in the progression of squamous cell carcinoma lung cancer [20].

In general, the number of T and B lymphocytes in the normal lung is less than the number of lymphocytes in the tumor microenvironment of lung adenocarcinoma, as lymphocyte infiltration progressively increases from premalignant lesions to the appearance of invasive lung adenocarcinoma. Interestingly, more lymphocytes were found in regressive in situ carcinoma, while there were fewer in situ carcinoma that progressed to squamous cell carcinoma, indicating reduced immune system surveillance and immune escape when premalignant lesions progressed to squamous cell carcinoma [18].

In this study, there was no association between NLR and MLR with bronchoscopic lesion appearance. A Korean study showed that direct contact between bronchial washing fluids had better diagnostic yield of NSCLC cytology. The amount of bronchial washing volume also did not result in different lung cancer diagnostic rates between 30 mL and 10 mL as used in this study, so the amount of bronchial washing volume may not be the cause of the non-significant analysis results [21]. The absence of a relationship between bronchoscopic lesion appearance and MLR may be due to bronchial lavage being able to reach the tumor area in the segmental bronchi, such as the analysis results of a study in Korea [4].

Monocytes in the inflammatory microenvironment are able to stimulate tumor cell growth, angiogenesis, and suppress the host's anticancer immune response. Monocytes also influence the development of malignant cells by producing proinflammatory cytokines including TNF-α , IL-1 and IL-6. In addition, cytokines and chemokines produced by tumor cells can induce the differentiation of monocytes into tumor-associated macrophages (TAM) which can weaken the anti-tumor immune response, stimulate the migration and metastasis of tumor cells [22].

The ratio between neutrophils and lymphocytes describes the innate and adaptive immune response in the body. Neutrophilic inflammation in cancer patients is increased due to stimulation of tumor-associated neutrophils (TAN). High NLR is a predictor of cancer-specific survival (CSS), progression free survival (PFS) and disease-free survival (DFS). In addition to survival, NLR is also potentially used in cancer stratification, associated with tumor size, tumor stage, metastatic potential and lymphatic invasion. For example, stage IV NSCLC patients with NLR > 4.95 have a correlation with higher rates of brain metastasis (Buonacera et al., 2022).. Increased NLR is associated with tumor progression and anti-tumor T cell exhaustion through inflammation in the tumor microenvironment mediated by T cells and tumor-associated macrophages producing IL-17. A low NLR threshold in blood (<5) prior to immunotherapy administration is associated with better survival (Kiriu et al., 2018).. The mean MLR that was not associated with the stage of NSCLC in this study could be due to all study subjects being in an advanced stage. The MLR value will increase as the stage of NSCLC increases, especially when grouped into early and advanced stages of NSCLC. (Zhang et al., 2023). Similar results were also obtained in the analysis of NLR values having predictive meaning for the diagnosis of CKD when compared with subjects who did not have lung cancer. The increase in NSCLC stage is directly proportional to the increase in NLR value [23].

Smoking behavior has been recognized as one of the main causes of lung cancer [24] Smoking status among the study subjects had an influence on the picture of bronchoscopic lesions of CKD as shown through correlation analysis, but differences in the classification of smoking status based on the Brinkman index did not affect the bronchoscopic lesions of CKD. There is no evidence of the influence of smoking status with the histopathological division and stage of NSCLC. Smoking status was also not shown to have an influence on the value of NLR, MLR, or TNF-α. The possibility that could explain the results of the analysis of no proven relationship between smoking status with NLR, MLR, and TNF-α is that the possibility of exposure to tobacco smoke from the environment (passive smoking) in the NSCLC patient population cannot be excluded.

Exposure to tobacco smoke will increase the number of alveolar macrophages due to an inflammatory response that increases the production of cytokines and chemokines. Chemoattractant molecules released by neutrophils IL-1β induce macrophage activation as well as the release of neutrophils from the bone marrow, so that inflammation in tissues in the airway continues [25]. Cigarette smoke exposure causes mutation of the p53 tumor suppression gene, which plays an important role in malignant transformation and histological progression in both adenocarcinoma and squamous cell carcinoma of the lung [26]. Cigarette smoke exposure leads to increased production of the cytokine TNF-α , either from alveoli epithelial cells of the alveoli or alveolar macrophages [25]. There is one study in Makassar with similar results to this study, which showed no relationship or influence between smoking status and TNF-α levels [9]. Of all the research subjects, there was 1 subject with comorbid asthma and 3 subjects with comorbid hypertension, but no analysis was carried out because statistically it could not be done due to the small number.

To the best of our knowledge, this study is one of the first to examine the relationship between cellular immune parameters in the tumor microenvironment and the appearance of intrabronchial lesions and staging based on TNM classification in bronchial washings of patients with CKD. This study illustrates that inflammatory parameters in the tumor microenvironment can be detected from bronchial lavage fluid samples even if cancerous lesions are not visible or a pathology diagnosis is not established from bronchial lavage analysis.

Some of the limitations of this study include not obtaining samples from subjects with early stage CKD, so it has not been able to describe the progression of the inflammatory process in lung cancer as a whole. The comorbid data of the subjects of this study were evaluated through anamnesis, not through gold standard / specific examinations to evaluate the presence of comorbid asthma, COPD, hypertension, and diabetes mellitus, so there is a possibility of the presence of comorbidities that were not detected in this study.

CONCLUSION

There are associations between MLR and NLR with histopathology of non-small cell carcinoma lung cancer. There is a relationship between NLR and the stage of non-small cell carcinoma lung cancer.

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