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VEGF and NLR as Diagnostic Biomarkers in Malignant, Non-Malignant, and Tuberculosis Pleural Effusion Patients

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Abstract

Differentiating malignant from non-malignant pleural effusions, as well as distinguishing malignancy from tuberculosis, remains challenging and carries significant diagnostic and therapeutic implications. This study aimed to analyze vascular endothelial growth factor (VEGF) level and the neutrophil-to-lymphocyte ratio (NLR) in pleural fluid as potential diagnostic biomarkers. A cross-sectional analytical observational study conducted using purposive sampling. A total 110 patients were divided into three groups, and VEGF and NLR levels were measured from pleural fluid samples. Data were analyzed using the Kruskal–Wallis test followed by Dunn's post-hoc test. Results demonstrated that VEGF levels among the three groups differed significantly (p=0.000). The VEGF level was highest in the malignant pleural effusion (MPE) group at 6,916.80 pg/mL, compared with 4,544.49 pg/mL in the Non-MPE (NMPE) and 1,936.61 pg/mL in the tuberculosis group. Receiver operating characteristic (ROC) curve analysis showed that VEGF had the highest diagnostic accuracy in differentiating malignant from non-malignant effusion (AUC: 80.8%, p=0.000), and for distinguishing malignant from tuberculosis effusion (AUC: 76.8%; p=0.000). In contrast, NLR did not demonstrate meaningful diagnostic performance. Although the VEGF levels were elevated across all groups (MPE, NMPE, and tuberculosis), they were significantly higher in malignant effusions. VEGF demonstrated high sensitivity and specificity as a diagnostic biomarker for differentiating MPE, NMPE, and tuberculosis-related effusions. Thus, VEGF shows good potential as a diagnostic biomarker for identifying MPE, whereas NLR does not demonstrate usefulness as a diagnostic biomarker.

Keywords: Malignant pleural effusion, neutrophil to lymphocyte ratio, vascular endothelial growth factor

Introduction

Pleural effusion (PE) is the accumulation of fluid within the parietal and visceral pleura. It is a common clinical condition associated with substantial morbidity and mortality, with an estimated incidence of approximately 320 cases per 100,000 population annually.[1,2] Exudative PEs are most frequently caused by malignancy, parapneumonic effusions, and tuberculosis. A study in China reported that the three leading causes are parapneumonic effusion and empyema (25.1%), malignancy (23.7%), and tuberculosis (12.3%).[3] Malignant PE (MPE) is a type of exudative effusion caused by the presence of malignant cells in the pleural space. It occurs in up to 15% of cancer patients, particularly those with lung cancer, breast cancer, lymphoma, gynecological malignancies, and malignant mesothelioma.[4]

Differentiating the causes of exudative PE remains a clinical challenge. The gold standard for diagnosing MPE is thoracoscopic pleural biopsy. Alternatively, pleural fluid cytology obtained via thoracocentesis is less invasive but has limited diagnostic accuracy, with a sensitivity of approximately 60%.[5] To enhance diagnostic precision, various laboratory biomarkers are being investigated. Vascular endothelial growth factor (VEGF) is a key pro-angiogenic molecule that promotes endothelial proliferation, inhibits apoptosis, and increases vascular permeability.[6] Elevated VEGF levels in pleural fluid have been recognized as one of the most important regulatory factors in tumor angiogenesis, which participates in the entire tumor growth process through its function to stimulate tumor angiogenesis, activate host vascular endothelial cells, and promote malignant proliferation.[7]

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Another potential marker is the neutrophil-to-lymphocyte ratio (NLR), a novel inflammatory indicator that has been investigated in pleural fluid to differentiate malignant from non-malignant (e.g., infectious) causes of exudative pleural effusion (EPE).[8] Given the diagnostic potential of both VEGF and NLR, further research is warranted to evaluate their clinical utility.

This study aimed to compare pleural fluid VEGF and NLR levels among (1) Malignant Pleural Effusion, (2) Non-Malignant in malignancy pleural effusion and (3) Tuberculosis pleural effusion. Additionally, the study seeks to assess the sensitivity and specificity of VEGF and NLR as diagnostic biomarkers in differentiating these conditions.

Methods

This research conducted with analytical observational study with a cross-sectional design. The study was conducted between June and December 2024 at Dr. Saiful Anwar Regional Hospital. The study population included patient diagnosed with EPE who were either hospitalized or attended the Pulmonary Outpatient Clinic.

Participants were recruited using a consecutive sampling method, based on the following inclusion criteria: age over 18 years old, diagnosed with EPE according to Light's criteria, and informed consent to participate in the study.

Meanwhile, the exclusion criteria included patients with transudative PE, those with pathogenic microorganisms found in pleural fluid culture, those who had received chemotherapy, and those with contraindications for thoracentesis.

A total of 300 participants had been screened with 110 research participants met inclusion and exclusion criteria. Each participant underwent thoracentesis to obtain pleural fluid samples, pleural biopsy, and rapid molecular testing of sputum to detect *Mycobacterium tuberculosis* infection. Pleural fluid samples were also subjected to cytological examination.

The study participants were then divided into three groups, namely, the MPE (with 40 samples), non-malignant in MPE (benign cytological pleural effusion in cancer patient) (n=37) and tuberculosis PE groups (n=33). The level of VEGF measured via ELISA and NLR via flowcytometry were conducted at the Clinical Pathology Laboratory of Dr. Saiful Anwar Malang Regional Hospital.

The collected data were first analyzed to obtain the baseline characteristics of the study population, presented as mean + standard deviation. Statistical analysis included the Kruskal–Wallis test, and differential test between groups with Dunn Test. Data processing and analysis were performed using IBM SPSS software version 24.0, that yielded a confidence level of 95%, significance level of α=0.05, and a statistical power of 80% and had been given ethical approved by Saiful Anwar Hospital

Table 1 Patient Characteristics

Characteristics (n=110)	n	Percentage	Mean ± SD
Sex			
Male	52	47.3%	
Female	58	52.7%	
Age			56,63 ± 13,27
<55 years old	48	43.6%	Median (Min–Max)
>55 years old	62	56.4%	57 (16–87)
Smoking Status			
Active	54	49.1%	
Passive	48	43.6%	
Not smoking	8	7.3%	
Cancer Diagnosed			
Unknown	27	24.5%	
Lung cancer/tumor	54	49.2%	
Others	29	26.3%	
Group			
MPE	40	36.4%	
Non MPE in malignancy	37	33.6%	
Tuberculosis PE	33	30.9%	

Health Research Ethics Committee with approval number 400/260/K.3/302/2023.

Results

Among the 110 study participants, 53 participants were male and 57 were female. Around 59 participants were older than 55 years, while 51 participants were under 55 years. The mean age was 56.63 years; the youngest being 16 and the oldest, 87 years. Smoking status was divided into active smokers, 55 participants, passive-smokers (n=53), and non-smokers (n=2).

Most participants experienced shortness of breath, cough being reported as an initial symptom, along with complaints like chest pain. Regarding cancer history, 27 participants had no history of cancer, while 83 did. Among them, 54 participants had a history of lung cancer or tumors and 29 participants had other types of cancer (Table 1).

Chi-square tests revealed no significant associations between age, sex, employment status, smoking status (including Brinkman Index), and common symptoms (dyspnea, cough, chest pain, weight loss) with the PE groups: malignant, non-malignant, and tuberculosis (all p>0.05). Furthermore, no clear trends were observed across these variables. However, a significant association was found between the history of cancer diagnosed, pleural fluid cytology, and the rapid molecular test results between groups (p=0.000) (Table 2).

Table 2 Demographic and Clinical Characteristics According to Pleural Effusion Group

Parameter	MPE (n=40)		Non-MPE in Malignancy (n=37)		Tuberculosis PE (n=33)		p-value
	n	%	n	%	n	%	
Sex							
Male	17	42.5	21	63.6	14	42.4	0.430
Female	23	57.5	16	46.4	19	57.6	
Age							
<55 years	19	47.5	16	43.3	13	39.4	0.663
>55 years	21	52.5	21	56.7	20	69.6	
Smoking Status							
Active	20	50.0	20	54.0	14	42.4	0.326
Passive	16	40.0	14	37.8	18	54.5	
Non-smoker	4	10.0	3	8.20	1	3.10	
Cancer Diagnosis							
Unknown	0	0.00	0	0.00	27	81.8	0.000
Lung cancer/tumor	30	75.0	24	64.9	0	0.00	
Breast carcinoma	3	7.50	2	5.40	3	9.10	
Ovarium tumor	4	10.0	4	10.8	3	9.10	
Ca cervix	1	2.50	1	2.70	0	0.00	
Prostate tumor	0	0.00	4	10.8	0	0.00	
Oropharyngeal cancer	1	2.50	0	0.00	0	0.00	
Non-Hodgkin lymphoma	1	2.50	0	0.00	0	0.00	
Hip tumor	0	0.00	2	5.40	0	0.00	
Pleural Fluid Cytology							
Class II	0	0.00	23	62.2	33	100	0.000
Class III	0	0.00	14	37.8	0	0.00	
Class IV adeno ca	19	47.5	0	0.00	0	0.00	
Class IV small cell	9	22.5	0	0.00	0	0.00	
Class V adeno ca	12	30.0	0	0.00	0	0.00	
Rapid Molecular Test Result							
Negative	40	100	37	100	0	0	0.000
Positive	0	0.00	0	0	33	100	

MPE = malignant pleural effusion, NHL = neutrophil-to-lymphocyte ratio; PE = pleural effusion; Ca = carcinoma; n = number

Table 3 Difference in the Mean Values of VEGF and NLR levels

Variable	MPE n = 40	Non MPE in Malignancy n = 37	Tuberculosis PE n = 33	p-value
	Mean ± SD	Mean ± SD	Mean ± SD	
VEGF (pg/mL)	6,916.80 ± 4318.38	4,544.49 ± 3,887.73	1,936.61 ± 1,924.40	0.000
NLR	1.17 ± 0.95	0.95 ± 0.80	0.84 ± 0.63	0.374

MPE = malignant pleural effusion; PE = pleural effusion; n = number; VEGF = vascular endothelial growth factor; NLR = neutrophil-to-lymphocyte ratio

The study found that VEGF levels significantly differed between the malignant, non-malignant, and tuberculosis-related PE (p = 0.000) groups. Further analysis showed that VEGF levels were significantly higher in malignant cases compared to both non-malignant and tuberculosis PE (Table 3).

In terms of sensitivity, VEGF outperformed NLR in distinguishing malignant from non-MPE, with a sensitivity of 82.5% (cut-off: 8,321), compared to 57.5% for NLR (cut off: 1,420). Similarly, VEGF also demonstrated higher sensitivity than NLR when differentiating malignant from tuberculous PE (72.73% vs. 54.55%) and non-malignant from tuberculous PE (75.76% vs. 57.58%). Among all comparisons, VEGF was most effective in identifying malignant versus non-malignant effusion. In contrast, NLR was most effective—though still limited—in distinguishing non-malignant from tuberculous effusion (sensitivity 57.58%). Regarding specificity, VEGF again demonstrated superior performance, especially in distinguishing non-malignant from tuberculous effusion, with a specificity of 77.5% (cut-off, 1908.5), versus NLR's 57.5%. In all scenarios, VEGF achieved higher specificity compared to NLR. The highest positive predictive value (PPV) for VEGF was observed in differentiating malignant from non-malignant (73.53%), and non-malignant

Table 4 Summary of Diagnostic Test Results

Diagnostic Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Positive Likelihood Ratio	Accuracy	Odds Ratio
VEGF in MPE and Non MPE in Malignancy (Cut off 8,321)	82.5%	75.7%	73.5%	65.1%	3.37	88.8%	10.76
VEGF in MPE and Tuberculosis PE (Cut off 1,420)	72.7%	74.0%	54.5%	86.5%	2.80	73.6%	7.60
VEGF in Non MPE in Malignancy and Tuberculosis PE (Cut off 1,908.5)	75.8%	77.5%	73.5%	79.5%	2.57	76.7%	5.19
NLR in MPE and Non MPE in Malignancy (Cut off 0.84)	57.5%	54.0%	57.5%	54.0%	1.25	55.8%	1.84
NLR in MPE and Tuberculosis PE (Cut off 0.775)	54.5%	53.2%	33.3%	73.2%	1.17	53.6%	1.37
NLR in Non MPE in Malignancy and Tuberculosis PE (Cut off 0.88)	57.6%	57.5%	52.8%	62.2%	1.35	57.5%	1.59

MPE=malignant pleural effusion; PE=pleural effusion; n=number; VEGF=vascular endothelial growth factor; NLR=neutrophil-to-lymphocyte ratio

from tuberculous PE (73.53%). Meanwhile, NLR had its best PPV (57.5%) in differentiating the malignant vs. non-malignant group (Table 4).

In terms of negative predictive value (NPV), VEGF showed the strongest performance in distinguishing MPE from tuberculous PE, with the highest NPV of 86.36%. NLR demonstrated best NPV (73.21%) in differentiating between malignant and tuberculous PEs. The highest likelihood ratio (LR) was observed for VEGF in distinguishing malignant from tuberculosis PE (LR=3.37). In contrast, NLR's best LR to predict between malignant and tuberculosis PE was 1.35. Accuracy was highest for VEGF in all comparisons compared to NLR. The best performance was seen in differentiating malignant from non-malignant effusion with an accuracy of 88.83%, while NLR was best seen in differentiating non-malignant from tuberculosis PE with an accuracy of 57.53%. VEGF had the highest odds ratio (OR) of 10.76 in predicting malignant vs. non-malignant effusion. NLR's best OR was significantly lower, at 1.84, which is in differentiating between malignant and non-MPE (Table 4).

Discussion

The demographic characteristics of the study population were generally comparable among the MPE, non-malignant pleural effusion in patients with malignancy, and TPE groups. No significant differences were observed in age, sex, or smoking status, suggesting that differences in biomarker levels were unlikely to be attributable to these demographic characteristics alone.

Previous studies have reported variation in the age and sex distribution of patients with PE. El-Sokkary et al. reported a mean age of 53.23±14.98 years, with most participants being men and living in urban areas.[9] In contrast, Anevlavis et al. reported a mean age of 67.3 years among hospitalized patients with MPE, most of whom were women.[10] These findings indicate that PE, particularly MPE, occurs predominantly in older adults. MPE may be more frequent among women because of breast and gynecologic malignancies, whereas malignant mesothelioma related to asbestos exposure is more common among men. Pleural effusion associated with systemic lupus erythematosus is also more common among women.[11] However, Saha et al. reported a predominance of men among patients with MPE.[12]

Nearly half of the participants (49.1%) were active smokers, and most smokers (79.6%) were classified as moderate smokers. No significant association was found between smoking intensity and PE group (p=0.448). Magkouta et al. reported that passive smoking increased the risk of lung cancer by 24% in women and 37% in men.[13]

Most MPE cases were associated with lung adenocarcinoma. Breast and ovarian cancers were also identified, particularly among women. Lung cancer is generally more common among men, although its occurrence among women may be associated with passive smoke exposure and hormonal influences on EGFR and gastrin-releasing peptide receptor pathways.[12] Cancer histology may also influence pleural fluid VEGF levels. Adenocarcinoma and other aggressive malignancies may increase VEGF activity to support tumor survival, invasion, and metastasis. Pleural fluid NLR may be influenced by lymphocyte migration from the circulation into the pleural cavity in response to increased vascular permeability. These processes are related to inflammation, tumor growth, and vascular permeability and contribute to pleural fluid accumulation in malignant disease.[5]

VEGF levels were significantly higher in the MPE group (6,916.80 pg/mL) than in the non-MPE group with malignancy (4,544.49 pg/mL) and the TPE group (1,936.61 pg/mL) (p<0.001). This finding suggests that pleural fluid VEGF may help differentiate malignant from non-malignant and tuberculous effusions. Psatha et al. similarly reported that elevated VEGF levels were associated with malignant processes and could support the diagnostic evaluation of PE.[14]

Khalil et al. also reported that VEGF had diagnostic value in differentiating malignant, non-malignant, and tuberculous pleural effusions.[15] VEGF promotes angiogenesis and increases vascular permeability. In MPE, tumor cells may upregulate VEGF to support invasion and metastasis. The resulting neovascularization is structurally fragile and highly permeable, facilitating pleural fluid accumulation. Thus, elevated pleural fluid VEGF may reflect tumor activity, angiogenesis, and metastatic involvement of the pleura.[14,15]

Although VEGF levels were lower in non-malignant and tuberculous effusions than in MPE, VEGF may still contribute to inflammatory responses by increasing vascular permeability, recruiting immune cells, activating endothelial cells, and interacting with cytokine pathways involved in the resolution of inflammation.[14,15] Therefore, VEGF participates in angiogenesis,

vascular permeability, and inflammatory regulation in both malignant and non-malignant conditions.

NLR did not differ significantly among the MPE (1.17), non-MPE with malignancy (0.95), and TPE (0.84) groups (p=0.374), indicating limited diagnostic utility in this population. This finding differs from that of Ewida et al., who reported that pleural fluid NLR could help identify malignant effusions.[16] However, it is consistent with the findings of Akturk et al., who reported limited discriminatory value of NLR among malignant, non-malignant, and tuberculous pleural effusions.[17]

Barata et al. also reported that NLR had limited diagnostic value for differentiating malignant, non-malignant, and tuberculous PE.[18] In the pleural tumor microenvironment, cytokines and immune mediators such as transforming growth factor-β, interleukin-10, and prostaglandin E2 may suppress lymphocyte activity. Tumors may also promote the expansion of regulatory T cells and myeloid-derived suppressor cells, further impairing adaptive immune responses. These mechanisms can alter pleural fluid leukocyte proportions and may reduce the diagnostic reliability of NLR.[19,20]

The diagnostic analyses further supported the superiority of VEGF over NLR. For differentiating MPE from non-MPE in patients with malignancy, VEGF showed a sensitivity of 82.5%, specificity of 75.7%, and an odds ratio of 10.76. It also demonstrated moderate diagnostic performance for differentiating MPE from TPE and non-MPE in patients with malignancy from TPE. In contrast, NLR consistently showed lower sensitivity, specificity, likelihood ratios, and odds ratios. These findings suggest that pleural fluid VEGF may serve as a useful adjunct to established diagnostic methods. However, it should not be considered a replacement for pleural fluid cytology or pleural biopsy without further prospective validation.

This study has several limitations. Its cross-sectional design prevented assessment of temporal changes in biomarker levels during the disease course. Potential confounding factors, including comorbidities, were not comprehensively evaluated. In addition, the single-center setting and purposive sampling may limit the generalizability of the findings.

In conclusion, pleural fluid VEGF levels were elevated in all three groups but were significantly higher in MPE than in non-MPE associated with malignancy and TPE. VEGF showed better diagnostic performance than NLR for differentiating these conditions and may be useful as an adjunctive biomarker. Pleural fluid NLR did not differ significantly among the groups and did not demonstrate adequate diagnostic value.

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