Prognostic significance of circulating lymphocyte subsets in patients with nasopharyngeal carcinoma

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Research Article

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

Posted Date: July 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-673390/v1

Abstract

Objective

The purpose of this study was to explore the prognostic value of circulating lymphocyte subsets in patients with nasopharyngeal carcinoma (NPC) before treatment and the impact of chemoradiotherapy on lymphocyte subsets.

Methods

934 patients with non-metastatic nasopharyngeal carcinoma were analyzed retrospectively. The percentage of circulating lymphocyte subsets was detected by flow cytometry and analyzed retrospectively. The correlation between lymphocyte subsets and prognosis was evaluated by the log-rank test and Cox proportional hazards model, and the differences of lymphocyte subsets before and after chemoradiotherapy was comparing by the paired t-test and Wilcoxon signed rank test.

Results

Univariate and multivariate analyses indicated that high $CD19^+$ B lymphocytes (HR 0.705, 95%CI 0.519–0.958, P = 0.026) and $CD4^+$ T lymphocytes (HR 0.560, 95%CI 0.331–0.946, P = 0.030) before treatment were independent favorable prognostic factors for overall survival (OS) and distant metastasis-free survival (DMFS) in NPC patients, respectively. After chemoradiotherapy, the percentage of $CD3^+$ T lymphocytes, $CD4^+$ T lymphocytes, CD4/CD8 ratio and $CD19^+$ B lymphocytes decreased significantly, while the percentage of $CD8^+$ T lymphocytes and NK lymphocytes increased significantly.

Conclusion

Patients with high $CD19^+$B lymphocytes and $CD4^+$T lymphocytes have a better prognosis, and perhaps the immune response can be improved to make the prognosis better by increasing the number of lymphocyte subsets. Chemoradiotherapy may reduce the immune function of patients with nasopharyngeal carcinoma.

Introduction

Nasopharyngeal carcinoma (NPC) is a cancer originating from the nasopharyngeal mucosal lining, which is epidemic and threatening in Southern China and Southeast Asia(Chen et al. 2019). The incidence and mortality of nasopharyngeal cancer are obviously different in different countries, and the death cases of nasopharyngeal carcinoma in China account for approximately 40% worldwide(Fu et al. 2018).

Radiotherapy is the main curative treatment for nasopharyngeal carcinoma, and concurrent chemoradiotherapy is recommendeded for locally advanced patients (Chen et al. 2019).

The TNM staging system is used to predict patients' prognosis in NPC. However, in patients with the same TNM stage who received similar treatment, the clinical results are quite different (Huang et al. 2020; Wu et al. 2019). This shows that the TNM staging system is not enough to accurately predict the prognosis of patients with NPC. Thus, new biomarkers are urgently demanded, which can reflect the biological and immunological heterogeneity of NPC, provide more accurate prognostic information, and further facilitate individualized treatment for this disease.

The occurrence, development, and outcome of tumors are closely related to the body's immune function. A wealth of attention has been paid to the important role of the immune system in affecting the prognosis of tumors. And the immune status of the body has the potential to be an important factor affecting prognosis(Fridman et al. 2017; Quigley and Kristensen 2015; Wenbo and Wang 2017). As an experimental marker, clinically circulating lymphocyte subsets are commonly used to reflect the body's immune function(de Pablo et al. 2014; Miller 2020). And previous studies have shown that specific circulating lymphocyte subset levels are related to poor prognosis in NPC patients(Chen et al. 2018; Hu et al. 2012; Lu et al. 2018; Tao et al. 2016; Xu et al. 2014). But 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 hold the advantages of easy accessibility, reproducibility and easy detection. Therefore, we expanded the study to explore the significance of circulating lymphocyte subsets for the prognosis of NPC and the changes of lymphocyte subsets after chemoradiotherapy in order to provide reference information for individualized treatment and follow-up strategies in nasopharyngeal carcinoma.

Materials And Methods

Patients

The information on the cases of nasopharyngeal carcinoma was collected at the Guangxi Medical University Cancer Hospital from 2010 to 2014. The inclusion criteria:（1）pathologically diagnosed as nasopharyngeal carcinoma without distant metastasis;（2）treat by intensity modulated radiotherapy (IMRT);（3）Karnofsky Performance Status (KPS) ≥ 70 points;（4）detect the percentage of circulating lymphocyte subsets before treatment. The exclusion criteria:（1）severe infection;（2）patients with immune system diseases or recent use of drugs affecting the body's immune function;（3）patients with severe liver and kidney dysfunction;（4）a synchronous malignancy. This study was approved by the Ethics Committee of Guangxi Medical University Cancer Hospital and conformed to the Declaration of Helsinki. But informed consent could not be obtained because of the retrospective nature. The data was either confidential or anonymized. All patients were staged again on the basis of the 8th edition of the American Joint Commission on Cancer (AJCC) staging system.

Treatment methods

The patients we included were treated with IMRT. The 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. In chemotherapy, patients with locally advanced NPC received concurrent chemoradiotherapy, combined induction chemotherapy (IC) or adjuvant chemotherapy (AC). And induction or adjuvant chemotherapy regimen consisted of docetaxel, cisplatin or 5-fluorouracil. Induction chemotherapy was administered for 1-4 cycles every 3 weeks. And adjuvant chemotherapy was administered for 1-6 cycles every 3 weeks. As for concurrent chemotherapy, platinum was applied for 1 to 3 cycles every 3 weeks.

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 subset were determined by flow cytometry using an epics XL flow cytometer (Beckman Coulter, USA) and the corresponding kits, including $CD3^+$ T lymphocytes, $CD3^+$ $CD4^+$ T lymphocytes, $CD3^+$ $CD8^+$ T lymphocytes, $CD19^+$ B lymphocytes and $CD16^+$ $CD56^+$ NK lymphocytes and the CD4/CD8 ratio was calculated.

Prognostic assessment and patient follow-up

The prognosis of patients is mainly evaluated by overall survival (OS), secondary by progression-free survival (PFS), locoregional relapse-free survival (LRFS) and distant metastasis-free survival (DMFS). The definition of OS was the time from the date of patient's diagnosis to the date of death for any cause or last follow-up, the definition of PFS was the time from the date of patient's diagnosis to the date of disease progression, death or last follow-up, the definition of LRFS was the time from the date of patient's diagnosis to the date of local and regional recurrence or last follow-up, and the definition of DMFS was the time from the date of patient's diagnosis to the date of distant metastasis or last follow-up.

After treatment, we paid a follow-up visit to patients every three months in the first two years, every six months for the next three years, and then once a year until death. The date of 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 cut-off point for lymphocyte subsets, and lymphocytes were grouped according to the cut-off value. The survival rates were calculated using the Kaplan-Meier method and survival curves were compared by the log-rank

test. Cox proportional hazards model was used for univariate and multivariate analysis to analyze the factors related to prognosis.

Differences between the percentages of lymphocyte subsets before and within 1 month after chemoradiotherapy were tested for normality using the Kolmogorov Smirnov test, paired t-test was used for data that conformed to normal distribution, and the Wilcoxon signed rank test was used for data that did not conform to normal distribution. All of the reported probability values were two-tailed, and $p< 0.05$ was considered statistically significant. All statistical analyses were carried out by the 25th edition of SPSS (IBM Corp, Armonk, NY, USA).

Results

Survival Outcomes

Our study included 934 patients with non-metastatic NPC, and patients' clinical characteristics are shown in table1. The median follow-up time for all patients was 75 months (ranging from 2 to 126 months). 190 patients (20.3%) died during follow-up. 51 (5.5%) patients developed local and regional recurrence, 100 (10.7%) patients developed distant metastasis, and 3 (0.3%) patients developed both recurrence and distant metastasis. The 5-year OS, PFS, LRFS, and DMFS for all 934 patients were 83.8%, 78.0%, 96%, and 89.7%, respectively.

Correlation Analysis

The percentages of $CD3^+$ T lymphocytes and $CD8^+$ T lymphocytes were slightly negatively correlated with patients' age ($r = -0.150$, $P < 0.001$; $r = -0.239$, $P < 0.001$, respectively). While the percentages of NK lymphocytes and the CD4/CD8 ratio were slightly positively correlated with patients' age ($r = 0.109$, $P = 0.001$; and $r = 0.223$, $P < 0.001$, respectively). The percentage of $CD19^+$ B lymphocytes were slightly negatively correlated with node stage, clinical stage ($r = -0.120$, $P < 0.001$; $r = -0.115$, $P < 0.001$, respectively).

Cutoff Values for circulating lymphocyte subsets

The optimal cutoff value for the prognosis was determined by ROC curve analysis. When the OS was taken as the endpoint of lymphocyte subset, the optimal cut-off value for the percentage of $CD19^+$ B lymphocytes was 9.55% (AUC=0.549, sensitivity 46.0%, specificity 67.0%, p=0.04). When DMFS was used as the endpoint, the optimal cut-off value for the percentage of $CD4^+$ T lymphocytes was 37.05% (AUC=0.584, sensitivity 37.0%, specificity 81.0%, P=0.007) and for the CD4/CD8 ratio was 1.35 (AUC=0.568, sensitivity 59.0%, specificity 56.0%, P=0.032). The ROC curve is shown in Fig.1.

Kaplan–Meier Curves

The cutoff values of $CD19^+$ B lymphocytes, $CD4^+$ T lymphocytes, and CD4/CD8 ratio were 9.55%, 37.05%, 1.35, and the median values of $CD3^+$ T lymphocytes, $CD8^+$ T lymphocytes, and NK lymphocytes were 65.1%, 23.25%, and 14.8%, respectively. Lymphocyte subsets were divided into high and low groups according to these values.

Compared with the low $CD19^+$ B cell group, the high $CD19^+$ B cell group (> 9.55%) had better 5-year OS (87.7% VS 80. 9%, P = 0.002), PFS (82% VS 75.1%, P = 0.013) and DMFS (92% VS 87. 9%, P = 0.045) (Fig.2). The 5-year DMFS of the high $CD4^+$ T cell group (> 37.05%) was significantly higher than that of the low $CD4^+$ T cell group (93.3% VS 87.8%, P=0.004) (Fig.3). The 5-year DMFS of the high CD4/CD8 ratio group (> 1.35) was significantly higher than that of the low CD4/CD8 ratio group (92% VS 86.7%, P=0.008) (Fig.4).

Univariate and multivariate analyses

The variable in univariate analysis was gender (male vs. female), age (≤ 46 years vs. > 46 years), smoking (yes vs. no), family history of NPC (yes vs. no), tumour stage (T1 + T2 vs. T3 + T4), node stage (N0 + N1 vs. N2 + N3), clinical stage (I + II vs. III + IVA), $CD3^+$ T lymphocytes (≤ 65.1% vs. > 65.1%), $CD4^+$ T lymphocytes (≤ 37.05% vs. > 37.05%), and $CD8^+$ T lymphocytes (≤ 23.25% vs. > 23.25%), CD4/CD8 ratio (≤ 1.35 vs. > 1.35), $CD19^+$ B lymphocytes (≤ 9.55% vs. > 9.55%), NK lymphocytes (≤ 14.8% vs. > 14.8%), respectively. In univariate analysis, the significant factors affecting OS included : $CD19^+$ B lymphocytes (HR 0.618, 95% CI 0.456-0.838, P = 0.002), age (HR 1.681, 95% CI 1.260-2.243, P = 0.000), tumour stage (HR 2.507, 95% CI 1.785-3.523, P = 0.000), node stage (HR 2.020, 95% CI 1.503-2.713, P = 0.000), clinical stage (HR 3.288, 95% CI 1. 997-5.412, P = 0.000). In univariate analysis, the significant factors affecting DMFS included: $CD4^+$ T lymphocytes (HR 0.503, 95% CI 0.311-0.814, P = 0.005), CD4/CD8 ratio (HR 0.589, 95% CI 0.397-0.875, P = 0.009), $CD19^+$ B lymphocytes (HR 0.655, 95% CI 0.431-0.994, P= 0.047), node stage (HR 2.225, 95% CI 1.471-3.367, P = 0.000), clinical stage (HR 4.145, 95% CI 1. 922-8. 937, P = 0.000) (Table 2).

In order to determine independent prognostic factors for OS and DMFS, multivariate Cox analysis was carried out on the significant variables found in univariate analysis. The analyses revealed that $CD19^+$ B lymphocytes (HR 0.705, 95% CI 0.519-0. 958, P = 0.026), age (HR 1.691, 95% CI 1.265-2.260, P = 0.000), tumour stage (HR 2.253, 95% CI 1.463-3.469, P = 0.000), and node stage (HR 1.877, 95% CI 1.341-2.626, P = 0.000) were independent prognostic factors for OS in NPC patients. $CD4^+$ T lymphocytes (HR 0.560, 95% CI 0.331-0. 946, P = 0.030), tumour stage (HR 2.522, 95% CI 1.383-4.599, P = 0.003), and node stage (HR 2.044, 95% CI 1.288-3.243, P = 0.002) were independent prognostic factors for DMFS in NPC patients (Table 2).

Effects of chemoradiotherapy on lymphocyte subsets

There were only 176 patients whose circulating lymphocyte subsets were reviewed within 1 month after the end of treatment. These patients were treated with concurrent chemoradiotherapy with or without

induction / adjuvant chemotherapy. The differences in lymphocyte subsets before and after chemoradiotherapy were tested for normality, and we found that the differences between CD4/CD8 ratios did not follow a normal distribution, and the differences in all other lymphocyte subsets followed a normal distribution. Wilcoxon signed rank test was used for CD4/CD8 ratio and paired t-test was used for other lymphocyte subsets. The results showed that the percentages of $CD3^+$ T lymphocytes, $CD4^+$ T lymphocytes, and $CD19^+$ B lymphocytes and the CD4/CD8 ratio decreased significantly after chemoradiotherapy, while the percentages of $CD8^+$ T lymphocytes and NK lymphocytes increased significantly ($P < 0.05$) (Table 3).

Discussion

The body's immune system is critically involved in tumor initiation, progression, treatment, and prognosis. The anti-tumor immune effect of the body is mainly mediated by cellular immunity(Fridman et al. 2017; Wenbo and Wang 2017). Inflammation and immune escape are key to tumor progression, and lymphocytes are involved in the whole process of tumor progression. Most lymphocytes have both dual functions of promoting tumor growth and inhibiting tumor growth. T lymphocytes are major players in the host immune response to tumors, which include $CD3^+CD4^+$ T lymphocytes and $CD3^+CD8^+$ T lymphocytes. $CD4^+$ T lymphocytes are described as helper T lymphocytes and play an important role in immunosurveillance, being able to help B lymphocytes produce antibodies and produce interleukin II to activate NK lymphocytes, thus exerting a powerful tumoricidal effect. $CD8^+$ T lymphocytes are described as cytotoxic T lymphocytes that can exert cell-mediated cytotoxic effects on target cells and suppress immune responses(Biswas 2015). Under normal circumstances, the ratio of CD4 /CD8 T lymphocytes keep a certain balance ratio to maintain the balance of immune function. When the ratio is higher than normal, the immune system is active, and the ratio decreased, or even inverted indicates that the immune state is very poor. B lymphocytes and NK lymphocytes have also been proved to correlate with tumor progression(Biswas 2015; Chraa et al. 2019; Fridman et al. 2017; Lakshmi Narendra et al. 2013). In many cancers, a correlation between the immune environment and clinical outcome has been demonstrated(Fridman et al. 2012). Recently, accumulating evidence has revealed that circulating lymphocyte subsets have a major impact on tumor prognosis, which may be potential biomarkers for tumor risk stratification(Cui et al. 2019; Yang et al. 2017).

In our study, we observed that the percentage of circulating lymphocyte subsets correlated slightly with age, and $CD19^+$B lymphocytes correlated negatively with node stage and clinical stage. There was a negative correlation between $CD3^+$ T lymphocytes and $CD8^+$ T lymphocytes and age in NPC patients, and a positive correlation between CD4/CD8 ratio and age, which may be related to the progressive degeneration of the thymus with age. It has been reported that after puberty, as the thymus involutes, the circulating T-cell reserve diminishes with age, especially with the most pronounced decline in the $CD8^+$ T-cell subset, accompanied by an increase in the circulating CD4/CD8 ratio(Kumar et al. 2018; Li et al. 2019). Consistent with previous findings(Hu et al. 2012; Tao et al. 2016), our study further confirmed that

the level of circulating $CD19^+$ B lymphocytes correlated with clinical stage. This suggests that $CD19^+$ B lymphocytes may play some critical role in the progression of NPC.

Interestingly, our results show that high $CD19^+$ B lymphocytes are a protective factor for OS in NPC. As we know, nasopharyngeal carcinoma is a malignant tumor associated with EBV infection. In EBV associated tumors, B lymphocytes are the primary objective of virus infection and participate in anti-tumor humoral response by producing antibodies (Tsao et al. 2017). In this study, univariate analysis indicated that NPC patients with high $CD19^+$ B lymphocytes had better OS, PFS, and DMFS. But multivariate analysis showed that $CD19^+$ B lymphocytes were only an independent prognostic factor for OS. Similarly, a previous study showed that Low $CD19^+$B lymphocytes was identified as a negative prognostic factor for 5-year PFS (Xu et al. 2014). The different results may be related to selection bias and mixed bias. Nevertheless, these studies collectively confirmed that NPC patients with high $CD19^+$ B lymphocytes had a better survival rate. In addition, previous studies have reported that B lymphocytes are associated with better survival when observed in gastric cancer and head and neck cancer(Liang et al. 2020; Pretscher et al. 2009; Yu et al. 2012).

On the other hand, Kaplan Meier survival curves showed that DMFS in the high $CD4^+$ T lymphocytes and high CD4/CD8 ratio groups were better than that in the low group. Univariate analysis indicated that the percentage of $CD4^+$ T lymphocytes and CD4/CD8 ratio were significantly associated with DMFS. And multivariate analysis showed that the percentage of $CD4^+$ T lymphocytes was an independent prognostic factor for distant metastasis in NPC patients. The results differed from a previous study (Tao et al. 2016). Tao et al thought that CD4/CD8 ratio was an independent prognostic factor for DMFS of nasopharyngeal carcinoma. And they considered that there was an overlap between $CD4^+$ T cells and CD4/CD8 ratio, so $CD4^+$ T cells were not included in the multivariate regression model. Whereas in our study, significant variables in univariate analysis were all included in the multivariate regression model. Our study suggested that patients with low $CD4^+$ T lymphocytes had a high risk of distant metastasis. The mechanism may be interpreted by various confounding factors. First, $CD4^+$ T cells play an important role in anti-tumor responses by participating in both cellular immune response and humoral immune response(Gerloni and Zanetti 2005). Second, previous study reported that the relative increase of $CD4^+$ T lymphocytes in the peripheral blood of patients with NPC may be related to immunosuppression and tumor progression(Liu et al. 2005). Besides, it was reported that a decrease of $CD4^+$ T lymphocytes may be associated with a very short life expectancy in metastatic cancer(Péron et al. 2013; Trédan et al. 2013). Similarly, our results suggested that patients with low $CD4^+$ T lymphocytes had a poor prognosis in NPC patients and circulating $CD4^+$ T lymphocytes might serve as a predictive biomarker for distant metastasis.

With the widespread application of IMRT, the local control of nasopharyngeal carcinoma has been significantly improved. Distant metastasis is the main pattern of treatment failure in NPC at present(Mao et al. 2016; Tian et al. 2019). Our study demonstrates that the $CD4^+$T lymphocytes may be an important

predictive factor of distant metastasis in nasopharyngeal carcinoma. The detection of circulating $CD4^+$ T lymphocytes is inexpensive and convenient, and it is one of the potential biomarkers that can be used for risk stratification to provide a reference for the development of individualized treatment and follow-up strategies for NPC patients. Since our study showed that patients with high $CD19^+$B cells and $CD4^+$T cells had a better prognosis, it may be a direction of immunotherapy in the future to improve the prognosis by increasing the number of lymphocyte subsets.

By comparing lymphocyte subsets in NPC patients before and within 1 month after chemoradiotherapy, we found that the percentages of $CD3^+$ T lymphocytes, $CD4^+$ T lymphocytes, and $CD19^+$ B lymphocytes and the CD4/CD8 ratio were significantly decreased, and the percentages of $CD8^+$ T lymphocytes and NK lymphocytes were significantly increased after chemoradiotherapy. The changes in lymphocyte subsets indicate that chemoradiotherapy may reduce the body's immune function in NPC patients. This is largely consistent with the findings of Hu et al(Hu et al. 2012). Chemoradiotherapy not only kills tumor cells, but also causes various degrees of killing and inhibition of normal cells (Lv et al. 2020; Sage et al. 2016). Besides, different lymphocyte subsets showed different sensitivity to chemoradiotherapy, which may account for the elevated levels of $CD8^+$ T lymphocytes and NK lymphocytes(Cesaire et al. 2020). A decline in patient immune function, in addition to increasing the risk of infection, is associated with a poor prognosis(Liu et al. 2018). Consequently, patients should be monitored for lymphocyte subsets during treatment, and the immune function of patients should be improved, which will be beneficial for improving patient outcomes.

The drawback of our study should 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 can't analyze the relationship between circulating lymphocyte subsets and EBV, because many people didn't detect EBV in this study.

In conclusion, circulating lymphocyte subsets correlated strongly with the prognosis of NPC patients. Patients with high $CD19^+$ B lymphocytes had a better survival, and patients with low $CD4^+$ T lymphocytes have a high risk of distant metastasis. Perhaps, lymphocyte subsets can be elevated by pharmacologic intervention, which may improve patient immune function to achieve improved outcomes. In addition, chemoradiotherapy may decrease the body's immune function in NPC patients. We should pay attention to monitoring circulating lymphocyte subsets in patients throughout the treatment period.

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Tables

Tables

Table 1: Baseline characteristics of patients (N = 934).

Characteristics	No of patients (range)	Percent
Age (Years)		
≤46	490	52.5
>46	444	47.5
Gender		
Males	692	74.1
Females	242	25.9
Smoking		
No	625	66.9
Yes	309	33.1
Family history of NPC		
No	845	90.5
Yes	89	9.5
Pathologic classification（1978 WHO）		
WHO I	3	0.3
WHO II	106	11.3
WHO III	825	88.3
Primary tumor (T) stage		
T1-2	367	39.3
T3-4	567	60.7
Regional lymph nodes (N) stage		
N0-1	474	50.7
N2-3	460	49.3
Clinical stage (AJCC 8th, 2016)		
I-II	203	21.7
III-IVa	731	78.3
Treatment received by patients		
Radiotherapy alone	46	4.9
CCRT	418	44.8

CCRT+IC/AC	468	50.1
Radiotherapy alone+IC/AC	2	0.2

Table 2: Multivariate Cox proportional hazards analysis of 934 patients with NPC

Variable	Univariate analysis			Multivariate analysis		
	HR	95%CI	P-value	HR	95%CI	P-value
OS						
Sex	0.702	0.490-1.006	0.054			
Age	1.681	1.260-2.243	0.000	1.691	1.265-2.260	0.000
Smoking	1.161	0.864-1.561	0.322			
Family history of NPC	0.618	0.344-1.110	0.107			
Tumour stage	2.507	1.785-3.523	0.000	2.253	1.463-3.469	0.000
Node stage	2.020	1.503-2.713	0.000	1.877	1.341-2.626	0.000
Clinical stage	3.288	1.997-5.412	0.000	1.124	0.562-2.247	0.742
$CD3^+$ T cells (%)	0.892	0.670-1.186	0.432			
$CD4^+$ T cells (%)	0.824	0.604-1.124	0.221			
$CD8^+$ T cells (%)	0.980	0.737-1.302	0.887			
CD4/CD8 ratio	0.874	0.657-1.162	0.354			
$CD19^+$ B cells (%)	0.618	0.456-0.838	0.002	0.705	0.519-0.958	0.026
NK cell (%)	1.038	0.781-1.380	0.797			
DMFS						
Sex	1.149	0.742-1.778	0.533			
Age	0.952	0.642- 1.412	0.806			
Smoking	0.994	0.655- 1.508	0.978			
Family history of NPC	0.584	0.256- 1.333	0.202			
Tumour stage	2.865	1.755-4.678	0.000	2.522	1.383-4.599	0.003
Node stage	2.225	1.471-3.367	0.000	2.044	1.288-3.243	0.002
Clinical stage	4.145	1.922-8.937	0.000	1.233	0.447-3.405	0.686
$CD3^+$ T cells (%)	0.871	0.588-1.291	0.491			
$CD4^+$ T cells (%)	0.503	0.311-0.814	0.005	0.560	0.331-0.946	0.030
$CD8^+$ T cells (%)	1.252	0.844-1.857	0.263			
CD4/CD8 ratio	0.589	0.397-0.875	0.009	0.756	0.490-1.169	0.209

$CD19^+$ B cells (%)	0.655	0.431-0.994	0.047	0.759	0.498-1.159	0.202
NK cell (%)	0.959	0.648-1.419	0.833			

Table 3: Effects of chemoradiotherapy on lymphocyte subsets

Lymphocyte subsets	Before chemoradiotherapy	After chemoradiotherapy	P-value
$CD3^+$ T cells (%)	64.48±11.64	62.56±12.65	0.044
$CD4^+$ T cells (%)	33.74±9.60	22.58±10.73	0.000
$CD8^+$ T cells (%)	23.98±7.43	31.23±11.40	0.000
CD4/CD8 ratio	1.40（1.10-1.98）	0.70（0.40-1.10）	0.000
$CD19^+$ B cells (%)	9.56±5.50	4.37±4.18	0.000
NK cell (%)	16.28±8.93	19.18±9.78	0.000

Figures

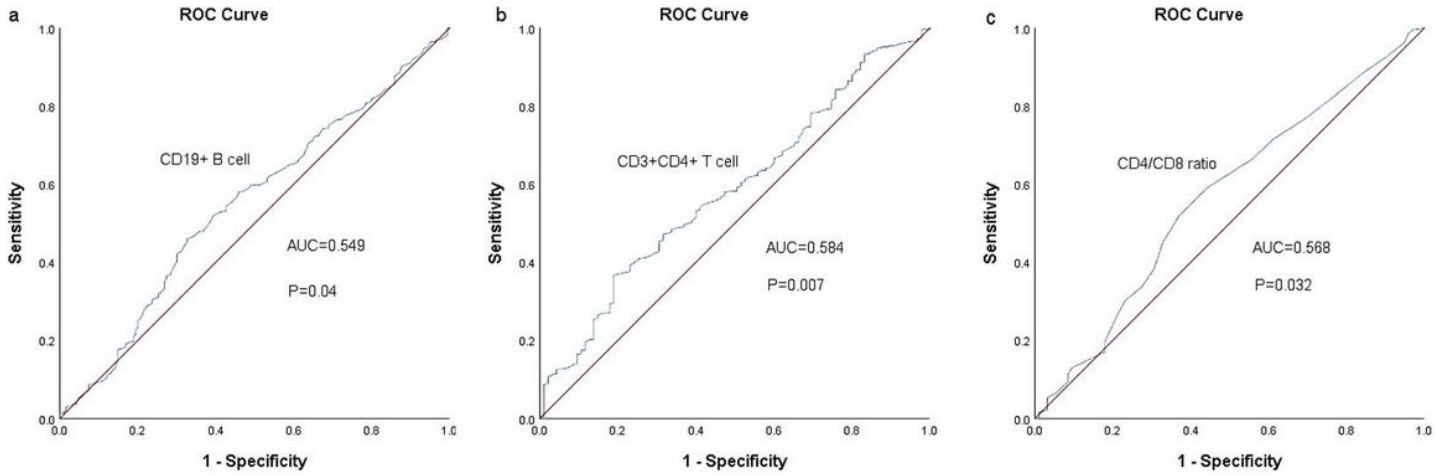

Figure 1

The ROC curve is shown in Fig.1.

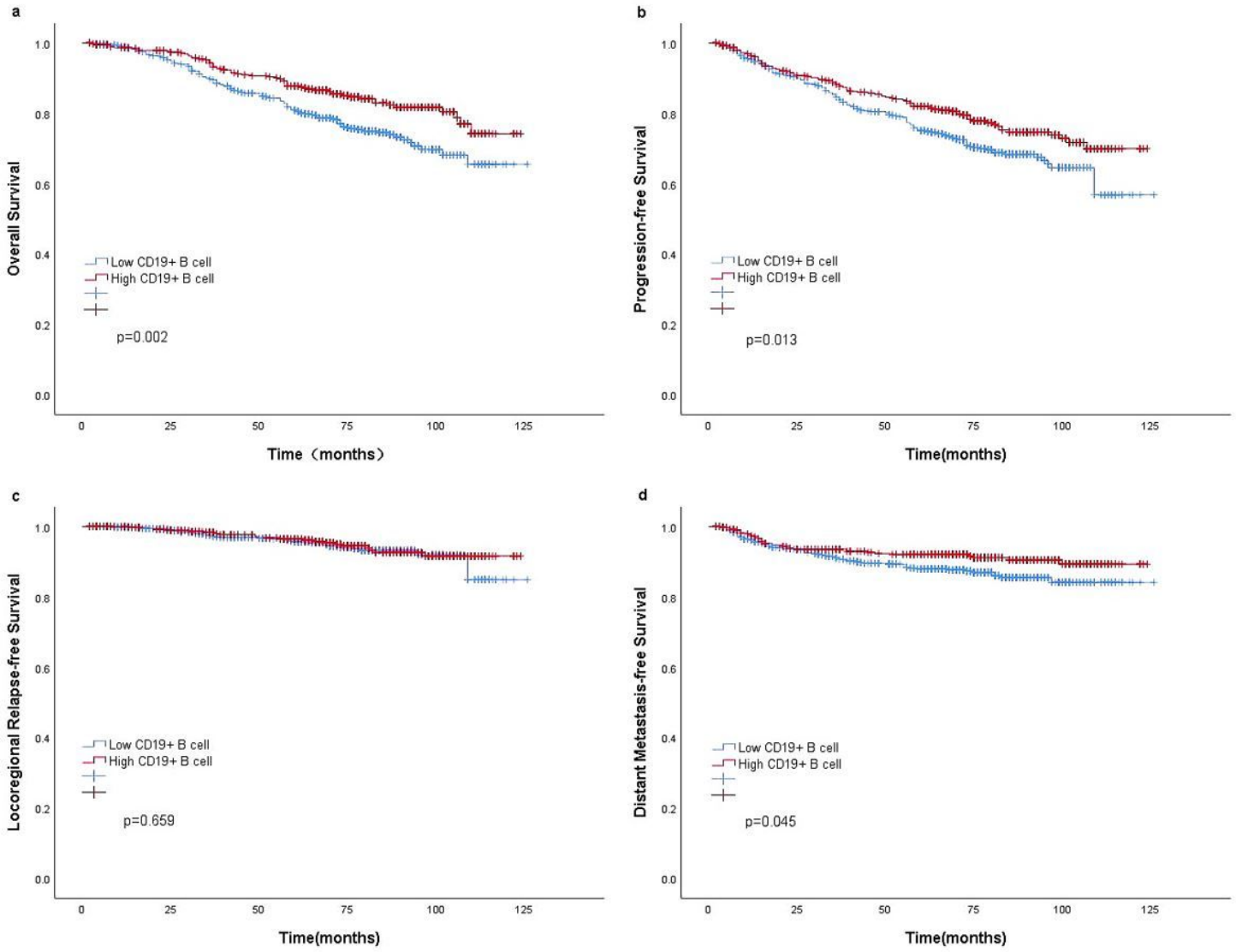

Figure 2

Compared with the low CD19+ B cell group, the high CD19+ B cell group (> 9.55%) had better 5-year OS (87.7% VS 80. 9%, P = 0.002), PFS (82% VS 75.1%, P = 0.013) and DMFS (92% VS 87. 9%, P = 0.045) (Fig.2).

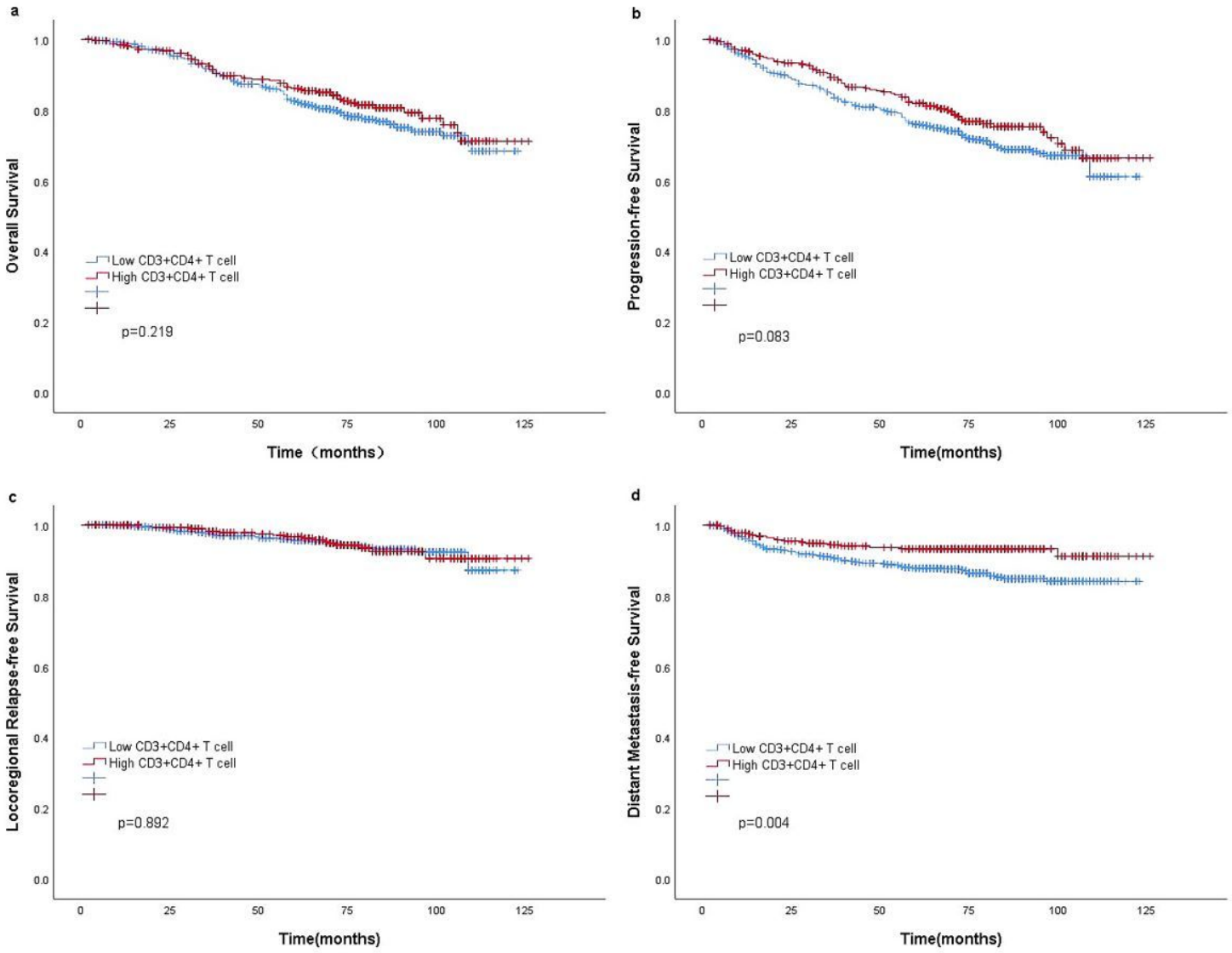

Figure 3

The 5-year DMFS of the high CD4+ T cell group (> 37.05%) was significantly higher than that of the low CD4+ T cell group (93.3% VS 87.8%, P=0.004) (Fig.3).

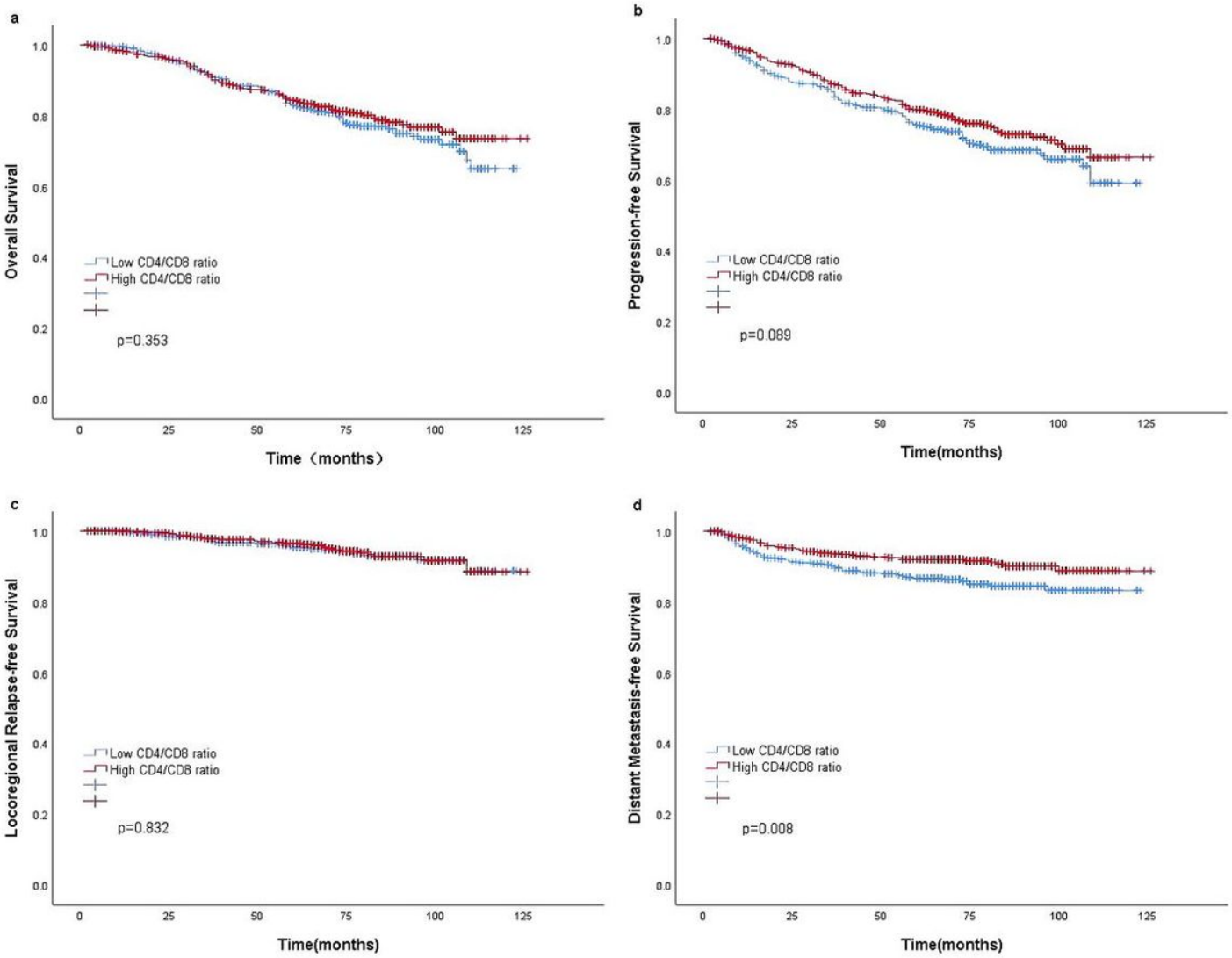

Figure 4

The 5-year DMFS of the high CD4/CD8 ratio group (> 1.35) was significantly higher than that of the low CD4/CD8 ratio group (92% VS 86.7%, P=0.008) (Fig.4).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PrognosticsignificanceoflymphocytesubsetsinNPC.pdf