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ORIGINAL RESEARCH

Integrated Early CRP Kinetics and Plasma EBV DNA Clearance as Prognostic Biomarkers in De Novo Metastatic Nasopharyngeal Carcinoma Treated with First-Line Chemoimmunotherapy

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Purpose: This study investigates the predictive value of early C-reactive protein (CRP) kinetics in patients with de novo metastatic nasopharyngeal carcinoma (dmNPC) receiving first-line chemotherapy combined with anti-PD-1 mAbs (first-line chemoimmunotherapy).

Patients and Methods: Patients were categorized into three groups based on early CRP kinetics within one and three months after the start of immunotherapy: (1) CRP flare-responders, with CRP levels rising to more than double the baseline within one month and subsequently falling below baseline within three months; (2) CRP non-flare-responders, with CRP levels decreasing by more than 30% within three months without initial flare; (3) CRP non-responders, with no significant CRP changes. Associations with objective response rate (ORR), and progression-free survival (PFS) were evaluated.

Results: The multicenter study included 149 patients with dmNPC (median follow-up: 22 months). The cohort comprised 39 (26.2%) CRP flare-responders, 76 (51%) CRP non-flare-responders, and 34 (22.8%) CRP non-responders. CRP flare-responders and non-flare-responders were combined into CRP responders, showing significantly improved ORR (94.8% vs 79.4%, P=0.009) and prolonged median PFS (20 vs 13 months, P=0.006) compared to CRP non-responders. Multivariable analysis identified early CRP kinetics as an independent prognostic factor for PFS (HR=2.688, 95% CI: 1.484–4.868, P<0.001). In subgroup analysis, patients with undetectable EBV DNA after three immunotherapy cycles showed higher median PFS among CRP responders compared to non-responders (28 vs 21 months, P=0.014), whereas no significant difference was observed in patients with detectable EBV DNA levels (13 vs 8 months, P=0.142).

Conclusion: CRP responders are associated with improved survival outcomes, particularly in patients achieving early EBV DNA clearance. Early CRP kinetics combined with early plasma EBV DNA clearance may be predictive of survival outcomes in dmNPC patients receiving first-line chemoimmunotherapy.

Keywords: de novo metastatic nasopharyngeal carcinoma, biomarker, immunotherapy, C-reactive protein, Epstein Barr virus DNA

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Introduction

Nasopharyngeal carcinoma (NPC) is a head and neck cancer originating in the nasopharynx, commonly associated with Epstein-Barr virus (EBV) infection.[1] The disease has a distinct geographical distribution, with the highest incidence in Asia and North Africa.[2] In 2022, approximately 120,416 new cases of NPC were reported globally,[3] with about 5% to 11% presenting as de novo metastatic nasopharyngeal carcinoma (dmNPC), a subtype characterized by a poor prognosis.[4–6] Survival in dmNPC varies widely due to significant individual differences, ranging from a few months to several years, with some patients achieving long-term survival.[7–10]

The advent of immune checkpoint inhibitors has transformed the treatment landscape of NPC. Three major Phase III clinical trials have shown that standard chemotherapy combined with anti-PD-1 monoclonal antibodies (anti-PD-1 mAbs) significantly improves progression-free survival (PFS) and overall response rate (ORR) compared to chemotherapy alone as first-line therapy for relapsed or metastatic NPC (RM-NPC).[11–13] Consequently, the combination of immunotherapy and palliative chemotherapy (PCT) is now recommended as the first-line treatment in the NCCN guidelines for RM-NPC.

However, not all RM-NPC patients respond to immunotherapy. Plasma EBV DNA load is currently used as a biomarker to assess prognosis in NPC immunotherapy.[14,15] Yet, variability in the standardization of EBV DNA testing across tumor centers leads to inconsistencies in data comparability and reproducibility, limiting its clinical utility. Identifying reliable predictive biomarkers to detect early treatment failure is crucial, as it would help avoid unnecessary side effects and costs of immunotherapy and optimize personalized treatment regimens. Therefore, exploring effective and stable biomarkers to predict the response to anti-PD-1 mAbs in RM-NPC patients is critical.

Immunotherapy success is largely dependent on the activation of an anti-tumor immune response. C-reactive protein (CRP), a widely recognized serum biomarker of systemic inflammation, reflects the acute phase response. Its levels increase in diseases characterized by chronic inflammation, such as cardiovascular disease, diabetes, and cancer.[16] Previous studies have reported that elevated CRP levels before treatment are associated with poor prognosis in various tumors, including colorectal cancer[17] and esophageal cancer.[18] Similarly, baseline and post-treatment elevated CRP, as well as sustained elevation during treatment, have been reported as adverse prognostic factors in patients with non-metastatic nasopharyngeal carcinoma.[19] Given that tumors can induce chronic inflammation, Ozawa et al also believe that the elevation of inflammatory cytokines in the very early phase indicated early activation of the immune-system.[20] It is speculated that CRP kinetics may have certain predictive value for the prognosis of immunotherapy in cancer patients. Several studies have demonstrated that changes in CRP kinetics following the initiation of immunotherapy are correlated with responses to anti-PD-1 monotherapy and combination therapy in metastatic renal cell carcinoma, non-small cell lung cancer, and advanced urothelial carcinoma.[21–24] However, there are no studies evaluating the effect of early elevated CRP after initiation of immunotherapy in patients with RM-NPC.

This study aims to evaluate whether early changes in C-reactive protein (CRP) kinetics can serve as predictive markers for therapeutic response and survival outcomes in patients with dmNPC receiving first-line chemotherapy combined with anti-PD-1 mAbs.

Material and Methods

Patient Selection

We retrospectively reviewed the medical records of patients with de novo metastatic nasopharyngeal carcinoma (dmNPC) who received platinum-based chemotherapy combined with anti-PD-1 monoclonal antibodies (mAbs) as first-line therapy between January 2019 and October 2021 at three centers in China: Sun Yat-sen University Cancer Center, Jiangxi Cancer Hospital, and Hubei Cancer Hospital. Inclusion criteria were pathologically confirmed NPC with newly diagnosed distant metastases, measurable metastatic lesions, complete baseline data, and availability of CRP and plasma EBV-DNA measurements for the first three courses after the initiation of immunotherapy. Baseline data were required to be collected within one month prior to starting immunotherapy. All included patients received at least three cycles of chemotherapy and anti-PD-1 mAbs, followed by locoregional radiotherapy (LRRT) as appropriate. This study was approved by the institutional review board of Jiangxi Cancer Hospital (No.2024ky106), institutional review board of Sun Yat-Sen University Cancer Center (SL-B2021-270-01), and institutional review board of Hubei Cancer Hospital

(LLHBCH2024YN-095). This study was conducted in accordance with the Declaration of Helsinki. The requirement for informed consent was waived by the Institutional Review Board due to the retrospective nature of the study. Patient confidentiality was strictly maintained, and all clinical data were anonymized to ensure patient privacy and compliance with ethical standards.

All patients underwent comprehensive pretreatment evaluations, including detailed medical history, physical examination, nasopharyngeal fiber endoscopy, blood counts, serum biochemistry, and plasma EBV DNA testing. Imaging assessments included magnetic resonance imaging (MRI) of the nasopharynx and neck, computed tomography (CT) scans of the chest, ultrasound, CT, or MRI of the abdomen, whole-body bone scans, and positron emission tomography/CT (PET/CT). Staging was performed according to the 8th Edition of the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) TNM staging manual. Metastatic sites and the number of lesions were evaluated based on imaging studies. Oligometastasis was defined as metastases involving ≤2 organs and ≤5 metastatic lesions.[25]

Treatments

Chemotherapy and Immunotherapy Regimens

Chemotherapy regimens included GP (gemcitabine and cisplatin/nedaplatin), TP (docetaxel and cisplatin/nedaplatin), PF (cisplatin/nedaplatin and 5-fluorouracil), TPF (docetaxel, cisplatin/nedaplatin, and 5-fluorouracil), and TPC (docetaxel, cisplatin/nedaplatin, and capecitabine). Anti-PD-1 monoclonal antibodies used in the study included sintilimab, camrelizumab, toripalimab, tislelizumab, pembrolizumab, and nivolumab. Both PCT and anti-PD-1 mAbs were administered every three weeks. Patients continued anti-PD-1 mAbs therapy until disease progression, intolerable side effects, or a clinical decision to terminate treatment.

Radiotherapy Regimens

Radiotherapy included locoregional radiotherapy (LRRT) and radiotherapy (RT) for metastatic lesions.LRRT (n=96) was performed using the intensity-modulated radiotherapy (IMRT) technique to target nasopharyngeal lesions and neck lymph nodes. Of which, 44 patients delivered received additional RT for metastatic lesions, including liver, bone, lung, and distant metastatic lymph nodes. Details of chemotherapy, anti-PD-1 mAbs regimens, and radiotherapy protocols are provided in Supplemental Methods and Supplemental Table S1.

Serum Measurement and Definition of Early CRP Dynamics

Baseline and longitudinal serum concentrations of C-reactive protein (CRP) were quantified using latex immunoturbidimetric assay in the routine laboratories at each center (Anti Bio, Zhengzhou, China). Data were analyzed using a fully automated biochemistry analyzer (Canon, model TBA-FX8, Dalian, China). CRP were measured at beginning, before the second immunotherapy (typically within 1 month after the first immunotherapy) and before the fourth immunotherapy (usually within 2 months after the second immunotherapy); LDH were measured at beginning. CRP kinetics were classified as follows: CRP flare-responders were defined as having a CRP level that increased to more than double the baseline within one month of initiating immunotherapy, followed by a decline below baseline within three months; CRP non-flare-responders were defined as patients whose CRP levels decreased by more than 30% within three months without a preceding flare. CRP non-responders were defined as patients in addition to the above two conditions.

Follow-up

Plasma EBV-DNA and serum CRP levels were collected after each course of anti-PD-1 mAbs treatment. Objective response rate (ORR) was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST V.1.1). Evaluations were conducted every two to four cycles during immunotherapy and every three to six months thereafter.

Statistical Analyses

PFS was defined as the time from the start of treatment to the onset of progression (imaging or clinical) or death, whichever occurred first. Due to differences in plasma EBV DNA detection techniques among the three centers, plasma EBV DNA was categorized based on the cut-off value specific to each center (detectable value: >10 copies/mL). Missing

plasma EBV DNA data were handled using complete case analysis. For between-group comparisons, the Chi-square test or Fisher's exact test was used for categorical variables, and the *t*-test was used for continuous variables. Survival curves were plotted using the Kaplan–Meier method, and median PFS differences between groups were tested by log-rank. For multivariable analysis of PFS, a Cox proportional hazards regression model was employed, including only variables with significant survival effects in univariate Cox regression. All statistical tests were two-sided, with a p-value <0.05 considered significant. The median follow-up time was calculated using the reverse Kaplan-Meier method. Model accuracy was evaluated using the Harrell-Concordance index (C-index). Analyses were performed using SPSS 26 and R software (version 3.6.1; http://www.R-project.org).

Results

Baseline Characteristics

We identified 149 eligible patients with de novo metastatic nasopharyngeal carcinoma (dmNPC) who received first-line chemotherapy combined with anti-PD-1 monoclonal antibodies (mAbs) between January 2019 and October 2021 at three centers in China: Sun Yat-sen University Cancer Center (n=128), Jiangxi Cancer Hospital (n=17), and Hubei Cancer Hospital (n=4). The median age of patients was 49 years (range: 20–74 years), with 114 male patients (76.5%). All patients had an ECOG score of ≤1, and 96 patients (64.4%) received locoregional radiotherapy (LRRT). Fifty percent (75 patients) presented with oligometastasis, and the median follow-up period was 22 months (interquartile range: 14–32 months). The clinical baseline characteristics of the patients are summarized in Table 1.

Table 1 Comparison of Baseline Parameters Between CRP Responders and CRP Non-Responders

Characteristic	Overall N=149 (%)	CRP Responders N=115 (%)	CRP Non-responder N=34 (%)	P-Value
Age, years				0.364
Median (IQR)	49	49	50	
Range	20–74	20–73	22–74	
Sex				0.169
Male	114 (76.5%)	85 (73.9%)	29 (85.3%)	
Female	35 (23.5%)	30 (26.1%)	5 (14.7%)	
ECOG				1
0	135 (90.6%)	104 (90.4%)	31 (91.2%)	
1	14 (9.4%)	11 (9.6%)	3 (8.8%)	
Smoking				0.469
Yes	47 (31.5%)	38 (33%)	9 (26.5%)	
No	102 (68.5%)	77 (67%)	25 (73.5%)	
Baseline LDH (U/L)				0.889
Normal	101 (67.8%)	78 (67.8%)	23 (67.6%)	
Abnormal	46 (30.9%)	36 (31.3%)	10 (29.4%)	
Missing	2 (1.3%)	1 (0.9%)	1 (3%)	

(*Continued*)

Table 1 (Continued).

Characteristic	Overall N=149 (%)	CRP Responders N=115 (%)	CRP Non-responder N=34 (%)	P-Value
RT				0.969
Yes	96 (64.4%)	74 (64.3%)	22 (64.7%)	
No	53 (35.6%)	41 (35.7%)	12 (35.3%)	
Oligometastatic				0.729
Yes	75 (50.3%)	57 (49.6%)	18 (52.9%)	
No	74 (49.7%)	58 (50/4%)	16 (47.1%)	
Baseline plasma EBV DNA levels				0.791
Negative	24 (16.1%)	18 (15.7%)	6 (17.6%)	
Positive	120 (96.6%)	93 (80.9%)	27 (79.4%)	
Missing	5 (3.3%)	4 (3.4%)	1 (3%)	
Baseline CRP (mg/L)				0.001
Normal	69 (46.3%)	40 (34.8%)	29 (85.3%)	
Abnormal	80 (53.7%)	75 (65.2%)	5 (14.7%)	
EBV-DNA $_{3\ cycles}$ levels				0.426
Undetectable	91 (61%)	74 (64.3%)	17 (50%)	
Detectable	36 (24.2%)	27 (23.5%)	9 (26.5%)	
Missing	22 (14.8%)	14 (12.2%)	8 (23.5%)	

Abbreviations: IQR, interquartile range; ECOG, Eastern Cooperative Oncology Group; RT, radiotherapy; LDH, Locoregional radiotherapy; EBV-DNA $_{3\ cycles}$ levels, EBV levels after 3 cycles of immunotherapy; CRP, C reactive protein; LDH, lactate dehydrogenase.

Early CRP Kinetics and Survival Analysis

Among the 149 patients, 39 (26.2%) were CRP flare-responders, 76 (51%) were CRP non-flare-responders, and 34 (22.8%) were CRP non-responders. There was no significant difference in the objective response rate (ORR) among the three groups (P=0.053; Supplementary Table S2). Survival analysis demonstrated that the median progression-free survival (PFS) was significantly shorter in CRP non-responders compared to CRP flare-responders and CRP non-flare-responders (13 months vs 21 months vs 20 months, P=0.018; Figure 1A). Since there was no significant difference in ORR (94.9% vs 94.7%; Supplementary Table S2) and median PFS (21 months vs 20 months, P=0.421; Figure S1) between CRP flare-responders and CRP non-flare-responders, these groups were combined into a single CRP responder group. Clinical characteristics of CRP responders (115, 77.2%) and CRP non-responders (34, 22.8%) were balanced except for baseline CRP levels. A summary of patient categorization by early CRP kinetics is provided in Table 1. The ORR of CRP responders was significantly higher than that of CRP non-responders (94.8% vs 79.4%, P=0.009, Table 2). Survival analysis further showed that the median PFS of CRP responders was superior to CRP non-responders (20 months vs 13 months, P=0.006; Figure 1B).

After adjusting for radiotherapy, oligometastasis, baseline EBV-DNA levels, and EBV-DNA levels at three cycles, multivariable Cox regression analysis revealed that early CRP kinetics remained an independent prognostic factor for PFS (HR=2.688, 95% CI: 1.484–4.868, P<0.001). Other independent prognostic factors for PFS included EBV-DNA levels at three cycles (HR=1.892, 95% CI: 1.147–3.123, P=0.013), radiotherapy (HR=0.495, 95% CI: 0.301–0.812, P=0.005), and baseline EBV-DNA levels (HR=2.852, 95% CI: 1.208–6.734, P=0.017;Table 3).

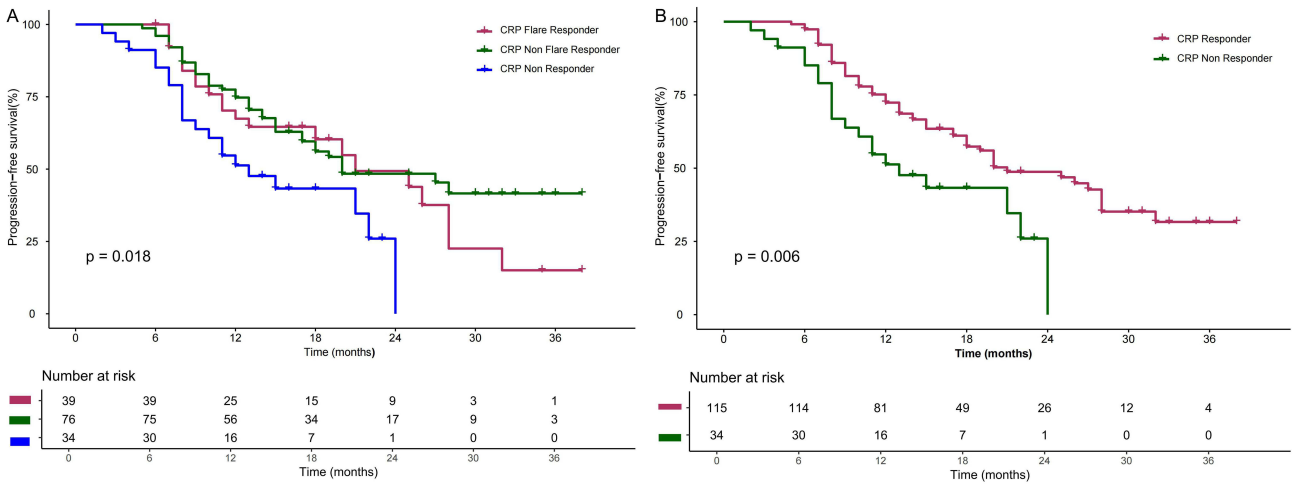

Figure 1 Distinct early on-treatment C reactive protein (CRP) kinetics correlates with progression-free survival (PFS) in the patients with dmNPC receiving first-line chemotherapy combined with anti-PD-1 mAbs. (**A**) Progression-free survival (PFS) for CRP flare-responder (N = 39), CRP non-flare-responder (N = 76) or CRP non-responder (N = 34). (**B**) Progression-free survival (PFS) for CRP responders (N = 115) and CRP non-responder (N = 34).

The Prognostic Value of Early CRP Kinetics and EBV DNA Levels at Three Cycles

Given that both EBV DNA levels at three cycles and early CRP kinetics were independent prognostic factors for PFS, we further evaluated their combined value in prognostic assessment. A subgroup analysis was conducted on 127 patients who underwent plasma EBV DNA testing after three cycles of immunotherapy. Patients were classified based on whether EBV DNA at three cycles was detectable The undetectable EBV DNA group included 91 patients (71.7%) with 74 CRP responders and 17 CRP non-responders, while the detectable EBV DNA group included 36 patients (28.3%) with 27 CRP responders and 9 CRP non-responders. Among patients with undetectable EBV DNA at three cycles, CRP responders had a significantly longer median PFS compared to CRP non-responders (28 months vs 21 months, P=0.014; Figure 2A). No statistically significant difference in PFS was observed between CRP responders and CRP non-responders in the detectable EBV DNA group (P=0.142; Figure 2B).

Development of a Prognostic Model Combining Early CRP Kinetics and EBV DNA Levels at Three Cycles

To improve the prognostic prediction of dmNPC patients undergoing first-line chemo-immunotherapy, we constructed a predictive model incorporating early CRP kinetics and EBV DNA levels at three cycles. The 127 patients were stratified into three groups: undetectable EBV DNA CRP responders (74, 58.4%), undetectable EBV DNA CRP non-responders (17, 13.3%), and detectable EBV DNA (36, 28.3%). Survival analysis revealed significant differences in PFS among the three groups (median PFS: 28 vs 21 vs 10 months, P<0.001; Figure 3). The C-index demonstrated that the combined model of early CRP kinetics and EBV DNA levels at three cycles provided more accurate prognostic predictions than either biomarker alone (combined model vs early CRP kinetics vs EBV DNA levels: 0.724 vs 0.679 vs 0.708).

Table 2 The Relationship Between Tumor Response and Early CRP Kinetics

Treatment Response	Overall, N=149	Responder, N=115	Non-Responder, N=34	P Value
ORR(CR+PR)	136(91.3%)	109(94.8%)	27(79.4%)	0.009
CR	13(8.7%)	11(9.6%)	2(5.9%)	
PR	123(82.6%)	98(85.2%)	25(73.5%)	
SD	11(7.4%)	6(5.2%)	5(14.7%)	
PD	2(1.3%)	0(0.0%)	2(5.9%)	

Abbreviations: CRP, C-reactive protein; ORR, overall response rate; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

Table 3 Uni- and Multivariable Cox Regression Analyses for Progression-Free Survival

		Univariate			Multivariate		
Characteristic	**N**	**HR**	**95% CI**	**P Value**	**HR**	**95% CI**	**P Value**
Sex	149						
Male		Ref.			–	–	
Female		0.586	0.323–1.063	0.078	–	–	
ECOG	149						
0		Ref.			–	–	
1		1.167	0.58–2.349	0.665	–	–	
Smoking	149						
No		Ref.			–	–	
Yes		1.396	0.886–2.201	0.150	–	–	
Baseline LDH	147						
Normal		Ref.			–	–	
Abnormal		1.503	0.942–2.397	0.087	–	–	
Baseline CRP	149						
Normal					–	–	
Abnormal		0.915	0.586–1.429	0.696	–	–	
RT	149						
No		Ref.					
Yes		0.425	0.273–0.661	0.0001	0.495	0.301–0.812	0.005
Oligometastatic	149						
No		Ref.					
Yes		0.596	0.383–0.936	0.024	0.75	0.437–1.250	0.259
Baseline plasma EBV DNA levels	144						
Negative		Ref.					
Positive		2.194	1.054–4.570	0.036	2.852	1.208–6.734	0.017
EBV-DNA $_{3\ cycles}$ levels	127						
Undetectable		Ref.					
Detectable		2.562	1.582–4.15	0.0001	1.892	1.147–3.123	0.013
CRP dynamics	149						
Responders		Ref.					
Non-responders		1.987	1.191–3.315	0.009	2.688	1.484–4.868	0.001

Abbreviations: ECOG, Eastern Cooperative Oncology Group; RT, radiotherapy; LDH, Locoregional radiotherapy; EBV-DNA$_{3cycles}$ levels, EBV levels after 3 cycles of immunotherapy; CRP, C reactive protein; LDH, lactate dehydrogenase; PFS, progression-free survival.

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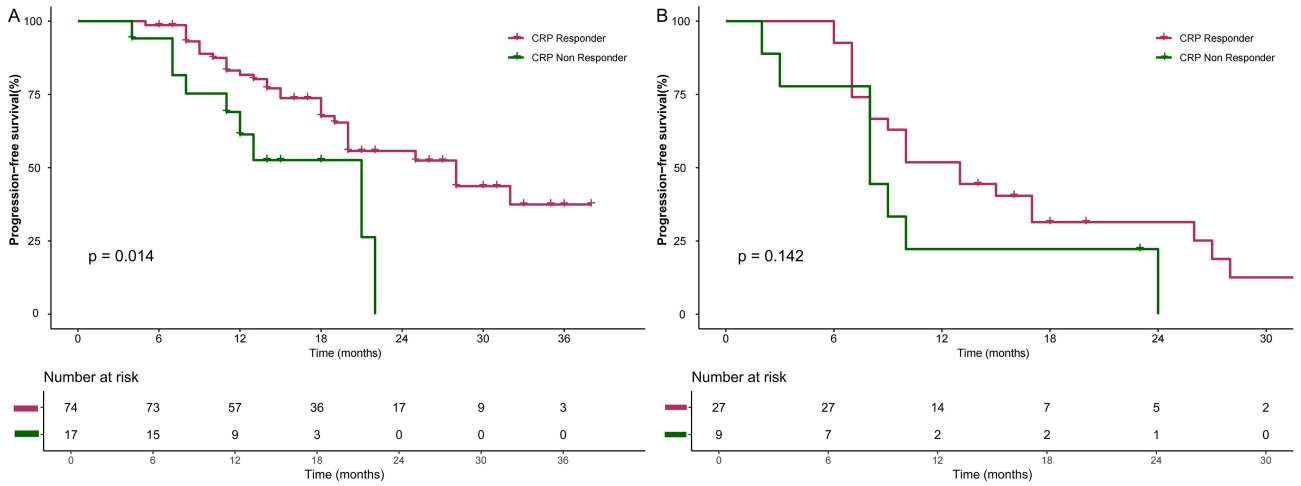

Figure 2 Prognostic association between EBV-DNA 3 cycles levels and early CRP kinetics in the patients with dmNPC receiving first-line chemotherapy combined with anti-PD-1 mAbs. (**A**) Progression-free survival (PFS) for CRP responder (N = 74) and CRP non-responder (N = 17) in the undetectable EBV-DNA 3 cycles group. (**B**) Progression-free survival (PFS) for CRP responder (N = 27) and CRP non-responder (N = 9) in the detectable EBV-DNA 3 cycles group.

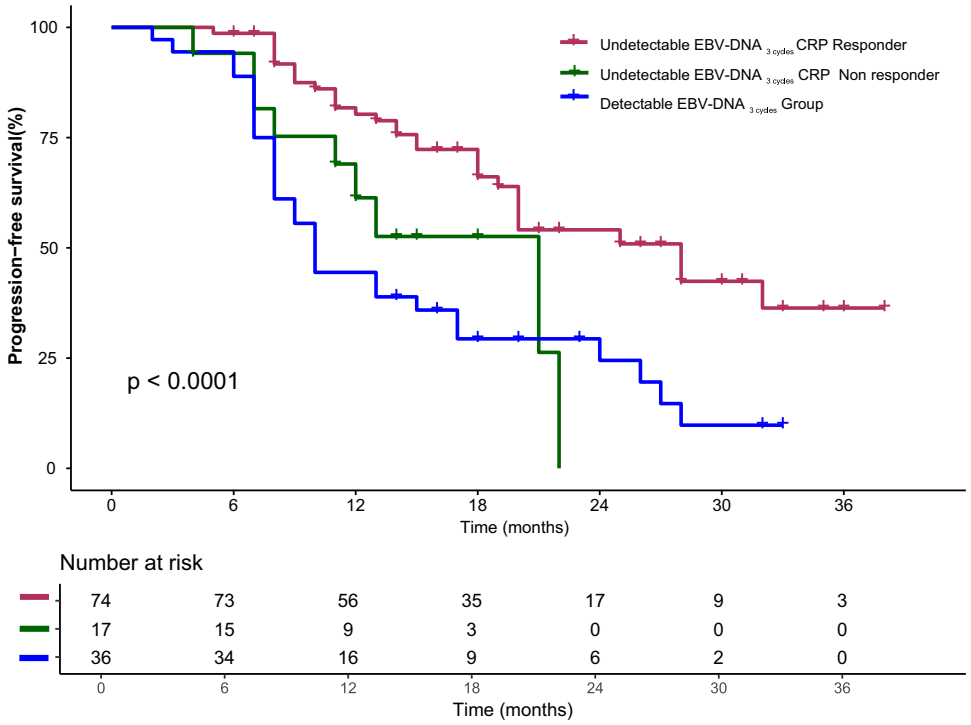

Figure 3 Early CRP kinetics combined with EBV-DNA$_{3\ cycles}$ levels to predict the prognosis of first-line chemotherapy combined with anti-PD-1 mAbs in patients with dmNPC. Progression-free survival (PFS) for undetectable EBV-DNA$_{3\ cycles}$ CRP responder (N=74), undetectable EBV-DNA$_{3\ cycles}$ CRP non-responder (N=17), or detectable EBV-DNA$_{3\ cycles}$ group (N=36).

Discussion

In this multicenter retrospective study, we found that early C-reactive protein (CRP) kinetics effectively predicted the prognosis of first-line chemoimmunotherapy in patients with de novo metastatic nasopharyngeal carcinoma (dmNPC). Furthermore, the combination of CRP and plasma Epstein-Barr virus (EBV) DNA levels enhanced the accuracy of prognostic predictions. Both CRP and plasma EBV DNA are readily available, low-cost, non-invasive biomarkers widely used in clinical practice, making their combined monitoring a valuable predictive tool for patients receiving first-line chemoimmunotherapy for dmNPC.

Our study demonstrated that CRP responders had significantly longer progression-free survival (PFS) and higher objective response rates (ORR) compared with CRP non-responders, highlighting the potential of early CRP kinetics as a prognostic marker in the era of immunotherapy for dmNPC. These findings are consistent with previous studies involving metastatic renal cell carcinoma, non-small cell lung cancer, and advanced urothelial carcinoma.[21–24] Notably, our study did not find a significant difference in median PFS between CRP flare-responders and CRP non-flare-responders, which contrasts with findings in other cancers. The reasons for the lack of prognostic significance of CRP flare kinetics in first-line chemoimmunotherapy for dmNPC are unclear; however, it is noteworthy that previous studies reported the efficacy of CRP flare response kinetics primarily in immunotherapy or immunotherapy combined with targeted therapy. In contrast, all patients in our study received first-line chemoimmunotherapy. The ORR in our study exceeded 90%, compared to 20.5%-43.0% in patients receiving immunotherapy alone for RM-NPC,[26–32] indicating a crucial role of chemotherapy in tumor control. Compared with other studies, the proportion of CRP flare-responders was lower in our cohort, possibly due to the immunosuppressive effects of chemotherapy on the immune response, which may inhibit CRP flare kinetics. This hypothesis requires further investigation in larger, prospective studies.

Locoregional radiotherapy target nasopharyngeal lesions and neck lymph nodes is the standard treatment for non-metastatic nasopharyngeal carcinoma and systemic therapy is the main treatment for R/M NPC. Interestingly, our study demonstrates that radiotherapy is an independent prognostic factor for PFS in patients with dmNPC receiving first-line chemoimmunotherapy. Our previously published work confirmed that LRRT following palliative chemotherapy combined with anti-PD-1 monoclonal antibodies significantly improved PFS in dmNPC patients.[33] Moreover, a Phase II clinical trial confirmed that dmNPC patients receiving first-line chemotherapy, when treated with a combination of LRRT and anti-PD-1 monoclonal antibodies, experienced prolonged PFS.[34] Further studies are needed to better understand the interactions between immunotherapy and chemoradiotherapy. In addition to radiotherapy, EBV DNA were also independent prognostic markers of PFS in multivariable Cox regression analysis. For plasma EBV-DNA,we always known that it was not only recognized as a diagnostic marker but also a strong prognostic biomarker for nasopharyngeal carcinoma, even in the era of immunotherapy.[35] Multiple studies have shown that high EBV DNA level was associated with poor prognosis.[36,37] Furthermore, the prospective CAPTAIN-1st clinical trial[12] and our prior retrospective work[33] also have shown that early clearance of plasma EBV DNA is associated with prolonged PFS in patients receiving combined chemotherapy and immunotherapy. However the varying testing standards for EBV DNA across different centers and the lack of unified method for quantitative detection yet. This prevents the calculation of a cut off value by combining the EBV DNA test results from multiple centers. To address this issue, we set a threshold of 10 copies/mL for EBV DNA detection at each center, which allows for more consistent classification of plasma EBV DNA levels and minimizes inter-center detection differences. Our subgroup analysis revealed that among patients with undetectable EBV DNA levels after three cycles of immunotherapy, CRP responders had significantly longer median PFS compared to CRP non-responders, a pattern not observed in the detectable EBV DNA group. These findings suggest that early CRP kinetics can further identify patients who benefit from immunotherapy, allowing clinicians to consider alternative or more effective treatments for those who do not benefit, even when EBV DNA is undetectable after three cycles.

Compared to EBV DNA, early CRP kinetics offers several advantages. Dynamic monitoring of CRP allows for the assessment of changes over time, both before and during treatment cycles. CRP kinetics captures the inflammatory response, which is a key factor in tumor progression and treatment efficacy. By evaluating the longitudinal changes in CRP, we can gain insights into the response of patients to first-line chemoimmunotherapy and better predict survival outcomes in patients with dmNPC receiving first-line chemoimmunotherapy. This dynamic monitoring can complement EBV DNA detection, offering a more comprehensive and time-sensitive approach to patient management. In our study, further analysis of the prognostic model combining early CRP kinetics and EBV DNA levels at three cycles demonstrated significant differences in PFS among the three groups, with accuracy testing (C-index) showing superior prognostic prediction compared to either biomarker alone. Unlike previous studies that combined baseline CRP and plasma EBV DNA for risk stratification in non-metastatic NPC,[16] our approach utilized longitudinal monitoring of these biomarkers during immunotherapy, offering a dynamic prediction of patient prognosis in dmNPC. Although acute CRP response alone or combined with early EBV DNA clearance was associated with improved PFS in our cohort, no

significant association with overall survival (OS) was observed, likely due to the limited number of events. A reanalysis of this cohort with extended follow-up is planned.

The underlying mechanisms linking systemic inflammatory responses to tumor responses to immune checkpoint inhibitors remain unclear. Previous studies have shown that chronic inflammation can create an immunosuppressive microenvironment,[38] and higher CRP levels have been associated with an immunosuppressive microenvironment characterized by M2 macrophage and regulatory T cell infiltration.[39] Yunpeng Yang et al has demonstrated that the results of immunological "hot" tumors identified through gene expression profiling (GEP) and activated DC characteristics may help identify R/M NPC patients who are most likely to benefit from immunotherapy.[11] Blank et al also indicates that CRP is one of the clinical markers of tumor-associated inflammation promoting tumor progression[40] Therefore, it is hypothesized that the CRP kinetics may be related to the dynamic changes in the tumor immune microenvironment in dmNPC patients receiving first-line chemoimmunotherapy, which could provide direction for subsequent mechanistic studies.

Despite being a rigorously designed retrospective study, there are some limitations. First, the retrospective nature of the study introduces potential selection bias. Second, the relatively short follow-up period limits the ability to assess long-term outcomes such as OS. Third, the study population was derived from EBV-endemic regions, which may differ in tumor characteristics compared to non-endemic areas. Therefore, large-scale prospective studies are needed to validate these findings in both endemic and non-endemic populations.

Conclusion

This study demonstrated that among patients with dmNPC receiving first-line chemoimmunotherapy, CRP responders were associated with significantly improved PFS, particularly in those with undetectable EBV DNA levels after three cycles of treatment. The combination of early CRP kinetics and EBV DNA levels provides a valuable tool for assessing the efficacy and prognosis of first-line chemoimmunotherapy in dmNPC patients. However, further prospective studies with larger sample sizes are necessary to validate these findings.

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Disclosure

The authors declare no conflicts of interest in this work.

References

1. Zhang LL, Huang MY, Fei X, et al. Risk stratification for nasopharyngeal carcinoma: a real-world study based on locoregional extension patterns and Epstein-Barr virus DNA load. *Ther Adv Med Oncol*. 2020;12:1758835920932052. doi:10.1177/1758835920932052
2. Chen YP, Chan ATC, Le QT, Blanchard P, Sun Y, Ma J. Nasopharyngeal carcinoma. *Lancet*. 2019;394(10192):64–80. doi:10.1016/S0140-6736(19)30956-0
3. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024;74(3):229–263. doi:10.3322/caac.21834
4. Lee AWM, Ng WT, Chan LK, et al. The strength/weakness of the AJCC/UICC staging system (7th edition) for nasopharyngeal cancer and suggestions for future improvement. *Oral Oncol*. 2012;48(10):1007–1013. doi:10.1016/j.oraloncology.2012.03.022
5. Pan JJ, Ng WT, Zong JF, et al. Proposal for the 8th edition of the AJCC/UICC staging system for nasopharyngeal cancer in the era of intensity-modulated radiotherapy. *Cancer*. 2016;122(4):546–558. doi:10.1002/cncr.29795
6. Lee AW, Ma BB, Ng WT, Chan AT. Management of Nasopharyngeal Carcinoma: current Practice and Future Perspective. *J Clin Oncol*. 2015;33(29):3356–3364. doi:10.1200/JCO.2015.60.9347
7. Jin Y, Cai XY, Cai YC, et al. To build a prognostic score model containing indispensible tumour markers for metastatic nasopharyngeal carcinoma in an epidemic area. *Eur J Cancer*. 2012;48(6):882–888. doi:10.1016/j.ejca.2011.09.004
8. Ong YK, Heng DM, Chung B, et al. Design of a prognostic index score for metastatic nasopharyngeal carcinoma. *Eur J Cancer*. 2003;39(11):1535–1541. doi:10.1016/S0959-8049(03)00310-1

9. Hui EP, Leung SF, Au JS, et al. Lung metastasis alone in nasopharyngeal carcinoma: a relatively favorable prognostic group. A study by the Hong Kong Nasopharyngeal Carcinoma Study Group. *Cancer*. 2004;101(2):300–306. doi:10.1002/cncr.20358
10. Setton J, Wolden S, Caria N, Lee N. Definitive treatment of metastatic nasopharyngeal carcinoma: report of 5 cases with review of literature. *Head Neck*. 2012;34(5):753–757. doi:10.1002/hed.21608
11. Yang Y, Pan J, Wang H, et al. Tislelizumab plus chemotherapy as first-line treatment for recurrent or metastatic nasopharyngeal cancer: a multicenter Phase 3 trial (RATIONALE-309). *Cancer Cell*. 2023;41(6):1061–1072.e4. doi:10.1016/j.ccell.2023.04.014
12. Yang Y, Qu S, Li J, et al. Camrelizumab versus placebo in combination with gemcitabine and cisplatin as first-line treatment for recurrent or metastatic nasopharyngeal carcinoma (CAPTAIN-1st): a multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol*. 2021;22 (8):1162–1174. doi:10.1016/S1470-2045(21)00302-8
13. Mai HQ, Chen QY, Chen D, et al. Toripalimab or placebo plus chemotherapy as first-line treatment in advanced nasopharyngeal carcinoma: a multicenter randomized phase 3 trial. *Nat Med*. 2021;27(9):1536–1543. doi:10.1038/s41591-021-01444-0
14. Qian X, Chen H, Tao Y. Biomarkers predicting clinical outcomes in nasopharyngeal cancer patients receiving immune checkpoint inhibitors: a systematic review and meta-analysis. *Front Immunol*. 2023;14:1146898. doi:10.3389/fimmu.2023.1146898
15. Xu JY, Wei XL, Ren C, et al. Association of Plasma Epstein-Barr Virus DNA With Outcomes for Patients With Recurrent or Metastatic Nasopharyngeal Carcinoma Receiving Anti-Programmed Cell Death 1 Immunotherapy. *JAMA Network Open*. 2022;5(3):e220587. doi:10.1001/jamanetworkopen.2022.0587
16. Tang LQ, Li CF, Chen QY, et al. High-sensitivity C-reactive protein complements plasma Epstein-Barr virus deoxyribonucleic acid prognostication in nasopharyngeal carcinoma: a large-scale retrospective and prospective cohort study. *Int J Radiat Oncol Biol Phys*. 2015;91(2):325–336. doi:10.1016/j.ijrobp.2014.10.005
17. Shibutani M, Maeda K, Nagahara H, et al. Prognostic significance of the preoperative serum C-reactive protein level in patients with stage IV colorectal cancer. *Surg Today*. 2015;45(3):315–321. doi:10.1007/s00595-014-0909-1
18. Guillem P, Triboulet JP. Elevated serum levels of C-reactive protein are indicative of a poor prognosis in patients with esophageal cancer. *Dis Esophagus*. 2005;18(3):146–150. doi:10.1111/j.1442-2050.2005.00474.x
19. Chen R, Zhou Y, Yuan Y, et al. Effect of CRP and Kinetics of CRP in Prognosis of Nasopharyngeal Carcinoma. *Front Oncol*. 2019;9:89. doi:10.3389/fonc.2019.00089
20. Ozawa Y, Amano Y, Kanata K, et al. Impact of early inflammatory cytokine elevation after commencement of PD-1 inhibitors to predict efficacy in patients with non-small cell lung cancer. *Med Oncol*. 2019;36(4):33. doi:10.1007/s12032-019-1255-3
21. Klümper N, Saal J, Berner F, et al. C reactive protein flare predicts response to checkpoint inhibitor treatment in non-small cell lung cancer. *J Immunother Cancer*. 2022;10(3):e004024. doi:10.1136/jitc-2021-004024
22. Tomisaki I, Harada M, Tokutsu K, et al. Impact of C-reactive Protein Flare Response in Patients With Advanced Urothelial Carcinoma Who Received Pembrolizumab. *Vivo*. 2021;35(6):3563–3568. doi:10.21873/invivo.12659
23. Klümper N, Schmucker P, Hahn O, et al. C-reactive protein flare-response predicts long-term efficacy to first-line anti-PD-1-based combination therapy in metastatic renal cell carcinoma. *Clin Transl Immunol*. 2021;10(12):e1358. doi:10.1002/cti2.1358
24. Fukuda S, Saito K, Yasuda Y, et al. Impact of C-reactive protein flare-response on oncological outcomes in patients with metastatic renal cell carcinoma treated with nivolumab. *J Immunother Cancer*. 2021;9(2):e001564. doi:10.1136/jitc-2020-001564
25. Zeng F, Lu T, Xie F, et al. Effects of locoregional radiotherapy in de novo metastatic nasopharyngeal carcinoma: a real-world study. *Transl Oncol*. 2021;14(11):101187. doi:10.1016/j.tranon.2021.101187
26. Chen X, Wang W, Zou Q, et al. 804 A phase II study of the anti-programmed cell death-1 (PD-1) antibody penpulimab in patients with metastatic nasopharyngeal carcinoma (NPC) who had progressed after two or more lines of chemotherapy. *J Immuno Ther Cancer*. 2020;8(Suppl 3):A481–A481.
27. Fang W, Yang Y, Ma Y, et al. Camrelizumab (SHR-1210) alone or in combination with gemcitabine plus cisplatin for nasopharyngeal carcinoma: results from two single-arm, Phase 1 trials. *Lancet Oncol*. 2018;19(10):1338–1350. doi:10.1016/S1470-2045(18)30495-9
28. Shen L, Guo J, Zhang Q, et al. Tislelizumab in Chinese patients with advanced solid tumors: an open-label, non-comparative, phase 1/2 study. *J Immunother Cancer*. 2020;8(1):e000437. doi:10.1136/jitc-2019-000437
29. Bby M, Lim WT, Goh BC, et al. Antitumor Activity of Nivolumab in Recurrent and Metastatic Nasopharyngeal Carcinoma: an International, Multicenter Study of the Mayo Clinic Phase 2 Consortium (NCI-9742). *J Clin Oncol*. 2018;36(14):1412–1418. doi:10.1200/JCO.2017.77.0388
30. Hsu C, Lee S-H, Ejadi S, et al. Safety and Antitumor Activity of Pembrolizumab in Patients With Programmed Death-Ligand 1–Positive Nasopharyngeal Carcinoma: results of the KEYNOTE-028 Study. *J clin oncol*. 2017;35(36):4050–4056. doi:10.1200/JCO.2017.73.3675
31. Wang FH, Wei XL, Feng J, et al. Efficacy, Safety, and Correlative Biomarkers of Toripalimab in Previously Treated Recurrent or Metastatic Nasopharyngeal Carcinoma: a Phase II Clinical Trial (POLARIS-02). *J Clin Oncol*. 2021;39(7):704–712. doi:10.1200/JCO.20.02712
32. Chan ATC, Lee VHF, Hong RL, et al. Pembrolizumab monotherapy versus chemotherapy in platinum-pretreated, recurrent or metastatic nasopharyngeal cancer (KEYNOTE-122): an open-label, randomized, phase III trial. *Ann Oncol*. 2023;34(3):251–261. doi:10.1016/j.annonc.2022.12.007
33. Hu YJ, Lu TZ, Zhang H, et al. Locoregional radiotherapy improves survival outcomes in de novo metastatic nasopharyngeal carcinoma treated with chemoimmunotherapy. *ESMO Open*. 2023;8(5):101629. doi:10.1016/j.esmoop.2023.101629
34. Chen S-Y, Chen M, Rui Y, Hua Y, Zou X, Wang Z-Q. Efficacy and safety of chemotherapy plus subsequent locoregional radiotherapy and toripalimab in de novo metastatic nasopharyngeal carcinoma. *J clin oncol*. 2022;40(16_suppl):6025. doi:10.1200/JCO.2022.40.16_suppl.6025
35. Li XY, Luo DH, Guo L, et al. Deintensified Chemoradiotherapy for Pretreatment Epstein-Barr Virus DNA-Selected Low-Risk Locoregionally Advanced Nasopharyngeal Carcinoma: a Phase II Randomized Noninferiority Trial. *J Clin Oncol*. 2022;40(11):1163–1173. doi:10.1200/JCO.21.01467
36. Du X-J, Tang -L-L, Mao Y-P, et al. Circulating EBV DNA, Globulin and Nodal Size Predict Distant Metastasis after Intensity-Modulated Radiotherapy in Stage II Nasopharyngeal Carcinoma. *Res Paper J Cancer*. 2016;7(6):664–670.
37. Sun X-S, Chen W-H, Liu S-L, et al. Individualized concurrent chemotherapy by pretreatment plasma Epstein-Barr viral DNA in II-III stage nasopharyngeal carcinoma: a propensity score matching analysis using a large cohort. *Cancer Med*. 2019;8(9):4214–4225. doi:10.1002/cam4.2343
38. Wang D, DuBois RN. Immunosuppression associated with chronic inflammation in the tumor microenvironment. *Carcinogenesis*. 2015;36 (10):1085–1093. doi:10.1093/carcin/bgv123

39. Nakayama T, Saito K, Kumagai J, et al. Higher Serum C-reactive Protein Level Represents the Immunosuppressive Tumor Microenvironment in Patients With Clear Cell Renal Cell Carcinoma. *Clin Genitourin Cancer*. 2018;16(6):e1151–e1158. doi:10.1016/j.clgc.2018.07.027
40. Blank CU, Haanen JB, Ribas A, Schumacher TN. Cancer Immunology: the cancer immunogram. *Science*. 2016;352(6286):658–660. doi:10.1126/science.aaf2834