Int J Clin Oncol (2016) 21:1038–1045
DOI 10.1007/s10147-016-1020-y

ORIGINAL ARTICLE

The value of circulating CYFRA21-1 expression in patients with nasopharyngeal carcinoma: a study of 529 subjects

Xin-mao Song[1] · Zhu-jian Wang[2] · Wen-jun Cao[2] · Ji-Li[1] · Fu Chen[1] · Sheng-zi Wang[1]

Received: 5 May 2016 / Accepted: 29 June 2016 / Published online: 12 July 2016

Abstract
Background Early diagnosis of nasopharyngeal carcinoma (NPC) needs more reliable biomarkers. The aim of this study was to investigate serum cytokeratin 19 fragment 21.1 (CYFRA21-1) as an NPC biomarker based on data from a large sample.
Methods From October 2010 to February 2014, 529 subjects were enrolled and divided into three groups—NPC group ($n = 274$), healthy control group ($n = 175$) and nasal inflammatory disease group ($n = 80$). Serum CYFRA21-1 levels were measured prior to radiotherapy/chemoradiotherapy, and their associations with T, N, and clinical classification were determined. Receiver operating characteristic curve analysis was performed to discriminate the NPC group from the healthy control and nasal inflammatory disease groups. Three Epstein–Barr virus (EBV) antibodies and their correlations with serum CYFRA21-1 levels were analyzed.
Results Pretreatment serum CYFRA21-1 levels were significantly elevated in the NPC group compared with the other groups ($p < 0.01$), Furthermore, serum CYFRA21-1 levels decreased significantly after radiotherapy ($p < 0.01$). Serum CYFRA21-1 levels were closely related to T, N, and clinical classifications. The area under the curve, sensitivity and specificity of the serum CYFRA21-1 levels in the NPC patients were 0.89, 0.87 and 0.83, respectively. Strong correlations were observed between serum CYFRA21-1 levels and EBV antibodies.
Conclusion Serum CYFRA21-1 may be a reliable and effective biomarker for NPC.

Keywords Cytokeratin 19 fragment 21.1 · Nasopharyngeal carcinoma · Epstein–Barr virus antibodies · Tumor biomarker

Introduction

Nasopharyngeal carcinoma (NPC) is more prevalent in southern China than elsewhere, with an incidence rate of 20–30 cases per 100,000 individuals [1]. Radiotherapy has been shown to be the most effective treatment, provided the disease is detected at an early stage. However, NPC is prone to early lymph node metastasis and difficult to detect because of its hidden location and its non-specific local symptoms in the early stage [2]. Most NPC patients have advanced disease at the time of diagnosis, which results in a poor prognosis and a low survival rate. Therefore, the discovery of biomarkers for detecting and monitoring NPC is critical for improving overall survival.

Previous studies have shown a correlation between the development of non-keratinizing undifferentiated NPC and Epstein–Barr virus (EBV) infection [3]. Several EBV antibodies have been used as reference factors for the clinical diagnosis and prognosis of NPC. However, in many cases, the degree of disease progression was not consistent with the change in EBV antibody titers, and some EBV antibody titers remained at high or even increased levels, with clear tumor regression and no signs of recurrence [4]. Plasma

X. Song and Z. Wang contributed equally and are co-first authors.

✉ Sheng-zi Wang
shengziwang@fudan.edu.cn

[1] Department of Radiation Oncology, Eye, Ear, Nose and Throat, FuDan University, Fenyang Road 83, Shanghai 200031, China

[2] Department of Clinical Laboratory, Eye, Ear, Nose and Throat, FuDan University, Fenyang Road 83, Shanghai 200031, China

EBV DNA load is one of the most well recognized biomarkers for NPC, and an expanding body of data suggests that the EBV DNA load is correlated with tumor load in NPC [1]. Notably, in patients who have a detectable residual EBV DNA load soon after completing a full course of radiotherapy or chemoradiotherapy, a very high risk of tumor recurrence was observed during the early follow-up period [5]. The use of the plasma EBV DNA load as a biomarker may improve the accuracy of NPC diagnosis in high-risk individuals, but it has shown limited value for screening patients with early stage NPC and for predicting NPC development [6]. An important question is why EBV antibody titers or DNA loads do not reflect the NPC tumor status very well. One reason may be related to whether the observed EBV antibody titers or DNA load were the result of EBV proliferation rather than the metabolism of NPC tumor cells. Therefore, better serum biomarkers for NPC must be discovered, similar to alpha fetal protein and carcinoembryonic antigen for liver cancer and gastrointestinal tumors, respectively. After preliminary experiments, the regularity and stability of cytokeratin 19 fragment 21.1 (CYFRA21-1) attracted our attention as a possible candidate among many serum proteins.

Cytokeratin 19 (CK-19) is a low-molecular-weight (40,000 Da) protein and the smallest member of its family. CK-19 is widely distributed in simple epithelia, including bronchial epithelial cells [7]. The activation of proteases accelerates the degradation of neoplastically transformed epithelial cells, resulting in the release of many degraded CK19 fragments into the blood. These fragments can specifically bind to soluble fragments of two monoclonal antibodies, KS19.1 and BM19.21. We chose one of these CK19 fragments, CYFRA21-1, because this fragment presented a regular changer with during chemoradiotherapy in a preliminary experiment [8]. CYFRA21-1 has a molecular weight of approximately 30,000 Da and is a useful auxiliary test and prognostic tool for non-small cell lung cancer and malignant lung tissue; in particular, it can be used to diagnose and monitor tumor metastasis in squamous cell carcinoma [9, 10]. Some studies have suggested the possible prognostic use of serum CYFRA21-1 in NPC [11, 12].

Our previous experiments confirmed that serum CYFRA21-1 levels in patients with NPC were significantly higher than those of healthy individuals and suggested that CYFRA21-1 could be used as a tumor marker of NPC [13]. To rigorously verify our results, we increased the sample size and included data on EBV antibodies in order to obtain more data for discussion. In this study, we measured the CYFRA21-1 levels of patients with NPC, patients with nasal/sinus inflammatory disease, and healthy volunteers. In addition, we also analyzed the serum CYFRA21-1 levels in the NPC group and their correlations with EBV antibodies. We measured serum CYFRA21-1 level changes immediately following treatment to explore the feasibility of serum CYFRA21-1 as a tumor biomarker of the presence and status of NPC.

Materials and methods

Subjects

Two hundred and seventy-four patients diagnosed with NPC were assessed between October 2010 and February 2014 at the Eye, Ear, Nose and Throat (EENT) Hospital of FuDan University. Patients with metastasis and malignancy in other organs were excluded. One hundred and seventy-five healthy volunteers and 80 patients with chronic nasal/sinus inflammatory disease (defined as inflammation of the nose and the paranasal sinuses characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge, for more than 12 weeks) were defined as the healthy control group and the nasal inflammatory disease control group, respectively. Patient evaluation included medical history, physical examination, and imageological and biochemical analyses to exclude other malignant tumors and serious diseases. Patients with NPC were required to be staged according to the American Joint Committee on Cancer, 2010 Edition (AJCC 2010). All subjects provided written informed consent. The study protocol was approved by the ethics committee of our institution. The study conforms with the Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the British Medical Journal (18 July 1964).

Treatment modalities

All patients received definitive intensity modulation radiation therapy (IMRT) or three-dimensional conformal radiotherapy (3D-CRT). Prescribed doses were administered according to tumor volume and stage according to the IMRT target dose designs and the expert consensus for NPC provided by the Chinese Clinical NPC Working Committee and Wang's target delineation protocol [14]. Patients with stage II-IV disease also received chemotherapy, including induction chemotherapy and concurrent chemotherapy. In addition, some patients also received at least six cycles of nimotuzumab (a humanized monoclonal antibody) target therapy.

Sample collection and assays

Venous blood samples were collected immediately before treatment and immediately at the end of radiotherapy. Peripheral blood samples (5 ml) were collected

Table 1 General baseline characteristics of 529 subjects

	NPC group (%)	Nasal inflammation disease group (%)	Healthy controls (%)
Age (years)			
>50	141 (51.5)	41 (51.3)	90 (51.4)
≤50	133 (48.5)	39 (48.7)	85 (48.6)
Gender			
Male	202 (73.7)	50 (62.5)	96 (54.9)
Female	72 (26.3)	30 (37.5)	79 (45.1)
T classification			
T1–2	151 (55.1)		
T3–4	123 (44.9)		
N classification			
N0	37 (13.5)		
N1	62 (22.6)		
N2	160 (58.4)		
N3	15 (5.5)		
AJCC stage			
I	23 (8.4)		
II	35 (12.8)		
III	145 (52.9)		
IV	71 (25.9)		

and stored in procoagulant tubes containing separating gel. The samples were left standing for 30 min at room temperature and then centrifuged at 3000*g* at 4 °C for 15 min. The supernatants were separated manually into freezing tubes and were frozen at −80 °C without any further treatment for a maximum of three years. CYFRA21-1 evaluation kits were purchased from Chemical Biotechnology Limited (Beijing, China). Serum CYFRA21-1 levels were automatically assessed using a chemiluminescent immunoassay (Chemclin Biotech, China) based on ELISA. All the operations and assessments were performed by clinical laboratory professionals at our hospital.

Methods for serological EBV antibody detection

The serum samples were tested blindly according to the manufacturer's instructions in the clinical laboratory of the EENT Hospital at FuDan University. Immunoglobulin A (IgA) antibodies against EBV capsid antigen (EBV-VCA-IgA) kits were purchased from the Euroimmun Medical Laboratory Diagnostics Stock Company (Germany). Immunoglobulin G (IgG) antibodies against EBV Rta (EBV-Rta-IgG) kits were purchased from Tarcine BioMed Limited (Beijing, China). IgA antibodies against EBV nuclear antigen 1 (EBV-NA1-IgA) were purchased from Zhongshan Biotechnology Limited (Guangzhou, China). The ELISA seromarker levels were assessed and standardized using photometric measurements according to the manufacturer's instructions.

Statistical analysis

The clinical characteristics of patients with NPC in different subgroups were compared using the chi-squared test or Fisher's exact test for categorical data. Student's *t* test or Mann–Whitney *U* test was performed to identify the differences in serum CYFRA21-1 levels for different T and N classifications and the differences among other continuous variables. A one-way analysis of variance (ANOVA) was used to compare the differences in serum CYFRA21-1 levels at different N classifications. Pearson's correlation analysis was used to assess correlations between serum CYFRA21-1 levels and the three EBV antibodies. Two-sided p values <0.05 were considered statistically significant. All statistical procedures were performed using SigmaPlot 12.3 and IBM SPSS 20 software. All continuous values in the text were represented as mean ± SD, and all categorical variables as absolute numbers (percentage, %).

Results

Characteristics and outcomes

We enrolled 274 cases in the NPC group, 80 cases in the nasal inflammatory disease group and 175 cases in the healthy control group. Table 1 shows the general characteristics of all subjects. Age and gender were matched among the three groups. The median ages of the three groups were 51 (16–81), 50 (25–70) and 50 (18–76) years, respectively.

Table 2 Correlations between CYFRA21-1, age and gender in different groups

Group	Age		*p* value	Gender		*p* value
	>50	≤50		Male	Female	
NPC	5.07 ± 1.97	5.02 ± 1.89	0.893	5.13 ± 1.91	4.88 ± 1.36	0.203
Healthy control	2.32 ± 1.36	2.41 ± 1.26	0.473	2.46 ± 1.28	2.24 ± 1.11	0.325
Nasal inflammatory disease	2.38 ± 1.41	2.30 ± 0.91	0.809	2.30 ± 1.16	2.39 ± 1.19	0.917

Serum CYFRA21-1 levels of each group

The baseline serum CYFRA21-1 levels in the NPC group, the nasal inflammatory disease group and the healthy control group were 5.05 ± 1.93 ng/ml (0.73–12.18 ng/ml), 2.34 ± 1.17 (0.89–6.40 ng/ml), and 2.36 ± 1.21 ng/ml (0.65–7.10 ng/ml), respectively, and were not significantly different in terms of age or gender (Table 2). The serum CYFRA21-1 level of the NPC group was higher than the other groups ($p < 0.01$); however, no significant difference was observed between the nasal inflammatory disease group and the healthy control group ($p = 0.712$; Fig. 1). The serum CYFRA21-1 levels of the NPC group decreased to 2.25 ± 0.81 ng/ml (0.75–6.50 ng/ml) after radiotherapy was completed, which was not significantly different from the baseline levels of the other groups ($H = 0.405$, $p = 0.817$; Fig. 2).

The diagnostic cut-off value for the serum CYFRA21-1 levels calculated using the receiver operating characteristics (ROCs) was 3.09 ng/ml. In 86.5 % (237/274) of the NPC group, the baseline serum CYFRA21-1 levels were >3.09 ng/ml; however, only 17.5 % (48/274) of the NPC group had higher levels after completing radiotherapy. In addition, 17.5 % (14/80) of the inflammation group and 17.7 % (31/175) of the healthy group had serum CYFRA21-1 levels higher than the 3.09 ng/ml cut-off; however, no significant differences were observed between the three groups ($\chi^2 = 6.0$, $p = 0.306$; Fig. 3).

ROC analysis between patients and healthy controls

The ROC curves based on the ELISA results were plotted to determine the diagnostic efficiency of serum CYFRA21-1 levels for NPC. We set the serum CYFRA21-1 level of the NPC group as the higher group and that of the healthy control group as the lower group. Prior to ROC analysis, the pretest probability and cost ratio were set to 0.05. The area under the curve (AUC) for serum CYFRA21-1 was 0.893 (95 % CI 0.865-0.921), and it satisfactorily discriminated between the patients with NPC and the healthy controls (Fig. 4). Based on the ROC curve and the dot histogram, we selected an optimum serum CYFRA21-1 cut-off value of 3.09 ng/ml for NPC diagnosis. Considering 3.09 ng/ml CYFRA21-1 as the cut-off value, the sensitivity and specificity of CYFRA21-1 for detecting NPC were 0.87 and 0.83, respectively (Fig. 5).

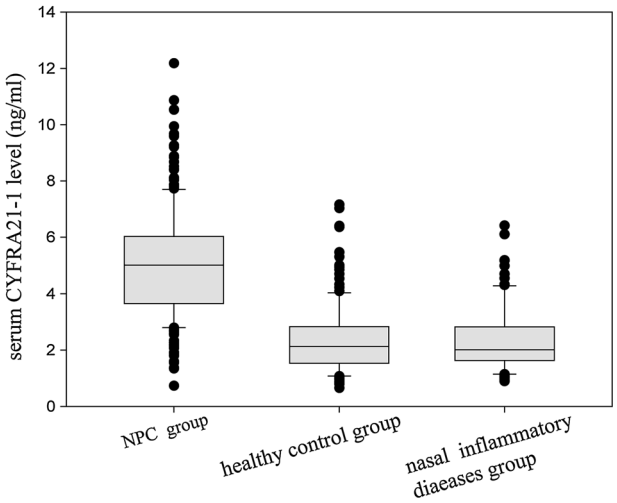

Fig. 1 Boxplots of the distribution of serum CYFRA21-1 (ng/ml) levels in the NPC group ($n = 274$), the healthy control group ($n = 175$) and the nasal inflammatory disease group ($n = 80$). The *vertical axis* represents the serum CYFRA21-1 levels determined with ELISA. The *upper hinge of the box* indicates the 75th percentile of the dataset, and the lower hinge indicates the 25th percentile. The *line in the box* indicates the median value. The *upper whiskers of the vertical lines* indicate the upper quartile +1.5 × quartile range, the *lower whiskers* indicate the lower quartile −1.5 × quartile range, and the *dots beyond the whiskers* are outliers

Correlation between serum CYFRA21-1 levels and T, N, and TNM stage

To compare the serum CYFRA21-1 levels among the different T stages, T1 and T2 were merged into an early T-stage group, and T3 and T4 were merged into an advanced T-stage group. The CYFRA21-1 level of the advanced T-stage patients was 5.95 ± 1.87 ng/ml, which was higher than the early T-stage patients (4.27 ± 1.62 ng/ml, $p < 0.01$). Pretreatment serum CYFRA21-1 level distribution differed significantly according to the N stage (N0–N3, 4.05 ± 1.88, 4.68 ± 1.58, 5.36 ± 1.94, and 5.58 ± 2.34 ng/ml, respectively; $H = 12.541$; $p = 0.006$). According to clinical classifications, all patients were divided into two groups—an early clinical stage (I–II) and an advanced clinical stage (III–IV) group, and the serum CYFRA21-1 level of the advanced clinical stage group was significantly higher than the early clinical stage group (5.39 ± 1.89 vs 3.73 ± 1.48 ng/ml, $p < 0.01$; Table 3).

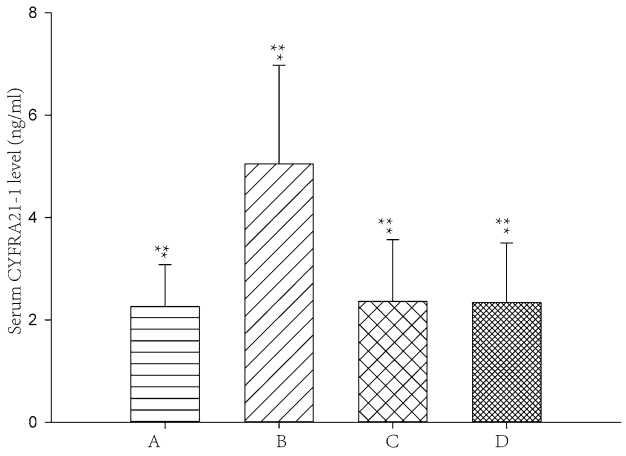

Fig. 2 Comparison of serum CYFRA21-1 levels of the different groups. *A*, *B*, *C*, and *D* represent the NPC group that received complete treatment ($n = 274$), the NPC group prior to treatment (baseline level, $n = 274$), the healthy controls ($n = 175$), and the nasal inflammatory disease group ($n = 80$), respectively. The *vertical axis* represents the serum CYFRA21-1 levels determined with ELISA. *indicates $p < 0.001$ between the NPC group and the other three groups; **indicates $p = 0.817$ between *A*, *C*, and *D*

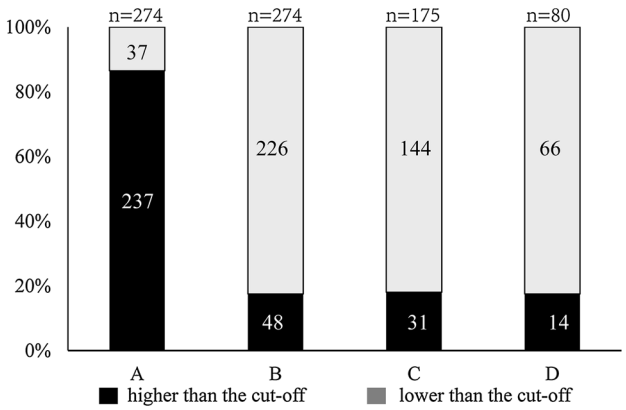

Fig. 3 The percentage of serum CYFRA21-1 levels higher than the cut-off value for all groups. *A*, *B*, *C*, and *D* represent the NPC group before treatment (baseline level, $n = 274$), the NPC group that received complete treatment ($n = 274$), the healthy control group ($n = 175$), and the nasal inflammatory disease group ($n = 80$), respectively. In 86.5 % (237/274) of the NPC group, the baseline serum CYFRA21-1 levels were higher than the cut-off value of 3.09 ng/ml; however, only 17.5 % (48/274) of patients in the NPC group received treatment. In total, 17.5 % (14/80) of the patients in the inflammatory group and 17.7 % (31/175) of patients in the healthy group had levels higher than the cut-off value of 3.09 ng/ml

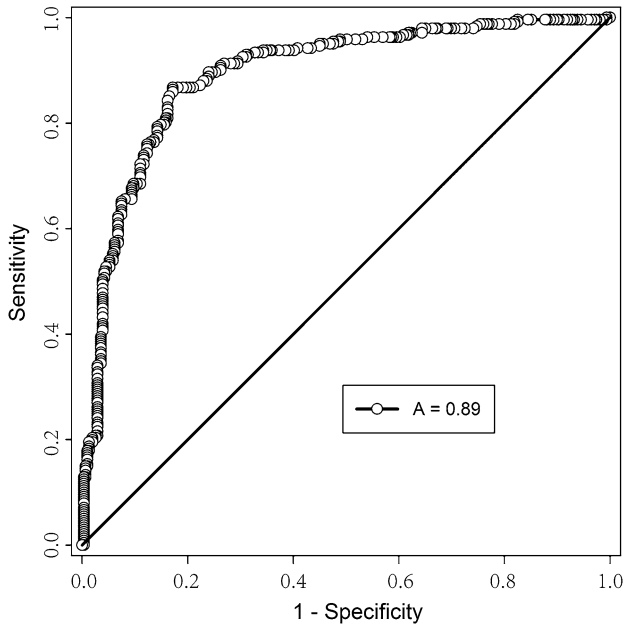

Fig. 4 Receive operator characteristic (ROC) curve analyses of the serum CYFRA21-1 levels in the NPC and healthy control groups to discriminate between patients with NPC and non-NPC controls. Comparison of the CYFRA21-1 levels of the two groups yielded an AUC of 0.89 (95 % CI 0.865–0.921)

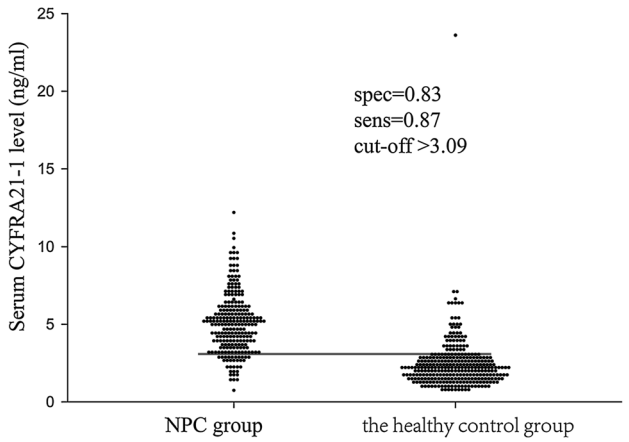

Fig. 5 Dot-histogram analysis shows the serum CYFRA21-1 concentrations in individuals in the NPC group and healthy control group. The *vertical axis* represents the serum CYFRA21-1 value determined by ELISA. The *red horizontal line* indicates the cut-off serum CYFRA21-1 level that differentiated the NPC group from the non-NPC healthy controls; this cut-off value of 3.09 ng/ml had a specificity of 0.83 and sensitivity of 0.87

Serum EBV Rta-IgG, EBNA1-IgA, and VCA-IgA antibody measurements

Of the 274 examined NPC cases, 183 were measured for three EBV antibodies—EBV-Rta-IgG, EBV-EBNA1-IgA and EBV-VCA-IgA. The relative optical density (rOD) values of the three EBV antibodies were 3.13 ± 3.11, 3.52 ± 2.69 and 4.78 ± 3.03, respectively (Fig. 6). According to the diagnostic criteria of the kits, the positive rates of Rta-IgG, EBNA1-IgA and VCA-IgA were 60.7 % (111/183), 85.8 % (157/183) and 91.3 % (167/183), respectively.

Table 3 Correlations between serum CYFRA21-1 level and tumor stage

Stage	Serum CYFRA21-1 level $M \pm$ SD (ng/ml)	p value
T1–2	4.27 ± 1.62	
T3–4	5.95 ± 1.87	<0.01
N0	4.05 ± 1.88	
N1	4.68 ± 1.58	
N2	5.36 ± 1.94	
N3	5.58 ± 2.34	0.006
I–II	3.73 ± 1.48	
III–IV	5.39 ± 1.88	<0.01

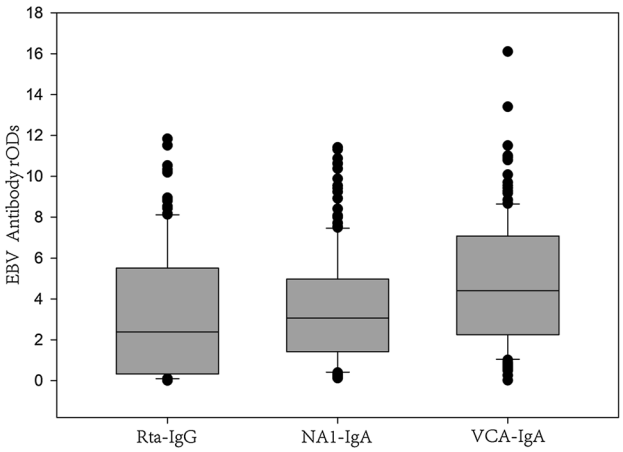

Fig. 6 Boxplots of the distribution of the relative optical density (rOD) values of serum EBV antibodies, including Rta-IgG, EBNA1-IgA, and VCA-IgA, in the NPC group ($n = 183$). The *vertical axis* represents the antibody titers determined with ELISA and indicates the rOD levels. The *upper hinge of the box* indicates the 75th percentile of the dataset, and the *lower hinge* indicates the 25th percentile. The *line in the box* indicates the median value. The *upper whiskers of the vertical lines* indicate the upper quartile + 1.5 × quartile range, the *lower whiskers* indicate the lower quartile + 1.5 × quartile range, and the *dots beyond the whiskers* indicate the outliers

Correlations between the baseline serum CYFRA21-1 level and the three EBV antibody rOD values were analyzed with Pearson's correlation test (two-tailed t test). The serum CYFRA21-1 levels were significantly correlated with Rta-IgG ($r = 0.210$, $p = 0.006$) and VCA-IgA ($r = 0.305$, $p = 0.000$) antibody rOD values but were not significantly correlated with EBNA1-IgA antibody rOD values ($r = 0.02$, $p = 0.796$).

Discussion

Early diagnosis of NPC can significantly improve patient survival. Because the anatomical location is mostly hidden and the early symptoms of NPC can be easily overlooked, the majority of patients were at the middle and late stages of the disease as diagnosis. Currently, EBV DNA/antibodies are the established and validated serum biomarker for NPC in clinical practice. Unfortunately, the use of EBV DNA/antibodies as markers for NPC diagnosis and monitoring has many limitations because a high proportion of the general population exhibits positive EBV antibodies, which increases the probability of false positive NPC diagnosis. Thus, other serum tumor biomarkers should be investigated to provide more information about tumor prognosis and treatment monitoring. Preliminary tests of dozens of serum markers yielded some potential candidates for NPC diagnosis and monitoring, including serum CYFRA21-1 levels. For better comparative analysis from different aspects, we also investigated serum CYFRA21-1 levels in the patients with nasal/nasal inflammatory disease and healthy volunteers.

The release of CYFRA21-1 has been closely associated with cellular apoptosis during tumor growth [15]. Previous studies have demonstrated a negative correlation between CK-19 and serum CYFRA21-1 levels in tumor cells [16]. Elevated serum CYFRA21-1 levels indicate greater CK-19 degradation into soluble fragments in the blood, which increases serum CYFRA21-1 levels and indicates the probability of abnormal epithelial cell differentiation into cell carcinoma. Serum CYFRA21-1 levels have been shown to have prognostic value in head and neck tumors [17], non-small lung cancer [18], esophageal carcinoma [19], pancreatic cancer [20], and urothelial carcinoma of the bladder [21].

Pretreatment serum CYFRA21-1 levels were significantly elevated in patients with NPC compared with healthy controls (5.07 ± 1.98 vs 2.36 ± 1.21 ng/ml) in our previous studies [13], and the serum CYFRA21-1 levels observed in the NPC group and healthy control group in the present study were consistent with the results of a previous study with large sample data [12, 22]. In addition, we also included the inflammatory disease group in this study to investigate whether the serum CYFRA21-1 levels also took into account the nasal/sinus inflammation. However, no difference was observed between the nasal inflammatory group and the healthy control group, suggesting that serum CYFRA21-1 levels increase showed a tumor-specific alteration. ROC curve analysis of the serum CYFRA21-1 levels in the NPC group and the healthy control group was able to distinguish between the two groups, and a threshold value of 3.09 ng/ml was obtained. The serum CYFRA21-1 levels were higher than the cut-off in 17.52 % (48/274) of patients in the NPC group who received complete radiotherapy, 17.50 % (14/80) of patients in the nasal inflammatory disease group and 17.71 % (31/175) of patients in the healthy

control group, and no differences were observed between the groups ($\chi^2 = 6.0$, $p = 0.306$); however, in 86.48 % (237/274) of patients in the NPC group, pretreatment serum CYFRA21-1 levels were higher than the cut-off. The serum CYFRA21-1 levels declined following treatment, suggesting a tumor cell status change as a result of the treatment. Thus, serum CYFRA21-1 levels may reflect the tumor burden in NPC.

To investigate this hypothesis, we collected detailed clinical information from NPC patients regarding their staging, treatment program and treatment efficacy. Serum CYFRA21-1 levels tended to be obviously higher in the advanced T-stage and clinical stage, indicating a significant correlation between serum CYFRA21-1 levels and T classification and clinical stage, and the results are consistent with previous studies [11, 22]. A significant difference in serum CYFRA21-1 levels was observed between different N classifications ($H = 12.541$, $p = 0.006$); however, Lin et al. [12] found no correlation between serum CYFRA21-1 levels and N classification in NPC patients. Thus, the close correlation between serum CYFRA21-1 levels and tumor stage suggested that serum CYFRA21-1 levels may be related to tumor severity. Tumor staging is an important prognostic factor. Furthermore, serum CYFRA21-1 levels also changed after radiotherapy was completed. This finding may indicate the feasibility of using serum CYFRA21-1 as a tumor biomarker. We then investigated the potential correlations between serum CYFRA21-1 levels and EBV antibodies.

EBV is closely related to the development of NPC, and many EBV virus-derived proteins have been used as tumor markers for NPC. Currently, VCA-IgA, EA-IgA, EBNA1-IgA, and Rta-IgG are routinely used in NPC screening and diagnosis. The increase in EBV Rta-IgG suggested that the EBV was in the lytic replication phase. Thus, the EBV Rta-IgG content may directly reflect the level of virus replication. Rta protein also increased with the expansion of tumor lesions and invasiveness. EBVNA1 is the only antigen expressed in cells with latent or activated EBV infection. EBVNA1 binding DNA is a key factor in maintaining latent EBV infection status and DNA replication in tumor cells [23]. A previous study showed that individual anti-EBV EBNA1-IgA seropositivity was the marker most strongly associated with NPC risk [24]. The combination of VCA-IgA and EBNA1-IgA detection with ELISA outperforms the traditional NPC screening scheme and could become the preferred serodiagnostic strategy for NPC screening in high-incidence areas [25].

Pearson's correlation analyses were performed to investigate correlations between serum CYFRA21-1 levels and EBV antibodies. Correlations between serum CYFRA21-1 and VCA-IgA ($p < 0.01$) and Rta-IgG ($p = 0.006$) were observed. This may suggest serum CYFRA21-1 as a biomarker for NPC in another aspect. The ROC showed that the AUC was 0.89, indicating that the serum CYFRA21-1 level has high accuracy as an NPC tumor biomarker. Sensitivity was 0.87, and specificity was 0.83, which were higher than in previous studies. Serum CYFRA21-1 levels decreased significantly with the progression of treatment from 5.05 ± 1.93 ng/ml at baseline to 2.25 ± 0.81 ng/ml after the completion of radiotherapy. These results are consistent with the short-term efficacy of treatment and could be described as accurately reflecting tumor status or monitoring the efficacy of treatment.

Conclusion

In summary, the baseline serum CYFRA21-1 levels in the NPC group were significantly higher than those of the healthy control and the nasal inflammatory group. As treatment progressed, the serum CYFRA21-1 levels decreased, suggesting that circulating CYFRA21-1 elevation and alteration were unique to tumors. Furthermore, the serum CYFRA21-1 levels of patients with nasopharyngeal tumors at different T, N, and clinical stages were obviously different. Serum CYFRA21-1 levels were significantly correlated with the EBV antibodies Rta-IgG and VCA-IgA. Therefore, serum CYFRA21-1 is an NPC diagnostic indicator with high accuracy, sensitivity, and specificity. Despite the many limitations of our study, its additional data from different perspectives verifies that serum CYFRA21-1 is a reliable, effective tumor biomarker for NPC. Further investigations should be performed to determine whether joint EBV-DNA detection can yield more accurate and effective results.

Acknowledgments This project was supported by funds from the People's Republic of China Ministry of Science and Technology. The Project Number is 2011ZX09101-001.

Compliance with ethical standards

Conflict of interest All authors declared they had no conflict of interest.

References

1. Wu SY, Wu YH, Yang MW et al (2014) Comparison of concurrent chemoradiotherapy versus neoadjuvant chemotherapy followed by radiation in patients with advanced nasopharyngeal carcinoma in endemic area: experience of 128 consecutive cases with 5 year follow-up. BMC Cancer 14:787
2. Su FR, Ding JH, Bo L et al (2014) Chromodomain helicase/ATPase DNA binding protein 1-like protein expression predicts poor prognosis in nasopharyngeal carcinoma. Exp Ther Med 8(6):1745–1750
3. Raab-Traub N (2002) Epstein–Barr virus in the pathogenesis of NPC. Semin Cancer Biol 12(6):431–441
4. Lee AW, Lin JC, Ng WT (2012) Current management of nasopharyngeal cancer. Semin Radiat Oncol 22(3):233–244

5. Leung SF, Chan KC, Ma BB et al (2014) Plasma Epstein–Barr viral DNA load at midpoint of radiotherapy course predicts outcome in advanced-stage nasopharyngeal carcinoma. Ann Oncol 24:1204–1208
6. Ji MF, Huang Qh, Yu X et al (2014) Evaluation of plasma Epstein–Barr virus DNA load to distinguish nasopharyngeal carcinoma patients from healthy high-risk populations in Southern China. Cancer 120(9):1353–1360
7. Arai T, Inoue Y, Sugimoto C et al (2014) CYFRA 21-1 as a disease severity marker for autoimmune pulmonary alveolar proteinosis. Respirology 19(2):246–252
8. Ono A, Takahashi T, Mori K et al (2013) Prognostic impact of serum CYFRA 21-1 in patients with advanced lung adenocarcinoma: a retrospective study. BMC Cancer 13:354
9. Hanagiri T, Sugaya M, Takenaka M et al (2011) Preoperative CYFRA 21-1 and CEA as prognostic factors in patients with stage I non-small cell lung cancer. Lung Cancer 74(1):112–117
10. Nisman B, Biran H, Heching N et al (2008) Prognostic role of serum cytokeratin 19 fragments in advanced non-small-cell lung cancer: association of marker changes after two chemotherapy cycles with different measures of clinical response and survival. Br J Cancer 98(1):77–79
11. Wei Z, Zeng X, Xu J et al (2014) Prognostic value of the pretreatment serum level of cytokeratin fraction 21-1 in undifferentiated nasopharyngeal carcinoma: a study of 332 cases. Head Neck 36(1):71–76
12. Lin WY, Yen TC, Cheng KY et al (1998) The value of CYFRA 21-1, a new tumor marker, in nasopharyngeal carcinoma. Neoplasma 45(1):21–24
13. Song XM, Wang SZ, Wang ZJ et al (2015) Serum CYFRA21-1 as an effective tumor biomarker for patients with nasopharyngeal carcinoma. Neoplasma 62(1):124–129
14. Wang SZ, Li J, Miyamoto CT et al (2009) A study of middle ear function in the treatment of nasopharyngeal carcinoma with IMRT technique. Radiother Oncol 93(3):530–533
15. Sheard MA, Vojtesek B, Simickova M et al (2002) Release of cytokeratin-18 and -19 fragments (TPS and CYFRA 21-1) into the extracellular space during apoptosis. J Cell Biochem 85(4):670–677
16. Kosacka M, Jankowska R (2009) Comparison of cytokeratin 19 expression in tumor tissue and serum CYFRA 21-1 levels in non-small cell lung cancer. Pol Arch Med Wewn 119(1–2):33–37
17. Liu L, Liu B, Zhu L-L et al (2013) CYFRA21-1 as a serum tumor marker for follow-up patients with squamous cell lung carcinoma and oropharynx squamous cell carcinoma. Biomark Med 7(4):591–599
18. Holdenrieder S, von Pawel J, Dankelmann E et al (2008) Nucleosomes, ProGRP, NSE, CYFRA 21-1, and CEA in monitoring first-line chemotherapy of small cell lung cancer. Clin Cancer Res 14(23):7813–7821
19. Cao X, Zhang L, Feng G-R et al (2012) Preoperative Cyfra21-1 and SCC-Ag serum titers predict survival in patients with stage II esophageal squamous cell carcinoma. J Transl Med 10:197
20. Boeck S, Wittwer C, Heinemann V et al (2013) Cytokeratin 19-fragments (CYFRA 21-1) as a novel serum biomarker for response and survival in patients with advanced pancreatic cancer. Br J Cancer 108(8):1684–1694
21. Satyam A, Singh P, Sharma M et al (2011) CYFRA 21-1: a potential molecular marker for noninvasive differential diagnosis of urothelial carcinoma of bladder. Biomarkers 16(5):413–421
22. Zhu WQ, Yu JM, Sun XD et al (2010) Serum CYFRA21-1 as a prognostic marker for patients with undifferentiated nasopharyngeal carcinoma. Biomarkers 15(7):602–607
23. Wu H, Kapoor P, Frappier L (2002) Separation of the DNA replication, segregation, and transcriptional activation functions of Epstein–Barr nuclear antigen 1. J Virol 76(5):2480–2490
24. Yu KJ, Hsu WL, Pfeiffer RM et al (2011) Prognostic utility of anti-EBV antibody testing for defining NPC risk among individuals from high-risk NPC families. Clin Cancer Res 17(7):1906–1914
25. Liu Y, Huang Q, Liu W et al (2012) Establishment of VCA and EBNA1 IgA-based combination by enzyme-linked immunosorbent assay as preferred screening method for nasopharyngeal carcinoma: a two-stage design with a preliminary performance study and a mass screening in southern China. Int J Cancer 131(2):406–416