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ORIGINAL RESEARCH

Prognostic Significance of Circulating Lymphocyte Subsets Before Treatment in Patients with Nasopharyngeal Carcinoma

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De-Song Shen[1]
Chang Yan[1]
Yu Liang[1]
Kai-Hua Chen[1]
Xiao-Dong Zhu[1,2]

[1]Department of Radiation Oncology, Guangxi Medical University Cancer Hospital, Nanning, Guangxi, 530021, People's Republic of China; [2]Department of Oncology, Wuming Hospital of Guangxi Medical University, Nanning, Guangxi, 530199, People's Republic of China

Purpose: We set out to explore the prognostic value of circulating lymphocyte subsets in patients with nasopharyngeal carcinoma (NPC) before treatment and to investigate changes in lymphocyte subsets resulting from chemoradiotherapy.

Patients and Methods: This retrospective study included 677 patients with non-metastatic NPC. The cutoff value of lymphocyte subsets was determined by the receiver operating characteristic curve (ROC), and the prognostic significance of lymphocyte subsets was evaluated by the Log rank test and Cox proportional hazards model. The endpoints were overall survival (OS), progression-free survival (PFS), locoregional relapse-free survival (LRFS) and distant metastasis-free survival (DMFS). Differences in lymphocyte subsets before and after chemoradiotherapy were analyzed by Wilcoxon signed rank test.

Results: NPC patients with high levels of $CD19^+$ B cells (>9.55%) had better 5-year OS (90.4% VS 76.8%, $P < 0.001$), 5-year PFS (85.3% VS 71.6%, $P < 0.001$) and 5-year DMFS (94% VS 86.8%, $P = 0.002$) than patients with low levels of $CD19^+$ B cells. Patients with high levels of $CD4^+$ T cells (> 37.05%) had better 5-year PFS (83% VS 74.2%, $P = 0.015$) and better 5-year DMFS (95.8% VS 86.7%, $P < 0.001$) than those with low levels of $CD4^+$ T cells. Multivariate analyses indicated that $CD19^+$ B cell was an independent prognostic factor for OS, PFS and DMFS in NPC. And $CD4^+$ T cell was an independent prognostic factor for PFS and DMFS. Within 1 month after chemoradiotherapy, the percentages of $CD4^+$ T cells, $CD19^+$ B cells, and the CD4/CD8 ratio decreased significantly, while the percentages of $CD8^+$ T cells increased significantly.

Conclusion: NPC patients with low levels of $CD19^+$ B cells or $CD4^+$ T cells before treatment have a poor prognosis. In addition, chemoradiotherapy may reduce the body's immune function in NPC patients.

Keywords: nasopharyngeal carcinoma, lymphocyte subsets, prognosis, chemoradiotherapy, immune function

Introduction

Nasopharyngeal carcinoma (NPC) is a cancer originating from the nasopharyngeal mucosal lining. NPC is major threat in Southern China and Southeast Asia, where the incidence is one of the highest in the world.[1] The incidence and mortality rate of NPC vary greatly in different countries, with the mortality from NPC in China accounting for approximately 40% of that worldwide.[2] Radiotherapy is the main curative treatment for NPC, and concurrent chemoradiotherapy is recommended for patients with locally advanced NPC.[1]

Correspondence: Xiao-Dong Zhu
Tel +86 771 533 1466
Email zhuxdonggxmu@126.com

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The TNM staging system is used to predict the prognosis of patients with NPC. However, patients with NPC that exhibit same TNM stage and who received similar treatment, can experience very different clinical outcomes,[3,4] suggesting that the TNM staging system on its own is insufficient to accurately predict the prognosis of these patients. Therefore, new biomarkers which reflect the true biological and immunological heterogeneity of NPC, and that can provide more accurate prognostic information and help facilitate individualized treatment are urgently needed.

The occurrence, development, and outcome of malignant tumors are closely related to the body's immune function. The response of the immune system impacts cancer prognosis, and therefore the immune status might be a valuable predictive biomarker.[5–7] Information of clinically circulating lymphocyte subsets, including the levels of T cells, B cells and NK cells, is commonly used as a hematological marker for the body's immune function.[8–10] T cells are divided into $CD3^+$ $CD4^+$ T cells and $CD3^+$ $CD8^+$ T cells according to differentiation antigens on the cell surface. CD19 is an important antigen and marker on the surface of B cells, while CD16 and CD56 are specific antigens on NK cells.[11–13] Previous studies have revealed an association between specific circulating lymphocyte subset levels and poor prognosis in patients with NPC.[14–18] However, the prognostic value of circulating lymphocyte subsets in NPC patients before treatment remains unclear. In contrast to tumor infiltrating lymphocytes, circulating lymphocyte subsets in the peripheral blood are easily detected and accessible, and exhibit measurement repeatability.

In this study, we set out to explore the prognostic significance of circulating lymphocyte subsets as well as to investigate changes of lymphocyte subsets after chemoradiotherapy, to provide insights that can help individualized treatments and follow-up strategies in patients with NPC.

Patients and Methods

Patients

We collected information on NPC cases at the Guangxi Medical University Cancer Hospital, China, between 2010 and 2014. The inclusion criteria used were: (1) patients pathologically diagnosed with NPC but without distant metastasis; (2) patients treated by intensity modulated radiotherapy (IMRT); (3) Karnofsky Performance Status (KPS) $\geq$ 70 points; (4) detection of the percentage of circulating lymphocyte subsets before treatment. The exclusion criteria were: (1) severe infection; (2) immune system diseases or recent use of drugs that could affect immune function; (3) severe liver and kidney dysfunction; (4) synchronous malignancy; (5) incomplete data. This study was approved by the Ethics Committee of Guangxi Medical University Cancer Hospital, China, and conformed to the Declaration of Helsinki. Informed consent was not obtained given the retrospective nature of the study. Data was either confidential or anonymized. All patients were re-staged based on the 8th edition of the American Joint Commission on Cancer (AJCC) staging system.

Treatments

All patients included were treated with IMRT. Delineation of the target area and organs at risk (OARs) was made with reference to the International Commission on Radiation Units and Measurements Reports 50 and 62. The prescribed radiation doses of the planned target volume (PTV) derived from the gross tumor volume (GTV), the nodal lesion GTV, high-risk clinical target volume (CTV1) and low-risk clinical target volume (CTV2) were 68–75.9 Gy, 64–73.6 Gy, 60–64 Gy and 54–57.6 Gy respectively. Patients received radiotherapy with five fractions per week and one fraction per day in 30–33 fractions. Patients with locally advanced NPC received concurrent chemoradiotherapy, combined induction chemotherapy or adjuvant chemotherapy. And induction or adjuvant chemotherapy regimen consisted of docetaxel, cisplatin or 5-fluorouracil. Induction chemotherapy was administered once every 3 weeks for 2–3 cycles. And adjuvant chemotherapy was administered once every 3 weeks for 1–3 cycles. In concurrent chemotherapy, platinum was used once every 3 weeks for 2–3 cycles. If necessary, the number of cycles for chemotherapy was adjusted by the clinician according to the patient's condition.

Detection of Circulating Lymphocyte Subpopulations

Before treatment and within 1 month after treatment, we collected peripheral venous blood samples from patients. The percentages of circulating lymphocyte subsets were detected by flow cytometry using an epics XL flow cytometer (Beckman Coulter, USA) and the corresponding

kits, including $CD3^+$ T cells, $CD3^+$ $CD4^+$ T cells, $CD3^+$ $CD8^+$ T cells, $CD19^+$ B cells and $CD16^+$ $CD56^+$ NK cells and the CD4/CD8 ratio was calculated.

Prognostic Assessment and Patient Follow-Up

The selected endpoints were overall survival (OS), progression-free survival (PFS), locoregional relapse-free survival (LRFS), and distant metastasis-free survival (DMFS). OS was defined as the time from patient's diagnosis to death of any cause or last follow-up; PFS was defined as the time of patient's diagnosis to disease progression, death or last follow-up; LRFS was defined as the time from patient's diagnosis to local and regional recurrence or last follow-up; and DMFS was defined as the time from patient's diagnosis to occurrence of distant metastasis or last follow-up.

After treatment, patients were followed-up every three months in the first two years, every six months in the subsequent three years, and then once a year until death. The date of the last follow-up was November 2020.

Statistical Analysis

Correlations between circulating lymphocyte subsets and clinical factors were evaluated by Spearman rank correlation. Through receiver operating characteristic (ROC) curves, the value corresponding to the highest Youden's index (Youden's index = sensitivity + specificity - 1) was selected as the optimal cutoff point for lymphocyte subsets, and lymphocytes were grouped according to the cutoff value. Patient survival rates were calculated using the Kaplan-Meier method and survival curves were compared using the Log rank test. The Cox proportional hazards model was used for univariate and multivariate analysis to analyze factors associated with prognosis. The Wilcoxon signed rank test was used to compare differences in lymphocyte subsets before and within 1 month after chemoradiotherapy. The reported probability values were two-tailed, and $p< 0.05$ was considered statistically significant. All statistical analyses were carried out using the 25th edition of SPSS (IBM Corp, Armonk, NY, USA).

Table 1 Baseline Characteristics of Patients (N =677)

Characteristics	No of Patients	Percent
Age (Years)		
≤46	351	51.8
>46	326	48.2
Gender		
Males	506	74.7
Females	171	25.3
Smoking		
No	444	65.6
Yes	233	34.3
Family history of NPC		
No	610	90.1
Yes	67	9.9
WHO pathologic classification		
WHO II	74	10.9
WHO III	603	89.1
Primary tumor (T) stage		
T1	32	4.7
T2	244	36
T3	256	37.8
T4	145	21.4
Regional lymph nodes (N) stage		
N0	36	5.3
N1	326	48.2
N2	263	38.8
N3	52	7.7
Clinical stage		
I	5	0.7
II	156	23
III	326	48.2
IVa	190	28.1
Treatment received by patients		
Radiotherapy alone	35	5.2
CCRT	312	46.1
CCRT+IC	134	19.8
CCRT+AC	175	25.8
IC+CCRT+AC	21	3.1

Note: According to the AJCC 8th edition staging.
Abbreviations: NPC, nasopharyngeal carcinoma; CCRT, concurrent chemoradiotherapy; IC, induction chemotherapy; AC, adjuvant chemotherapy.

Results

Survival Outcomes

Based on the inclusion criteria, 677 patients with non-metastatic NPC were included in our study. The clinical characteristics of the included patients are shown in Table 1. The median follow-up time was 79 months (ranging from 3 to 126 months). 162 patients (23.9%) died during follow-up. 42 patients (6.2%) developed local and regional recurrence, 75 patients (11.1%) developed distant metastasis, and 3 patients (0.4%) developed

Table 2 Correlation of Circulating Lymphocyte Subsets with Clinical Variables

Clinical Variables	Circulating Lymphocyte Subsets					
	CD3	CD4	CD8	CD4/CD8	CD19	NK
Sex	r =0.030 P =0.439	r =0.032 P =0.399	r =−0.021 P =0.592	r =0.037 P =0.342	r =0.087 P =0.024	r =−0.010 P =0.800
Age	r =−0.160 P <0.001	r =0.049 P =0.204	r =−0.244 P <0.001	r =0.230 P <0.001	r =−0.053 P =0.171	r =0.129 P =0.001
Pathology	r =0.056 P =0.148	r =0.033 P =0.392	r =0.070 P =0.070	r =−0.034 P =0.371	r =0.011 P =0.779	r =−0.076 P =0.048
Tumor stage	r =−0.010 P =0.794	r =−0.011 P =0.783	r =−0.017 P =0.660	r =−0.017 P =0.662	r =−0.100 P =0.010	r =0.054 P =0.163
Node stage	r =0.049 P =0.203	r =−0.002 P =0.966	r =0.038 P =0.328	r =−0.019 P =0.621	r =−0.171 P <0.001	r =−0.004 P =0.922
Clinical stage	r =−0.014 P =0.719	r =−0.009 P =0.818	r =−0.016 P =0.676	r =0.019 P =0.630	r =−0.174 P <0.001	r =0.046 P =0.234

both recurrence and distant metastasis. The 5-year OS, PFS, LRFS, and DMFS for all 677 patients were 82.3%, 77.1%, 95.8%, and 89.7%, respectively.

Correlation Analysis

The percentages of $CD3^+$ T cells and $CD8^+$ T cells were negatively correlated with patients' age ($r = -0.160$, $P < 0.001$, and $r = -0.244$, $P < 0.001$, respectively). The percentages of NK cells and the CD4/CD8 ratio were positively correlated with patients' age ($r = 0.129$, $P = 0.001$, and $r = 0.230$, $P < 0.001$, respectively). The percentage of $CD19^+$ B cells was negatively correlated with tumor stage, node stage and clinical stage ($r = -0.100$, $P = 0.010$; and $r = -0.171$, $P < 0.001$; $r = -0.174$, $P < 0.001$, respectively) (Table 2).

Cutoff Values for Circulating Lymphocyte Subsets

The optimal cutoff value for factors associated with prognosis was determined by ROC curve analysis. When OS was the endpoint of lymphocyte subsets, the optimal cutoff value for $CD19^+$ B cells was 9.55% (AUC = 0.604, sensitivity 46%, specificity 78%, $P < 0.001$) (Figure 1A). When PFS was the endpoint, the $CD19^+$ B cell threshold was 9.55% (AUC = 0.585, sensitivity 46%, specificity 73%, $P = 0.001$), and the $CD4^+$ T cell threshold was 37.05% (AUC = 0.557, sensitivity 36%, specificity 74%, $P = 0.020$) (Figure 1B). When DMFS was used as the endpoint, the $CD4^+$ T cell threshold was 37.05% (AUC = 0.620, sensitivity 36%, specificity 88%, P=0.001) and the CD4/CD8 ratio threshold was 1.35 (AUC = 0.602, sensitivity 60%, specificity 60%, P=0.004) (Figure 1C).

Kaplan–Meier Curves

Lymphocyte subsets were divided into high and low groups according to cutoff values. Compared with the low $CD19^+$ B cell group, the high $CD19^+$ B cell group (> 9.55%) had better 5-year OS (90.4% VS 76.8%, $P < 0.001$), PFS (85.3% VS 71.6%, $P < 0.001$) and DMFS (94% VS 86.8%, $P = 0.002$) (Figure 2). The high $CD4^+$ T cell group (> 37.05%) had better 5-year PFS (83% VS 74.2%, $P = 0.015$) and DMFS (95.8% VS 86.7%, $P < 0.001$) than the low $CD4^+$ T cell group (Figure 3). The high CD4/CD8 ratio group (> 1.35) had better 5-year PFS (79.9% VS 73.4%, $P = 0.024$) and DMFS (93% VS 85.3%, $P = 0.002$) than low CD4/CD8 ratio group (Figure 4).

Univariate and Multivariate Analyses

Pre-treatment neutrophil-to-lymphocyte ratio (NLR), albumin, hemoglobin, and lactate dehydrogenase have been previously reported to be associated with patient's prognosis in NPC.[19–21] Therefore, these prognostic factors were also included in our Cox regression model. The median values for NLR, albumin, hemoglobin, and lactate dehydrogenase were 2.18, 4.7g/L, 140g/L, 170U/L, respectively. And the median levels for $CD3^+$ T cells, $CD8^+$ T cells, and NK cells were 64.9%, 23.1%, and 15%, respectively.

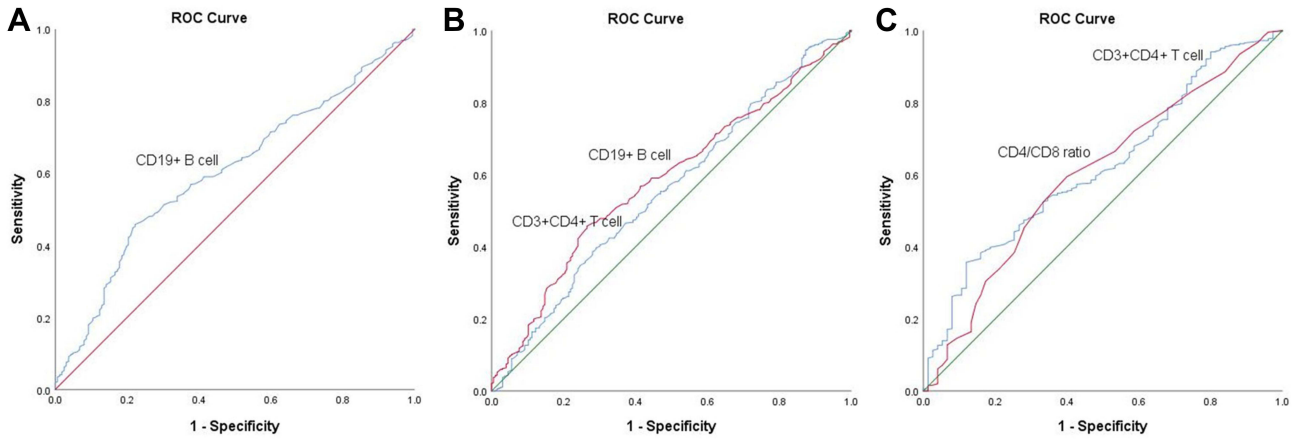

Figure 1 ROC curves of $CD19^+$ B cells based on overall survival (**A**), $CD3^+CD4^+$ T cells and $CD19^+$ B cells based on progression-free survival (**B**), $CD3^+CD4^+$ T cells and CD4/CD8 ratio based on distant metastasis-free survival (**C**).

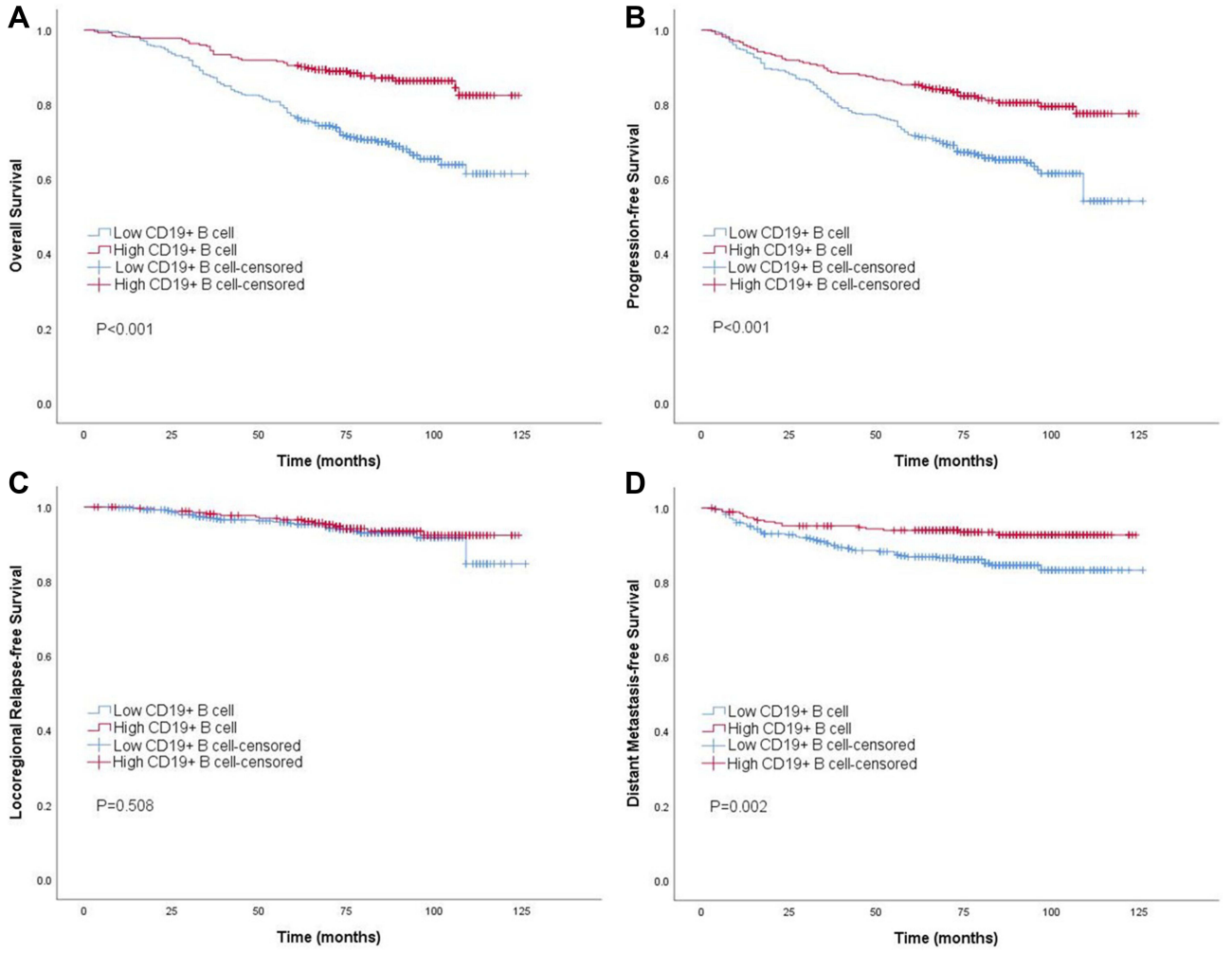

Figure 2 Kaplan–Meier survival curves of high and low $CD19^+$ B cell groups based on overall survival (**A**), progression-free survival (**B**), locoregional relapse-free survival (**C**), distant metastasis-free survival (**D**) in NPC patients.

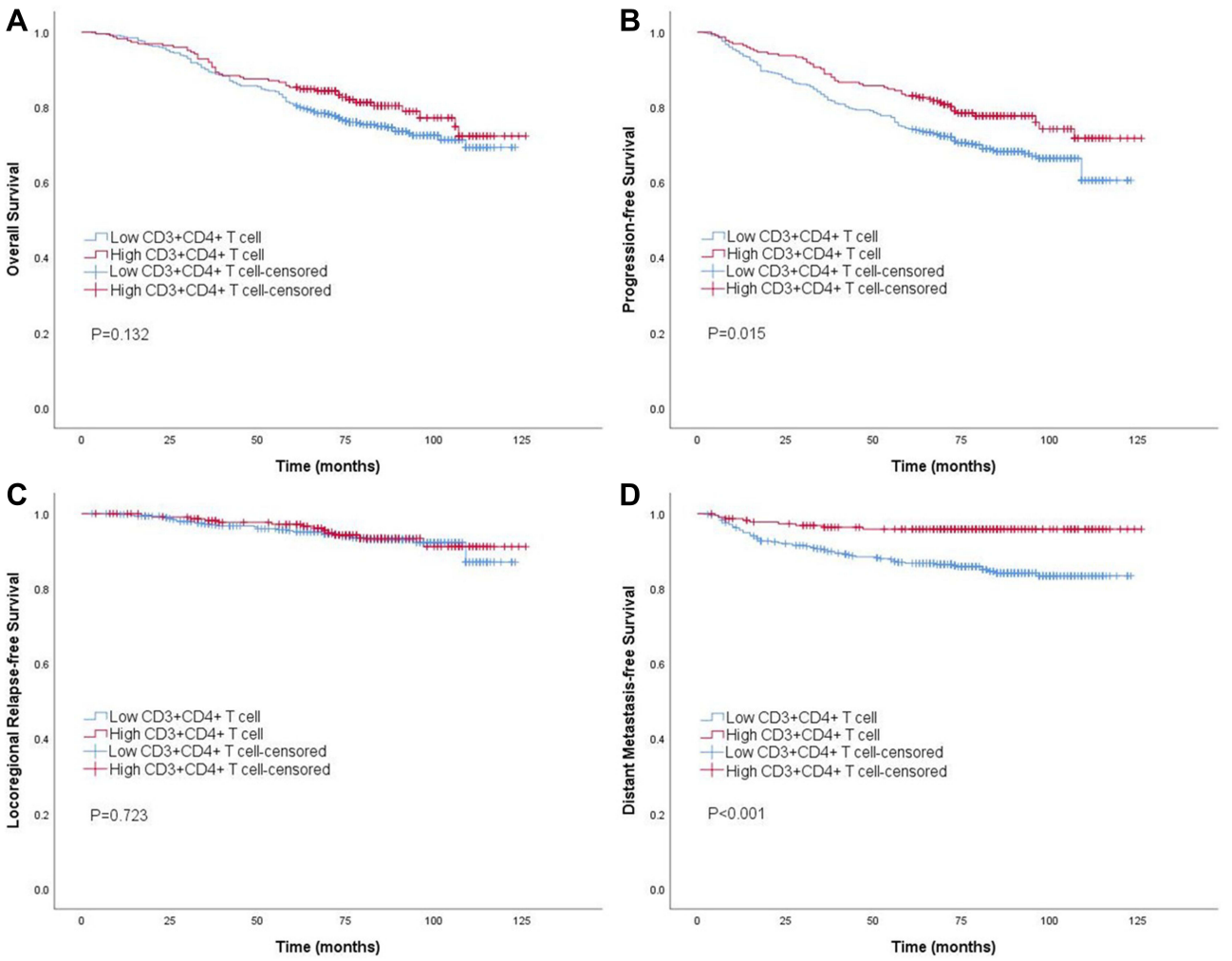

Figure 3 Kaplan–Meier survival curves of high and low $CD3^+CD4^+$ T cell groups based on overall survival (**A**), progression-free survival (**B**), locoregional relapse-free survival (**C**), distant metastasis-free survival (**D**) in NPC patients.

In univariate analysis, the significant factors for OS were age, tumor stage, node stage, clinical stage, NLR, albumin, hemoglobin, lactate dehydrogenase, and $CD19^+$ B cells. The significant factors for PFS were age, tumor stage, node stage, clinical stage, NLR, albumin, hemoglobin, lactate dehydrogenase, $CD4^+$ T cells, CD4/CD8 ratio, and $CD19^+$ B cells. No factors were found to be significantly associated with LRFS in univariate analysis. The significant factors for DMFS were tumor stage, node stage, clinical stage, lactate dehydrogenase, $CD4^+$ T cells, CD4/CD8 ratio, and $CD19^+$ B cells (Table 3).

To determine independent factors of prognosis in patients with NPC, multivariate COX regression analysis was carried out on the significant variables found in univariate analysis ($P < 0.1$). Our analyses revealed that sex (HR 1.797, 95% CI 1.185–2.725, $P = 0.006$), age (HR 0.618, 95% CI 0.449–0.850, $P = 0.003$), tumor stage (HR 0.517, 95% CI 0.323–0.827, $P = 0.006$), node stage (HR 0.545, 95% CI 1.341–2.626, $P = 0.001$), albumin (HR 1.509, 95% CI 1.076–2.116, $P = 0.017$), hemoglobin (HR 1.704, 95% CI 1.196–2.427, $P = 0.003$), lactate dehydrogenase (HR 0.493, 95% CI 0.355–0.684, $P < 0.001$), and $CD19^+$ B cells (HR 2.147, 95% CI 1.468–3.139, $P < 0.001$) were independent predictors for OS. Age (HR 0.691, 95% CI 0.517–0.923, $P = 0.012$), tumor stage (HR 0.532, 95% CI 0.354–0.799, $P = 0.002$), node stage (HR 0.497, 95% CI 0.354–0.698, $P < 0.001$), albumin (HR 1.393, 95% CI 1.024–1.895, $P = 0.035$), hemoglobin (HR 1.435, 95% CI 1.065–1.933, $P = 0.018$), lactate dehydrogenase (HR 0.540, 95% CI 0.401–0.729, $P < 0.001$), $CD4^+$ T cells (HR 1.462, 95% CI 1.018–2.099, $P = 0.040$), and $CD19^+$ B cells (HR 1.736, 95% CI 1.251–2.409, $P = 0.001$) were independent predictors for PFS. Tumor stage (HR 0.363, 95% CI 0.178–0.738, $P = 0.005$), node stage (HR 0.446, 95% CI 0.259–0.768, $P = 0.004$), lactate dehydrogenase (HR 0.444, 95% CI 0.269–0.733, $P = 0.002$), $CD4^+$ T cells (HR 3.124, 95% CI 1.487–

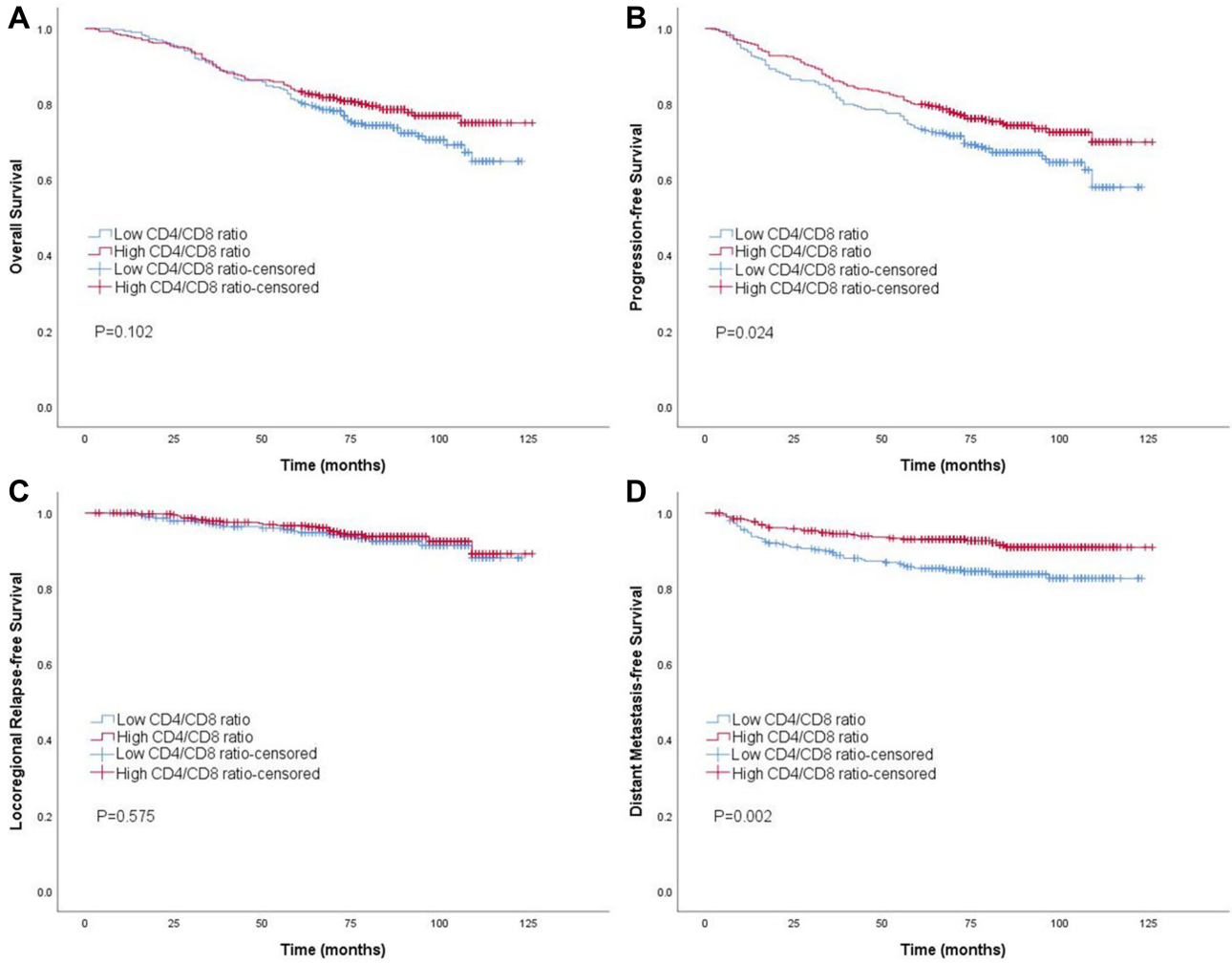

Figure 4 Kaplan–Meier survival curves of high and low CD4/CD8 radio groups based on overall survival (**A**), progression-free survival (**B**), locoregional relapse-free survival (**C**), distant metastasis-free survival (**D**) in NPC patients.

6.562, P = 0.003), and $CD19^+$ B cells (HR 1.847, 95% CI 1.077–3.165, P = 0.026) were independent predictors for DMFS (Table 3). The results showed that low $CD19^+$ B cell and high lactate dehydrogenase were independent prognostic factors associated with poor OS, PFS, and DMFS. Low $CD4^+$ T cell was an independent prognostic factor associated with poor PFS and DMFS. Low albumin and hemoglobin were independent prognostic factors associated with poor OS and PFS. NLR was not an independent prognostic factor in multivariate analysis, although it was significant in univariate analysis for OS and PFS.

Effects of Chemoradiotherapy on Lymphocyte Subsets

Lymphocyte subsets were detected again in 138 patients within 1 month after the end of treatment. These patients received concurrent chemoradiotherapy with or without induction/adjuvant chemotherapy. We observed that the percentages of $CD4^+$ T cells, the percentages of $CD19^+$ B cells, and the CD4/CD8 ratio decreased significantly after chemoradiotherapy, while the percentages of $CD8^+$ T cells and NK cells increased significantly by Wilcoxon signed rank test ($P < 0.05$) (Table 4). The change in NK cells was statistically, although not dramatically, different.

Discussion

The immune system plays a critical role in tumor initiation, progression, treatment, and prognosis. The anti-tumor immune effect of the body is mainly mediated by cellular immunity.[5,6] Studies suggested that inflammation and immune escape are key to tumor progression. Lymphocytes are involved in the whole process of tumor progression and can promote or inhibit tumor growth.[7,22] T cells are main

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Table 3 Multivariate Cox Proportional Hazards Analysis of 677 Patients with NPC

Variable	OS				PFS			
	Univariate		Multivariate		Univariate		Multivariate	
	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)
Sex	0.098	1.387(0.942–2.042)	0.006	1.797(1.185–2.725)	0.399	1.155(0.826–1.616)		
Age	0.000	0.555(0.405–0.761)	0.003	0.618(0.449–0.850)	0.003	0.649(0.489–0.862)	0.012	0.691(0.517–0.923)
Smoking	0.219	1.220(0.888–1.675)			0.400	1.133(0.847–1.515)		
Family history of NPC	0.179	0.657(0.356–1.212)			0.268	0.742(0.438–1.257)		
Pathology	0.297	0.787(0.502–1.234)			0.302	0.804(0.532–1.216)		
Tumor stage	0.000	0.392(0.272–0.566)	0.006	0.517(0.323–0.827)	0.000	0.443(0.321–0.610)	0.002	0.532(0.354–0.799)
Node stage	0.000	0.460(0.334–0.632)	0.001	0.545(0.379–0.786)	0.000	0.462(0.346–0.617)	0.000	0.497(0.354–0.698)
Clinical stage	0.000	0.297(0.175–0.506)	0.954	1.022(0.487–2.146)	0.000	0.327(0.206–0.519)	0.987	1.005(0.528–1.915)
NLR	0.009	0.658(0.481–0.900)	0.273	0.837(0.609–1.151)	0.010	0.689(0.519–0.914)	0.263	0.847(0.634–1.133)
Albumin	0.001	1.695(1.233–2.330)	0.017	1.509(1.076–2.116)	0.009	1.458(1.097–1.939)	0.035	1.393(1.024–1.895)
Hb	0.002	1.650(1.200–2.267)	0.003	1.704(1.196–2.427)	0.003	1.557(1.168–2.074)	0.018	1.435(1.065–1.933)
LDH	0.000	0.490(0.355–0.675)	0.000	0.493(0.355–0.684)	0.000	0.512(0.383–0.684)	0.000	0.540(0.401–0.729)
$CD3^+$ T cells	0.629	1.079(0.793–1.469)			0.437	1.118(0.844–1.480)		
$CD4^+$ T cells	0.134	1.301(0.922–1.835)			0.016	1.481(1.076–2.038)	0.040	1.462(1.018–2.099)
$CD8^+$ T cells	0.417	0.880(0.647–1.198)			0.391	0.885(0.668–1.171)		
CD4/CD8 ratio	0.104	1.291(0.949–1.757)			0.025	1.378(1.042–1.824)	0.125	1.283(0.933–1.764)
$CD19^+$ B cells	0.000	2.641(1.822–3.827)	0.000	2.147(1.468–3.139)	0.000	2.058(1.498–2.828)	0.001	1.736(1.251–2.409)
NK cells	0.596	0.920(0.676–1.252)			0.650	0.937(0.708–1.240)		
Treatment	0.396	0.702(0.311–1.588)			0.287	0.664(0.312–1.412)		
	LRFS				DMFS			
	Univariate		Multivariate		Univariate		Multivariate	
	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)
Sex	0.798	1.097(0.539–2.232)			0.548	0.857(0.518–1.419)		
Age	0.535	1.216(0.656–2.255)			0.690	0.912(0.580–1.434)		
Smoking	0.190	0.621(0.305–1.265)			0.553	1.152(0.722–1.840)		
Family history of NPC	0.640	1.250(0.491–3.187)			0.173	0.497(0.181–1.360)		
Pathology	0.115	0.537(0.248–1.163)			0.423	1.406(0.610–3.239)		
Tumor stage	0.323	0.727(0.387–1.368)			0.000	0.333(0.189–0.587)	0.005	0.363(0.178–0.738)
Node stage	0.245	0.698(0.381–1.280)			0.000	0.397(0.246–0.642)	0.004	0.446(0.259–0.768)
Clinical stage	0.191	0.582(0.258–1.310)			0.001	0.257(0.112–0.592)	0.963	1.027(0.328–3.215)
NLR	0.249	0.698(0.379–1.287)			0.416	0.828(0.526–1.304)		

Albumin	0.970	1.012(0.552–1.854)			0.806	1.058(0.673–1.665)	
Hb	0.323	1.361(0.738–2.510)			0.472	1.182(0.750–1.862)	
LDH	0.901	0.962(0.525–1.764)			0.000	0.369(0.224–0.607)	0.002	0.444(0.269–0.733)
$CD3^+$ T cells	0.784	1.089(0.594–1.996)			0.480	1.178(0.748–1.856)	
$CD4^+$ T cells	0.723	1.126(0.585–2.166)			0.000	3.716(1.852–7.457)	0.003	3.124(1.487–6.562)
$CD8^+$ T cells	0.881	0.955(0.521–1.749)			0.196	0.740(0.469–1.168)	
CD4/CD8 ratio	0.575	1.189(0.649–2.180)			0.002	2.056(1.295–3.264)	0.225	1.359(0.828–2.230)
$CD19^+$ B cells	0.509	1.234(0.661–2.306)			0.002	2.278(1.340–3.873)	0.026	1.847(1.077–3.165)
NK cell	0.931	1.027(0.561–1.881)			0.458	0.842(0.534–1.326)	
Treatment	0.518	1.474(0.455–4.772)			0.158	0.242(0.034–1.739)	

Notes: Sex (male/female), Age (≤46 years/>46 years), Smoking (Yes/No), Family history of NPC (Yes/No), Pathology (undifferentiated/differentiated), Tumour stage (T1+T2/T3+T4), Node stage (N0+N1/N2+N3), Clinical stage (I+II/III +Iva), NLR (≤2.18/>2.18), Albumin (≤44.7g/L/>44.7g/L), Hb (≤140g/L/>140g/L), LDH (≤170U/L/>170U/L), $CD3^+$ T cells (≤64.9%/>64.9%), $CD4^+$ T cells (≤37.05%/>37.05%), $CD8^+$ T cells (≤23.1%/>64.9%), CD4/CD8 ratio (≤1.35/>1.35), $CD19^+$ B cells (≤9.55%/>9.55%), NK cell (≤15%/>15%), Treatment (RT/RT+Chemotherapy).

Abbreviations: NPC, nasopharyngeal carcinoma; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PFS, progression-free survival; LRFS, locoregional relapse-free survival; DMFS, distant metastasis-free survival; Hb, hemoglobin; LDH, lactate dehydrogenase; RT: radiotherapy.

players in the host immune response to tumors. In particular, $CD4^+$ T cells have been described as helper T lymphocytes as they play an important role in immunosurveillance by helping B cells to produce antibodies, and produce interleukin II to activate NK cells, thus exerting a powerful tumoricidal effect. $CD8^+$ T cells are described as cytotoxic T lymphocytes that can exert cell-mediated cytotoxic effects on target cells and suppress immune responses.[22] Under normal physiological circumstances, the ratio of CD4 /CD8 helps a certain ratio to maintain the balance of immune function. When the ratio is higher than normal, the immune system is active, whereas decreased, or even inverted, ratios indicate poor immune function. B cells and NK cells have also been shown to be involved in tumor progression.[6,22–24] Associations between the immune environment and clinical outcome have been demonstrated in many cancers.[25] Accumulating evidence has revealed that levels of circulating lymphocyte subsets have a major impact on tumor prognosis, and may be potential biomarkers for tumor risk stratification.[26,27]

In our study of NPC patients, we observed that the percentage of circulating lymphocyte subsets correlated with patient age, and $CD19^+$ B cells correlated negatively with tumor stage, node stage, and clinical stage. $CD3^+$ T cells and $CD8^+$ T cells correlated negatively with age, and the CD4/CD8 ratio correlated positively with age in NPC patients, which may be related to the progressive degeneration of the thymus with age. After puberty, as the thymus involutes, the circulating T-cell reservoir diminishes, with the most pronounced decline being in the $CD8^+$ T-cell subset, accompanied by an increase in the circulating CD4/CD8 ratio.[28,29] Consistent with previous findings,[15,17] our study further showed a correlation between the level of circulating $CD19^+$ B cells and the clinical stage. These findings suggest that $CD19^+$ B cells may play some critical role in the progression of NPC. Interestingly, our results also indicated that a low level of $CD19^+$ B cells was an independent unfavorable prognostic factor for OS, PFS, and DMFS in NPC.

The association between NPC and Epstein-Barr virus (EBV) infection has been well-established. In EBV associated tumors, B cells are the primary target of virus infection and participate in anti-tumor humoral response by producing antibodies.[30] NPC patients with low $CD19^+$ B cells have a poor prognosis, which may be related to this. Coincidentally, a previous study showed that low $CD19^+$ B cells was a negative prognostic factor for 5-year PFS.[16] Although different results may be related

Table 4 Effects of Chemoradiotherapy on Lymphocyte Subsets

Lymphocyte Subsets	Before Chemoradiotherapy (Median)	After Chemoradiotherapy (Median)	P-value
$CD3^+$ T cells (%)	63.35	64.15	0.536
$CD4^+$ T cells (%)	32.60	21.35	0.000
$CD8^+$ T cells (%)	23.05	29.6	0.000
CD4/CD8 ratio	1.40	0.70	0.000
$CD19^+$ B cells (%)	8.75	3.1	0.000
NK cell (%)	15.65	16.75	0.007

to selection bias and mixed bias, overall, evidence suggests that NPC patients with high $CD19^+$ B cells had a better survival rate. Previous studies have also reported that high B cells are associated with better survival when observed in gastric cancer and head and neck cancer.[31–33]

In our study, Kaplan-Meier survival curves showed that groups with high $CD4^+$ T cells and high CD4/CD8 ratio had better PFS and DMFS than low groups. However, multivariate analysis indicated that $CD4^+$ T cell was an independent prognostic factor for PFS and DMFS in NPC patients, and CD4/CD8 ratio was not an independent prognostic factor. Our results differed from a previous study.[15] Tao et al thought that there was an overlap between $CD4^+$ T cells and CD4/CD8 ratio, only the CD4/CD8 ratio entered into the multivariate analysis, which might explain differences in the results.[15] Our study suggested that patients with low $CD4^+$ T cells had a high risk of developing distant metastasis, although this link could be a result of confounding factors, for a number of reasons. First, $CD4^+$ T cells play an important role in anti-tumor responses by participating in both cellular immune response and humoral immune response.[34] Second, it was reported that the level of $CD4^+$ T cells in the peripheral blood of patients with NPC may be related to immunosuppression and tumor progression.[35] On the other hand, a decrease of $CD4^+$ T cells may be associated with a very short life expectancy in metastatic cancer.[36,37] Our results indicated that NPC patients with low level of $CD4^+$ T cells had a poorer prognosis, suggesting that circulating $CD4^+$ T cells could serve as a predictive biomarker for distant metastasis. With the widespread application of IMRT, the local control of NPC has improved significantly. Currently, the occurrence of distant metastasis is most common reason of treatment failure in patients with NPC.[38,39] The detection of circulating lymphocyte subsets is inexpensive and noninvasive, and may be a potential biomarker for risk stratification to help provide a reference for the development of individualized treatment and follow-up strategies for NPC patients. In addition, the levels of lymphocyte subsets could be increased by means of pharmacologic intervention, offering a new immunotherapy strategy that might help improve patients' outcomes.

Additionally, we analyzed the prognostic value of a number of nutritional and inflammatory indicators. In agreement with previous studies,[19–21] our multivariate analysis indicated that low albumin, low hemoglobin, and high lactate dehydrogenase were associated with a poor prognosis.

By comparing lymphocyte subsets in NPC patients before and within 1 month after chemoradiotherapy, we found that $CD4^+$ T cells, $CD19^+$ B cells, and the CD4/CD8 ratio were significantly decreased, while $CD8^+$ T cells were significantly increased after chemoradiotherapy. These findings are largely consistent with those from Hu et al.[17] Changes in lymphocyte subsets in NPC patients could reflect a reduction of the body's immune function. Chemoradiotherapy not only kills tumor cells, but also causes various degrees of killing and inhibition of normal cells.[40,41] Moreover, different lymphocyte subsets showed different sensitivity to chemoradiotherapy, which may account for increased levels of $CD8^+$ T cells and NK cells.[42] A decline in immune function, increases the risk of infection and is associated with a poor prognosis.[43] Therefore, patients should be monitored for lymphocyte subsets during treatment, and improving the patients' immune function by pharmacologic intervention, likely to result in improvement in patient outcomes.

The limitations of our study should also be noted. Due to the nature of a single-center retrospective study, the findings may be affected by confounding factors and selection bias. Secondly, we were unable to analyze the relationship between circulating lymphocyte subsets and EBV, given that EBV testing was not performed in some of the patients.

Conclusion

In this study, we observed that levels of circulating lymphocyte subsets correlated strongly with the prognosis of patients with NPC. Patients with low levels of $CD19^+$ B cells or $CD4^+$ T cells experienced poorer prognosis compared to those with high levels. In addition, chemoradiotherapy may decrease the body's immune function in NPC patients. Circulating lymphocyte subsets should be monitored throughout the treatment period.

Abbreviations

NPC, Nasopharyngeal carcinoma; ROC, Receiver operating characteristic; OS, Overall survival; PFS, Progression-free survival; LRFS, Locoregional relapse-free survival; DMFS, Distant metastasis-free survival; KPS, Karnofsky Performance Status; IMRT, Intensity modulated radiotherapy; AJCC, American Joint Committee on Cancer; GTV, Gross tumor volume; PTV, Planning target volume; CTV, Clinical target volume; NLR, Neutrophil-to-lymphocyte ratio.

Data Sharing Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

This study was approved by the Ethics Committee of Guangxi Medical University Cancer Hospital (LW2021023), in compliance with the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflict of interest.

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