Original Article

Abnormal variation and prognostic significance of circulating immune cells in patients with nasopharyngeal carcinoma treated with chemoradiotherapy: a prospective cohort study

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Background: Circulating immune cells are associated with tumor development and poor prognosis in multiple solid tumors. However, the circulating immune-cell profile of nasopharyngeal carcinoma (NPC) remains largely unknown. Therefore, we aimed to determine the changes in immune status and the prognostic significance of circulating immune cells before and after chemoradiotherapy (CRT) in patients, which can provide clinicians with valuable insights to optimize treatment strategies, monitor immune function, and personalize interventions, ultimately improving patient outcomes.

Methods: Circulating immune cells before and after CRT in 77 patients with NPC and in 30 healthy controls were measured with flow cytometry. A thorough follow-up was conducted to assess prognosis outcomes, including local failure-free rate (LFFR), distant failure-free rate (DFFR), disease-free survival (DFS), and overall survival (OS). The differences of the subpopulation distribution in the two groups were determined by *t*-tests or Mann-Whitney tests. The paired *t*-test or Wilcoxon matched-pairs signed rank test was used to compare differences in lymphocyte subsets before and after CRT. The prognostic significance of lymphocyte subsets was evaluated by Kaplan-Meier analysis and Cox proportional hazards model.

Results: Compared with the control group, the NPC group showed significant decreases in the proportions of $CD3^+$ cells, $CD4^+$ T cells, $CD8^+CD28^+$ T cells, and $CD19^+$ B cells as well as the $CD4^+$:$CD8^+$ ratio ($P<0.05$) but a significant increase in the proportion of natural killer (NK) cells ($P<0.05$). After CRT, the proportions of $CD4^+$ cells, $CD8^+CD28^+$ T cells, and $CD19^+$ B cells as well as the $CD4^+$:$CD8^+$ ratio were markedly decreased ($P<0.05$), while the proportions of $CD8^+$ T cells and NK cells were significantly increased ($P<0.05$). Multivariate analysis showed that a lower percentage of $CD19^+$ B cells [hazard ratio (HR) 6.550, 95% CI: 1.661–25.831; P=0.007] and a positive test for Epstein-Barr virus (EBV) DNA (HR 0.261, 95% CI: 0.074–0.926; P=0.038) before treatment independently predicted worse 5-year OS ($P<0.05$).

Conclusions: The disproportion of circulating immune cells was observed in patients with NPC before treatment. CRT further aggravated immune dysfunction. Notably, a lower percentage of $CD19^+$ B cells and EBV DNA-positive status before treatment were independent predictors of a worse prognosis. Thus, the measurement of circulating immune cells may help elucidate immune function status and predict the outcomes of patients with NPC.

Keywords: Nasopharyngeal carcinoma (NPC); circulating immune cells; chemoradiotherapy; immune function; prognosis

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Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor that originates from the epithelium lining the nasopharynx. Concurrent chemoradiotherapy has improved the 5-year overall survival rates of this disease in intermediate and advanced cases (1). However, distant metastasis and local recurrence remain the main causes of treatment failure in NPC. Residual tumors, metastatic lesions, and circulating tumor cells are considered to be the key agents responsible for treatment failure (2). Immune escape is a phenomenon in which the host immune system cannot mount a response against an infectious or malignant agent (3). Patients suffering from malignancy may have an impaired immune function, which chemoradiotherapy (CRT) can further compromise (4) and thereby contribute to tumor progression.

The role of the immune system in antitumor defense is widely recognized. The occurrence, development, and treatment response of NPC are inextricably linked with immune status. Circulating immune cells may reflect the local host response in the tumor microenvironment and thus provide valuable information regarding the tumorigenesis and progression of NPC (5). However, the circulating immune profile of NPC remains largely unknown. The proportion of lymphocyte subsets in the peripheral blood is a key indicator of the antitumor immune status. Although studies (6-9) have revealed that changes in circulating immune cells are associated with tumor development and poor prognosis in diverse tumors, including NPC, no consensus has yet been reached concerning the extent of changes in different circulating immune cells during treatment. CRT, the main treatment for NPC, not only kills tumor cells but also affects immune status. Nevertheless, it is not yet clear whether CRT-induced lymphocytopenia adversely influences the prognosis of patients with NPC.

The current American Joint Committee on Cancer (AJCC) (Tumor-Node-Metastasis, TNM) staging system is based on anatomy and may not sufficiently assess the prognosis of patients with NPC, as a great deal of heterogeneity in prognosis is observed within individual TNM stages (10). Besides tumour burden as reflected by the TNM stage classification, other clinical and molecular prognostic variables have also been proposed. Low-penetrance allelic variation of DNA repair genes, overexpression of serglycin and p53, chromatin modification, epidermal growth factor receptor-phosphoinositide 3-kinase signaling, and elevated plasma Epstein-Barr virus (EBV) DNA are among several notable prognostic factors in NPC (11). Investigation of additional prognostic factors may provide more accurate prognostic information and help facilitate individualized treatment. The response of the immune system is inextricably linked to cancer prognosis. Circulating immune cells, specifically lymphocyte subsets, had been investigated as potential prognostic biomarkers in various cancer. Studies had revealed that changes in circulating immune cells were associated with tumor development and poor prognosis in diverse tumors (5,12-14). Therefore, circulating immune cells may potentially be valuable predictive biomarkers for NPC patients.

The peripheral blood is a noninvasive, low-risk, and

Highlight box

Key findings

- The disproportion of circulating immune cells was observed in nasopharyngeal carcinoma (NPC) before treatment. Chemoradiotherapy (CRT) further aggravated immune dysfunction. The lower percentage of $CD19^+$ B-cells and Epstein-Barr virus (EBV) DNA-positive status before treatment were independent predictors of a worse prognosis.

What is known and what is new?

- The proportion of $CD3^+$ cells, $CD4^+$ T-cells, and $CD19^+$ B cells as well as the $CD4^+$:$CD8^+$ ratio were significantly downregulated in the NPC group as compared to the control group. Testing positive for EBV DNA before treatment independently predicted worse 5-year overall survival.
- The proportion of $CD8^+CD28^+$ cells was markedly decreased both before treatment and after CRT. After CRT, the proportions of $CD8^+$ T cells and natural killer cells were significantly increased. A lower percentage of $CD19^+$ B cells before treatment independently predicted worse 5-year overall survival.

What is the implication, and what should change now?

- Circulating immune cells might have potential as valuable predictive biomarkers and may be used to assess immune function status and predict the outcomes of patients with NPC.

convenient source for repetitive sampling. And we can use it in a real-world clinical practice. Furthermore, circulating immune cells in the peripheral blood has the potential to provide a more holistic insight into the host immune status, which is a decisive factor for the efficacy of cancer immunotherapy (15). Hence, this study aimed to investigate the changes in immune status and the possible prognostic significance of circulating immune cells before and after CRT in patients with NPC. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2024/rc).

Methods

Patients and controls

All patients with non-metastatic NPC confirmed by histopathology and prospectively treated at Fujian Cancer Hospital were included in this prospective study. Originally, we enrolled 80 patients and 30 controls for the study. However, due to 3 patients discontinuing the treatment prematurely and not completing the full course of radiotherapy and chemotherapy, they were subsequently excluded from the study. All patients underwent disease staging using the staging system of the American Joint Committee on Cancer (seventh edition). The inclusion criteria for the NPC group were as follows: age, 18–70 years; Karnofsky performance status ≥80 points; no history of malignant tumors or autoimmune diseases; and no signs of infection. The exclusion criteria were as follows: previous radiotherapy; distant metastasis; severe infection; immune system diseases; severe liver and kidney dysfunction; pregnancy.

We also recruited healthy control participants, who were age- and gender-matched to the patients, from the physical examination center of our hospital. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All procedures were approved by the ethics committee of Fujian Cancer Hospital (No. K201426). Written informed consent was obtained from all participants or their families. The information of the participants was anonymized and deidentified prior to analysis.

Treatments

All patients were treated with intensity-modulated radiotherapy (IMRT). The target volumes were delineated using an institutional treatment protocol defined by our center. IMRT was performed with a total radiation dose of 68–70 Gy to the planning target volume containing the gross tumor volume of the primary tumor and the gross tumor volume of the affected lymph nodes, 60–66 Gy to the planning target volume containing the clinical target volume (CTV)-1, and 54–56 Gy to the planning target volume containing the CTV-2 and CTV-lymph nodes. All prescribed doses were delivered in 31–33 fractions.

In addition to IMRT, patients with locally advanced NPC received concurrent chemotherapy, combined induction chemotherapy, or adjuvant chemotherapy. The Induction or adjuvant chemotherapy regimens consisted of cisplatin (80 mg/m^2 in 3 daily doses) plus gemcitabine (1,000 mg/m^2 on day 1 and day 8) or paclitaxel (135 mg/m^2 in day 1) for 2–3 cycles. In concurrent chemotherapy, cisplatin (80 mg/m^2 in 3 daily doses) was used once every 3 weeks for 2 cycles. All chemotherapy plans took 21 days as 1 cycle.

Blood collection and flow cytometry

Peripheral blood was drawn from the patients before the initiation of treatment and immediately after the completion of CRT. Blood samples collected from both the patients and the controls were immediately stored at 4 ℃ and subjected to flow cytometry within 6 h. Peripheral blood mononuclear cells (PBMCs) were separated using Ficoll-Paque density gradient centrifugation (Ficoll-Paque Plus, GE HealthCare, Chicago, IL, USA), according to the manufacturer's instructions. The blood samples in heparin-containing anticoagulant tubes were shaken well, diluted with an equal volume of Dulbecco's phosphate-buffered saline (D-PBS), transferred to a centrifuge tube containing 3 mL of lymphocyte isolate, and centrifuged at 700 g for 25 min with a capillary tube. The supernatant was discarded, and the pellet was washed twice with D-PBS. PBMCs from the patients with NPC and healthy control participants were subjected to flow cytometry using the FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The following antibodies were used to classify the cells: anti-CD3-PC5/CD4-PC7/CD8-ECD (Beckman Coulter, Brea, CA, USA), anti-5.5/CD4 PE-C7/CD19 APC/CD8 APC-Cy7 (BD Biosciences), 6-color TBNK (CD3-FITC/CD16-PE+CD56-PE/CD45-PerCP-CyTM5.5/CD4-PE-CyTM7/CD19APC/CD8-APC-Cy7; BD Biosciences), and anti-CD8/CD28 (BD Biosciences). We also stained the cells with the corresponding isotype control antibodies purchased from the same company. The percentage of

Table 1 Characteristics of the patients and healthy control participants

Characteristic	No. of patients	No. of controls	χ^2	P
Age (years)				
≥50	36	14	0.374	0.541
<50	41	16		
Gender				
Male	55	22	0.039	0.844
Female	22	8		
TNM stage		–	–	–
T1/T2/T3/T4	11/12/27/27			
N0/N1/N2/N3	9/39/18/11			
I/II/III/IVa	2/12/28/35			

TNM stage, Tumor-Node-Metastasis staging.

fluorescent cells was calculated using flow cytometry (BD Biosciences). Each measurement was performed 3 times, and the mean value was calculated for statistical analysis.

Detection of EBV DNA

Plasma samples were prepared by centrifugation of the peripheral blood sample at 1,000 g for 10 min and stored at −80 ℃ until use. Polymerase chain reaction assays were used to measure the concentration of EBV DNA in the plasma. According to the manufacturer (Daan Gene Co. Ltd., Guangzhou, China), a positive result was defined as an EBV DNA load ≥500 copies/mL.

Follow-up

Patients were evaluated within 3 months after the completion of radiotherapy. The patients were then followed up every 3 months for 3 years, every 6 months for the next 2 years, and once a year thereafter. During the follow-up period, patients undergo clinical assessments, radiological evaluations, and laboratory tests, including but not limited to head and neck contrast-enhanced magnetic resonance imaging (MRI) scan, chest computed tomography (CT) scan, abdominal color ultrasound, and Peripheral blood EBV-DNA testing. The 5-year local failure-free rate (LFFR), 5-year distant failure-free rate (DFFR), 5-year disease-free survival (DFS), and 5-year overall survival (OS) from the date of diagnosis to the date of the first recurrence/metastasis or the date of the last follow-up examination or death was calculated.

Statistical analysis

Statistical analysis was carried out using SPSS statistical software (version 19.0, IBM, Armonk, NY, USA). Differences between two groups were analyzed using the *t*-test in the case of normal distribution or the Mann-Whitney test in the case of nonnormal distribution. The paired *t*-test or Wilcoxon matched-pairs signed rank test was used to compare differences in lymphocyte subsets before and after CRT. Correlations between two groups were analyzed using the Pearson test (normal distribution) or Spearman test (nonnormal distribution). Receiver operating characteristic curve analysis was used to assess the rationality of different cutoff points of circulating immune cells. Survival rates were calculated using the Kaplan-Meier method, and univariate comparisons of the differences in survival were performed using the log-rank test. The Cox proportional hazards model was used to analyze multiple prognostic factors for survival. Statistical significance was set at $P<0.05$ (two-sided).

Results

Patient characteristics

This study included 77 patients with NPC and 30 healthy controls. The NPC group consisted of 55 men and 22 women, with a median age of 47.9 years (range, 18–69 years). The healthy control participants were age- and gender-matched to the patients, and consisted of 22 men and 8 women, with a median age of 46.6 years (range, 20–69 years). The characteristics of the study participants are listed in *Table 1*.

Comparison of circulating immune cells between patients and controls

Compared with the control group, the NPC group showed significant decreases in the proportions of $CD3^+$ cells, $CD4^+$ cells, $CD8^+CD28^+$ T-cells, and $CD19^+$ B cells as well as the $CD4^+$:$CD8^+$ ratio ($P<0.05$). In contrast, the proportion of natural killer (NK) cells was significantly increased in the NPC group ($P<0.05$). No significant difference was found in the proportion of $CD8^+$ T cells between the two groups

Table 2 Proportions of circulating immune cells in patients with NPC and healthy controls

Cell type	Patients with NPC	Healthy controls	*t*/z	P
$CD3^+$, %	64.21±11.86	68.76±4.74	−2.835	0.005*
$CD4^+$, %	30.50±8.8	38.61±6.23	−4.571	<0.001*
$CD8^+$, %	26.14±7.92	25.35±4.77	0.633	0.528
$CD4^+$:$CD8^+$	1.19 (0.90, 1.55)	1.45 (1.18, 1.91)	−2.982	0.003*
Natural killer, %	23.73±11.71	17.56±5.43	3.709	<0.001*
$CD19^+$, %	10.43±3.15	12.57±4.23	−2.851	0.005*
$CD8^+CD28^+$	10.70 (7.60, 12.90)	13.55 (9.50, 16.15)	−2.698	0.007*

Data are presented as mean ± standard deviation, or median (IQR). *, P<0.05. NPC, nasopharyngeal carcinoma; IQR, interquartile range.

(P>0.05; *Table 2*).

Comparison of circulating immune cells before and after CRT

We evaluated the effects of CRT on immune function in patients with NPC. After CRT, the proportions of T-cell subsets, NK cells, and B cells significantly changed (*Figure 1*). The proportions of $CD4^+$ cells, $CD8^+CD28^+$ cells, and $CD19^+$ B cells as well as the $CD4^+$:$CD8^+$ ratio were remarkably decreased (P<0.05), while the proportions of $CD8^+$ T cells and NK cells were significantly increased after CRT (P<0.05).

Correlation between circulating immune cells and clinical parameters

We analyzed the correlations of the immune indexes with gender, age, EBV DNA, T stage, N stage, and clinical TNM stage. The results indicated that the immune indexes, including $CD3^+$, $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, NK, $CD19^+$ B, and $CD8^+CD28^+$ lymphocytes, were not significantly correlated with the TNM classification (P>0.05). Age was negatively correlated with the proportion of $CD3^+$ cells (r=−0.257; P<0.05) and $CD8^+CD28^+$ T cells (r=−0.381; P<0.05) and positively correlated with the proportion of NK cells (r=0.246; P<0.05). EBV DNA-positive status was positively associated with N stage (r=0.262; P<0.05) and clinical TNM stage (r=0.363; P<0.01). The proportion of $CD19^+$ B cells was significantly lower in EBV DNA-positive patients than in EBV DNA-negative patients {median [interquartile range (IQR)], 8.1 (7.4, 11.6) *vs.* 10.5 (8.4, 12.0); P<0.05}.

Correlation between circulating immune cells and prognosis of patients with NPC

The median follow-up duration was 56.0 months for all patients. The 5-year LFFR, DFFR, DFS, and OS of all patients were 90.9%, 88.3%, 70.1%, and 83.1%, respectively. The optimal cutoff values for factors associated with prognosis were determined using receiver operating characteristic curve analysis. For the endpoint of 5-year OS, the optimal cutoff value of $CD19^+$ B cells before treatment was 9.5% (area under the curve =0.745; sensitivity =0.609; specificity =0.846; P=0.006). Kaplan-Meier survival analysis and the log-rank test revealed that patients with a low percentage of $CD19^+$ B cells had poorer 5-year OS than did patients with a high percentage of $CD19^+$ B cells (71.4% *vs.* 92.9%; P=0.005) (*Figure 2A*). EBV DNA-positive status before treatment was significantly associated with worse 5-year DFS (52.2% *vs.* 77.8%; P=0.024) and 5-year OS (65.2% *vs.* 94.4%; P=0.001) (*Figure 2B*,*2C*). Other immune-cell subsets had no prognostic significance for survival time.

Evaluation of immune parameters after CRT predicted prognosis. We found no optimal cutoff value for posttreatment levels associated with NPC prognosis. Kaplan-Meier analysis was used to compare the OS and DFS of patients with high and low levels of immune cells after treatment, and it revealed no statistically significant differences in OS and DFS between the two groups.

The Cox proportional hazards regression model was constructed to calculate the relative risks and confidence intervals (CIs) for different prognostic factors. Multivariate analysis revealed that EBV-DNA positivity and a lower percentage of $CD19^+$ B cells before treatment were adverse prognostic factors for OS (P<0.05). After adjustment for

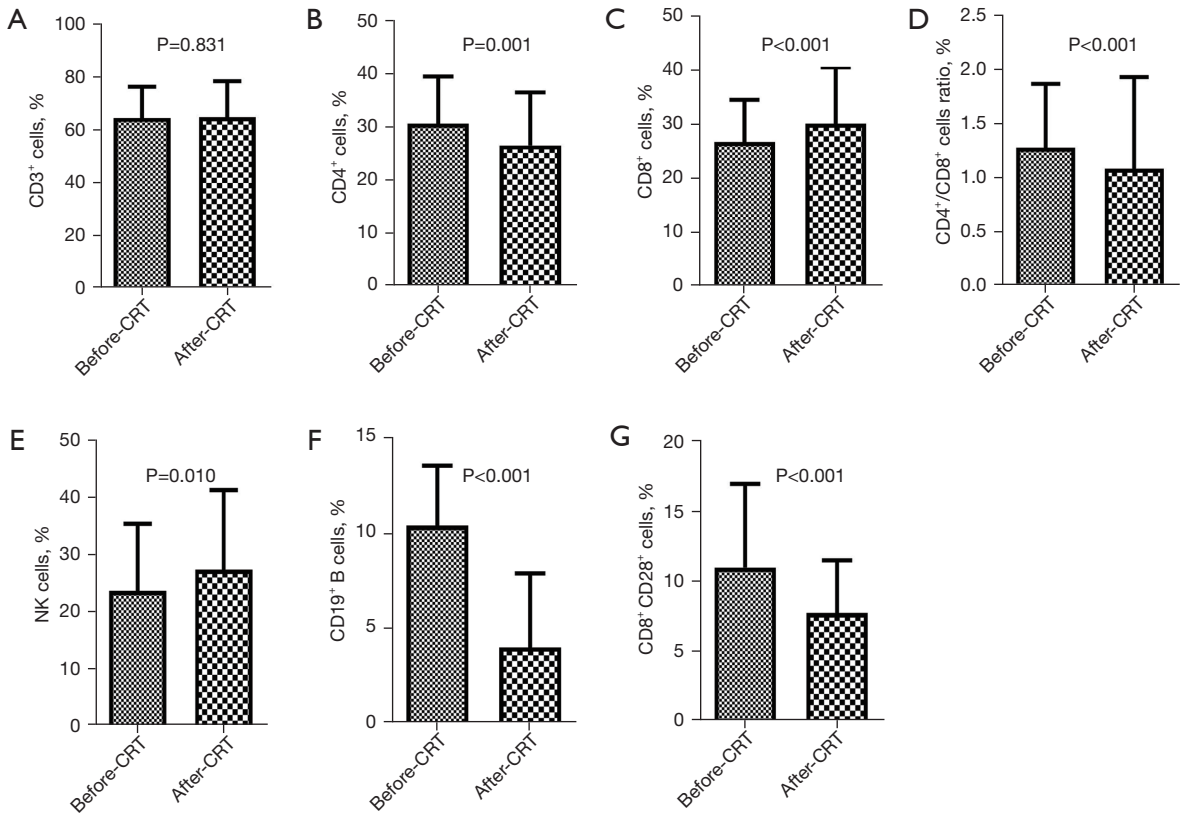

Figure 1 Variations before and after CRT on (A) $CD3^+$ cells; (B) $CD4^+$ cells; (C) $CD8^+$ cells; (D) $CD4^+/CD8^+$ cells ratio; (E) NK cells; (F) $CD19^+$ B cells; (G) $CD8^+CD28^+$ cells. CRT, chemoradiotherapy; NK, natural killer.

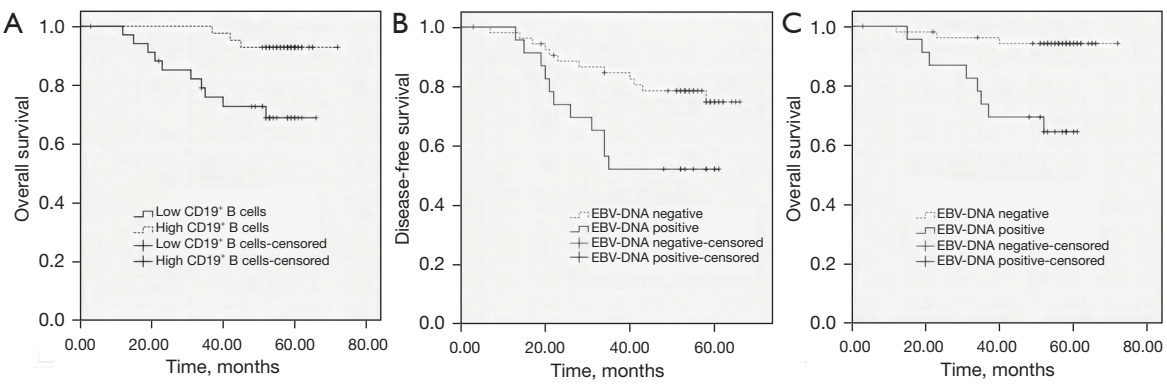

Figure 2 Kaplan-Meier survival curves based on (A) the 5-year OS of patients with a high *vs.* low percentage of $CD19^+$ B cells. (B) The 5-year DFS of patients with negative *vs.* positive EBV-DNA status. (C) The 5-year OS of patients with negative *vs.* positive EBV-DNA status. EBV, Epstein-Barr virus; OS, overall survival; DFS, disease-free survival.

competing risk factors, including for sex, age, and disease stage, EBV DNA-positive status [hazard ratio (HR) 0.261, 95% CI: 0.074–0.926; P=0.038] and a low percentage of $CD19^+$ B cells (HR 6.550, 95% CI: 1.661–25.831; P=0.007) were found to be independent predictors of worse OS.

Discussion

The development and progression of NPC are closely associated with EBV infection and immune dysfunction (1,16). Yet, EBV infection alone may not fully explain the carcinogenesis of NPC. EBV infection has a prevalence about 90–95% of global adult population (17). Most of the EBV infected adults remain asymptomatic during their lifetime. This is because with the programmed cell death like apoptosis, the human innate immunity performs the "cell self-destruction, debris clearing and cell rebuilding" strategy to clear the infection-damaged cells (18,19). Immune dysfunction such as the dysfunction in dead cell debris clearance and apoptosis-resistance induces chronic inflammation and causing gene mutation and NPC. Tumor-infiltrating immune cells may reflect the local immune response in the tumor microenvironment, and peripheral circulating immune cells are key indicators for assessing overall immune status *in vivo*. To a certain extent, the abundance of intratumoral tumor-reactive T cells is proportional to the number of tumor-reactive T cell clones in the peripheral blood (20,21). Hence, circulating immune cells could provide valuable information about immune function in patients with NPC (22). It is worth noting that the detection of circulating immune cells is less expensive and less invasive than is the direct detection of tumor-infiltrating lymphocytes. In this study, the changes in circulating immune cells and the prognostic value of circulating immune cells before and after CRT were analyzed in patients with NPC. The results revealed significant changes in circulating immune cells both before and after treatment. Notably, a higher percentage of $CD19^+$ B cells before treatment was correlated with a better prognosis.

Circulating immune cells, including T cells, B cells, and NK cells, are commonly used as hematological markers of immune function and have been shown to be related to tumor progression and immunotherapy responses (6,9). The $CD3^+$, $CD4^+$, and $CD8^+$ lymphocyte subsets are relatively constant, and they coordinate with and restrict each other, which ensures the stability of immune function. Maintaining the dynamic balance of the $CD4^+$:$CD8^+$ ratio is crucial for the stability of immune function (23). The levels of circulating immune cells have been confirmed to undergo significant changes in multiple types of cancers. In patients with colorectal carcinoma, $CD4^+$ cells, $CD8^+$ cells, $CD19^+$ B cells, and NK cells in the peripheral blood were found to be significantly decreased compared to healthy controls (8). Patients with stage IV inflammatory breast cancer show lymphopenia with significant reductions in circulating T, B, and NK cells (24). In this study, the proportion of $CD3^+$ cells, proportion of $CD4^+$ T cells and the $CD4^+$:$CD8^+$ ratio were significantly downregulated in the NPC group as compared to the healthy control group, which is consistent with the result of a previous study in NPC (25). The proportion of T-cell subsets is a key indicator of the antitumor immune status *in vivo*. Most patients with NPC are in a state of immune functional decline or imbalance. Most evaluations thus far have centered on the role of $CD8^+$ T cells in the peripheral blood and the tumor microenvironment in various tumors, with no further classification of $CD8^+$ cells into $CD28^+$ and $CD28^-$ subgroups. CD28 and B7 are a pair of positive costimulatory molecules that play an important role in initiating T-cell activation. When both signals are engaged, T cells are activated and exert immune responses to tumors. T cells are crucial to the efficacy of tumor immunotherapy, including immune checkpoint inhibitors (26). The development of cancer is associated with T-cell exhaustion, a hypofunctional state characterized by the progressive loss of T-cell effector functions and self-renewal capacity (20), until finally, the T cells become less responsive to tumor antigens and lose control of tumor cells. The proportion of circulating immune cells, including $CD8^+CD28^+$ cells, was markedly decreased in patients with NPC. The phenomenon of T-cell exhaustion in NPC might be attributed to chronic stimulation in the setting of persistent exposure to tumor antigens (20,27). To assess the clinical significance of circulating immune cell subsets, we assessed the correlation of circulating immune cells before CRT with the TNM stage, and the results indicated no significant association.

NK cells, which mediate innate immunity and contain perforin and granzyme granules, play an important role in cancer immunosurveillance (28). In the early stage of EBV-associated infection, the marked expansion of NK cells and EBV-specific T cells figure prominently in controlling EBV infection (29). Consistent with a previous report (25), this study found that the proportion of NK cells was significantly higher in patients with NPC than in the healthy control participants. This finding might be attributed to EBV

infection or to the activation of the innate immune system by the malignant process in patients with NPC (25). B cells play a dominant role in humoral immunity through antibody production. They also contribute to cellular immunity by serving as antigen-presenting cells that enhance T cell-mediated immunity and by modulating immune responses through cytokines or regulatory B cells (30,31). Many studies have shown that B cells are involved in antitumor immunity and could improve the survival of patients with cancer. $CD20^+$ tumor-infiltrating B cells have been shown to correlate with a favorable prognosis in patients with non-small cell lung cancer and ovarian cancer (32,33). In this study, the percentage of circulating $CD19^+$ B cells was markedly decreased in patients with NPC and was negatively correlated with the pretreatment plasma EBV DNA level. EBV is a dual-tropic virus that infects both B lymphocytes and epithelial cells in NPC, and EBV-infected B cells are more susceptible to NK cell-mediated lysis in the lytic phase than in the latent phase (29). Notably, this study revealed that in patients with NPC, a lower percentage of $CD19^+$ B cells and EBV DNA-positive status were independent prognostic factors for a worse 5-year OS. Other study has also reported that a lower percentage of $CD19^+$ B cells is associated with worse survival (7). It means the population of $CD19^+$ B cells might be a valuable indicator of the prognosis of patients with NPC. Patients with lower levels of $CD19^+$ B cells and EBV DNA-positivity may be considered at higher risk for poorer overall survival. This risk stratification can help identify patients who may require closer monitoring or additional interventions. Thus, assessing the levels of circulating lymphocyte subsets before treatment in NPC patients can provide insight into the immune status and potential prognosis.

As the main treatment of NPC, CRT is a double-edged sword because it destroys both tumor cells and the immune system. Compared with the lymphocyte subsets before treatment, the proportions of $CD4^+$ cells, $CD8^+CD28^+$ T cells, and $CD19^+$ B cells as well as the $CD4^+$:$CD8^+$ ratio were remarkably decreased, whereas the proportions of $CD8^+$ T cells and NK cells were significantly increased after CRT. $CD8^+$ T cells are composed of $CD8^+CD28^+$ T cells and $CD8^+CD28^-$ T cells. CD28 is a costimulatory molecule that is required for the activation of $CD8^+$ T cells, which play an important role in antitumor immunity via their cytotoxic effects (34). However, the proportion of $CD8^+CD28^+$ T cells was significantly decreased after CRT. This indicates that radiotherapy might induce immunosuppressive responses that manifested as the loss of T-cell function activation and the inhibition of T-cell proliferation. The decrease in the proportion of $CD19^+$ B cells indicates that humoral immunity was also impaired after CRT. A study has shown that radiotherapy can alter tumor immunogenicity and increase the NK cell-induced killing of different tumor types (35). NK cells are more radioresistant than are T and B lymphocytes in rats (36). Low-dose radiation can augment the natural cytotoxicity of NK cells against tumor-cell targets (37), which might account for the increased levels of NK cells after treatment. Interestingly, it has been proven that radiotherapy sensitizes NPC cells to NK cell-mediated killing, and blocking of the programmed cell death ligand 1 (PD-L1)/programmed cell death protein 1 (PD-1) checkpoint further increases the killing of NPC cells by NK cells in the context of radiotherapy (37). This study reveals changes in the proportions of various immune cell subsets, indicating potential immunomodulatory effects of radiotherapy. For example, the increase in NK cells after CRT may contribute to enhanced tumor cell killing in combination with PD-1 checkpoint inhibitors. Certainly, further research is needed to explore the underlying mechanisms. This study also attempted to explore the potential prognostic value of circulating immune cells in patients with NPC after CRT. However, none of the circulating immune cell subsets after treatment showed any prognostic significance for survival time.

This paper clearly highlights the importance and relevance of the study, demonstrating its value within the field. We conduct rigorous data analysis using appropriate statistical methods and tools, reinforcing the credibility of our research conclusions. However, one limitation to acknowledge is the inadequate sample size, which may affect the generalizability of our findings. Future research with a larger sample size could further support and strengthen our conclusions.

Conclusions

In this study, the disproportion of circulating immune cells was observed in patients with NPC before treatment. CRT further aggravated immune dysfunction in patients with NPC. Notably, a lower percentage of $CD19^+$ B cells and EBV DNA-positive status before treatment were independent predictors of a worse prognosis in patients with NPC. Therefore, measurement of circulating immune cells may help assess the immune function status and predict the outcomes of patients with NPC. By incorporating circulating immune cell subsets and their prognostic

significance, clinicians can optimize treatment strategies, monitor immune function, and personalize interventions to improve patient outcomes.

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Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2024/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2024/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2024/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2024/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Approval was granted by the ethics committee of Fujian Cancer Hospital (No. K201426). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from all participants or their families.

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