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ORIGINAL ARTICLE

Serum CYFRA21-1 as a prognostic marker for patients with undifferentiated nasopharyngeal carcinoma

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Abstract

The purpose of this study was to evaluate the potential of serum CYFRA21-1 and carcinoembryonic antigen (CEA) as prognostic markers in patients with undifferentiated nasopharyngeal carcinoma. Sixty-one patients who received definitive radiotherapy/chemoradiotherapy were analysed retrospectively. We investigated the association of the follow-up results with pretreatment level, post-treatment level and change of serum CYFRA21-1 and CEA, respectively. Patients with low pretreatment CYFRA21-1 had a significantly better overall survival. There were no significant associations among the remaining serum markers, and the survival and recurrence rates on multivariate analysis. The present study shows that pretreatment CYFRA21-1 level is a potential factor for predicting long-term survival.

Keywords: *Nasopharyngeal carcinoma; undifferentiated; CYFRA21-1; CEA; prognosis*

Introduction

Undifferentiated nasopharyngeal carcinoma (UNPC) is the most frequent histological entity of nasopharyngeal carcinoma. Moreover, compared with other types of nasopharyngeal carcinoma, it has higher invasive and metastatic potential. The mainstay treatment for UNPC has been radiotherapy as it is highly radiosensitive. Recently, intensity-modulated radiotherapy (IMRT) has been shown to improve tumour coverage and reduce adverse side-effects compared with conventional techniques. Treatment outcome in UNPC remains heterogeneous; therefore, identification of novel prognostic factors that potentially predict outcome is of great interest. Many prognostic factors have been identified as useful in evaluating the long-term outcome (Xie et al. 2009, Makdissi et al. 2009, Xi et al. 2009). The test of circulating biomarkers is a convenient way to achieve these objectives.

Serum markers, such as cytokeratin fraction 21-1 (CYFRA21-1) and carcinoembryonic antigen (CEA), have shown promise as diagnostic indicators for lung cancer and other cancers (Mrázová et al. 2009, Tsuchiya et al. 1999, Gkialas et al. 2008). CYFRA21-1 is a cytoplasmic protein fragment of cytokeratin 19 that is found in a variety of epithelial malignancies (Kremer et al. 2006). It is released into the serum as soluble fragments after cell death, and its level may reflect the degree of tumour necrosis (Doweck et al. 2000). CEA is one of the tumour markers that have been exploited extensively in clinic. It is a complex glycoprotein that is associated with the plasma membrane of tumour cells, and which has been reported to have diagnostic and prognostic value in peripheral adenocarcinoma of lung cancer (Deyasi et al. 1974, Sakao et al. 2004).

The aims of the present study were to validate the levels of serum CYFRA21-1 and CEA before and after treatments with overall survival (OS), progression free survival (PFS), time to locoregional recurrence (TLR) and time to distant recurrence (TDR) after radical treatment.

Patients and methods

Patients

We retrospectively analysed the datasets of 61 patients with biopsy-proven UNPC whose serum levels of CYFRA

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and CEA were measured before and after treatments. All the patients were referred for definitive IMRT to the Department of Radiation Oncology, Shandong Cancer Hospital and Institute from March 2004 to July 2008. They were initially evaluated with a complete medical history and physical examination, full blood count, baseline serum biochemistry and fibreoptic nasopharyngoscopy with tumour biopsy or neck lymph nodal biopsy. Other routine staging modalities included chest radiography, computed tomography (CT) scan or magnetic resonance imaging (MRI) of the head and neck, abdominal ultrasonography, and whole-body bone scan. Tumour stage was determined according to the 6th edition of the American Joint Committee on Cancer (AJCC) staging system.

Test of serum markers level

Blood samples were routinely obtained by venipuncture at 1 week before treatment and at 1–2 weeks after the treatments. Serum CYFRA21-1 and CEA levels were measured by CYFRA21-1 and CEA enzyme immunoassay kits (Boehringer-Mannheim GmbH, Mannheim, Germany), respectively.

Treatment

All patients received definitive IMRT, and the patient with StageII_B-IV also received concomitant chemotherapy followed by three cycles of chemotherapy. During treatment planning and radiotherapy, patients were immobilized in the supine position with the custom-made thermoplastic mask. The CT simulation was performed with administration of intravenous contrast, and images were acquired at intervals of 3–5 mm from the skull base to the level of the carina. Radiation was administered by using 6-MV photon beams for 1.8–2.0 Gy per fraction, every fraction per day, 5 days per week. The radiotherapy area included the gross tumour area with at least 1 cm margins and the whole neck for 44–46 Gy, and then a cone-down boost to the initial gross tumour area to a total of 70–72 Gy for gross target volume 60–65 Gy for clinical target volume of high risk, and 50–60 Gy for elective nodal. For concomitant chemotherapy, fluorouracil (500 mg m^{-2} daily, days 1–5) and cisplatin (12–15 mg m^{-2} daily, days 1–5) were given every 4 weeks on day 1 and day 29. For adjuvant chemotherapy, fluorouracil (600 mg m^{-2} daily, days 1–5) and cisplatin (80 mg m^{-2}, day 1) were given every 3 weeks.

Outcome determination and statistical analysis

OS (PFS) were measured from the date of diagnosis to the date of death (disease progression) or when patients were recorded at the last report date if they remained alive. TLR was defined from diagnosis to the time of first local recurrence if this occurred before the diagnosis of distant metastasis or death; otherwise, it would be recorded at the date of the first event or the last report date. Similarly, TDR represented the time from diagnosis to the time of first distant recurrence if it happened before the diagnosis of death or local recurrence. Serum marker levels and ages were analysed as binary variables using the median values of each marker as cut-off levels, which is more than median as the high group and less than or equal to median as the low group. Tumour (T) classification and lymph node (N) classification were analysed as categorical variables. Survival probabilities were estimated using the Kaplan–Meier method, and the differences between survival curves in relation to low and high levels of each prognostic factors (pretreatment and post-treatment serum levels of CYFRA21-1 and CEA) were tested using the log-rank test in the univariate analysis. Multivariate analysis was performed to identify the prognostic factors influencing (age, sex, chemotherapy, pretreatment and post-treatment serum levels of CYFRA21-1 and CEA, a decrease in CYFRA21-1 and CEA, T classification, N classification),and the survival and recurrence rates (OS, PFS, TDR, TLR) using Cox's proportional hazards regression model. According to TNM stage, the pretreatment CYFRA21-1 levels of various groups were expressed as the mean ± SD. One-way ANOVA test and the least significant difference (LSD) test were used to test for differences among the subgroups. SPSS (version 17.0) was used for statistical analysis. All statistical tests were conducted at a two-sided level of significance of 0.05.

Results

Patient characteristics and outcome

Patient characteristics are shown in Table 1. The mean age was 45.91 ± 13.68 years (range 17–69; median 46; interquartile range 37–58). The median follow-up for surviving patients was 45.2 months (range 15.4–65.0). Forty-nine patients were alive at the last follow-up and 12 had died. Of the 61 patients, 22 (34.9%) developed progressive disease (local recurrence or/and distant metastases), 10 (15.9%) had local recurrence during the observation period and 14 (22.2%) showed distant metastases; the remainder had no recurrence or metastases.

The correlation between serum CYFRA21-1 and stage

The pretreatment serum CYFRA21-1 level distribution significantly differed according to T and stage (T_1 vs T_4, $p = 0.001$; T_2 vs T_4, $p < 0.001$; stage I vs stage IV, $p = 0.045$; stage II vs stage IV, $p = 0.013$; Table 2). Serum CEA level did not significantly differ according to age, nodal status, etc.

Table 1. Patients characteristics.

Characteristics	No. of patients (%)	Pretreatment (mean ± SD)		Post-treatment (mean ± SD)	
		CYFRA21-1	CEA	CYFRA21-1	CEA
Age					
High (>46)	30 (49.1)	5.63 ± 7.58	3.48 ± 5.04	2.64 ± 2.28	2.57 ± 2.23
Low (≤46)	31 (50.9)	3.20 ± 2.43	2.70 ± 2.17	2.22 ± 1.93	2.50 ± 2.02
		$p = 0.096$	$p = 0.433$	$p = 0.432$	$p = 0.905$
Sex					
Male	51 (81.0)	4.11 ± 4.74	3.28 ± 4.17	2.47 ± 2.24	2.58 ± 2.13
Female	10 (15.9)	5.84 ± 9.33	2.05 ± 0.75	2.22 ± 1.19	2.28 ± 2.08
		$p = 0.383$	$p = 0.360$	$p = 0.737$	$p = 0.685$
Chemotherapy					
With	42 (68.9)	5.50 ± 6.55	3.65 ± 4.48	2.79 ± 2.42	3.02 ± 2.34
Without	19 (31.1)	1.96 ± 0.80	1.81 ± 1.12	1.62 ± 0.54	1.45 ± 0.79
		$p = 0.023$	$p = 0.084$	$p = 0.042$	$p = 0.006$
Smoker					
Yes	32 (52.5)	4.46 ± 5.37	3.13 ± 4.97	2.52 ± 1.89	2.47 ± 2.29
No	29 (47.5)	4.32 ± 6.09	3.02 ± 2.09	2.32 ± 2.33	2.60 ± 1.93
		$p = 0.922$	$p = 0.717$	$p = 0.911$	$p = 0.817$
Tumour classification					
T1	11 (17.5)	1.99 ± 1.11	2.95 ± 2.39	1.39 ± 0.52	1.62 ± 1.52
T2	30 (47.6)	2.84 ± 1.91	2.30 ± 1.20	2.42 ± 1.70	2.30 ± 1.31
T3	10 (15.9)	6.20 ± 9.18	3.05 ± 2.37	2.61 ± 1.25	2.96 ± 2.50
T4	10 (15.9)	9.90 ± 8.06	5.58 ± 8.55	3.41 ± 3.96	3.84 ± 3.46
		$p = 0.001$	$p = 0.140$	$p = 0.173$	$p = 0.079$
N classification					
N0	16 (25.4)	4.50 ± 8.05	1.66 ± 1.06	1.81 ± 1.30	1.50 ± 0.90
N1	18 (28.6)	3.06 ± 1.91	3.23 ± 2.68	1.91 ± 0.67	2.70 ± 2.34
N2	15 (23.8)	4.73 ± 5.54	3.13 ± 1.75	2.91 ± 2.48	2.68 ± 1.70
N3	12 (19.0)	5.84 ± 6.09	4.67 ± 7.64	3.423.28	3.48 ± 2.90
		$p = 0.620$	$p = 0.238$	$p = 0.107$	$p = 0.088$
AJCC stage					
Stage I	6 (9.5)	1.52 ± 0.76	2.02 ± 1.64	1.17 ± 0.30	1.10 ± 0.64
Stage II	18 (28.6)	2.20 ± 0.65	2.09 ± 1.27	1.91 ± 0.50	1.91 ± 1.24
Stage III	17 (27.0)	5.05 ± 7.38	3.53 ± 2.23	2.81 ± 2.27	2.88 ± 1.92
Stage IV	20 (31.7)	6.68 ± 6.50	3.90 ± 6.28	2.94 ± 2.85	3.23 ± 2.80
		$p = 0.048$	$p = 0.431$	$p = 0.171$	$p = 0.069$
Total (median)		4.38 ± 5.68 (2.49)	3.08 ± 3.85 (2.17)	2.43 ± 2.10 (2.05)	2.53 ± 2.11 (1.97)

The correlation between serum CYFRA21-1 and CEA and outcome

From the multivariate analysis (Table 3), only the pretreatment CYFRA21-1 level was an independent prognostic factor for poor OS (hazard ratio (HR) 8.460; 95% confidence interval (CI) 1.246–57.444; $p = 0.029$). As shown in Figure 1, patients who had low pretreatment CYFRA levels had better OS compared with patients who had high pretreatment CYFRA levels ($p = 0.019$; log-rank test). However, there was no significant correlation between the change of pre- and post-treatment serum CYFRA level in any direction or magnitude with the OS. In the univariate analysis, pretreatment CYFRA21-1 level ($p = 0.013$), post-treatment CYFRA21-1 level ($p = 0.020$), post-treatment CEA ($p = 0.008$) and chemotherapy ($p = 0.01$) were associated significantly with PFS in addition to the T classification ($p = 0.006$) and N classification ($p = 0.001$). In the multivariate analysis, the relationship between pretreatment, post-treatment CYFRA21-1 and post-treatment CEA level with PFS did not reach statistical significance. Only T classification (HR 4.061; 95% CI 1.816–9.078; $p = 0.001$) and N classification (HR 4.117; 95% CI 1.764–9.611; $p = 0.001$) were independent prognostic factors for poor PFS (Table 3).

No correlation was observed between the remaining serum markers and TLR on univariate analysis, but T classification ($p = 0.006$) and a decrease in CYFRA21-1 level after treatment ($p = 0.046$) correlated significantly with TLR. In the multivariate analysis, only T classification reached significance (HR 5.149; 95% CI 1.282–20.683; $p = 0.021$). N classification was associated significantly

Table 2. Results of pretreatment CYFRA21-1 levels of subgroups: *post hoc* multiple comparisons.

Variables		*Post hoc* multiple comparisons		
		Compare T1/N1/I	Compare T2/N2/II	Compare T3/N3/III
T	$p_{total} = 0.001$			
T1		-	0.623	0.061
T2		0.623	-	0.076
T3		0.061	0.076	-
T4		0.001	<0.001	0.106
N	$p_{total} = 0.607$			
N0		-	0.462	0.914
N1		0.462	-	0.405
N2		0.914	0.405	-
N3		0.534	0.191	0.608
Stage	$p_{total} = 0.046$			
I		-	0.797	0.178
II		0.797	-	0.125
III		0.178	0.125	-
IV		0.045	0.013	0.358

with distant recurrence in the univariate analysis ($p = 0.007$) and multivariate analysis (HR 2.757; 95% CI 1.187–6.403; $p = 0.018$).

Discussion

It has been reported that some prognostic factors can be investigated by immunohistochemical staining in the tumour tissue. However, biopsy samples do not represent the genetic information or protein expression of the entire tumour (Krishna et al. 2006, Nakao et al. 2006, Wang et al. 2006). Thus, more effective and convenient means of identifying patients at high risk are needed. The pretreatment elevated serum CYFRA21-1 levels predicted a poor prognosis in patients with certain malignancies such as the lung, esophagus, ovary and non-NPC head and neck squamous carcinoma independent of disease stage (Tsuchiya et al. 1999, Blankenburg et al. 2008, Kandiloros et al. 2006). It has been reported previously that the serum CYFRA21-1 level was increased significantly in untreated patients with UNPC compared with healthy subjects (Jmal et al. 2004, Lin et al. 1998). However, the long-term prognostic effect of serum CYFRA21-1 levels in undifferentiated non-metastatic NPC was not determined.

Our study analysed biological and clinical variables in 61 patients with UNPC, the prognostic value of CYFRA21-1 was confirmed, and the hazard ratio yielded by a high pretreatment serum level of this marker was established at 8.46 (OS). These results reinforce both the putative usefulness of this marker in prognostication of the disease and its relative place in comparison with paramount covariables (i.e. TNM). Furthermore,

Table 3. Results of Cox's multivariate analysis (overall survival and progression-free survival).

Significant prognostic factor	χ^2	*p*-Value	HR	95% CI for HR	
				Lower	Upper
Overall survival					
T classification	1.056	0.304	1.574	0.663	3.737
N classification	3.291	0.070	2.445	0.931	6.423
Sex	0.001	0.972	0.961	0.101	9.133
Age	0.049	0.825	1.168	0.295	4.614
Smoking	0.214	0.643	1.457	0.296	7.159
Chemotherapy	0.001	0.996	1.007	0.068	14.811
Pretreatment CYFRA	4.774	0.029	8.460	1.246	57.444
Post-treatment CYFRA	2.966	0.085	0.084	0.005	1.407
Pretreatment CEA	1.348	0.246	0.319	0.046	2.193
Post-treatment CEA	1.431	0.232	3.26	0.470	22.603
CYFRA decreased[a]	2.993	0.084	6.459	0.780	53.468
CEA decreased[b]	0.715	0.398	0.466	0.079	2.736
Progression free survival					
T classification	10.99	0.001	3.599	1.688	7.674
N classification	10.849	0.001	4.054	1.763	9.324
Sex	0.211	0.646	1.426	0.314	6.476
Age	2.835	0.092	0.405	0.141	1.160
Smoking	0.005	0.943	0.963	0.346	2.682
Chemotherapy	3.517	0.061	0.103	0.010	1.108
Pretreatment CYFRA	0.219	0.640	0.730	0.195	2.732
Post-treatment CYFRA	0.767	0.381	2.094	0.401	10.95
Pretreatment CEA	1.332	0.248	2.062	0.603	7.050
Post-treatment CEA	0.094	0.759	1.248	0.303	5.142
CYFRA decreased[a]	0.674	0.412	1.768	0.453	6.898
CEA decreased[b]	2.093	0.148	2.527	0.720	8.875

HR, hazards ratio; CI, confidence interval; CEA, carcinoembryonic antigen. [a]The serum level of CYFRA21-1 decreased after treatment; [b]the serum level of CEA decreased after treatment.

the simultaneous evaluation of all putative clinical and routine biological variables in the proportional hazards model suggests that CYFRA21-1 deserves to be considered as a prognostic factor of UNPC. As CYFRA21-1 was related to disease stage, it was not surprising that patients with a high CYFRA21-1 level proved to have a worse prognosis. However, Cox's model retained the CYFRA21-1 as an independent prognostic determinant of survival. These data suggest that the prognostic significance of a high CYFRA21-1 level is not exclusively explained by its correlation to disease stage. Although pretreatment and post-treatment CYFRA21-1 level associated significantly with PFS in the univariate analysis, multivariate analysis failed to confirm any prognostic correlation. The poor prediction to the PFS might be mainly due to the limited number of patients in our study. Moreover, in the retrospective study, unlike survival, the progression time may be overestimated or underestimated. Consequently, bias might be introduced when comparing treatments (Dancey et al. 2004).

In terms of disease progression, serum CYFRA21-1 levels did not correlate with TDR or TLR on the univariate

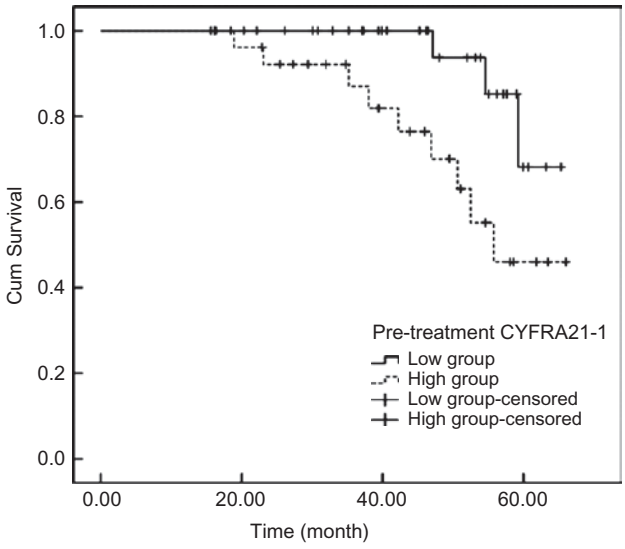

Figure 1. Overall survival according to pretreatment CYFRA21-1 levels (median as cut-off value); $p = 0.019$.

analysis and multivariate analysis. Furthermore, we cannot say the decrease in CYFRA21-1 is a good prognostic predictor. In fact, most cases had a declining trend. However, some positive associations were reported not only between subsequent disease recurrence and a single, elevated pretreatment CYFRA21-1 level but also between distant metastases and progressively rising, serial, post-treatment CYFRA21-1 levels in patients with non-NPC HNSCC (Hoffmann-Fazel et al. 2003, Deng et al. 2003). The difference among the above malignancies may be explained by the following reasons. Firstly, serum CYFRA21-1 levels are not sensitive enough to detect the subclinical location or microscopic residual disease (Ho et al. 1996). Secondly, the kinetics of post-treatment CYFRA21-1 levels in patients with NPC may differ from the kinetics in patients with non-NPC, assuming that CYFRA21-1 is released into the bloodstream through cell death (Doweck et al. 2000). In theory, the radiation-induced microvascular damage may restrict passage of tumour markers into the circulation (Leung et al. 2003). Furthermore, most studies in non-NPC HNSCC are relatively heterogeneous in terms of cancer types, which will influence the results. In sum, if the level elevates before the primary treatment, the chance of poor OS is very high.

CEA, a good prognostic marker of adenocarcinoma (Sakao et al. 2004), was also evaluated in the study, but no relationships between serum levels of CEA with survival and recurrence rates were found. Similar conclusions are established in head and neck squamous cell carcinoma and esophageal squamous cell carcinoma (Shimada et al. 2005, Laarman et al. 1991). Because of the complex pathogenesis of CEA (Bauch et al. 2009, Cojocaru et al. 2008, Nart et al. 2008), its level is not associated with the depth of tumour invasion, the tumour diameter or lymph node metastasis (Shimada et al. 2005). Furthermore, CEA is mainly sensitive to adenocarcinomas (Yamada et al. 1992).

Currently, stage is the most important prognostic factor in predicting the outcomes of patients with malignant tumours (van Rens et al. 2000). However, stage does not predict well in all cases, especially when the stage is the same. Therefore, it is necessary that many non-anatomical biomarkers should be taken into account to assess the tumour accurately. The serum level of CYFRA21-1 is a more powerful prognostic marker than T and N classification, and the serum CYFRA21-1 assay requires only a commercially available and relatively affordable immunoassay kit, so the detection is very cost-effective and is readily available.

In conclusion, the main limitations of our study are the relatively low number of patients in our cohort, the heterogeneity of the patients and treatments, and the retrospective design. However, despite these limitations, it was demonstrated that the pretreatment serum CYFRA21-1 level is a valuable prognostic factor in patients with undifferentiated NPC. Future prospective studies with more cases will be helpful to confirm the prognostic and predictive value of CYFRA21-1 and other factors in UNPC.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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