

The neutrophil/lymphocyte ratio is an independent prognostic indicator in patients with bone metastasis

SHOUYU WANG^{1*}, ZHEN ZHANG^{1*}, FENGQI FANG², XUE GAO³, WEI SUN⁴ and HUANRAN LIU¹

Departments of ¹Surgery, ²Phymatology, ³Diagnostic Pathology and ⁴Nephrology,
First Affiliated Hospital of Dalian Medical University, Dalian 116011, P.R. China

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Abstract. Patients with the most common advanced human cancers such as lung, breast, uterus, and cancers of the digestive system almost always develop bone metastases, with painful and untreatable consequences. This study aimed to determine the prognostic implications of the neutrophil/lymphocyte (N/L) ratio in the peripheral blood of patients with malignant bone metastasis. Study participants were identified from a prospective cohort of cancer patients with bone metastasis. Data for the N/L ratios were obtained from clinical and pathological records and were analyzed together with other known prognostic factors in the multivariate and univariate analyses. The results showed the average N/L ratio of all 497 patients to be 4.25 ± 2.44 (range 0.54-45.50 years). Multivariate analysis revealed that tumor type and a high N/L ratio were significantly associated with poor prognosis. For the high N/L ratio group, the estimated hazard ratio of death was 1.348 [95% confidence interval (CI), 1.062-1.712] compared with the low N/L ratio group. The average N/L ratio of the 225 patients in the surgery group was 2.79 ± 2.46 (range 0.77-22.75 years). Multivariate analysis revealed that a preoperatively high N/L ratio ($P=0.013$; HR=2.945; 95% CI, 1.256-6.906) was significantly associated with poor prognosis after bone metastasis in the surgery group. In conclusion, the N/L ratio was confirmed to be an independent prognostic factor in patients with bone metastasis. Thus, the N/L ratio may serve as a clinically accessible and useful biomarker for patient survival.

Introduction

Metastasis of the bone is a common and serious problem in a number of malignant neoplasms, such as breast, thyroid, uterus

and lung carcinoma (1). Numerous patients suffering from these advanced cancers eventually succumb to the disease (2-4). Various molecular biomarkers have been used to identify the baseline parameters predicting patient prognosis in various studies, but technical factors and excessive costs preclude their clinical use.

Studies have reported that certain immune cells may serve as good indicators of prognosis in the progression of cancer. An imbalance in the ratio of neutrophils/lymphocytes (N/L) in the peripheral blood of cancer patients may be associated with neoplasm development (5,6). The N/L ratio indicates fluctuations in cell numbers and plays a role in the prognosis of cancer. Studies on gastrointestinal cancer have supported the hypothesis that the N/L ratio is significantly associated with overall survival (OS) in patients with colorectal cancer (7,8). In a previous study, we found that the peripheral blood N/L ratio is associated with tumor size and is an independent indicator of survival in patients with rectal carcinoma (9). The independent prognostic role of the peripheral blood N/L ratio for malignant bone metastasis at diagnosis remains to be confirmed. However, the N/L ratio can be measured relatively easily and can serve as a valuable index (indicator of the progression of malignant bone metastasis) in the clinical setting for determining patients that would benefit from clinical treatment. Consequently, the aim of the present study was to investigate whether the baseline N/L ratio is an independent prognostic indicator in patients with malignant bone metastasis.

Materials and methods

Study design. Between 1999 and 2006, 497 patients who were diagnosed with bone metastasis from a variety of carcinomas in our hospital were randomly selected (291 males and 206 females; average age 61.51 ± 10.14 years; range 14-91 years). A total of 225 patients had undergone surgery for various types of tumor including breast (73 cases, 69 underwent surgery), thyroid (7 cases, 6 underwent surgery), uterus (76 cases, 48 underwent surgery), lung (238 cases, 40 underwent surgery) and digestive system cancer (76 cases, 46 underwent surgery), as well as other types of cancer (29 cases, 16 underwent surgery) prior to the diagnosis of metastatic malignant carcinoma. Bone metastasis was the primary symptom in 275 patients. Patients lost to follow-up and patients with synchronous or metachronous multiple cancers were excluded. Slides stained by hematoxylin

Correspondence to: Dr Huanran Liu, Department of Surgery, First Affiliated Hospital of Dalian Medical University, Zhongshan Road 222, Dalian 116011, P.R. China
E-mail: lhlhr9050@126.com

*Contributed equally

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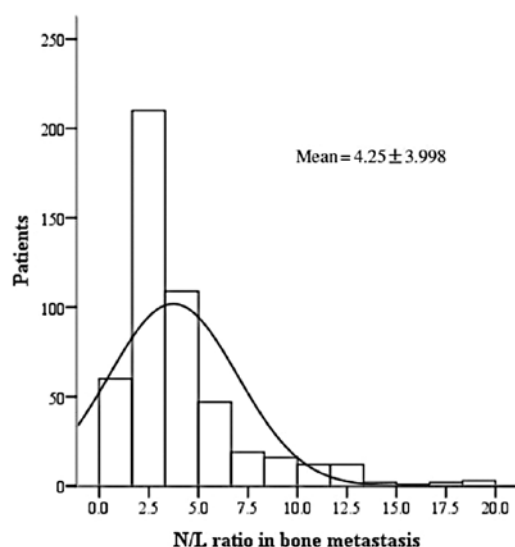


Figure 1. Histogram of the baseline N/L ratio in the peripheral blood of 497 patients with malignant bone metastases.

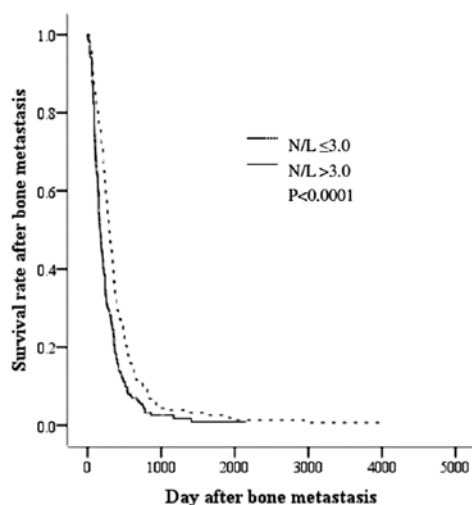


Figure 2. Survival curves based on the baseline N/L ratio in all patients with bone metastasis. The survival time of the high N/L ratio group is shorter than that in the low N/L ratio group ($P < 0.0001$).

and eosin were examined and classified according to the tumor node metastasis classification recommended by the International Union Against Cancer. Patients had received postoperative adjuvant therapy according to NCCN guidelines. Disease-free survival (DFS), time before bone metastasis and OS were calculated from the date of diagnosis until the date the patient succumbed to the disease or the last follow-up time. Patients who succumbed to unrelated causes with no evidence of disease were censored at death.

Statistical analysis. Data including gender, age, type of tumor, stage of disease, and pathological factors were obtained from clinical and pathological records and included in the univariate and multivariate analyses. Significant differences were determined by the Kruskal-Wallis and Mann-Whitney tests. In the univariate analysis, parameters that affect survival were assessed using the log-rank test, and the Cox proportional

Table I. The neutrophil/lymphocyte ratio according to tumor parameters and markers in all patients with bone metastasis.

Parameters	Neutrophil/lymphocyte ratio		P-value
	≤3.0	>3.0	
Age			
≤60	124	98	0.069
>60	131	144	
Gender			
Male	133	158	0.003
Female	122	84	
Tumor type			
Breast	46	27	0.014
Thyroid	2	4	
Uterine	44	31	
Lung	115	123	
Digestive system	35	41	
Other	13	16	
Surgery			
No	134	138	0.317
Yes	121	104	
Other metastasis			
No	147	128	0.288
Yes	108	114	
Level of CEA at BM	94.29±132.9	146.24±195.67	0.038
Level of ALP at BM	197.25±151.77	237.36±195.88	0.214

Surgery, with or without surgical treatment; Other metastasis, with or without other metastasis; BM, bone metastasis; CEA, carcinoembryonic antigen; ALP, alkaline phosphatase.

hazard regression model was used in the multivariate analysis. $P < 0.05$ was considered to be statistically significant. Statistical analyses were performed using SPSS software (version 16).

Results

Association of N/L ratio with patient parameters. The distribution of the baseline N/L ratio of the 497 patients is shown in Fig. 1. The average N/L ratio was 4.25 ± 2.44 (range, 0.54–45.50 years), with the 25th and 75th percentiles at 2.06 and 4.81, respectively. The N/L ratio of normal controls is generally < 2.0 since the proportion of neutrophils and lymphocytes in WBCs is approximately 50–60 and 30–40%, respectively. Thus, compared with the normal controls, the N/L ratio values of patients with bone metastasis exhibited a substantial upward shift. The subjects were dichotomized at the N/L ratio value of 3.0 to yield one group with N/L ratio > 3.0 (high N/L ratio group, $n = 255$) and another with N/L ratio ≤ 3.0 (low N/L ratio group, $n = 242$). Findings of our analysis revealed that the N/L ratio was significantly associated with gender ($P = 0.003$), tumor type ($P = 0.014$) and level of carcinoembryonic antigen (CEA) ($P = 0.038$). However, there was no correlation between the N/L ratio and surgery or other metastasis.

Table II. Cox regression analysis for survival in all patients with bone metastasis.

Parameters	Overall survival		
	Univariate	Multivariate	
	P-value	RR, 95% CI	P-value
Age	0.011	1.228 (0.963-1.565)	0.098
Gender	<0.0001	0.658 (0.508-0.852)	0.002
Tumor type	<0.0001	1.157 (1.036-1.292)	0.009
Surgery	<0.0001	0.843 (0.643-1.106)	0.217
Other metastasis	0.001	1.665 (1.311-2.115)	<0.0001
N/L ratio at BM	<0.0001	1.348 (1.062-1.712)	0.014
CEA at BM	<0.0001	1.000 (1.000-1.001)	0.752
ALP at BM	<0.0001	1.000 (1.000-1.000)	0.57
Adjuvant therapy	0.026	0.767 (0.590-0.999)	0.049

BM, bone metastasis; surgery, with or without surgical treatment; other metastasis, with or without other metastasis; adjuvant therapy, with or without adjuvant therapy. RR, risk ratio; CI, confidence interval.

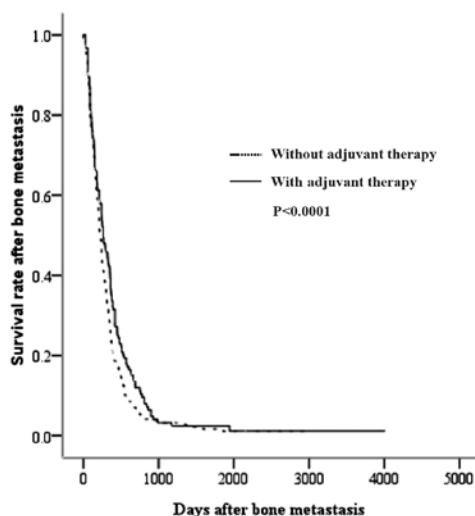


Figure 3. Survival curves based on adjuvant therapy in all patients with bone metastasis. The survival time of the group receiving adjuvant therapy is longer than that of the group not receiving adjuvant therapy ($P<0.0001$).

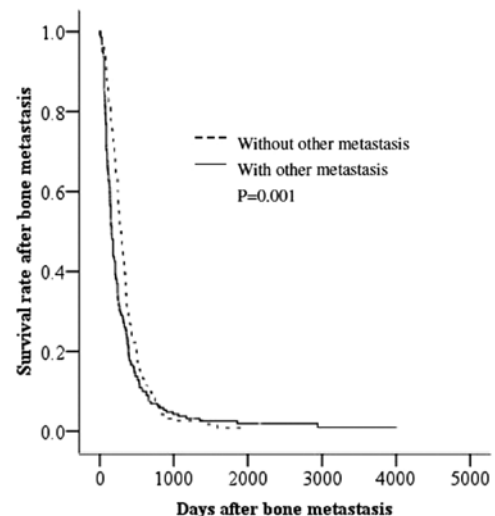


Figure 4. Survival curves based on other metastasis in all patients with bone metastasis. The survival time of the group without other metastases is longer than that of the group with other metastases ($P=0.001$).

Survival rates. The median survival time of all the patients with bone metastasis was 875.73 days, and the 3- and 5-year survival rates were 15.9 and 9.3%, respectively. The OS after bone metastasis differed significantly between the two groups (log-rank test, $P<0.0001$, Fig. 2). The 3-year survival rates were 17.3 and 12.2%, respectively, in the low N/L ratio group and the 5-year survival rates were 14.5 and 6.7%, respectively, in the high N/L ratio group. On the other hand, the survival time was significantly different based on adjuvant therapy (Fig. 3) and other metastases (Fig. 4). The multivariate analysis revealed that tumor type and a high N/L ratio were significantly associated with poor prognosis. For the high N/L ratio group, the estimated hazard ratio of death was 1.348 [95% confidence interval (CI), 1.062-1.712] compared with the low N/L ratio group, i.e., a 134.8% increase in the risk of death (Table II).

Using other cut-off values for low vs. high N/L ratio, such as 3.5 or 4.0, they did not alter the results described here.

The distribution of the baseline N/L ratio of the 225 patients in the surgery group was then analyzed (Fig. 5). The average N/L ratio was 2.79 ± 2.46 (range 0.77-22.75 years), with the 25th and 75th percentiles at 1.87 and 3.06, respectively. Table III shows that the N/L ratio was significantly associated with age ($P=0.001$), gender ($P=0.013$), tumor type ($P<0.0001$) and depth of invasion ($P<0.0001$), but was not associated with lymph node metastasis or high preoperative levels of CEA and ALP. The median DFS, survival time after bone metastasis and OS of the patients who underwent surgery for a primary tumor were analyzed, were shown to be 1041.5 ± 984.9 days for DFS, 347.0 ± 291.8 days for bone metastasis and 1428.2 ± 1075.0 days for OS.

Table III. The neutrophil/lymphocyte ratio according to tumor parameters and markers in surgery group.

Parameters	Neutrophil/lymphocyte ratio		P-value
	≤3.0	>3.0	
Age			
≤60	70	41	0.001
>60	94	20	
Gender			
Male	87	21	0.013
Female	77	40	
Tumor type			
Breast	63	6	<0.0001
Thyroid	5	1	
Uterine	34	14	
Lung	22	18	
Digestive system	28	18	
Other	12	4	
Invasion			
T1	29	3	<0.0001
T2	49	15	
T3	65	20	
T4	11	20	
Lymph node			
N0	51	16	0.247
N1	71	26	
≥N2	31	16	
Preoperative CEA	27.29±41.17	27.53±39.16	0.993
Preoperative ALP	145.90±116.90	129.71±70.93	0.766

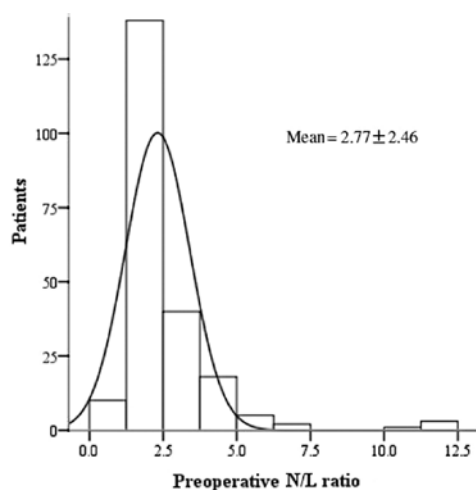
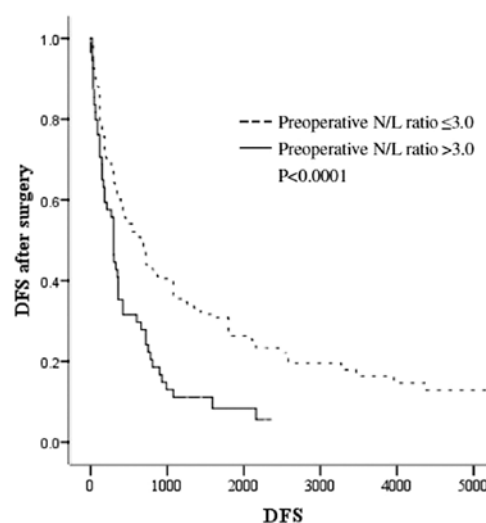
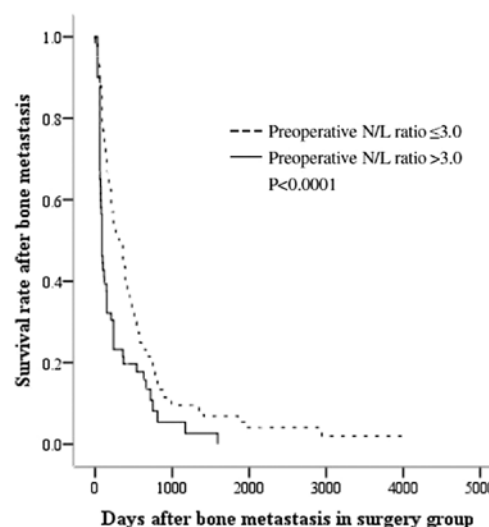


Figure 5. Histogram of the baseline N/L ratio in the peripheral blood of 225 patients with malignant bone metastasis.

In the group in which patients underwent surgery, the DFS in the high N/L ratio group (N/L ratio >3.0) was found to be significantly shorter than that in the low N/L ratio group (log-rank test: $P<0.0001$, Fig. 6). Similar results were observed for

Figure 6. Survival curves based on the baseline N/L ratio in the surgery group. The DFS of the high N/L ratio group is shorter than that of the low N/L ratio group ($P<0.0001$).Figure 7. Survival curves based on the baseline N/L ratio in the surgery group. The survival time after bone metastasis is shorter in the high N/L ratio group than that in the low N/L ratio group ($P<0.0001$).

bone metastasis ($P=0.009$, Fig. 7) and OS ($P<0.0001$, Fig. 8) of the surgery group. Multivariate analysis revealed that the T stage of the tumor ($P=0.049$; HR=1.979; 95% CI, 1.003-3.905) and lymph node metastasis ($P=0.023$; HR=2.451; 95% CI, 1.131-5.311) were significantly associated with shorter DFS (Table IV). Tumor type ($P=0.003$; HR= 1.622; 95% CI, 1.179-2.230) and a high preoperative N/L ratio ($P=0.013$; HR=2.945; 95% CI, 1.256-6.906) were significantly associated with poor prognosis after bone metastasis in the surgery group. Lymph node metastasis ($P=0.002$; HR=3.426; 95% CI, 1.550-7.571) and high preoperative levels of ALP ($P=0.013$; HR=1.003; 95% CI, 1.001-1.005) were significantly associated with poor prognosis in the surgery group. Using other cut-off values for low vs. high N/L ratio, such as 3.5 or 4.0, did not alter the results described here.

Table IV. Cox regression analysis for survival in surgery group.

Features	Disease-free survival			Survival after bone metastasis		Overall survival	
	Univariate	Multivariate		Multivariate		Multivariate	
	P-value	RR, 95% CI	P-value	RR, 95% CI	P-value	RR, 95% CI	P-value
Age	0.002	1.341 (0.557-3.231)	0.513	1.384 (0.592-3.234)	0.453	0.950 (0.364-2.484)	0.917
Gender	<0.0001	1.622 (0.576-4.568)	0.360	1.094 (0.512-2.340)	0.816	0.553 (0.202-1.517)	0.250
Tumor type	<0.0001	1.279 (0.861-1.899)	0.222	1.622 (1.179-2.230)	0.003	1.119 (0.786-1.593)	0.533
T stage	<0.0001	1.979 (1.003-3.905)	0.049	0.983 (0.624-1.548)	0.941	1.176 (0.666-2.077)	0.576
Lymph node	<0.0001	2.451 (1.131-5.311)	0.023	1.041 (0.624-1.738)	0.878	3.426 (1.550-7.571)	0.002
High preoperative N/L ratio	<0.0001	0.851 (0.336-2.157)	0.734	2.945 (1.256-6.906)	0.013	1.605 (0.585-4.406)	0.358
Preoperative CEA	<0.0001	0.996 (0.991-1.000)	0.075	0.999 (0.995-1.004)	0.720	1.000 (0.995-1.005)	0.914
Preoperative ALP	<0.0001	1.001 (0.999-1.004)	0.170	1.002 (1.000-1.004)	0.108	1.003 (1.001-1.005)	0.013
Adjuvant therapy	0.912	1.816 (0.692-4.768)	0.225	0.864 (0.364-2.052)	0.741	0.455 (0.166-1.246)	0.126

Adjuvant therapy, with or without adjuvant therapy. RR, risk ratio; CI, confidence interval.

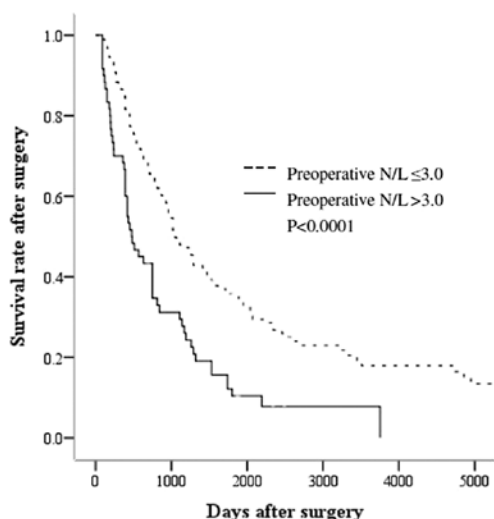


Figure 8. Survival curves based on the baseline N/L ratio in the surgery group. The overall survival time is shorter in the high N/L ratio group than that in the low N/L ratio group ($P < 0.0001$).

Discussion

A high count of peripheral neutrophils is considered to be an independent prognostic factor for short survival in a variety of advanced cancers (10-13). However, granulocytes (G) in humans show an increase in the daytime, while T cells, B cells, alphabet T cells and $CD4^+$ lymphocytes show an increase at night (14). Fluctuations in the number of granulocytes are not always in line with those of lymphocytes. The relative value of the N/L ratio can correctly reflect fluctuations between granulocytes and lymphocytes in order to reflect the antitumor efficacy of the host immune system more precisely. Recent studies have indicated that the N/L ratio is a good independent indicator for evaluating the condition of a tumor-bearing host and survival in patients with advanced cancers (3,9,13). However, there was no further evidence to indicate that the

N/L ratio could be used as an independent indicator for the prognosis of patients with malignant bone metastasis.

In the present study, we investigated patients with malignant bone metastasis who suffered from various types of cancer such as breast, thyroid, urine, lung or digestive system cancers. The baseline N/L ratio value in the peripheral blood of patients with malignant bone metastasis was found to be associated with survival. In all patients, our results showed that a high N/L ratio was significantly associated with poor prognosis. It is known that the initial steps involved in the development of bone metastases are similar to those of other metastases (15,16). The primary malignant neoplasm promotes new blood vessel formation, and these blood vessels carry the cancer cells to capillary beds in the bone. Aggregates of tumor cells and other blood cells form embolisms that are inhibited in distant capillaries in the bone. These cancer cells are capable of adhering to the vascular endothelial cells to escape the blood vessels. As they enter the bone, malignant bone metastasis occurs. Certain studies have identified that neutrophils stimulate tumor angiogenesis by producing proangiogenic factors, including vascular endothelial growth factor (17), interleukin-8 (18), matrix metalloproteinases (19) and elastases (20). It can be concluded that an array of cytokines or other molecules, which are proangiogenic factors produced by neutrophils, contribute to a stimulating microenvironment for bone metastasis in cancer cells. Thus, a high density of circulating neutrophils may lead to unfavorable effects on the tumor-bearing host. A negative correlation between neutrophil density and prognosis of patients with malignant bone metastases was therefore noted.

In the present study, we analyzed the distribution of the baseline N/L ratio of the patients in the surgery group. The results showed that the N/L ratio is significantly associated with age, gender, tumor type and depth of invasion. The prognosis of younger or female patients is better than that of older or male patients. The DFS in the high N/L ratio group (N/L ratio > 3.0) is significantly shorter than in the low N/L ratio group. Tumor type and preoperatively high N/L ratio

were significantly associated with poor prognosis after bone metastasis in the surgery group. A possible explanation of the association between high N/L ratio values and poor prognosis in the surgery group is that neutrophils inhibit the immune system of patients with bone metastasis. Studies have shown that neutrophils may suppress the cytolytic activity of a series of immune cells such as lymphocytes, activated T cells, and natural killer cells following the co-culture of neutrophils and lymphocytes from healthy donors, and the degree of suppression is proportional to the number of neutrophils added (21-24). Therefore, an increased N/L ratio may be associated with low immunocompetence in cancer patients with malignant bone metastasis. Furthermore, a high preoperative N/L ratio is an independent prognostic factor for short survival. These parameters are useful as stratification factors in clinical trials.

In conclusion, the results indicate that the prognosis of patients with malignant bone metastasis is independently associated with the peripheral blood N/L ratio; a high N/L ratio that is significantly associated with poor prognosis. Whether neutrophils enhance the process of malignant bone metastasis or impede the immune reaction of protection. Variations in the tumor type and the individual background of patients as well as other factors possibly affect the N/L equilibrium and prognosis for various patients. In this study, unavoidable heterogeneity exists in the length of survival among cancer patients with malignant bone metastasis whose age, gender, tumor type and clinical treatment are different. This study may aid in improving the accuracy of risk evaluation in cancer patients with malignant bone metastasis, as well as lead to more appropriate clinical treatment for such patients. The N/L ratio may serve as a potential biomarker of outcome in the clinical setting since it is cost-effective. However, further analysis of the molecular mechanism and prognostic implications of the N/L ratio in various types of malignant bone metastasis is required.

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