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ORIGINAL RESEARCH

Treatment-Related Lymphopenia is Possibly a Marker of Good Prognosis in Nasopharyngeal Carcinoma: a Propensity-Score Matching Analysis

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Purpose: The aims of the study were to monitor circulating lymphocyte subset counts before and after therapy for nasopharyngeal carcinoma (NPC), and investigate their relationships with patient outcomes.

Patients and Methods: Subjects comprised patients with TNM stage I–IVA NPC who underwent radiotherapy. Peripheral venous blood samples were collected before and after treatment. Lymphocyte subset counts were analyzed by flow cytometry. Differences between post-treatment and baseline counts were calculated to determine Δ values. Patients were divided into high and low groups, based on median lymphocyte subset counts; propensity score matching was applied to balance groups. Progression-free survival (PFS) and overall survival (OS) were plotted using Kaplan-Meier curves and compared using a Log rank test. Relationships between lymphocyte subset counts and patient survival were subjected to Cox regression analysis.

Results: Patients with NPC (n=746) were enrolled from 2012–2022. Higher $CD8^+$ and total T cell baseline counts were associated with better 5-year PFS (73.7% vs 63.1%, P=0.002 and 73.8% vs 64.1%, P=0.005, respectively). Similarly, higher Δ values of $CD4^+$ and total T cells were associated with higher 5-year PFS (76.2% vs 63.5%, P=0.001; 74.3% vs 65.4%, P=0.010) and OS (89.8% vs 81.6%, P=0.005; 88.6% vs 82.5%, P=0.009). Multivariate Cox regression revealed that $CD8^+$ (hazard ratio (HR) 0.651, P=0.002) and total T (HR 0.600, P<0.001) cells were significantly associated with PFS. $CD4^+$ (HR 0.708, P=0.038) and total T (HR 0.639, P=0.031) cells were independent prognostic factors for OS.

Conclusion: NPC patients with low total or $CD8^+$ T cell counts before treatment had worse prognosis; however, those with more significant decreases in total or $CD4^+$ T cells possibly had better outcomes. T cell counts can be reliable indicators to predict prognosis.

Keywords: chemoradiotherapy, T cell counts, progression-free survival, overall survival, propensity score matching

Introduction

Nasopharyngeal carcinoma (NPC) is a type of cancer that occurs in the epithelium of the nasopharynx and is particularly common in southern China.[1] For many years, radiation therapy and chemotherapy have been the primary treatments for NPC,[1,2] and have achieved 5-year OS rates around 80%;[3] however, approximately 15% of patients experience recurrence or metastasis within five years,[4] with median progression-free survival (PFS) and overall survival (OS) rates after salvage treatment of around 7.9 and 15.7 months, respectively.[5] TNM stage is the most commonly used method for predicting the prognosis of patients with NPC; however, tumor heterogeneity and treatment-related changes can lead to considerable variability in the outcomes observed among patients who share the same staging classification.[6,7] Recent research has shown that, in addition to TNM staging, several factors can be used to predict the prognosis of NPC. These factors include the level of Epstein-Barr virus (EBV) DNA, primary and nodal gross tumor volume, neutrophil-lymphocyte ratio,

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C-reactive protein/albumin ratio, anemia, platelet count, lactate dehydrogenase, maximum primary tumor standardized uptake value, and total lesion glycolysis; however, the correlation between the diverse stratification system and immunotherapy remains inadequately robust.[8] Hence, there is a pressing need to develop a prognostic approach that can accurately predict patient prognosis and identify those at high risk, which is also linked to the efficacy of future immunotherapy.

The immune system is a crucial determinant in the prevention of cancer development and progression. Lymphocytes, which reflect patient immune function, coordinate cellular immunity and are the primary mediators of antitumor immune responses. In the tumor microenvironment, tumor-infiltrating lymphocytes (TILs) are heterogeneous, dynamic, and exhausted. These cells are abundant within "hot" tumors, examples of such tumors include certain melanomas, lung cancers, and bladder cancers, which demonstrating favorable responses to immunotherapy. Conversely, "cold" tumors, such as pancreatic cancers, glioblastomas, and ovarian cancers, exhibit a sparse presence of TILs, correlated with diminished efficacy of immunotherapy interventions.[9] Recent research findings have highlighted a positive association between TILs and outcomes in diverse cancers, including head and neck, breast, hepatocellular, colorectal, non-small cell lung cancer, and melanoma;[10–15] however, the prognostic impact of TILs varies, due to differences in their distribution and subpopulation characteristics. Mei et al reported that TILs located at tumor margins are correlated with improved OS and disease-free survival (DFS). In contrast, TILs within the tumor center or stroma do not significantly affect OS or DFS.[16] The functional diversity of $CD8^+$ TILs within the tumor microenvironment further complicates interpretation.[17] While specific distinctions among $CD4^+$ T cell subsets were not made, $CD4^+$ T cell infiltration has been positively associated with the prognosis of patients with head and neck cancer.[18] Conversely, high levels of $FoxP3^+$ regulatory T cells in the tumor microenvironment have generally been linked to inferior outcomes across various cancers.[19] Interestingly, elevated $Foxp3^+$ levels were found to predict better 5-year disease-specific survival in patients with oropharyngeal squamous cell carcinoma, but no such correlation was observed in patients with NPC.[20,21] In recent years, immunotherapies based on immune checkpoint inhibitors, particularly antibodies targeting programmed cell death 1 (anti-PD1), have demonstrated promising clinical efficacy and become a critical alternative in the treatment of NPC.[22,23] Nonetheless, anti-PD1 therapy has limited efficacy in patients with lower peripheral blood lymphocyte levels,[24] highlighting the significance of treatment-related lymphopenia (TRL). High lymphocyte numbers of pre-treatment are significantly associated with favorable DFS and OS,[25,26] while TRL can predict a poor outcome;[27] however, conflicting results have been reported concerning the prognostic value of TRL, with some studies reporting that patients with grade 3–4 TRL have longer survival than patients with lower grade or without TRL.[28] Therefore, further investigation is required to confirm the prognostic value of TRL, due to inconsistent findings among studies of patients with NPC.

The aims of this study were to explore associations between dynamic changes in lymphocyte subsets and patient outcomes and the prognostic value of TRL, by excluding confounding factors through propensity score matching in a large group of patients.

Materials and Methods

Patient Population

Data on cases of NPC from May 2012 to January 2022 were compiled. Eligibility criteria were: (1) 18 to 75 years old; (2) pathological diagnosis with NPC; (3) Eastern Cooperative Oncology Group performance score ≤ 2; (4) before treatment, all of the following three conditions must be met: hemoglobin $\geq$ 90 g/L, platelet count $\geq 100 \times 10^9$/L, neutrophil count $\geq 1.5 \times 10^9$/L; (5) nasopharyngeal area and cervical lymph nodes treated using intensity-modulated radiation therapy; and (6) peripheral blood lymphocyte subsets were analyzed before and after treatment. Exclusion criteria were: (1) severe infection; (2) primary bone marrow hematopoietic dysfunction; (3) severe liver and kidney dysfunction; (4) bone subjected to radiation therapy; (5) ever had another type of cancer or synchronous malignancy; (6) a dose < 66 Gy was administered to the gross tumor volume (GTV); and (7) incomplete data.

As the staging system was being revised during the study period, the 8th American Joint Commission on Cancer (AJCC) TNM staging system was used to restage patients diagnosed before 2017.[29,30] This study was approved by the Affiliate Chongqing University Cancer Hospital Ethics Committee (ethical code:CZLS2023323-A), China, and

conformed to the Declaration of Helsinki. Considering the non-interventional nature of the study, and as it was conducted retrospectively, individual patient informed consent was not obtained.

Treatment Regimens

Planned target volume, derived from primary lesion GTV, nodal lesion GTV, high-risk clinical target volume, and low-risk clinical target volume were irradiated with 66–72.6, 66–72.6, 60–64, and 54–56 Gy, respectively, as 1.8–2.2 Gy/fraction, once a day and five times a week.

Reports 83 of the International Commission on Radiation Units and Measurements were used to define the target area.[31] Target design and dose constraints for organs at risk were determined according to the 2010 Chinese expert consensus.[32]

Patients received radiotherapy or concurrent chemoradiotherapy (CCRT), combined neoadjuvant chemotherapy (NACT) or adjuvant chemotherapy (ACT), depending on the tumor stage. Platinum-based chemotherapy combined with paclitaxel, docetaxel, or 5-fluorouracil as an induction or ACT regimen, was administered once every three weeks for 1–3 cycles. In CCRT, platinum or paclitaxel was used for 6 cycles weekly or for 2–3 cycles tri-weekly. If necessary, the number of cycles was adjusted according to the patient's condition.

Detection of Lymphocyte Subsets

Peripheral venous blood samples were collected within one month before therapy and 14–30 days after all treatments. Absolute lymphocyte counts, T cell subsets ($CD4^+$ and $CD8^+$), B cells, and natural killer cells (NK) were quantified by immunofluorescence analysis using flow cytometry. The blood samples underwent treatment with six-color fluorescent monoclonal antibodies (Supplementary Table 1), namely CD3-FITC, CD16+56-PE, CD45-PerCP-Cy5.5, CD19-APC, CD8-APC-cy7, and CD4-PE-cy7 (Beijing Tongsheng Shidai Biotechnology, Beijing, China). Following the treatment, the samples were incubated in a dark 37°C incubator for 10 minutes with FACS Lysing Solution(Beijing Tongsheng Shidai Biotechnology, Beijing, China). The PH value was adjusted to between 7.2–7.4 using PBS. The samples were then subjected to measurement using BD FACS Calibur or BD FACS Canto and analyzed using FACStation or FACS Canto Clinical Software (BD Biosciences, San Jose, CA, USA). Lymphocytes were selected based on a combination of side scatter and CD45 positivity, and a minimum of 2000 events for lymphocytes was required.

Follow-Up

Following treatment, patients continued to be evaluated every 1–3 months for the first year, every 3–6 months for the second year, every 6–8 months for the third to fifth year, and every year thereafter. OS and PFS were calculated. OS was the interval between the time of diagnosis and death from any cause or the date of last follow-up. PFS was defined as the time from diagnosis to disease progression, death, or last follow-up.

Statistical Analysis

All analyses were conducted using R software (4.3.1) packages, including survival, MatchIt, dplyr, and rstatix. P values < 0.050 were considered significant. A correlation analysis was conducted between baseline counts and Δ values. The patients were divided into two groups based on their lymphocyte subset counts. This was done at three different stages - baseline, after treatment, and Δ values. Univariate and multivariate Cox regression models were used to examine the factors associated with OS and PFS. To ensure fair comparison, the groups with higher counts were 1:1 matched with those with lower counts based on their estimated propensity score (PS). This was done within each prespecified covariate that affected survival (the results of Cox regression analysis) or represented treatment intensity (GTV dose, NACT, ACT, CCRT, and chemotherapy cycle). Using the nearest neighbor approach with a caliper width of 0.2 on the PS scale. Standard mean differences were calculated to assess the balance of covariates; a standard mean difference of 0.1 was considered a good balance, while 0.2 was deemed an acceptable balance. For continuous and categorical variables, Wilcoxon and Chi-squared tests, respectively, were used to evaluate differences between the two matched groups. Following matching, survival analysis was conducted and Kaplan-Meier survival curves were plotted. Subsequently, Cox regression analysis was applied to examine relationships between lymphocyte subset counts and patient survival.

Results

Patient Characteristics

Data from a total of 746 eligible patients with NPC were analyzed. The clinical characteristics of all patients at diagnosis are summarized in Table 1; detailed information is provided in Supplementary Table 2. Among all patients, 69.7% were male and 77.1% were < 60 years old. According to the World Health Organization classification, 73.7% of patients had type 2 NPC. TNM stages 1, 2, 3, and 4 were recorded in 13 (1.7%), 166 (22.3%), 293 (39.3%), and 274 (36.7%) patients, respectively. Patients with EBV DNA levels < 500 and ≥ 500 copies/mL comprised 25.7% and 74.3%, respectively. In 535 patients (71.7%), NACT or ACT (or both) was given, and 348 (46.6%) patients received CCRT, while 61 (8.2%) received radiation alone.

Changes in Lymphocyte Counts Post-Therapy Relative to Pretherapy

Lymphocytes were categorized into different subtypes based on their cluster of differentiation expression markers. These subtypes include T lymphocytes ($CD3^+$), B lymphocytes ($CD19^+$), helper T lymphocytes ($CD3^+CD4^+$), suppressor/cytotoxic T lymphocytes ($CD3^+CD8^+$), and NK cells (CD3-$CD16^+$ and/or $CD56^+$). Following treatment, most patients exhibited declines in numbers of lymphocytes and their subsets; specifically, 92%, 97%, 80%, 93%, and 66% of patients

Table 1 Baseline Characteristics of Patients (N = 746)

Characteristic	Number of Patients	Percent (%)
Gender		
Male	520	69.7
Female	226	30.3
Age(years)		
≥ 60	171	22.9
< 60	575	77.1
ECOG score		
0–1	699	93.7
2	47	6.3
Pathology		
WHO type 1	184	24.7
WHO type 2	550	73.7
WHO type 3	12	1.6
TNM stage		
I	13	1.7
II	166	22.3
III	293	39.3
IV	274	36.7
EBV DNA(copies/mL)		
≥ 500	554	74.3
< 500	192	25.7
Treatment model		
RT alone	61	8.2
CCRT	150	20.1
NACT+RT	105	14.1
NACT+CCRT	59	7.9
RT+ACT	73	9.8
CCRT+ACT	71	9.5
NACT+RT+ACT	159	21.3
NACT+CCRT+ACT	68	9.1

Abbreviations: ECOG, Eastern Cooperative Oncology Group; WHO, World Health Organization; EBV, Epstein-Barr virus; RT, radiotherapy; CCRT, concurrent chemoradiation; NACT, neoadjuvant chemotherapy; ACT, adjuvant chemotherapy.

exhibited declines in B, $CD4^+$ T, $CD8^+$ T, total T, and NK cells, respectively. Patients with higher baseline counts exhibited comparatively greater reductions than those with lower baseline counts (P<0.050 for all subsets) (Table 2). Furthermore, correlation analysis of each subset demonstrated positive linear correlations between baseline and Δ values (Table 3).

Survival and Associated Factors

Follow-up was completed through May 11, 2023, with a mean (range) duration of 55.6 (4.6–142.9) months. Among the total 746 patients enrolled, 3- and 5-year OS rates were 93.7% and 84.4%, respectively, while corresponding PFS rates were 76.5% and 68.8%. OS and PFS median values were not reached. After univariate analysis, statistically significant variables were incorporated into multivariate Cox regression analysis, which revealed that gender, TNM stage, and EBV DNA were associated with PFS. In contrast, GTV dose, NACT, age, TNM stage, and EBV DNA were associated with OS.

Propensity Score Matching

To perform propensity score matching, treatment-related covariates, including GTV dose, NACT, ACT, CCRT, number of chemotherapy cycles; and survival-related covariates, including age, gender, TNM stage, and EBV DNA were considered. Additionally, seven continuous parameters, including total lymphocyte, total T cell, $CD4^+$ T cell, $CD8^+$ T cell, B cell, and NK cell numbers, and $CD4^+/CD8^+$ T cell ratio, were used to divide patients into high and low groups, according to median pre- and post-therapy and Δ values. In total, 21 matched sample pairs were included in the analysis. Before matching, 18, 18, and 22 background variables differed significantly (P<0.050) between these two groups for pre-therapy, post-therapy, and Δ values, respectively. When all covariates were matched, there were no significant differences (Supplementary Table 3).

Table 2 Comparison Between High and Low Lymphocyte Count Groups (Wilcoxon Test)

Lymphocyte Subset[a]	Group	Decreased Proportion	Baseline Median (Q1–Q3)	P	Δ Value Median (Q1–Q3)	P
B cell	H	0.73	225 (183–294)	<0.001	165 (125–228)	<0.001
	L	0.62	101 (76–127)		63 (29–93)	
$CD4^+$ T cell	H	0.74	712 (632–867)	<0.001	528 (452–651)	<0.001
	L	0.60	403 (318–472)		244 (148–325)	
$CD8^+$ T cell	H	0.48	499 (428–619)	<0.001	238 (130–339)	<0.001
	L	0.20	257 (206–319)		51 (–25–117)	
Ratio	H	0.56	1.97 (1.66–2.48)	<0.001	1.10 (0.79–1.58)	<0.001
	L	0.40	1.10 (0.91–1.26)		0.44 (0.24–0.64)	
Total T cell	H	0.60	1283 (1137–1516)	<0.001	767 (602–999)	<0.001
	L	0.45	738 (601–886)		335 (154–471)	
NK cell	H	0.35	340 (284–462)	<0.001	119 (31–212)	<0.001
	L	0.00	154 (106–193)		0 (–49–48)	
Lymphocyte	H	0.58	1833 (1644–2110)	<0.001	1063 (829–1309)	<0.001
	L	0.39	1148 (940–1318)		450 (219–641)	

Note: [a]The unit for lymphocyte subset cell count is 10E+6 cells/L. Ratio, $CD4^+/CD8^+$ T cell ratio; H, group with above median value; L, group with below median value; Q1–Q3, interquartile range.

Table 3 Correlations Between Baseline and Δ Values (N = 746)

Baseline and Δ Value	B Cell	$CD4^+$ T Cell	$CD8^+$ T Cell	Ratio	T cell	NK Cell	Lymphocyte
Correlation index	0.843	0.919	0.707	0.842	0.840	0.674	0.833
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Notes: Ratio, $CD4^+/CD8^+$ T cell ratio; T cell, Total T cell.

Survival Analysis After Propensity Score Matching

No significant differences in survival were detected between patients in high and low B cell, NK cell, and total lymphocyte count groups at baseline, post-therapy, or Δ values (Supplementary Table 4).

Associations of Patient Survival Outcomes with Baseline Lymphocyte Subset Counts

Survival analysis was then conducted using the matched dataset. The group with high $CD8^+$ T cell counts at baseline had better 5-year PFS rates (73.7% vs 63.1%, P=0.002; hazard ratio (HR), 0.63, 95% confidence interval (CI), 0.47–0.84) and OS (88.5% vs 81.6%, P=0.024; HR, 0.63, 95% CI, 0.41–0.94) than the corresponding low $CD8^+$ T cell count group. Similarly, the high total T cell group had better 5-year rates of PFS (73.8% vs 64.1%, P=0.005, HR 0.65, 95% CI, 0.49–0.88) and OS (89.7% vs 82.5%, P=0.039, HR 0.64, 95% CI, 0.42–0.98) than the low total T cell group. In contrast, although patients with high $CD4^+$ T cell counts had an excellent 5-year OS rates (88.5% vs 82.0%, P=0.022; HR 0.61, 95% CI, 0.40–0.94), there was no significant difference in PFS between the high and low $CD4^+$ T cell count groups (Figure 1).

Associations of Patient Survival Outcomes with Post-Treatment Lymphocyte Subset Counts

After treatment, the high $CD8^+$ T cell count group had a 5-year PFS rate of 72.3%, which was better than that of the low $CD8^+$ T cell count group (64.1%) (P=0.030; HR 0.73, 95% CI, 0.55–0.97). Further, the high $CD4^+/CD8^+$ T cell ratio group had lower 5-year PFS than the low $CD4^+/CD8^+$ T cell ratio group (62.9% vs 72.8%, P=0.024; HR 1.38, 95% CI, 1.04–1.83); however, there was no significant difference in 5-year OS between these two groups (Figure 2).

Associations of Patient Survival Outcomes with Difference (Δ) in Pre- and Post-Treatment Lymphocyte Subset Counts

Patients in the high $CD4^+$ T cell Δ value group had better 5-year rates of PFS (76.2% vs 63.5%, P=0.001; HR 0.61,95% CI, 0.45–0.82) and OS (89.8% vs 81.6%, P=0.005; HR 0.54,95% CI, 0.35–0.83) than those in the low $CD4^+$ T cell Δ value group. Similarly, the group with high total T cell Δ values also had higher 5-year PFS rates (74.3% vs 65.4%, P=0.010; HR 0.68, 95% CI, 0.51–0.91) and OS rates (88.6% vs 82.5%, P=0.009; HR 0.57, 95% CI, 0.37–0.87).

Additionally, PFS (72.3% vs 65.0%, P=0.043; HR 0.68, 95% CI, 0.51–0.91) was significantly higher in the high $CD8^+$ T cell Δ value group compared to the low $CD8^+$ T cell Δ value group, while there was no significant difference in OS (Figure 3).

Cox Analysis

In univariate analysis, significant factors associated with survival were baseline and Δ values for $CD4^+$, $CD8^+$, and total T cells. Further, $CD4^+/CD8^+$ T cell ratio and $CD8^+$ T cells were significant factors post-therapy. All factors from univariate analysis and previously identified prognostic factors, including gender, age, TNM stage, EBV DNA, GTV dose, and NACT, were integrated into the multivariate analysis.

Based on a multivariate analysis of baseline data, $CD8^+$ (HR 0.651, 95% CI 0.495–0.857, P=0.002) and total (HR 0.600, 95% CI 0.456–0.789, P<0.001) T cells were identified as significant independent prognostic factors for PFS (Figure 4A and B). Moreover, $CD4^+$ (HR 0.708, 95% CI 0.470–0.966, P=0.038) and total (HR 0.639, 95% CI 0.425–0.959, P=0.031) T cells were independent prognostic factors for OS.

In contrast, multivariate analyses of post-therapy data revealed that neither $CD4^+/CD8^+$ T cell ratio nor $CD8^+$ T cells were independent prognostic factors for PFS or OS (Figure 4C and D). In multivariate analysis of Δ values, differences in $CD4^+$ and total T cells were identified as independent prognostic factors for both PFS (HR 0.642, 95% CI 0.487–0.848, P=0.002; HR 0.650, 95% CI 0.494–0.855, P=0.002; respectively) and OS (HR 0.574, 95% CI 0.378–0.872, P=0.009; HR 0.646, 95% CI 0.431–0.969, P=0.035, respectively), whereas difference in $CD8^+$ T cells (HR 0.701, 95% CI 0.533–0.920, P=0.011) was a significant prognostic factor only for PFS (Figure 4E and F).

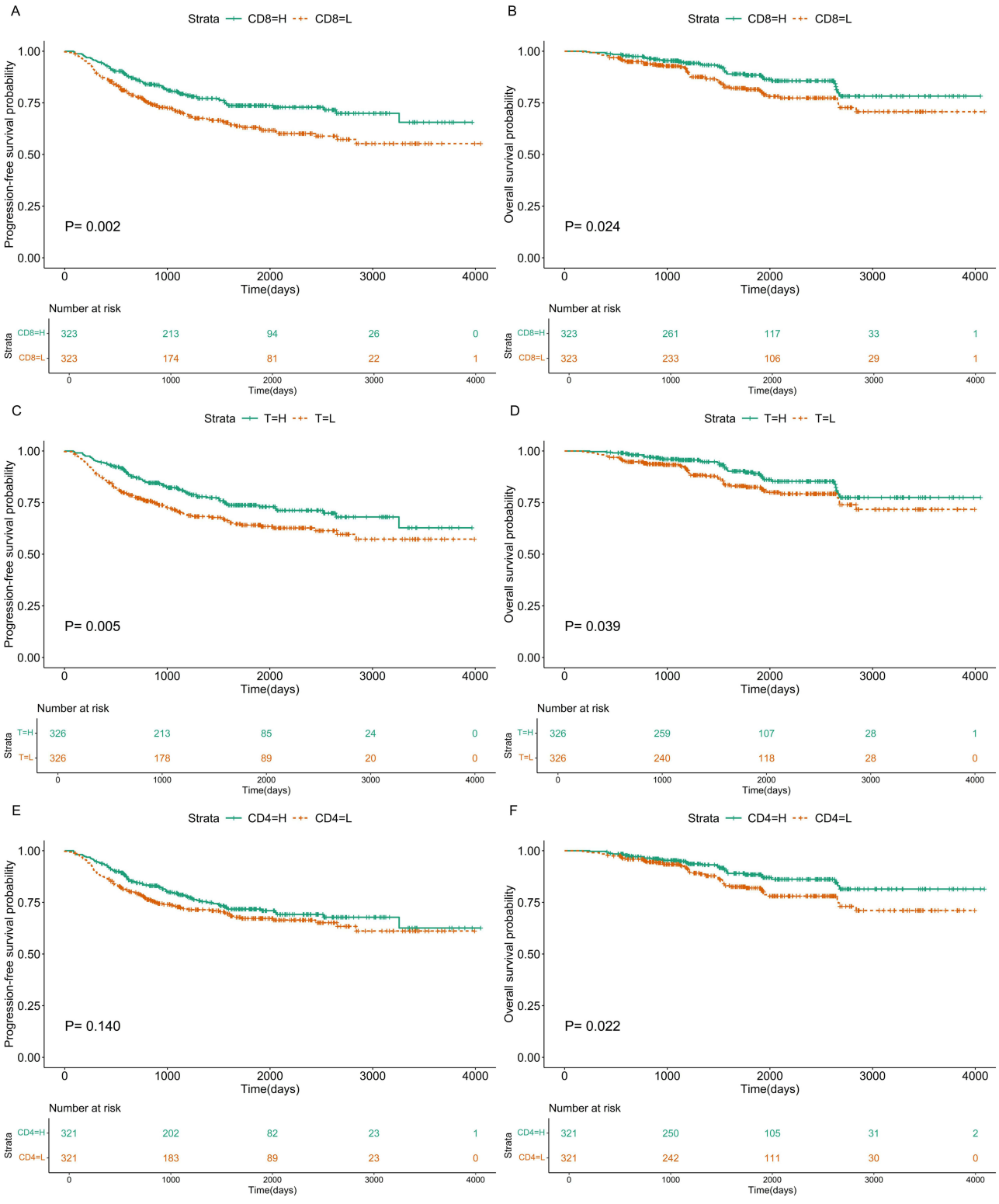

Figure 1 Kaplan-Meier survival curves based on high and low baseline counts of $CD8^+$ (**A** and **B**), total (**C** and **D**), and $CD4^+$ (**E** and **F**) T cells.

Discussion

Our research findings suggest that higher peripheral blood baseline counts of total, $CD4^+$, and $CD8^+$ T cells are potential indicators of favorable outcomes in patients with NPC. After treatment, the numbers of these cells tended to decrease;

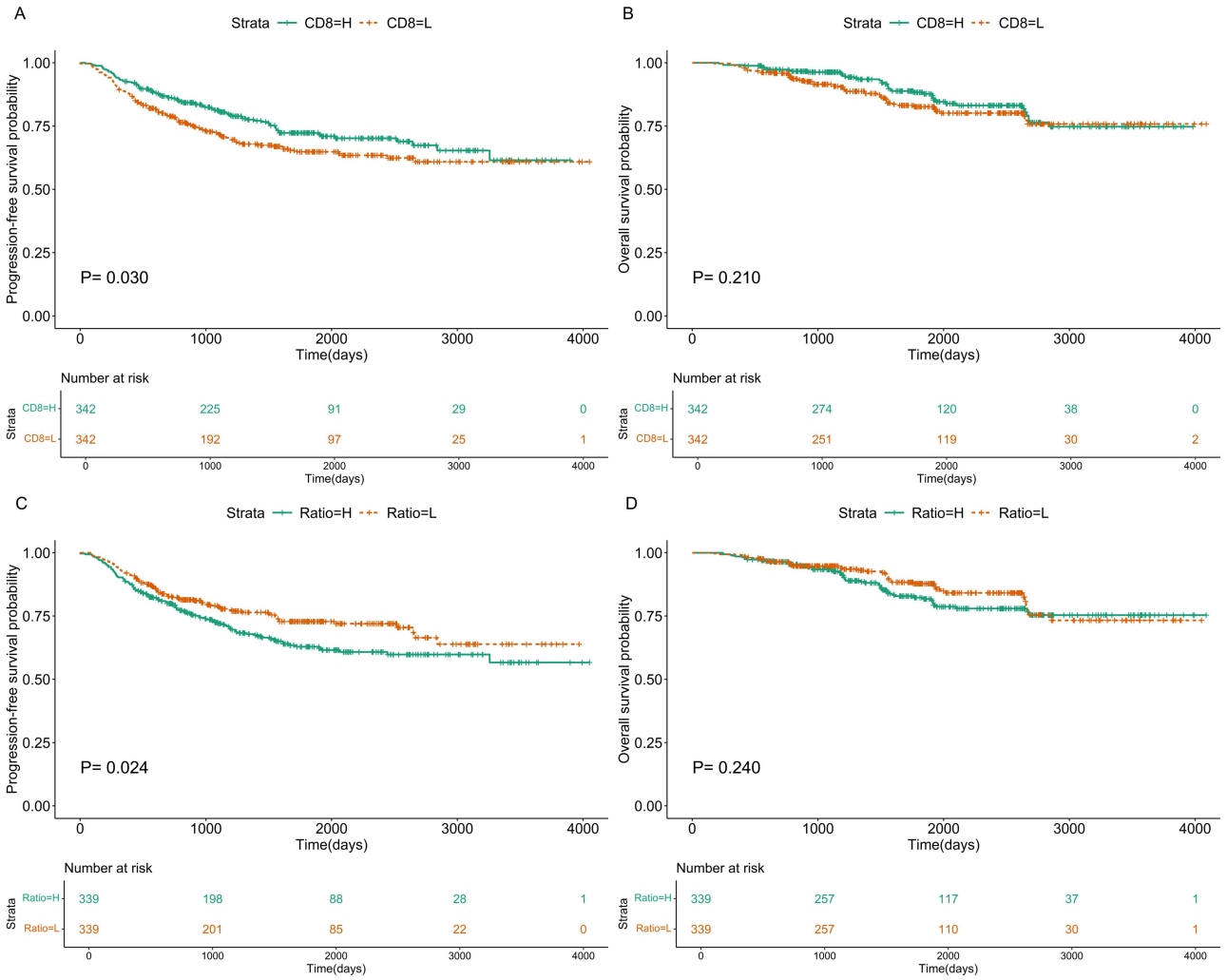

Figure 2 Kaplan-Meier survival curves for patients in the high and low $CD8^+$ T cell count (**A** and **B**) and $CD4^+/CD8^+$ T cell ratio (**C** and **D**) groups after treatment.

however, this decrease may be a sign of better prognosis, and it may be preferable for clinicians to focus more on providing sufficient treatment, rather than concerning the adverse consequences of lymphopenia. Further, combined analysis of multiple lymphocyte subsets and detection timepoints could facilitate better prediction of prognosis.

Interaction between the innate and adaptive immune systems is crucial in combatting cancer. Both types of immunity work together to recognize and destroy cancerous cells; the innate immune system provides the initial response, while the adaptive immune system provides a more targeted approach.[33] When exposed to cytokines, $CD4^+$ T lymphocytes can adopt diverse effector phenotypes, to coordinate the adaptive and innate immune responses.[34] These effector $CD4^+$ T lymphocytes release cytokines upon encountering antigens presented by antigen-presenting cells, thereby activating other immune cells. Additionally, certain specialized $CD4^+$ T cells possess cytotoxic properties and can eliminate infected or transformed cells directly.[35] Finally, regulatory T cells, a specific $CD4^+$ T cell subset, function in regulating immune responses and preserving immune balance.[36] $CD8^+$ T cells differentiate into cytotoxic effector cells upon activation. These effector cells directly kill target cells through mechanisms such as cell lysis or apoptosis. Importantly, $CD8^+$ T cells also develop into memory $CD8^+$ T cells, which provide long-term protection by rapidly responding to re-infection or re-exposure.[37] In this study, we found that NPC patients with high baseline $CD8^+$ (HR 0.651, P=0.002) and total (HR 0.600, P<0.001) T cell counts had better PFS, while high baseline counts of $CD4^+$ (HR 0.708, P=0.038) and total (HR 0.639, P=0.031) T cells were associated with superior OS. Similarly, Shen et al[38] concluded that high levels of $CD4^+$ T cells at treatment outset is a good predictor of favorable 5-year

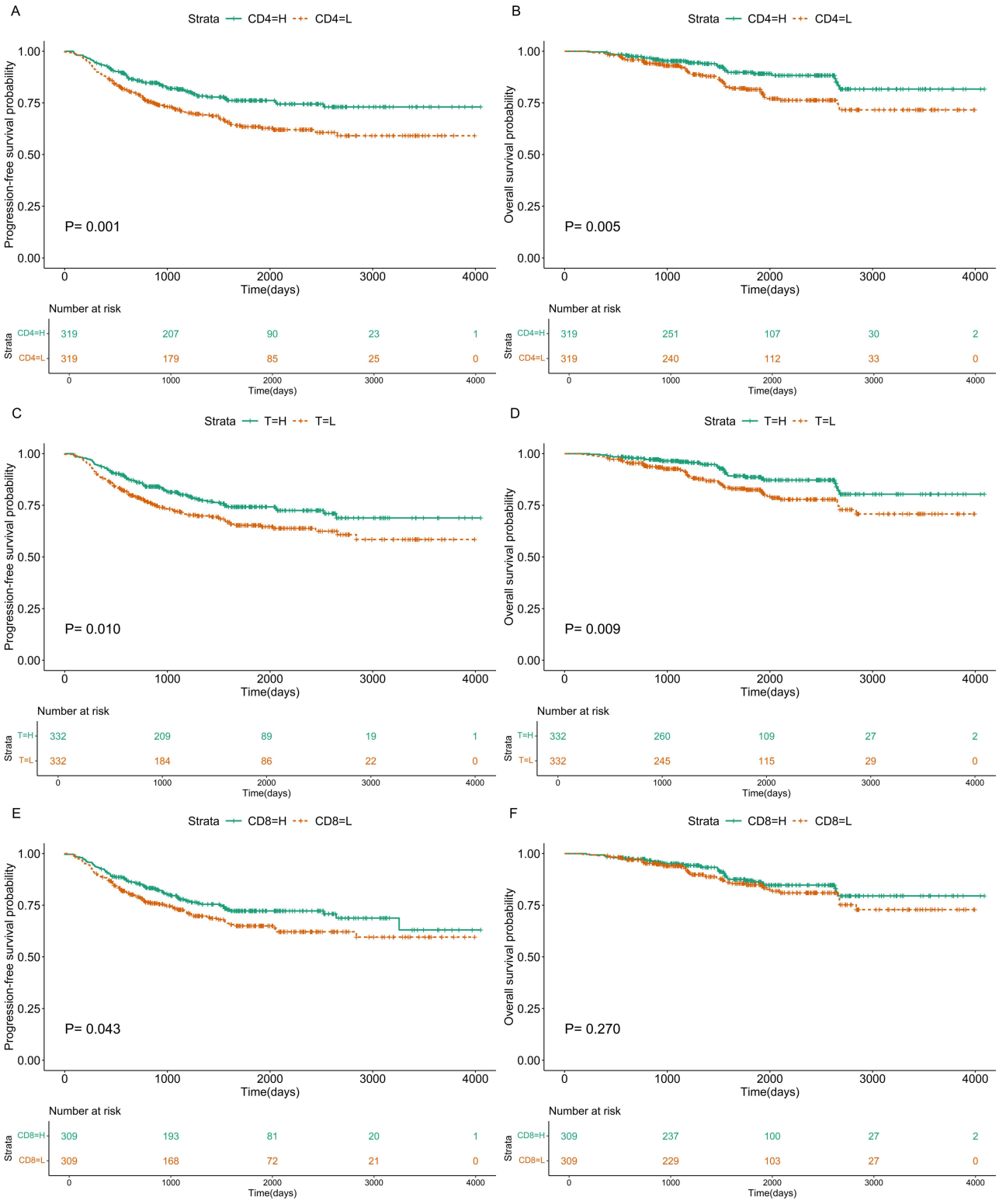

Figure 3 Kaplan-Meier survival curves for patients in the high and low $CD4^+$ (**A** and **B**), total (**C** and **D**), and $CD8^+$ (**E** and **F**) T cell groups, based on Δ values.

PFS (83% vs 74.2%, P=0.015). Further, Zhu et al[39] reported that a higher proportion of $CD8^+$ T cells is linked to longer median OS (39.53 vs 25.00 months P=0.003).

After treatment completion we detected significant decreases in absolute numbers of lymphocyte cell subtypes relative to baseline. Most patients (> 80%) had lower absolute B, $CD4^+$ T, and $CD8^+$ T cell counts, while there was

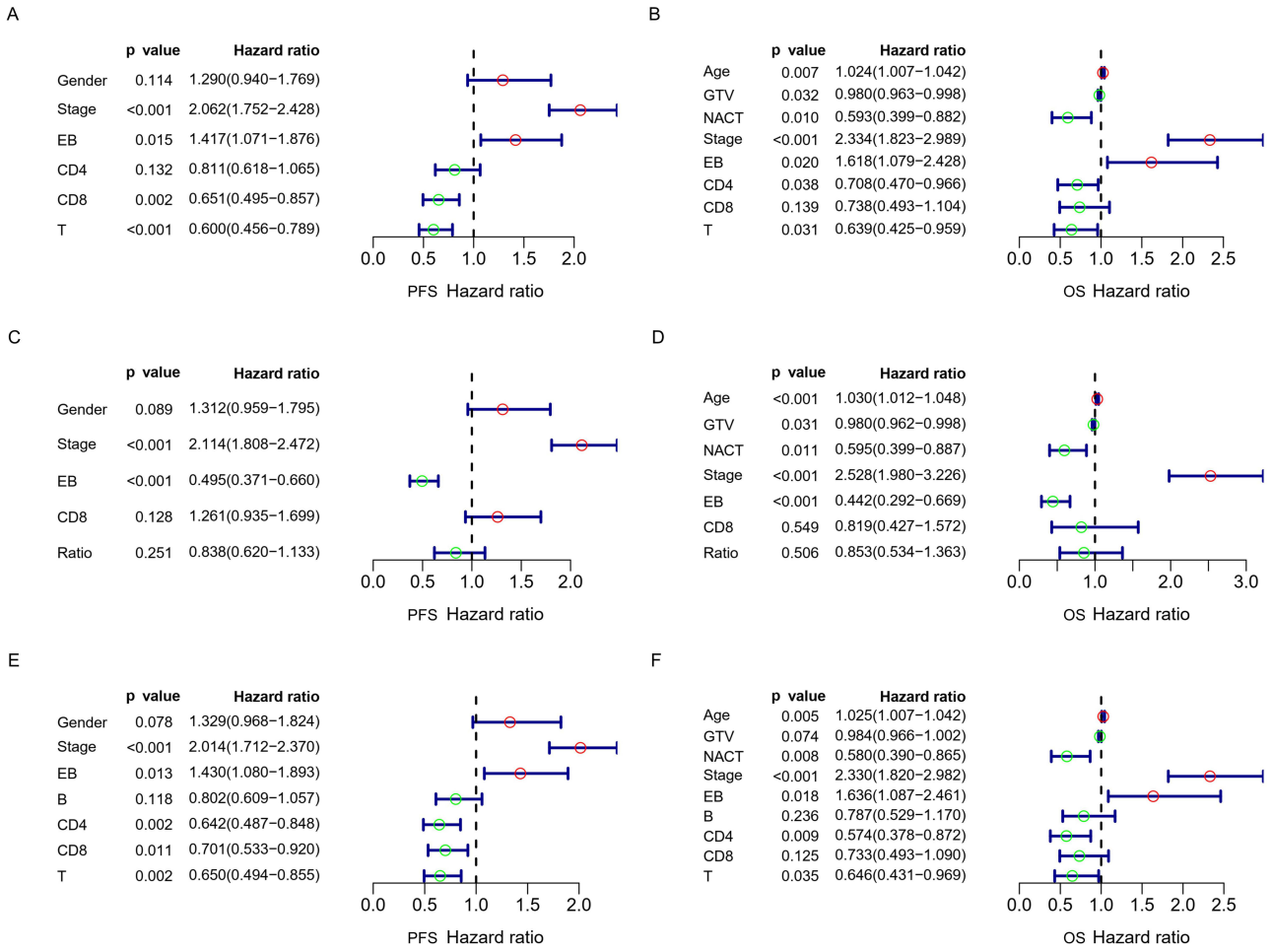

Figure 4 Multivariate Cox logistic regression analysis of baseline (**A** and **B**), post-therapy (**C** and **D**), and Δ (**E** and **F**) values.
Notes: EB, EBV DNA; B, B cells; CD4, $CD4^+$ T cells; CD8, $CD8^+$ T cells. Gender (female/male); Age (18–75 years); TNM stage (I, II, III, IV); NACT (Yes/No); GTV (66–72.6 Gy); EB, B, CD4, CD8, T (Low/High group).
Abbreviations: T, total T cells; GTV, gross tumor volume dose; NACT, neoadjuvant chemotherapy.

a less marked decrease in NK cells. Lymphocytes are highly susceptible to the effects of radiation, with significant impacts observed at relatively low doses. In vitro experiments have demonstrated that exposure to radiation at doses as low as 0.5, 2.0, and 3.0 Gy led to reductions in $CD4^+$ and $CD8^+$ T lymphocyte populations to 90%, 50%, and 10% of their original counts, respectively.[40] Even minimal doses of radiation therapy can induce substantial DNA fragmentation and destroy mature circulating lymphocytes.[41] Further, chemotherapy often causes lymphopenia, which is an adverse hematologic event. Patients with NPC had lower numbers of terminally differentiated $CD4^+$ and $CD8^+$ T cells on day 21 after gemcitabine and cisplatin induction chemotherapy,[42] and a prospective study demonstrated a significant reduction in lymphocyte count by 77.8% following concurrent chemotherapy.[26]

In this research, we investigated the relationships between changes in lymphocyte numbers and patient prognosis. Intriguingly, the results differed somewhat from our initial expectations. Although some patients experienced discernible decreases in their absolute lymphocyte counts following treatment, but lower absolute lymphocyte counts post-treatment did not significantly worsen patient prognosis. Conversely, patients with more significant lymphocyte count disparities (measured by Δ values) exhibited a more favorable prognosis. Patients with high Δ values for $CD4^+$ T cells had a clear advantage of 12.7% in 5-year PFS (P=0.001), as well as higher OS rates (89.8% vs 81.6%; P=0.005). Further, patients with high Δ values for total T cells had an 8.9% higher PFS rate (P=0.010), and OS rates were also 6.1% better (P=0.009) than those in the low Δ value group; however, this result was inconsistent with those of previous studies.[43,44]

One possible hypothesis is that patients with higher values of Δ received more intense treatment, leading to better efficacy. However, this hypothesis was ruled out by our application of propensity score matching. All treatment factors were matched one-to-one according to propensity score. Variables such as GTV dose, NACT, ACT, CCRT, and number of chemotherapy cycles did not differ between the two groups after propensity score matching. Therefore, patients in both low and high Δ value groups received the same treatment intensity, indicating that treatment intensity was not the main cause of lymphopenia. Furthermore, it was not an interfering factor in the different prognoses between the two groups in our data.

The second hypothesis is that patients who have a significant Δ value may have had high baseline counts. Initially, the patient data was divided into two groups, with each group based on the median baseline lymphocyte subset counts. The groups with higher baseline counts showed greater Δ values in all lymphocyte subsets, which is similar to previous reports.[26] This suggests that the effect of Δ values on prognosis cannot be separated from the baseline value.

The third hypothesis suggests that lymphocyte redistribution and associated injuries might lead to a decrease in peripheral blood lymphocyte count, but its positive effects could improve the prognosis. Jin et al developed a dynamic model illustrating how lymphocytes move between five compartments of the immune system, and proposed that circulating blood is an important at-risk organ during radiation therapy. The effect of radiation on lymphocytes depends on several factors, including the dose, duration of exposure, and range of irradiation; however, results also vary according to flow rate and lymphocyte concentration, assuming that other parameters are constant.[45] Blood lymphocyte counts are typically expressed as numbers of cells per milliliter (ie, describe cell numbers per unit volume of blood). When lymphocyte concentration is high, a larger number of lymphocytes may be damaged as blood flows through an irradiated area during treatment, which may partially explain the large reductions observed in this study, given the high baseline cell counts.

The immune response to cancer is directed against somatic mutation-derived neoantigens and, during therapy, tumor-specific neoantigens are released as a consequence of chemoradiotherapy-induced tumor cell death.[46] Antigen-presenting cells recognize neoantigens and become activated, then migrate to lymph nodes where they present antigens to $CD4^+$ T cells (in the context of MHC class II molecules) and $CD8^+$ T cells (on MHC class I proteins). This process ultimately activates neoantigen-specific T cells, which then infiltrate into the tumor tissue and initiate tumor killing. The immune response mediated by $CD4^+$ T cells can change the tumor microenvironment and facilitate the recognition of tumor cells by the immune system. $CD8^+$ T cells can directly lyse tumor cells through T cell-mediated killing, and the lysed tumor cells will release more tumor neoantigens, leading to a wider spectrum of anti-tumor immune responses in the elimination phase;[47] however, not all cancer cells are eradicated during this process, leading to the equilibrium stage, in which a delicate balance between immune surveillance and tumor adaptation ensues. Some cancer cells persist, but remain dormant due to immune pressure. The immune system remains vigilant, preventing tumor escape. In the escape phase, tumor cells evolve mechanisms to evade immune detection and destruction, including downregulation of antigen presentation, alteration of immune checkpoints, and creation of an immunosuppressive microenvironment. Consequently, tumors become less immunogenic and more adept at evading immune responses.[48,49] During the majority of the tumor immune response period, immune cells gather around the tumor. Furthermore, immune cells can form tertiary lymphoid structures around tumors,[50] which are organized immune aggregates with distinct T and B cell zones, in response to persistent antigens. According to recent studies, an assortment of immune cells, including $CD4^+$ T, $CD8^+$ T, memory B, plasma B, dendritic, and follicular dendritic cells, are present in these structures,[51] and contribute to induction or reactivation of antitumor immunity.[52,53]

Immune responses against tumors trigger the departure of substantial numbers of lymphocytes from the bloodstream to migrate toward the tumor site. This clustering of lymphocytes in proximity to tumors amplifies their ability to eliminate them. Typically, approximately 70% of the body's lymphocytes are situated in lymphoid tissues, with only 2% present in the circulating peripheral blood.[45] Nevertheless, antitumor immune activation may lead to variation in lymphocyte spatial distribution, which could substantially alter peripheral blood cell counts. Previous studies have reported the development of peripheral blood lymphocytopenia as a result of this phenomenon, associated with a favorable prognosis.[54–56] Furthermore, radiation will particularly damage lymphocytes that remain stationary in proximity to the tumor site, as such lymphocytes will receive higher doses of irradiation for a longer duration, increasing the likelihood of cell death. Patients with high baseline lymphocyte counts and successful induction of tumor immune responses tend to have more lymphocytes clustered around the tumor, resulting in better treatment efficacy, but they also lose more lymphocytes during treatment.[57] In this study, we

observed that the subgroup of patients who lost more lymphocytes after treatment had better prognosis, likely because they mounted superior anti-tumor immune responses and had higher basal lymphocyte populations.[28] In summary, a higher concentration of lymphocytes in the circulating volume and more redistribution of the tumor area may result in severe treatment-related injury, causing a more severe treatment-related lymphopenia for those with high baseline counts. Paradoxically, this reduction may initiate a more robust anti-tumor reaction. Moreover, given their higher baseline values, there are still more lymphocytes to maintain basic function and sustained immune response after depletion, culminating in favorable long-term outcomes. Given the challenges associated with frequent and repeated pathological biopsies, monitoring dynamic changes in peripheral blood lymphocyte counts may prove valuable as a means of predicting patient prognosis.

Limitation of the Study

The present study has several limitations. First, the retrospective and single-center study design may have introduced confounding and selection bias. Second, the study enrolled patients over an extended period of time and from a wide age range, which could also have impacted the accuracy of our findings. Finally, the chemotherapy regimens used were not entirely consistent across patients, which could have introduced some bias.

Conclusion

Based on the findings of our study, we observed a strong correlation between circulating lymphocyte subset counts and the prognosis of patients with NPC. Patients who had low total T or $CD8^+$ T cell counts at baseline had a worse prognosis than those with high counts of these cells. Moreover, chemoradiotherapy may lead to a decrease in circulating lymphocyte subsets in patients with NPC; however, patients with greater Δ values between baseline and post-treatment for total or $CD4^+$ T cells possibly had better outcomes. Hence, while high baseline lymphocyte count is associated with superior prognosis in patients with NPC; a decrease in lymphocyte numbers after treatment does not necessarily indicate poor prognosis.

Abbreviations

NPC, nasopharyngeal carcinoma; PFS, progression-free survival; OS, overall survival; anti-PD1, antibodies targeting programmed cell death 1; TRL, treatment-related lymphopenia; GTV, gross tumor volume; AJCC, American Joint Commission on Cancer; CCRT, concurrent chemoradiotherapy; NACT, neoadjuvant chemotherapy; ACT, adjuvant chemotherapy; ECOG, Eastern Cooperative Oncology Group; WHO, World Health Organization; EBV, Epstein-Barr virus.

Data Sharing Statement

In response to reasonable requests, the corresponding author will provide access to the data generated and/or analyzed during the current study.

Ethics Statement

This study was approved by the Affiliate Chongqing University Cancer Hospital Ethics Committee (ethical code: CZLS2023323-A), China. The study was conducted according to the principles of the Helsinki Declaration.

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 report no conflicts of interest in this work.

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