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High Levels of Interleukin-6, Vascular Endothelial Growth Factor, And High Expression of B-Cell Lymphoma 2 as Risk Factors for Bone Metastasis in Lung Cancer Patients

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ABSTRACT

Introduction: Bone metastasis is malignancy originates from other cancers and accounts for an estimated 70% of bone malignancy cases. Previous studies have found that the incidence of bone metastasis cases in lung cancer patients is approximately 16.89% of the total patients. In Indonesia, lung cancer ranks among the top three cancers with a 5-year prevalence rate reaching 13.77 per 100,000 population in 2018. Interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), and B-cell lymphoma 2 (Bcl-2) have been extensively studied and potential as predictors of bone metastasis occurrence in lung cancer patients. Researcher aimed to determine whether high levels of IL-6, VEGF, and Bcl-2 expression are risk factors for bone metastasis in lung cancer patients.

Methods: This study involved 40 lung cancer patients at Prof. Dr. I.G.N.G. Ngoerah General Government Hospital. It included 20 patients lung cancer with metastatic bone disease as the case group and 20 patients lung cancer without metastatic bone disease as the control group. Data on IL-6, VEGF, and Bcl-2 expression levels were collected from patients in both groups and statistically analyzed to determine whether these parameters are risk factors for bone metastasis in lung cancer patients.

Results: In bivariate analysis, this study shows that high levels of IL-6, high levels of VEGF, and high expression of Bcl-2 are statistically significant risk factors for bone metastasis in lung cancer patients (OR_{IL-6}=107,67(10,21-1135,59); OR_{VEGF}= 76,0(7,69-750,49); OR_{Bcl-2}=22,67(4,37-117,47), p=<0.001). Multivariate analysis showed that IL-6 levels and Bcl-2 expression were the most influential risk factors for bone metastases in lung cancer patients (AOR_{IL-6}=6,78, p=0,038; AOR_{Bcl-2}=13,78, p=0,003).

Discussion: This study proves that high levels of IL-6, high levels of VEGF, and high expression of Bcl-2 are risk factors for bone metastasis in lung cancer patients. This study showed minimal confounding factors as there were no differences in characteristics between the two groups. However, if all three

factors are present in a patient at the same time, the most influential factors are the levels of IL-6 and Bcl-2.
Conclusion: High levels of IL-6, high levels of VEGF, and high expression of Bcl-2 are risk factors for bone metastasis in lung cancer patients.
Level of Evidence: III B

Keywords: IL-6 levels, VEGF levels, Bcl-2 expression, bone metastasis, lung cancer

INTRODUCTION

Bone metastases are malignancies in the bones that originate from other cancers. It is estimated that 70% of bone malignancy cases originate from metastases. The location of metastases that is often found is in the joints rather than the cortical bone. The femur bone is often the location of cortical bone metastases and generally originates from bronchogenic carcinoma.(1)

Bone is the most common site of metastasis for most malignancies. Deaths due to bone metastases in the United States reach 350,000 cases per year, with the number of cases diagnosed varying between 20,000-400,000 cases per year.(2) Zhou Yang*et al* in 2017, his study reported that 23.9% of 2021 patients with lung cancer had metastases from bone cancer. The most common types of lung cancer that cause metastases are adenocarcinoma and carcinomas*mall cell*.(3)

Lung cancer is also a disease that occurs quite often in the Asian region. There were 1,033,881 cases (71.13% men and 28.87% women) and 936,051 deaths from this incident (71.45% men and 28.55% women) in 2012. The five countries with the most cases were: China (652,842 cases), Japan (94,855 cases), India (70,275 cases), Indonesia (34,694 cases), and Turkey (24,489 cases).(4,5) In Indonesia itself, lung cancer is in the top three cancers with a 5-year prevalence reaching 13.77 per 100,000 population in 2018. The incidence of new cases of lung cancer in 2020 reached 8.8% across all ages and genders.(3)

Tumor marker is a substance that can be used to detect changes that occur due to cancer. Over expression of Bcl-2 increases the tumorigenicity and metastatic potential of some tumors which may stimulate a switch to an angiogenic phenotype in response to low oxygen conditions. Expression of *vascular endothelial growth factor* (VEGF) can be triggered by hypoxic conditions and oncogene signaling. In addition, VEGF ligands in the extracellular matrix can also be activated by*matrix degrading protease* (MMP-9) (D'Oronzo, 2017). Interleukin-6 (IL-6) is a pleiotrophic cytokine that is usually expressed in response to injury, inflammation, and infection. Initially IL-6 was cloned as B *cell stimulatory factor* and is designated by β2. IL-6 is produced by osteoblasts, monocytes, macrophages, and BMMCs. IL-6 under physiological conditions is very low or may not be detected (Ara T., et al., 2010). IL-6 was the only inflammatory cytokine that was independently associated with CRP concentrations in patients with *non-small cell lung cancer* (NSCLC) further, showing that CRP is a useful surrogate marker of IL-6 activity in patients with NSCLC (Iuliani, et al., 2020). These three markers can be tumor markers in the incidence of bone metastases in lung cancer.(4)

Therefore, the authors intend to find out whether levels of IL-6, VEGF, and Bcl-2 are risk factors for bone metastasis in patients with lung cancer. In the field of orthopedics, duration and risk factors can help to determine the possibility of bone metastases so that they can predict short and long term mortality due to these metastases. It is hoped that this can provide appropriate management decisions and provide a prognostic picture for patients with lung cancer.

METHODS

Study design

This research is a *case control*. The case control design is an observational research design by grouping or classifying groups with outcomes (case group) with groups

without outcomes (control group), and then observed to see if there are differences in risk factors, which are summarized mathematically in odds ratios. This research was conducted to determine the relationship between IL-6, VEGF, and Bcl-2 with the incidence of bone metastases in lung cancer patients. In this research design, two groups were used, namely the group diagnosed with lung cancer without metastases and the lung cancer group with bone metastases.

Data Collection

The research was conducted at RSUP Prof. Ngoerah carried out in November 2023 – February 2024. Data was obtained through the Orthopedic and Pulmonary Polyclinic at RSUP Prof. Dr. Dr. I.G.N.G. Research and laboratory data on blood and tumor tissue samples were obtained through the Laboratory of the Udayana Medical Faculty and the Anatomical Pathology Laboratory of Prof. RSUP. Dr. Dr. I. G. N. G. Ngoerah.The research sample was all patients suffering from lung cancer with metastatic bone disease or withoutmetastatic bone disease at RSUP Prof. Ngoerah, who met the inclusion criteria and was willing to participate in the research by signing the sheetinformed consent in November 2023 – February 2024.

STASTISTICAL ANALYSIS

After the data has been collected completely and a re-evaluation is carried out, an analysis of the collected data is carried out. First, a descriptive statistical analysis is presented in a cross distribution table, so that the comparability of subject analysis between groups can be assessed. Second, a proportion comparison analysis by making a 2x2 cross tabulation and calculating the size of association in the form *Odds Ratio (OR).* For unpaired categorical comparative data with 2x2 tabulation, if the x2 condition is met, then the test is used *Chi square* with Yates correction. If the condition x2 is not met, then the test is used *Fisher*. Third, Multiple Logistic Regression Test by including all independent variable indicators and the magnitude of their influence on the dependent variable. The size of the association obtained is *Adjust Odds Ratio.* The level of significance (α) of this research was set at a probability value (p) of less than 0.05. All statistical analyzes were performed using the SPSS software program *for Windows* (Version 24; IBM Corp, Armonk, NY, USA).

RESULTS

Research Subject Characteristics

Patient demographic characteristics are listed in Table 1. The number of lung cancers in this study was 40 (100%), with 20 bone metastases and 20 people without bone metastases. The average age of the patients in this research sample was 66.18 ± 8.98 years with the largest age group in the 61–70-year age category with a total of 22 people (55%). Gender has an equal distribution of men and women with 20 people each. Research subjects had a family history of lung cancer (70%). Research Subject Characteristics showed in table 1.

Table 1. Basic Characteristics of the Sample

Variable	Total (N=70)
Age (Mean±)	66.18 ± 8.98
Age: n (%)	
50-60 year	7 (17.5)
61-70 year	22 (55.0)
71-80 year	7 (17.5)
>80 year	4 (10.0)
Sex: n (%)	
Male	20 (50.0)
Female	20 (50.0)
Family history: n (%)	
Yes	28 (70.0)
No	12 (30.0)

Level of IL-6	
High (>6pg/ml)	22 (55.0)
Low (<6pg/ml)	18 (45.0)
Expression of Bcl-2	
High (immunoreactive >10%)	19 (47.5)
Normal (imminoreactive ≤10%)	21 (52.5)
Level of VEGF	
High (>62-601 pg/mL)	17 (42.5)
Normal (62-601 pg/mL)	23 (57.5)

Result Analysis

The results of the analysis of subject characteristics showed that the numerical variables age, categorical age, gender, and family history did not have a statistically significant difference in proportion between the groups without bone metastases and those with bone metastases in lung cancer patients. Data is presented in table 5 below. Thus, these variables are not confounding variables.

In the bivariate test, it was found that the variables that met the requirements for multivariate logistic regression analysis were the IL-6 category variable, the VEGF category variable, and the Bcl-2 expression variable ($p<0.25$). The results of this multivariate analysis can be seen in table 6. Test results Hosmer and Lemes how test shows that the equation above has good calibration ($x2= 3.907$, $df=2$, $p=0.142$). Then, the analysis continued for the AUC predictive model on the ROC curve showing strong accuracy with a sensitivity of 95% and a specificity of 80% (AUC=0.885, 95% CI: 0.770 – 1.000). The results are presented in Table 7 and Figure 1.

Table 2. Characteristics of Case and Control Research Subjects

Variable	**Group (N=40)**		***p***
	With Metastase n=20	**Without Metastases N=20**	
Age (mean±SD)	65,45 ± 9,15	66,90 ± 8,98	0,616*
Age: n (%)			0,928**
50-60 Year	4 (20,0)	3 (15,0)	
61-70 Year	10 (50,0)	12 (60,0)	
71-80 Year	4 (20,0)	3 (15.0)	
>80 Year	2 (10,0)	2 (10,0)	
Sex: n (%)			0,527***
Male	9 (45,0)	11 (55,0)	
Female	11 (55,0)	9 (45,0)	
Familiy history: n (%)			0,168***
Yes	12 (60,0)	16 (80,0)	
No	8 (40,0)	4 (20,0)	

Table 3. Chi-Square test results for IL-6 in groups of lung cancer patients with bone metastases and those without bone metastases

Variable	**Cases Group (With Bone Metastases) (n=20)**	**Control Group (Without bone metastases) (n=20)**	**p**	**OR (95% CI)**
IL-6: n (%)			<0,001	107,67 (10,21- 1135,59)
High (>6pg/ml)	19(95,0)	3(15,0)		
Normal (<6pg/ml)	1(5,0)	17(85,0)		

Table 4. Chi-Square test results for Bcl-2 expression in groups of lung cancer patients with bone metastases and those without bone metastases

Variable	Case Group (With Bone Metastases) (n=20)	Control Group (Without bone Metastases) (n=20)	*p*	OR (95% CI)
Bcl-2: n (%)			<0,001	22,67 (4,37- 117,47)
High (immunoreactive >10%)	16 (80,0)	3(15,0)		
Normal (immunoreactive ≤10%)	4 (20,0)	17 (85,0)		

Table 5. Chi-Square test results for VEGF in groups of lung cancer patients with bone metastases and those without bone metastases

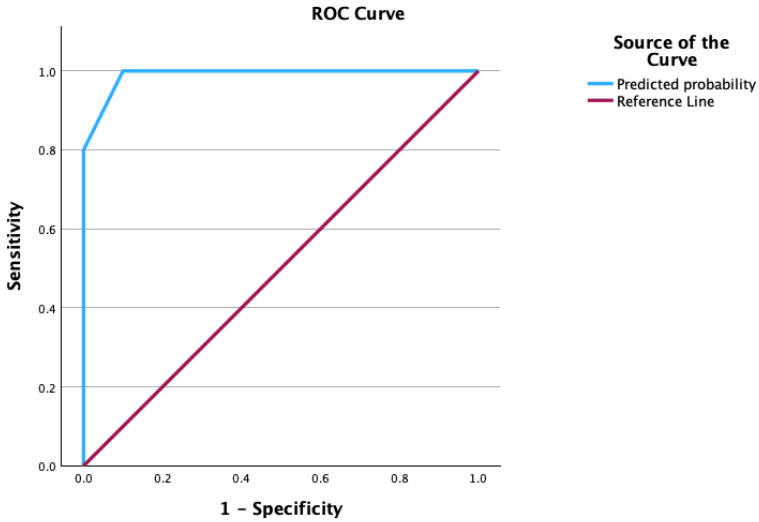
Variable	Case Group (With Bone Metastases) (n=20)	Control Group (Without Bone Metastases) (n=20)	*p*	OR (95% CI)
VEGF: n (%)			<0,001	76,00 (7,69 - 750,49)
High (>62-601 pg/mL)	16(80,0)	1(5.0)		
Normal (62-601 pg/mL)	4(20,0)	19(95,0)		

Table 6. Logistic Regression Tests on risk factors for bone metastases in lung cancer

Predictors	B Coefficient	SE	Wald Statistics	p	Exp(B)	Odds Ratio (95%CI)
Step 1						
Constant	-2,77	1,19	5,45	0,020	0,063	
Level of IL-6(1)	1,83	0,92	3,95	0,047	6,241	1,03-37,94
Level of VEGF (1)	1,12	1,15	0,95	0,330	3,07	0,32-29,39
Expression of Bcl-2 (1)	2,48	0,91	7,47	0,006	11,963	2,02-70,96
Step 2						
Constant	-1,97	0,68	8,45	0,004	0,138	
Level of IL-6(1)	1,91	0,92	4,29	0,038	6,78	1,11-41,38
Expression of Bcl-2 (1)	2,62	0,90	8,53	0,003	13,78	2,37-80,11

Table 7. Area Test Results Under the ROC Curve Multivariate Logistic Regression Analysis Results

Area	Std. Error	*p*	95%CI
0,885	0,059	<0,001	0,77 – 1,00



DISCUSSION

The investigation into potential risk factors for bone metastasis in lung cancer patients yielded insightful findings, shedding light on demographic and familial correlations. Analysis of age distribution revealed a

consistent trend among studies, with the majority indicating an older age bracket, typically over 65 years, as being more susceptible to bone metastases. While our study echoes these findings, with an average age of 66.17±8.98 years, the lack of statistical significance underscores the need for further exploration. Gender distribution displayed parity, contrasting with previous research that often noted a male preponderance.(6) Notably, our findings align with recent studies, emphasizing gender as an insignificant factor in bone metastasis occurrence. Intriguingly, familial history emerged as a notable consideration, with 60% of cases exhibiting such a connection. However, disparities with analogous investigations in breast cancer patients underscore the complexity of familial predispositions across different malignancies. This discussion underscores the multifactorial nature of bone metastasis in lung cancer and underscores the need for comprehensive risk assessment frameworks integrating demographic, familial, and molecular factors.(7)

The investigation into interleukin-6 (IL-6) levels as a potential risk factor for bone metastasis in lung cancer patients presents intriguing insights into the intricate interplay of cytokine signaling in cancer progression. Consistent with previous research, our findings indicate a significant association between elevated IL-6 levels and the incidence of bone metastases in lung cancer. Notably, studies by Liu et al. provide compelling evidence linking heightened IL-6 levels to increased metastatic potential and disease progression in lung cancer patients. Additionally, logistic regression analysis underscores the prognostic utility of IL-6 in predicting disease advancement, further corroborating its role as a key biomarker in lung cancer management. Moreover, the translational relevance of these findings necessitates further clinical validation, particularly regarding the direct causative role of IL-6 in metastatic dissemination, as highlighted by Liu et al. This discussion underscores the pivotal role of IL-6 in the pathogenesis of bone metastasis in lung cancer, warranting continued exploration to elucidate its therapeutic implications and potential as a prognostic marker.(8)

In this study, it was found that high levels of VEGF are a risk factor for bone metastases in patients with lung cancer. Study by Chen et al. found that the levels of VEGF, MVD, MMP-2/9 in serum and lung cancer bone metastasis group were significantly higher than those in the primary bone tumor group and benign bone tumor group ($P < 0.05$). COX-2 and VEGF levels in the serum of both groups were significantly lower compared to before treatment. Until now there have been no studies showing that VEGF is not related to the incidence of bone metastases. This is because tumor invasion and metastasis requires sufficient nutrients that are circulated through the blood vessels.(9)

The exploration of B-cell lymphoma 2 (Bcl-2) expression as a potential risk factor for bone metastasis in lung cancer patients reveals significant associations with disease progression and metastatic propensity. Our study corroborates previous research findings suggesting that elevated Bcl-2 expression, particularly when exceeding a threshold of 10%, is indicative of heightened metastatic potential in lung cancer. Notably, Bcl-2 has been implicated in various malignancies, including hematologic and solid tumors, where its over expression is often linked to advanced disease states and resistance to conventional therapies. Additionally, insights from Alam et al. highlight Bcl-2's involvement in crucial cellular processes like cell survival and mitochondrial dynamics regulation, further emphasizing its significance in cancer progression. However, the context-dependent nature of Bcl-2 expression, as elucidated by Fu et al., underscores the complexity of its role across diverse patient populations. Furthermore, experimental evidence from elucidates the direct involvement of Bcl-2 in mediating metastasis, particularly to bone, underscoring its clinical relevance in understanding metastatic dissemination

patterns in cancer. Collectively, these findings underscore the pivotal role of Bcl-2 in the metastatic cascade of lung cancer, warranting further investigation into its therapeutic targeting and prognostic implications in bone metastasis management.(10)

The examination of biomarker parameters in this study elucidated the pivotal role of B-cell lymphoma 2 (Bcl-2) expression as the most significant contributor to bone metastasis risk in lung cancer patients, underscored by its notably low p-value of 0.003. This finding aligns with existing research, such as that of Tawara et al. and Razkova et al., which emphasize the critical involvement of the IL-6 signaling pathway in cancer progression, particularly in metastasis formation. Additionally, insights from Nature et al. highlight the relevance of the EGFR pathway in various cancers, including non-small cell lung cancer (NSCLC), shedding light on potential therapeutic strategies targeting the EGFR-mediated Bax/Bcl-2 cascade pathway to mitigate bone metastasis risk. Conversely, while vascular endothelial growth factor (VEGF) did not exhibit a significant effect in this study, its relevance in bone metastasis development is underscored by research such as that of Chen et al., indicating elevated VEGF levels at sites of bone metastases compared to primary tumors. These findings collectively underscore the multifaceted interplay of molecular pathways in the pathogenesis of bone metastasis in lung cancer, offering valuable insights for the development of targeted therapeutic interventions aimed at mitigating metastatic spread and improving patient outcomes.(9)

CONCLUSSION

The general conclusion from this research is that High IL-6 levels are a risk factor for bone metastases 07.67 times in patients with lung cancer.High levels of VEGF are a risk factor for bone metastases 76 times in patients with lung cancer.High Bcl-2 expression is a risk factor for bone metastasis 22.67 times in patients with lung cancer.The specific conclusion obtained from this study is that IL-6 levels and Bcl-2 expression are the biomarker parameters that have the most influence on the risk factors for bone metastasis in patients with lung cancer.

Declaration Of Interest Statement

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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