INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 172
2024 March; 30 (2): 172-176
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

Analysis of BloPrognostic Analysis of NLR, PLR, and, LMR in Osteosarcoma at Dr. Wahidin Sudirohusodo Hospital

Febriani Helda Pongbala, Uleng Bahrun, Mansyur Arif

Department of Clinical Pathology, Faculty of Medicine, Hasanuddin University/Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia.
E-mail: drheldapongbala@gmail.com

ABSTRACT

Osteosarcoma is a primary pediatric bone malignancy with an annual incidence of 5.6 cases per million children under the age of 15. The high mortality rate leads to necessary for prognostic biomarkers of the disease. NLR, PLR, and LMR can be considered as prognostic predictors of osteosarcoma patients. This study aimed to determine the difference and correlation between NLR, PLR, and LMR based on grade and outcome in osteosarcoma patients. The study used medical record data from 122 osteosarcoma patients at Dr. Wahidin Sudirohusodo Hospital, Makassar. Samples were grouped by stage according to Enneking criteria (grade I, II, and III) and by outcome (deceased and not deceased), then analyzed based on NLR, PLR, and LMR values using the Kruskal-Wallis test and the Mann-Whitney test (significant if $p<0.05$). There was a significant difference in NLR, PLR, and LMR values by grade, ($p=0.05$). There was a significant difference in the value of NLR, and LMR based on output ($p=0.00$), but not in PLR ($p=0.954$). There was a correlation between the values of NLR, PLR, and LMR and the stage of osteosarcoma ($p=0.05$). Based on the outcome, a correlation with the NLR and LMR values was obtained ($p=0.00$), but there was no correlation with the PLR value ($p=0.955$). Cut-off NLR, PLR, and LMR were 4.43; 0.21; and 0.44, respectively, with sensitivity of 76%, 56%, and 76% and a specificity of 76%, 63.9%, and 68%, respectively. There were differences in NLR, PLR, and LMR values based on the stage and outcome of osteosarcoma. Higher NLR, PLR, and LMR values will lead to a higher stage of osteosarcoma and a worse outcome. Cut-off NLR, PLR, and LMR optimal for distinguishing stage of osteosarcoma were 4.43; 0.21, and 0.44, respectively.

Keywords: NLR, PLR, LMR, osteosarcoma

INTRODUCTION

Osteosarcoma is the most common primary pediatric bone malignancy originating from primitive bone-forming mesenchymal cells and is divided into primary and secondary accounting for approximately 20% of all bone tumors. The manifestations of osteosarcoma are very heterogeneous, which allows categorization into several subtypes according to the degree of differentiation, location within the bone, and histological variations. These subtypes vary in radiological imaging, demographics, and biological behavior. Osteosarcoma is a rare sarcoma, which has histological findings of osteoid production associated with malignant mesenchymal cells.[1]

The annual incidence of osteosarcoma is 5.6 cases per million children under 15 years old, with the highest incidence reported in the second decade of life. Before the age of five years, osteosarcoma is rare.[2] The most common areas of osteosarcoma are the femur (42%), tibia (19%), and humerus (10%), while the rare locations are the skull or jaw (8%) and hip (8%).[3] The 5-year survival rate using a multidisciplinary approach is 60-70%; most patients still face poor outcomes due to the development of drug resistance, tumor metastases, and recurrence.[4] Therefore, prognostic factors are very important to predict high-risk patients and initiate treatment to increase the survival rate of osteosarcoma patients. Among prognostic factors, tumor size, metastatic disease at diagnosis, histological grade, histological response to neoadjuvant chemotherapy, and adequate surgical margins have consistently shown a strong correlation with survival in osteosarcoma patients.[5]

The study used serum biomarkers as a prognostic factor in 57 osteosarcoma patients who had received treatment at Dr. Cipto Mangunkusumo Hospital (RSCM), Jakarta showed a high mortality rate. Of the various prognostic factors used, the Neutrophil-Lymphocyte Ratio (NLR), Platelet-Lymphocyte Ratio (PLR), and Lymphocyte-Monocyte Ratio (LMR) are considered good prognostic factors.[5] A study by Vasquez *et al.* confirmed that NLR is an independent prognostic factor for pediatric sarcoma, which plays an important role in

the immune system. The clinical benefit of this prognostic biomarker should be validated in larger studies.[6] Another study by Xia showed that advanced stage and metastasis were significantly associated with high NLR and PLR. This study concluded that NLR and PLR are good prognostic factors in predicting overall survival in patients with osteosarcoma.[7] However, a study by Liu *et al.* in 327 patients undergoing surgical treatment for osteosarcoma between 2006 and 2010 showed that lymphocyte low monocyte ratio is associated with shorter survival.[8]

The diagnosis of osteosarcoma can be established by history, physical and radiological imaging, and biopsy (definite diagnosis). However, not all health facilities can perform a biopsy, indicating a need for easier and faster tests, including NLR, PLR, and LMR. Based on this background, the authors were interested in determining the role of NLR, PLR, and LMR in osteosarcoma patients.

METHODS

This research was a retrospective study with a cross-sectional method using medical record data of osteosarcoma patients at Dr. Wahidin Sudirohusodo Hospital from January 2017 to December 2021. The research subjects were all osteosarcoma patients diagnosed by clinicians based on histopathological results and staging based on Enneking criteria. The inclusion criteria were the patients who were not pregnant had NLR, PLR, and LMR test results, and had not received chemotherapy.

The variables used in this study were age, gender, outcome (died and survived), stage (grade I, II, and III) as well as NLR, PLR, and LMR values, which were calculated using absolute cell count. The Kruskal-Wallis test, Mann-Whitney test, and SPSS version 25 were used for statistical analysis ($p<0.05$ was significant). Approval for the ethical feasibility of this study was obtained from the Health Research Ethics Commission (KEPK) Faculty of Medicine of Hasanuddin University Hospital (RSUH) /Dr. Wahidin Sudirohusodo Hospital, Makassar with number of LB.02.04/2.2/11368/2022.

RESULTS AND DISCUSSIONS

The research was conducted at the Medical Record Installation of Dr. Wahidin Sudirohusodo Hospital using data from 122 osteosarcoma patients.

Table 1 shows that most subjects were males (76.4%), the most age group was <25 years (80.33%), the most common grade of osteosarcoma was grade II (45.9%), and most subjects survived 79.5%.

The Kolmogorov-Smirnov normality test showed that the data of lymphocytes, neutrophils, monocytes, and platelets were not normally distributed ($p<0.05$) (Table 2).

The Kruskal-Wallis test showed that the median of NLR, PLR, and LMR was higher in grade III compared to grade II and I ($p<0.05$) and there were significant differences in the median of NLR, PLR, and LMR among the three grades (Table 3).

The Spearman test showed that there was a significant correlation between NLR, PLR, and LMR with the grade of osteosarcoma ($p<0.05$), with strong (r=0.8), weak (r=0.45) and moderate (- 0.545) correlation, respectively

Table 1. Characteristics of subjects

Criteria	Total (n=122)	Percentage (%)
Gender		
Male	76	76.4
Female	46	38.8
Age (years)		
<25 years	98	80.33
>25 years	24	19.67
Grade of osteosarcoma		
Grade I	15	12.3
Grade II	56	45.9
Grade III	51	41.8
Outcome		
Survived	97	79.5
Death	25	20.5

Table 2. Laboratory test results

Variable	Minimum	Maximum	Median	SD	p*
Cell count (x10^3/µL)					
Lymphocyte	2.60	62.30	321.09	11.15	0.000
Neutrophil	6.60	94.50	68.31	14.19	0.005
Monocyte	0.80	33.30	7.49	4.10	0.000
Platelet	15	819	377.98	164.32	0.170
NLR	0.11	35.6	3.74	5.20	0.000
PLR	0.004	1.19	0.17	0.16	0.000
LMR	0.06	3.08	0.39	0.39	0.000

*Kolmogorov-Smirnov test

Table 3. Difference in NLR, PLR, and LMR according to grade of osteosarcoma

Variable	Grade of Osteosarcoma						p*
	Grade I		Grade II		Grade III		
	Median	SD	Median	SD	Median	SD	
NLR	1.23	0.42	2.89	1.12	5.87	6.40	0.000
PLR	0.12	0.05	0.15	0.08	0.25	0.20	0.000
LMR	5.36	2.29	3.16	1.86	1.92	2.25	0.000

*Kruskal-Wallis test

Table 4. Difference in NLR, PLR, and LMR according to clinical outcome

Variable	Outcome				p*
	Survival		Death		
	Median	SD	Median	SD	
NLR	3.42	3.81	5.88	7.71	0.000
PLR	0.17	0.14	0.23	0.21	0.954
LMR	3.02	2.39	1.58	1.20	0.000

*Mann-Whitney test

The Mann-Whitney test showed that there was a significant difference between the NLR, LMR, and the outcome ($p<0.005$), while there was no significant difference in PLR ($p>0.005$) (Table 4).

The Spearman test showed a significant correlation between NLR, LMR, and the clinical outcome ($p<0.05$), all with weak correlation strength, whereas there was no correlation between PLR and the outcome ($p>0.05$).

Based on the results of the ROC curve for analyzing NLR, PLR, and LMR values as markers to predict outcomes, the optimal cut-off value of NLR was 4.43 with a sensitivity of 76% and a specificity of 76%, optimal cut-off PLR was 0.21 with a sensitivity of 56% and a specificity of 63.9%, and an optimal cut-off LMR was 0.44 with a sensitivity of 76% and a specificity of 68% (Figure 1).

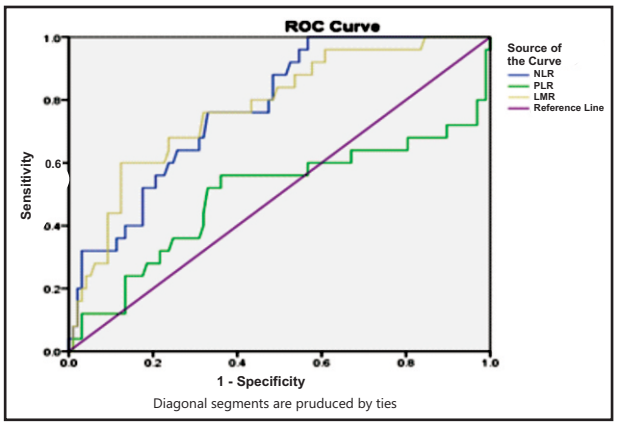

Figure 1. The AUC for NLR, PLR, and LMR in osteosarcoma patients

Based on the results of this study, it was known that the incidence of osteosarcoma in males was higher than in females (76.4% vs. 38.8%). The results of this study were in accordance with the 2018 Oncology Services Osteosarcoma Handbook, which states that conventional osteosarcoma is more common in males than in females with a ratio of 3:2.[9] This can be due to the longer bone growth period in males compared to females. This tumor most often affects children in the second decade of life; more than 60% of patients are less than 25 years of age. The incidence of osteosarcoma can increase again at the age of 60 years, indicating a bimodal distribution.[9] Ottavani and Jaffe stated that osteosarcoma has a bimodal age distribution, with the first peak occurring during adolescence and the second peak occurring in older age. The first peak occurs in the age group of 10-14 years, coinciding with the pubertal growth spurt. This condition suggests a close relationship between adolescent growth spurt and osteosarcoma. The second peak of osteosarcoma occurs in adults older than 65 years. This condition is associated with secondary malignancy, which is associated with Paget's disease.[10]

The highest NLR and PLR values in this study were found in grade III osteosarcoma, while the highest LMR value was found in grade I osteosarcoma (Table 3). The results of this study were in line with a study by Song *et al.*, which suggested the role of NLR as an inflammatory marker related to the progression of osteosarcoma tumors despite unclear pathomechanism.[5,11] In contrast, the highest LMR

value was found in grade I osteosarcoma. This was in line with a study by Nakano *et al*., which found a relationship between the degree of malignancy and LMR in carcinoma. This can be attributed to the increase in immune cells along with the increasing degree of malignancy, presumably due to an increase in tumor cell antigens.[12,13]

Based on the outcome, the results of this study showed that the NLR, PLR, and LMR values had significant differences with p <0.05. The NLR and PLR values in the group who died were higher than those who survived (NLR=5.88 vs. 3.42; PLR=0.23 vs. 0.17). This was in line with a study by Yapar *et al*., which showed a significant difference in the median NLR and PLR values (p <0.05). This study shows that inflammatory markers can change around the tumor microenvironment, indicating its potential to be an effective prognostic factor for tumors.[13] Higher NLR and PLR values will lead to a worse prognostic. In contrast, the results of this study showed that the LMR was higher in the non-deceased group (3.02) compared to the deceased group (1.58). A study by Gajewski *et al*. suggested that in various malignancies, specific and nonspecific immune cell infiltration has a role in the tumor microenvironment through communication between NK cells, antigen-presenting cells (macrophages), and lymphocytes in controlling tumor growth. Abnormal immune function contributes to increased tumor growth by creating a conductive environment for tumor growth through local inflammatory processes.[14,15] The results of this study indicated that LMR cannot be used as a prognostic marker in osteosarcoma patients.

Based on the ROC curve, the cut-offs of NLR, PLR, and LMR as predictors of osteosarcoma progression were 4.43, 0.21, and 0.44, respectively, with a sensitivity of 76%, 56%, 76%, and a specificity of 76%, 63.9%, 68%, respectively.

The limitation of this study was the use of secondary data with a retrospective design at a single center, leading to limited information.

CONCLUSIONS AND SUGGESTIONS

It was concluded that there were significant differences between NLR, PLR, and LMR values based on the stage and outcome of osteosarcoma. Higher NLR, PLR, and LMR values will lead to a higher grade of osteosarcoma; higher NLR value will lead to a worse PLR outcome. A high LMR indicates a better prognosis. Optimal cut-offs of NLR, PLR, and MLR to differentiate the stage of osteosarcoma were 4.43, 0.21, and 0.44 with a sensitivity of 76%, 56%, 76% and a specificity of 76%, 63.9%, 68%, respectively.

It was recommended to use NLR, PLR, and LMR as prognostic markers, and NLR and PLR as markers to predict outcome in osteosarcoma patients. A large-scale and prospective multi-center study was needed to confirm the results of studies regarding predictors of prognosis in osteosarcoma patients.

REFERENCES

1. Lindsey BA, Markel JE, Kleinerman ES. Osteosarcoma overview. Rheumatol Ther, 2017; 4(1): 25-43.
2. Misaghi A, Goldin A, Awad M, Kulidjian AA. Osteosarcoma: A comprehensive review. SICOT J, 2018; 4: 12.
3. Jiang ZY, Liu JB, Wang XF, Ma YS, Fu D. Current status and prospects of clinical treatment of osteosarcoma. SAGE, 2022; 21: 1-12. Available from: https://10.1177/15330338221124696 (accessed May 3, 2022).
4. Faisham WI, Saad AZM, Alsaigh LN, Azman MZN, Imran MK, *et al*. Prognostic factors and survival rate of osteosarcoma: A single-institution study. Asia. Pac. J. Clin. Oncol, 2017; 13(2): e104-e110.
5. Xin S, Wei G. Prognostic factors in osteosarcoma: A study level meta-analysis and systematic review of current practice. J. Bone Oncol, 2020; 21: 100281.
6. Kamal A, Prasetyo R. Association between laboratory markers and oncological outcomes in patients with osteosarcoma-A review of osteosarcoma treatment in Indonesia. Formos. J. Surg, 2018; 51(3): 111-117.
7. Xia WK, Liu ZL, Shen D, Lin QF, Su J, Mao WD. Prognostic performance of pre-treatment NLR and PLR in patients suffering from osteosarcoma. World J. Surg. Oncol, 2016; 14(1): 1-8.
8. Liu T, Fang XC, Ding Z, Sun ZG, Sun LM, Wang YL. Pre-operative lymphocyte-to-monocyte ratio as a predictor of overall survival in patients suffering from osteosarcoma. FEBS Open Bio, 2015; 5: 682-687.
9. Mehlman CT, Cripe TP, Talavera F, Binitie O. Pedoman osteosarkoma. Medscape, 2022; 1-4. Available from: https:// emedicine.medscape.com/ article/ 1256857-guideline#g3 (accessed May 3, 2022).
10. Kim C, Davis LE, Albert CM, Samuels B, Roberts JL, Wagner MJ. Osteosarcoma in pediatric and adult populations: Are adults just big kids?. MDPI, 2023; 15(20): 5044. Available from: http://10.3390/cancers 15205044 (accessed May 3, 2023).
11. Song X, Zhang H, Yin F, Guo P, Yang X, *et al*. Review article systemic inflammatory markers for predicting overall survival in patients with osteosarcoma?: A systematic review and meta-analysis. PMC, 2021; 21: 1-16. Available from: https:// doi.org/ 10.1155/2021/ 3456629 (accessed May 3, 2022).
12. Leoncini E, Ricciardi W, Cadoni G, Arzani D, Petrelli L, *et al*. Adult height and head and neck cancer: A pooled analysis within the INHANCE consortium. Springer, 2023; 36(10): 1391-2014. Available from: https:// 10.1007/s10654-013-9863-2 (accessed May 3, 2023).
13. Yapar A, Tokgöz MA, Yapar D, Atalay IB, Ulucaköy C, Güngör BŞ. Diagnostic and prognostic role of

neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, and lymphocyte/monocyte ratio in patients with osteosarcoma. Jt. Dis. Relat. Surg, 2021; 32(2): 489-496.
14. Corrales L, Matson V, Flood B, Spranger S, Gajewski TF. Innate immune signaling and regulation in cancer immunotherapy. Cell Research, 2017; 27: 96-108. Available from: https:// 10.1038/cr.2016.149 (accessed May 3, 2023).
15. Peng LP, Li J, Li XF. Prognostic value of neutrophil/lymphocyte, platelet/lymphocyte, lymphocyte/monocyte ratios and glasgow prognostic score in osteosarcoma: A meta-analysis. World J. Clin. Cases, 2022; 10(7): 2194-2205.