Prognostic Significance of Lymphocyte to Monocyte Ratio in Gallbladder Carcinoma

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Research article

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Abstract

Background: Increasing evidence indicates cancer-associated inflammatory biomarkers show great promise for predicting prognosis of cancer patients. The objective of this study aims to evaluate the prognostic significance of the lymphocyte-to-monocyte ratio in patients with gallbladder carcinoma.

Methods: Receiver operating characteristic curves was used to determine cut-off values for the LMR at detecting death. The primary outcome was overall survival, which was estimated by the Kaplan-Meier method. Univariate survival analysis was performed using a log rank test. Multivariate analysis using the Cox regression proportional hazard model was performed to identify the factors associated with the prognosis.A retrospective cohort of 80 GBC followed by operation was recruited between March 2008 and August 2014 at the Qingdao Municipal Hospital. Counts for absolute lymphocytes and monocytes were obtained and used to calculate the LMR.

Results: For the LMR, the area under the ROC curve was 0.675 (95%CI: :0.530-0.820).The cut-off value for the LMR was determined to be 4.62. Patients in the high-LMR group experienced significant improvements in median survival time compared with patients in the low-LMR group(P = 0.03).The univariate analysis demonstrated that LMR, differentiation degree, TNM stage,CA199, CEA, Resection margin and operative methods were associated with overall survival (P < 0.05). The multivariate analysis identified that Differentiation grade , TNM stage, and CRP as independent prognostic factors in the patients with GBC(P <0.05).

Conclusion: Our study demonstrated that LMR is closely correlated with GBC prognosis and could be useful for the evaluation of prognosis of patients with GBC.

Background

Gallbladder carcinoma (GBC) is a common malignant tumor of the biliary tract .The Surveillance, Epidemiology, and End Results (SEER) program estimates the incidence of GBC at 2.5 per 100,000 persons[1].Despite the relatively low incidence rate, GBC-associated mortality is higher than that of other cancers [2].GBS constitutes a rare set of malignancies with dismal survival rate overall, which is due to early metastasis via lymphatic, perineural, and hematogenous routes, as well as direct invasion into the liver [3]. Although the tumor, node, and metastasis (TNM) staging system of the American Joint Committee on Cancer(AJCC) is the most widespread applied system, there is no global consensus on the optimal system or indicators for predicting the prognosis of GBC patients.Previous research has suggested that CA125[4] and CA199 levels[5], and tumor-node-metastasis (TNM) stage[6] might affect the survival of these patients. Hence, there is an urgent need for exploring more specific and sensitive factors for the prognosis of GBC.

It has been reported that systemic inflammation promotes tumor progression and metastasis via the inhibition of apoptosis, promotion of angiogenesis, and damaging of DNA[7] . The inflammatory response involves lymphocytes,monocyte,neutrophils, platelets, and acute-phase proteins, including

albumin in peripheral blood.Lymphocyte and monocyte play an important role in inflammatory reaction, reflecting body immune status. Lymphocyte-to-monocyte ratio is a prognostic evaluation system based on the theory of tumor inflammation microenvironment[8].In recent years,a growing number of clinical researches show that pretreament of LMR in the patients with maligant tumor may have a certain value in evaluating the prognosis of cancer patients such as breast cancer[9], lung cancer [10], colorectal cancer [11],classical Hodgkin's lymphoma[12]and nasopharyngeal carcinoma [13,14].Thus, we hypothesized that LMR may also play an important role in GBC.

This study evaluated the correlations between LMR and clinicopathological features of GBC for the evaluation of their prognostic value in GBC patients.Moreover, we also investigated the optimal cut-off values of LMR for predicting prognosis.

Methods

Study population

80 collected patients with histologically confirmed gallbladder carcinoma, who have been operated between March 2008 and August 2014 at the Qingdao Municipal Hospital were included in this retrospective study. Patients diagnosed with gallbladder carcinoma, treated only with surgery, including radical operations, cholecystectomy, and palliative resection. The exclusion criteria were as follows:(1) patients who previously received preoperative chemotherapy or radiotherapy, (2) patients who were combined with other tumors , and (3) patients with clinical evidence of infection or other bone marrow, hematological,or autoimmune disease.

Laboratory data

As part of the physical examinations, Peripheral venous blood samples was collected within one week before treatment, and both peripheral lymphocytes and monocytes were counted. And the peripheral LMR was calculated by dividing the absolute lymphocyte count by the absolute monocyte count. Then we extracted information of total bilirubin, C-reactive protein (CRP), carbohydrate antigen (CA199) and carcinoembryonic antigen (CEA).Further, the following data were collected: age and gender, surgical margin, and operative methods, and the results of pathological reports. We adopted The TNM staging system (7th edition), based on the criteria of the American Joint Committee on Cancer[15].

Follow-up

Follow-up data were collected until August 2016 or death. Follow-up

were put into effect every 2 months since start of treatment. Overall survival(OS) defined as the time between operation and death, loss to follow-up or the last follow-up.

Statistical analysis

Receiver operating curve (ROC) curve analysis and area under the curve (AUC) was performed to select the optimal cut-off value of LMR to assess prognosis based on their utility as a marker for the clinically relevant binary outcome of death/survival. The OS was estimated by the Kaplan-Meier method, and compared using the log-rank test. Univariate survival analysis was performed using a log rank test. multivariate analysis using the Cox regression proportional hazard model was performed to determine the prognostic predictors for survival. These analyses were performed with SPSS software (version 22.0,SPSS Inc, Chicago, IL, USA). A P value less than 0.05 was considered statistically significant.

Results

Determination of the cut-off value for the LMR

We used ROC curve analysis to determine the optimal cut-off value for LMR. The optimal cut-off value of the LMR was 4.62 with a sensitivity of 66.7% and a specificity of 64.6% by the Youden index. (Fig. 1). The area under the ROC curve (AUC) indicates the diagnostic power of LMR((AUC = 0.675, 95%CI:0.530-0.820,) There were 47 and 33 cases, respectively, in the low(LMR≤4.62) and high (LMR>4.62) LMR groups.

Patients' characteristics with GBC in the LMR group

The cohort included 35 men and 45 women, with a median age 63 years(range, 40-81 years). According to the LMR, We summarized clinicopathological characteristics(gender, age, differentiation grade, pTNM stage, Total bilirubin,CEA,CA199, CRP, surgical margin, and operative methods) in Table 1. As shown, there was no significant difference between the groups in age, gender, TNM stage, Total bilirubin, CEA, Resection margin and operative methods of the patients (all P>0.05). There was an obvious difference between the groups in the degree of differentiation (P =0.017) and C-reactive protein(P = 0.026).

Elevated LMR indicates better clinical outcome in GBC

Kaplan–Meier survival analysis and long-rank analysis were used to carry out the relationship between OS and LMR, which was statistically significant (log-rank P = 0.03) (Figure 2). Furthermore, patients in the high-LMR group experienced significant improvements in median survival time [18 months (95% CI 1.1–34.853) vs. 6 months (95% CI 4.408–7.916)] compared with patients in the low-LMR group.

Univariate and multivariate analysis of clinical and biochemical parameters

The univariate analysis was performed using the Kaplan-Meier method to assess the predictive parameters. The analysis showed that LMR(P =0.03),differentiation grade(P =0.03), TNM stage(p<0.001), CA199(p=0.028), CEA(p=0.012),CRP(p<0.001),Resection margin(p=0.011) and operative methods(p<0.001) were as significant tools for predicting OS in patients with GBC(Table 2). All parameters which were statistical significance in univariate analysis were included in multivariate analysis(Cox proportional hazard models) . To assess the independent prognostic value of the LMR for OS , the multivariate analysis identified that Differentiation grade (P=0.011), TNM stage(P=0.001),and

CRP(P = 0.008)as independent prognostic factors in the patients with GBC (Table 3). Whereas LMR is not(p=0.203),then it was a significant prognosis factor of GBC.

Discussion

In recent years, more and more studies have shown that chronic inflammation is closely related to malignant tumors. Cancer immunoediting points out that the tumor-promoting effect of Inflammation has been well demonstrated[16-18].Systemic inflammation is a key component of tumor progression, because it can not only do great damage to cancer cells but also establish tumor microenvironment, which contributes to proliferation, migration and immune escape of malignant cells[19]. Literatures have reported hematological markers of systemic inflammation response, such as, LMR,NLR, and PLR might serve as independent prognostic markers of survival in various cancers. Previous studies have demonstrated that several systemic inflammatory factors can be used to predict the prognosis of GBC patients, such as platelet-to-lymphocyte ratio[20] and neutrophil-to-lymphocyte ratio[20-21]. As one of the indexes reflecting inflammation,the correlation between LMR and survival has been covered[9-14]. As far as we know, this is the first study to evaluate the prognostic significance of the LMR in patients with GBC.

In our study, Patients with low-LMR had a worse prognosis than those with high-LMR.The mechanism that LMR affects the prognosis of cancer patients is not yet clear, and further discussion is still needed.Low LMR often suggests that patients with low lymphocyte count, or higher monocyte count, which is often associated with the poor prognosis of the patients.The connection between the lymphocytes and monocytes may be explained by the suppression of the immune cells infiltrating in the tumor tissue or the growth of the tumor.LMR can reflect the immune status and load of the tumor.Tumor-infiltrating lymphocytes(TILs) and tumor-associated macrophages(TAMs) are immune cells that have been found to play an important role in many malignant tumor tissues and can play a specific role in predicting the prognosis. Lymphocyte is a kind of cell with anti-tumor and regulatory immunitiy functions, which plays an important role in immune surveillance in vivo[22], and the change of its content can reflect the immune function of organism.TILs control tumor progression by participating in cellular and humoral immunity. Low lymphocyte count may be a sign of poor immune function,which weakens the control of tumor immunity by immunization and leads to poor prognosis.Decrease of lymphocytes respectively positively with CD4+ cytotoxic T cells and CD8+ cytotoxic T cells,which is the contributory factor to the decrease of immunologic surveillance and immune clearance function.Protective immunity in LMR depended on CD4+ and CD8+ T cells. Actually, Nakakubo [23]suggested that a low presence CD4+ and CD8+ T-cells in the tumor correlate with poor prognosis after surgery for GBC. Recent studies have demonstrated that an lymphocyte count alone[24]are predictive of poor survival in patients with GBC.

On the other hand, when malignant tumors occurs, peripheral blood monocytes can be recruited into the tumor storma, where they gradually differentiate into Tumor-associated macrophages(TAMs) .

Tumor-associated macrophages (TAMs) were inflammatory cells[25],which promotes tumor cell invasion and migration[26], and their number is related to malignant degree and poor prognosis. TAMs are active in the secretion of tumor chemokines around tumor tissues. TAMs can also accelerate tumor progression by stimulating tumor angiogenesis and generate anti-immune responses through the production of growth factors and cytokines.Related studies have confirmed that patients with high TAMs infiltration have a poor prognosis[27–28]. Absolute peripheral blood monocytes can be used as a biomarker for the replacement of TAMs.Therefore, low LMR indicates deficiency of anti-tumor immunity and a tumor promoted inflammatory microenvironment.

Nevertheless, in our cohort, the LMR which is a simple combined index, was not identified as an independent prognostic factor. Univariate analysis showed that LMR was significantly correlated to the progosis($p<0.05$).Multivariate analysis showed that LMR was not independent prognostic predictors($p>0.05$). Interestingly, Inflammatory markers such as C-reactive protein have been found to be a Independent predictors of prognosis in our research. Elevated CRP as a sensitive indicator of systemic inflammatory reaction, which is closely related to the prognosis of many cancers[29–31].

It's possible that factors not considered in the analyses could account for the results.First of all our study was obtained from data collected at a single center and a relatively small retrospective study of 80 patients, and there is a need for prospective, multicenter cohort studies to validate o prognostic value of LMR in gallbladder carcinoma. Moreover, this outcome could be partly explained by the difference among several differentiation grades of GBC, which leads to different effects of the standard prognostic variables. Further studies and analyses are

needed on this issue by lamination design. Last but not least, Because of patients and their family members with poor medical compliance, our follow-up indicators did not include progression-free survival (PFS), local progression-free survival (LPFS) and distant metastases free survival (DMFS). Despite these limitations, we believe that our results provide valuable support for the prognostic role of LMR in GBC patients.Further large-scale studies are needed to clarify the prognostic value of preoperative LMR in GBC patients .

Conclusions

Taken together, LMR is closely correlated with GBC prognosis and could be useful for the evaluation of prognosis of patients with GBC. Low LMR was associated with poor overall survival. The LMR could be a widely available and low price prognostic biomarker of GBC in clinical practice.

Abbreviations

LMR :Lymphocyte-to-monocyte ratio GBC: Gallbladder carcinoma ROC :Receiver operating characteristic curves SEER :The Surveillance, Epidemiology, and End Results TNM :Tumor, node, and metastasis AJCC :the American Joint Committee on Cancer CRP :C-reactive protein CA199:Carbohydrate antigen CEA:

Carcinoembryonic antigen OS:Overall survival AUC :Area under the curve TAMs :Tumor-associated macrophages PFS:Progression-free survival LPFS: Local progression-free survival DMFS: Distant metastases free survival.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of the Qingdao Municipal Hospital. Written informed consent was obtained from all patients prior to treatment.

Consent for publication

Not applicable.

Availability of data and materials

The data sets generated and analysed during the current study are not publicly available due to privacy reasons, but anonymized data may be available from the corresponding author on reasonable request and after approval of the regional ethical committee.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZY and YSL designed the project, interpreted data and wrote the manuscript. XXM, JXM, WSY and ZY collected samples. XXM and JXM performed the statistical analyses. XXM, WSY and ZY contributed to helpful discussion. JXM and YSL revised the manuscript. All authors reviewed and approved the manuscript.

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Tables

Table 1.Association of LMR with the parameters of 80 gallbladder cancer patients

Characteristics	Total	LMR≤4.62	LMR>4.62	P
Sex				0.264
Male	35	23	12	
Female	45	24	21	
Age(yr)				0.326
≤60	36	19	17	
60	44	28	16	
Differentiation grade				0.017
low-differentiation	37	27	10	
middle-differentiation+high-differentiation	43	20	23	
TNM stage				0.860
I+II+III	50	29	21	
IV	30	18	12	
Total bilirubin (lmol/L)				0.077
≤17.1	49	25	24	
>17.1	31	22	9	
CA199 (U/ml)				0.089
≤37	37	18	19	
>37	43	29	14	
CEA (ng/mL)				0.308
≤3.5	48	26	22	
>3.5	32	21	11	
CRP (mg/L)				0.026
≤5	32	14	18	
>5	48	33	15	
Resection margin				0.264
negative	70	39	31	

positive	10	8	2	
operative methods				0.107
Radical operation	39	21	18	
cholecystectomy	14	6	8	
palliative resection	27	20	7	

Table 2. Univariate analysis of factors associated with overall survival of gallbladder carcinoma patients

Table 3. Multivarate Cox regression analysis of overall survival in gallbladder cancer patient

Variable	HR (95%CI)	P value
Differentiation grade	0.478(0.270-0.845)	0.011
TNM stage	2.652(1.476-4.768)	0.001
CA199	0.890(0.464-1.707)	0.726
CEA	1.301(0.716-2.364)	0.387
CRP	2.459(1.265-4.778)	0.008
Resection margin	2.000(0.947-4.222)	0.069
operative methods	1.319(0.950-1.832)	0.099
LMR	1.471(0.812-2.665)	0.203

Figures

Variable	N	Median survival time (months)	P value	X2
Sex			0.834	0.043
Male	35	14		
Female	45	11		
Age(yr)			0.732	0.118
≤60	36	10		
60	44	11		
Differentiation grade			0.03	8.991
low-differentiation	37	6		
middle-differentiation+high-differentiation	43	18		
TNM stage			<0.001	24.360
I+II+III	50	22		
IV	30	4		
Total bilirubin (lmol/L)			0.336	0.925
≤17.1	49	14		
>17.1	31	7		
CA199 (U/ml)			0.028	4.842
≤37	37	18		
>37	43	6		
CEA (ng/mL)			0.012	6.326
≤3.5	48	18		
>3.5	32	5		
CRP (mg/L)			<0.001	16.452
≤5	32	31		
>5	48	6		
Resection margin			0.011	6.465
negative	70	14		
positive	10	5		

operative methods			<0.001	63.865
Radical operation	39	17		
cholecystectomy	14	43		
palliative resection	27	4		
LMR			0.03	8.560
≤4.62	47	6		
>4.62	33	18		

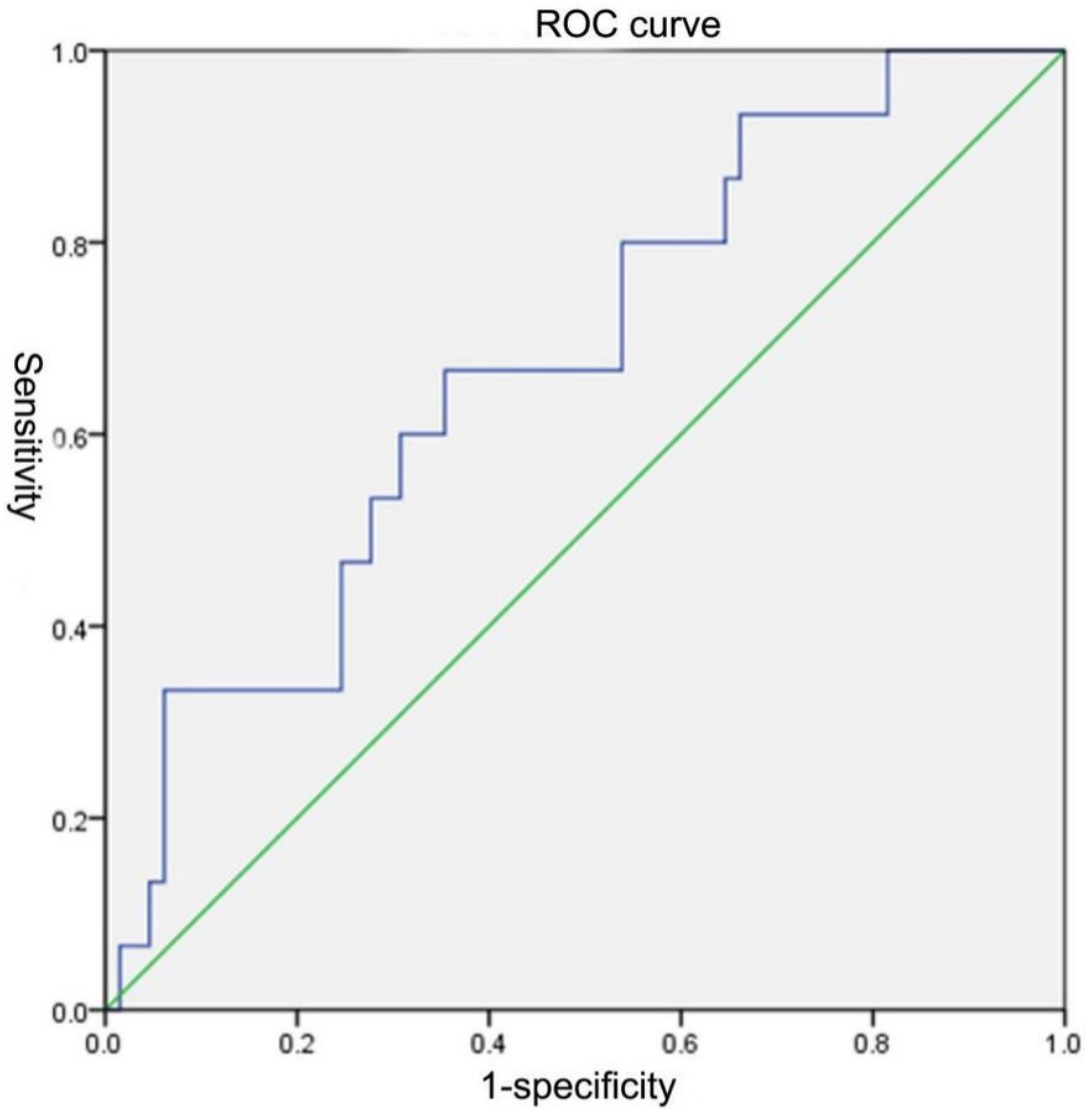

Figure 1

The optimal cut-off value of the LMR was 4.62 with a sensitivity of 66.7% and a specificity of 64.6% by the Youden index. (Fig. 1).

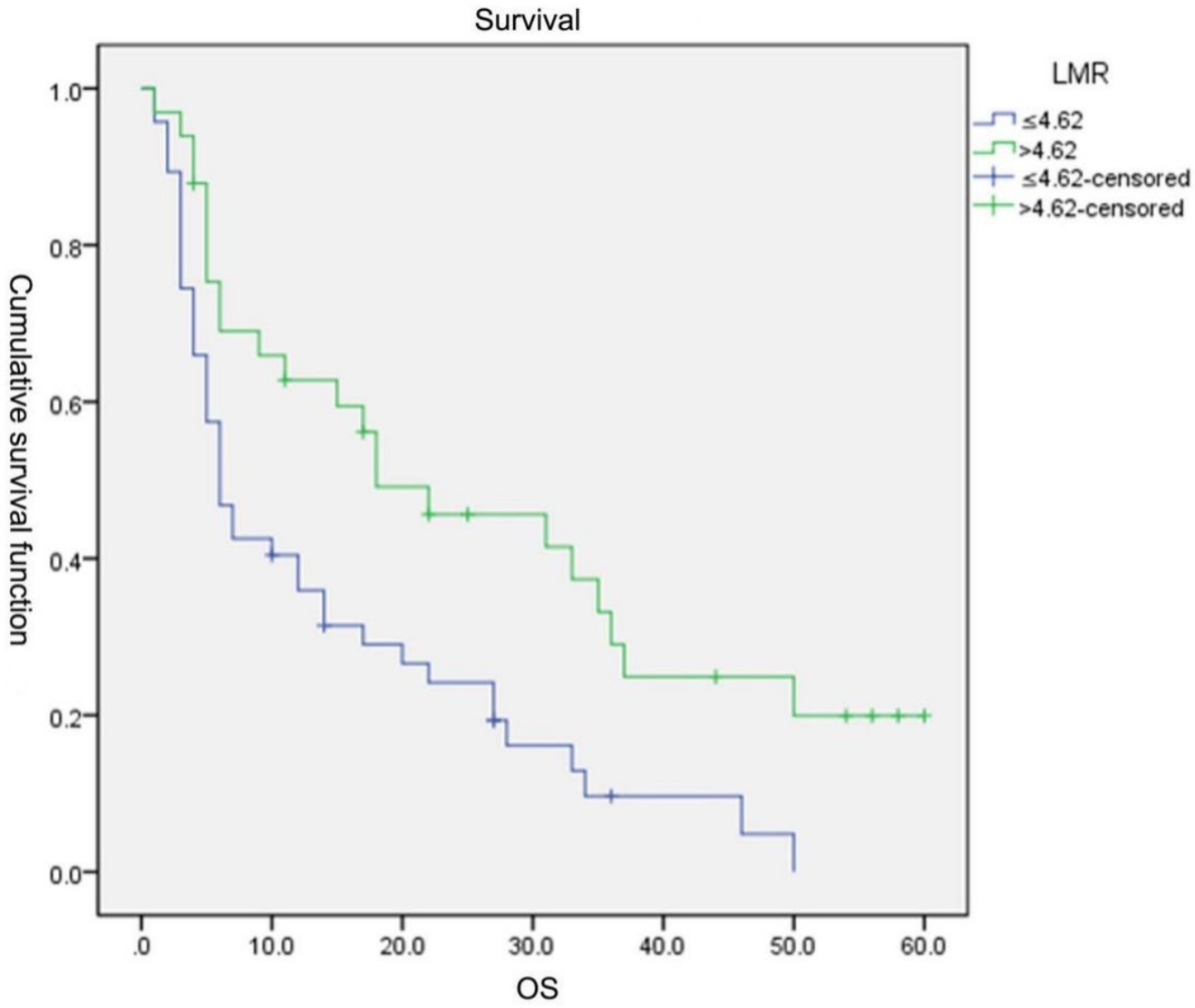

Figure 2

Kaplan–Meier survival analysis and long-rank analysis were used to carry out the relationship between OS and LMR, which was statistically significant (log-rank P = 0.03) (Figure 2).