RESEARCH COMMUNICATION

Prognostic Significance of CYFRA21-1, CEA and Hemoglobin in Patients with Esophageal Squamous Cancer Undergoing Concurrent Chemoradiotherapy

Hai-Qin Zhang, Ren-Ben Wang*, Hong-Jiang Yan, Wei Zhao, Kun-Li Zhu, Shu-Mei Jiang, Xi-Gang Hu, Jin-Ming Yu

Abstract

Purpose: To evaluate the prognostic value of serum CYFRA21-1, CEA and hemoglobin levels regarding long-term survival of patients with esophageal squamous cell carcinoma (ESCC) treated with concurrent chemoradiotherapy (CRT). Methods: Age, gender, Karnofsky Performance Status (KPS), tumor location, tumor length, T stage, N stage and serum hemoglobin, and CYFRA21-1 and CEA levels before concurrent CRT were retrospectively investigated and related to outcome in 113 patients receiving 5-fluorouracil and cisplatin combined with radiotherapy for ESCC. The Kaplan-Meier method was used to analyze prognosis, the log-rank to compare groups, the Cox proportional hazards model for multivariate analysis, and ROC curve analysis for assessment of predictive performance of biologic markers. Results: The median survival time was 20.1 months and the 1-, 2-, 3-, 5- year overall survival rates were 66.4%, 43.4%, 31.9% and 15.0%, respectively. Univariate analysis showed that factors associated with prognosis were KPS, tumor length, T-stage, N-stage, hemoglobin, CYFRA21-1 and CEA level. Multivariate analysis showed T-stage, N-stage, hemoglobin, CYFRA21-1 and CEA level were independent predictors of prognosis. By ROC curve, CYFRA21-1 and hemoglobin showed better predictive performance for OS than CEA (AUC= 0.791, 0.704, 0.545; P=0.000, 0.000, 0.409). Conclusions: Of all clinicopathological and molecular factors, T stage, N stage, hemoglobin, CYFRA21-1 and CEA level were independent predictors of prognosis for patients with ESCC treated with concurrent CRT. Among biomarkers, CYFRA21-1 and hemoglobin may have a better predictive potential than CEA for long-term outcomes.

Keywords: Esophageal carcinoma - prognosis - hemoglobin - carcinoma embryonic antigen - keratoprotein 21-1

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Introduction

Although good results are obtained with the current multimodal treatment of ESCC, ''patient-tailored'' treatments are expected to give greater benefit. The long-term survival of patients with ESCC is still quite poor. The overall 5-year survival rate for patients treated with surgery alone was less than 20%, with a median survival of 13 to 17 months (Urba et al., 2001; Medical Research Council Oesophageal Cancer Working Party, 2002; Bedenne et al., 2007; Hu et al., 2010). There is increasing evidence that oesophageal cancer responds to 5-fluorouracil and cisplatin (FP)-based concurrent CRT. Park et al. (2011) reported the long outcome of patients who had a pathologic complete response after preoperative concurrent CRT. The median follow-up time was 45.2 months. The 5-year overall survival (OS) and disease-free survival rates (DFS) were 60.2% and 80.4%, respectively. Tepper et al. (2008) also reported that 5-year survival rate was 39% with trimodality therapy with cisplatin, fluorouracil, radiotherapy in patients with nonmetastatic esophageal cancer. This indicated that concurrent CRT should be considered for patients with resectable cancer of the esophagus.

The development of molecular biology, tumor markers are becoming more and more widely used. Various target molecules have been identified and their relations with chemo- or radiosensitivity and the prognosis have been evaluated. In this study, we analyzed the prognostic significance of CYFRA21-1, CEA, hemoglobin and other clinicopathologic data in patients with ESCC treated with concurrent CRT using the FP regimen.

Materials and Methods

patients

A total of 107 patients with locally advanced ESCC who treated with definitive CRT between September 2002 and September 2006 at Shandong Tumor Hospital were included in this retrospective study. All patients fulled

*Department of Radiation Oncology, Shandong Cancer Hospital, Jinan, China *For correspondence: wangrenben@tom.com*

the following criteria: (1) histologically documented esophageal cancer; (2) no previous treatment; (3) clinically diagnosed T1-4, N any, and M any on the International Union Against Cancer tumor-node-metastasis (TNM) classification; (4) Karnofsky Performance Status (KPS) scale 60–100; (5) no significant medical disease; (6) those with physical examination, computed tomography (CT), hematologic and biochemical profiles performed before and after treatment; (7) informed consents were obtained before treatment.

Treatment schedule

All patients were treated with definitive CRT using 5-fluorouracil and cisplatin combined with radiotherapy. Chemotherapy and radiotherapy were started on the same day. All patients received a total radiation dose of 60 Gy given in 30 fractions (2 Gy per fraction) using conformal radiotherapy or intensity modulated radiotherapy. Cisplatin 25 mg/m2/day as a continuous intravenous drip on days 1 to 3 and 5-fluorouracil (5-FU) 750mg/m^2/day infusion on days 1 to 5 were administered. Two cycles of chemotherapy were done during radiotherapy at 4-week intervals. Two more cycles of FP chemotherapy with the same dose were given at 3-week intervals three weeks after completion of radiotherapy.

Follow-up and observational indices

Blood samples were obtained by venipuncture before CRT. The cut-off values of CYFRA21-1, CEA and hemoglobin were defined according to the 95% confidence intervals of non-cancer Chinese patients: 3.4 ng/ml, 3.3 ng/ml, and 110 g/L (female), 120 g/L (male) respectively. Follow-up data after CRT were available for all patients. Endoscopy, computed tomography, or both, were carried out at regular intervals (every 3-6 months) after CRT. The means of followup and data collection included regular outpatient followup, mailings, and telephone followup. Overall survival was defined as the interval between the date of the beginning of CRT and the date of death or last follow-up.

Statistical analysis

Data were analyzed using SPSS version 17.0. Overall survival were calculated for each potential prognostic factor with the Kaplane Meier method. Differences between the Kaplane Meier curves were evaluated in a univariate manner with the Log-rank test. Potential prognostic factors found to be significant in the univariate analysis were evaluated in a multivariate analysis, which was carried out with the Cox proportional hazard model. To further evaluate and compare the predictive performance of biologic markers, we employed ROC curve for censored data and the area under the ROC curve (AUC) as the criterion. Larger AUC indicates better predictability of therapeutic effect. AUC of 0.5 indicates no predictive ability, whereas a value of 1 represents perfect predictive ability. Surviving patients and patients that died from causes other than the carcinoma were regarded as censored data. Data were recognized as statistically significant when <0.05.

Table 1. Univariate Analysis: 1-, 2-, 3-, and 5-year Overall Survival Rate Obtained from the KaplaneMeier Analysis, and P values, Obtained from the Log-rank Test, Related to the Potential Prognostic Factors

Potential prognostic factor	Patient No. (%)	OS(%) 1-year	2-year	3-year	5-year	P
Age (years)						
≤60	54(48)	74.1	50	35.2	16.7	0.483
>60	59(52)	59.3	37.3	28.8	13.6	
Gender						
Female	49(43)	69.4	44.9	32.7	14.3	0.376
Male	64(57)	64.1	42.2	31.3	15.6	
KPS						
>70	71(63)	70.4	49.3	36.6	18.3	0.045
≤70	42(37)	59.5	33.3	23.8	9.5	
Tumour location						
Upper third	38(34)	73.7	47.4	31.6	15.8	0.23
Middle third	52(46)	67.3	44.2	32.7	15.4	
Lower third	23(20)	52.2	34.8	30.4	13	
Tumour length (cm)						
<7	78(69)	74.4	50	35.9	17.9	0.038
≥7	35(31)	48.6	28.6	22.9	8.6	
T-stage						
T1-2	21(19)	90.5	71.4	47.6	28.6	0.01
T3	56(50)	66.1	41.1	33.9	14.3	
T4	36(32)	52.8	30.6	19.4	8.3	
N-stage						
N0	39(35)	76.9	56.4	41	17.9	0.017
N1	28(25)	71.4	39.3	35.7	17.9	
N2	25(22)	52	36	32	12	
N3	21(19)	47.1	33.3	19.5	9.5	
haemoglobin (g/L)						
≥120(110)	59(52)	72.9	55.9	44.1	23.7	0.003
<120(110)	54(48)	59.3	29.6	18.5	5.6	
CYFRA21-1 (ng/ml)						
<3.4	42(37)	78.6	57.1	47.6	28.6	0.001
≥3.4	71(63)	59.2	35.2	22.5	7	
CEA (ng/ml)						
<3.3	48(42)	79.2	54.2	45.8	27.1	0.002
≥3.3	65(58)	56.9	35.4	21.5	6.2	

Results

Follow up

The 113 patients with locally advanced ESCC were followed to August 24, 2011. The followup period was 2-105 months. A total of 10 patients were still alive at the end of the followup, and 2 patients were lost to followup. Of the 111 patients, 99 died from the carcinoma and 2 patients died from other causes.

Survival conditions

The median survival time was 20.1 months. The 1-, 2-, 3-, 5- year overall survival rates were 66.4%, 43.4%, 31.9% and 15.0%, respectively (Figure 1).

Univariate analysis

The results of the univariate analysis for overall survival relate to the potential prognostic factors summarized in Table 1. Univariate analysis showed that factors associated with prognosis were KPS, tumor length, T-stage, N-stage, hemoglobin, CYFRA21-1 and CEA

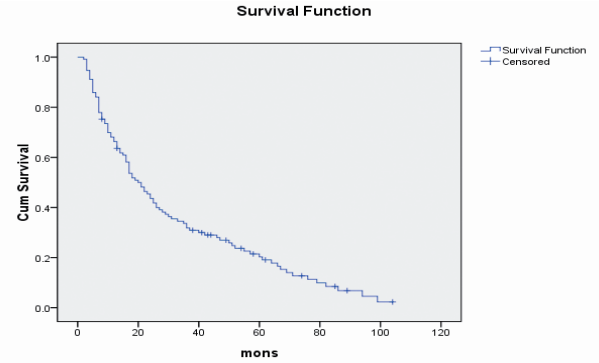

Figure 1. The Overall Survival Curve of 113 Patients

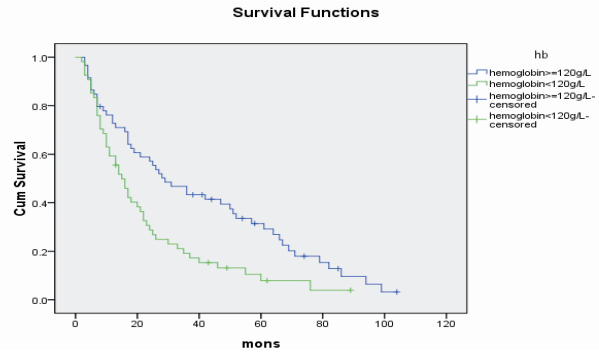

Figure 2. Survival Curves of Patients at Different Hemoglobin Level

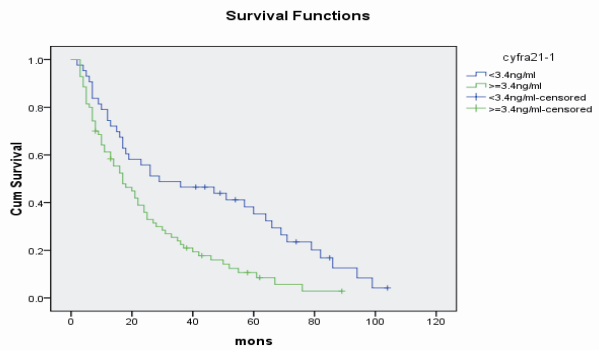

Figure 3. Survival Curves of Patients at Different Serum Level of Cyfra21-1

level. Hemoglobin levels of greater than 120(110) g/L, serum CYFRA21-1 and CEA levels of less than 3.4ng/ml and 3.3 ng/ml before concurrent CRT were associated with better outcomes than hemoglobin levels of less than 120(110) g/L, serum CYFRA21-1 and CEA levels of greater than 3.4 ng/ml and 3.3 ng/ml, respectively. The Kaplan-Meier curves for the hemoglobin, CYFRA21-1 and CEA levels for overall survival are shown in Figure 2-4.

Multivariate analysis

Factors that were correlated with prognosis as analyzed by univariate analysis were introduced into the Cox model, showing that T-stage, N-stage, hemoglobin, CYFRA21-1 and CEA level were independent predictors of prognosis. The results of the multivariate analysis are summarized in Table 2.

ROC curve analysis

ROC curve analysis showed the AUC for each follow-up duration (Figure 5). CYFRA21-1 and hemoglobin

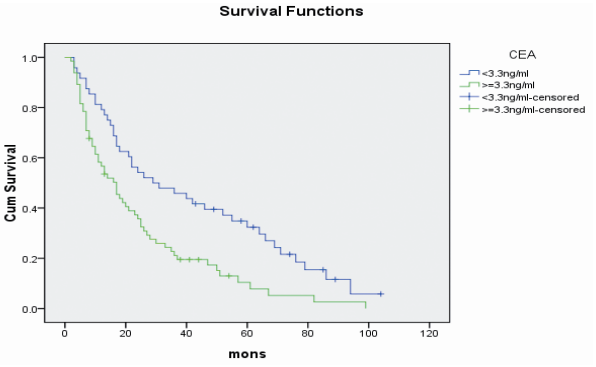

Figure 4. Survival Curves of Patients at Different Serum Level of CEA

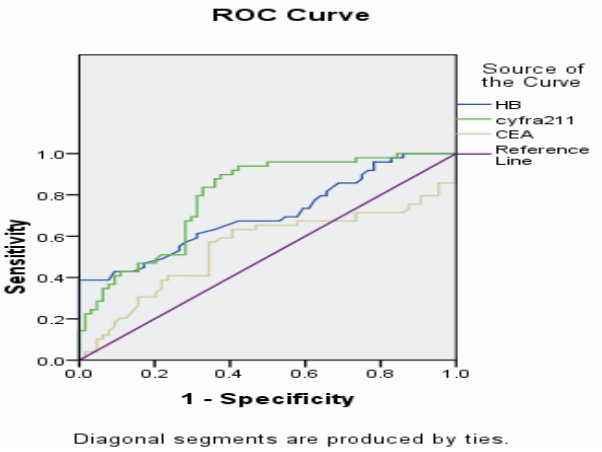

Figure 5. ROC Curve Analysis for Three Biological Markers. CYFRA21-1 and hemoglobin showed better predictive performance for OS than CEA (AUC= 0.791, 0.704, 0.545; P=0.000,0.000,0.409)

Table 2. Results of the Multivariate Analysis (Cox Proportional Hazards Model), Including the Potential Prognostic Factors Found to Be Significant in the Univariate Analysis

parameter	B	SE	Wald	df	Sig.	Exp(B)
KPS	0.013	0.235	0.104	1	0.471	1.013
tumor length	0.507	0.436	4.894	1	0.024	1.66
T-stage	0.645	0.313	8.452	1	0.005	1.906
N-stage	1.206	0.184	17.846	1	0	3.34
hemoglobin	0.569	0.23	6.136	1	0.013	1.767
CEA	0.44	0.238	3.427	1	0.044	1.553
Cyfra21-1	0.729	0.217	11.242	1	0.001	2.073

showed good predictive performance for OS, consistently better than CEA. While CEA showed a very low accuracy in predicting overall survival. The AUC at therapeutic effect was 0.791 with CYFRA21-1, 0.704 with hemoglobin and 0.545 with CEA.

Discussion

Recently, definitive CRT is being offered for patients with stage I and II disease who essentially hope for preservation of the esophagus, as well as for patients with potentially nonresectable ESCC. Therefore the patients' background factors, including clinicopathologic and molecular factors, have been investigated for patients selection who were suitable for definitive CRT without surgery. In the present study, we reviewed age, gender,

KPS, tumor location, tumor length, T-stage, N-stage and serum hemoglobin, CYFRA21-1 and CEA levels before CRT with ESCC who underwent concurrent CRT in our institution. Our study findings strongly support that T-stage, N-stage, hemoglobin, CYFRA21-1 and CEA level were independent predictive factors of prognosis.

Due to the recent developments in molecular biology, various target molecules have been identified and their relations with chemo- or radiosensitivity and the prognosis have been evaluated. In this study, patients with detected serum levels of hemoglobin, CYFRA21-1 and CEA before CRT were enrolled into our list. Both univariate and multivariate analyses showed these three factors were significantly associated with OS. ROC curve analysis showed that CYFRA21-1 have a relative better predictive effect for OS than hemoglobin and CEA, while CEA showed a very low accuracy in predicting prognosis (AUC=0.310). It indicated that serum CYFRA21-1 and hemoglobin levels may be more helpful in predicting prognosis to CRT of ESCC.

A number of studies have demonstrated that high CYFRA 21-1 levels in patients with different types of carcinomas are associated with poor prognosis. CYFRA 21-1 has been reported as a useful tumor marker for ESCC (Yamamoto et al., 1997; Brockmann et al., 2000). Yamamoto et al. (1997) reported that the levels of CYFRA21-1 were correlated with tumor size, tumor depth and pTNM stage. The specificity, sensitivity and accuracy of CYFRA21-1 were 100%, 47.9% and 66.7%, respectively. Nakamura et al. (1998) reported that there is a correlation between CYFRA21-1 levels and clinical responses in patients who received chemotherapy or CRT. CYFRA 21-1 correlates better with the pathologic TNM stage. In Yi et al.'s study, the CR rates in CYFRA21-1 high and low groups were significantly different (p=0.002), and the effective rates (CR+PR rate) were also significantly different (p=0.013). ESCC with a high level of CYFRA21-1 is less sensitive to CRT (Yi et al., 2009). Previous studies showed a high serum CYFRA level may be predictive of an adverse therapeutic outcome. Shimada et al. (2003) reported that a high CYFRA 21-1 level is associated with tumor progression and poor survival in patients with esophageal squamous cell carcinoma. Our results are concordant with aboving finding: there was a significant correlation between serum levels of CYFRA21-1 before CRT and overall 5-year survival. The prognosis of patients with CYFRA21-1 levels great than 3.4 ng/ml was markedly worse than that of patients with CYFRA21-1 levels less than 3.4 ng/ml. Lowering its cut-off point to 3.4 ng/ml might be more useful in current clinical practice. We also found that CYFRA21-1 level ≤ 3.4 ng/mL was the most significant independent predictor of good OS (P=0.001). By ROC curve, we found that CYFRA21-1 is a better predictor of OS than hemoglobin and CEA in patients with ESCC.

CEA is the most widely used and readily available tumor marker for the management of colorectal carcinoma (Hamada et al., 1985; Midiri et al., 1985; Wiggers et al., 1986). CEA immunoreactivity was frequently detected in the carcinoma cells as well as in the stroma around the cancer tissues. Previous study have shown that CEA may function as a metastatic potentiator by different pathways. Assessment of CEA distribution in neoplastic tissue is the most direct method by which to predict malignant potential. Several investigators have reported that CEA in neoplastic tissue shows a relationship to histological grade, malignant potential and may be of prognostic value in colorectal carcinoma. As for esophageal cancer, CEA was found to be of little benefit in clinical settings. Previous study demonstrated the efficacy of CEA as a diagnostic and prognostic factor in patients with esophageal cancers. Kijima et al. (2000) reported stromal CEA expression plays important roles in lymphatic invasion of ESCC. CEA has been reported to be a sensitive predictor of ESCC to CRT (Yi, 2009). While there was no report to support its clinical significance as a predictor of prognosis until now. In our study, we found that CEA is one of significant factors associated with overall 5-year survival in ESCC. However, it showed a lower accuracy in predicting overall survival than CYFRA21-1 and hemoglobin by ROC curve (AUC=0.545).

Tumor hypoxia leads to an increased resistance to radiation-induced tumor cell kill caused by less radiation-induced production of cytotoxic-free radicals and less fixation of DNA damage. Thus, low hemoglobin level may result in a worse therapeutic effect and prognosis. In our study, hemoglobin levels before concurrent CRT was showed to be associated with overall 1-, 2-, 3- and 5-year survival. The accuracy of hemoglobin is 61.3%. It is concordant with previous study: hemoglobin levels of 12-14 g/dl and greater than 14 g/dl during concurrent CRT provided better outcomes than hemoglobin levels less than 12 g/dl. The 2-year overall survival rates were 34%, 35% and 16%, respectively (Rades D, 2006).

As would be expected, KPS, tumor length and T-/N- stage significantly correlated with overall survival according to univariate analysis. In the current study, there was certainly an influence of tumor length and KPS on overall survival rates, this influence were no longer significant when using multivariate analysis. This could be due to a relative small number of patients.

Several clinical investigations show that depth of infiltration and lymph-node metastasis are independent risk factors of poor prognosis (Rice et al., 1998; Kunisaki et al., 2005; Tachibana et al., 2005). Hu et al. (2010) used the number of lymph node metastasis, the degree of metastasis, and the number of involved lymphatic regions to stratify the patients. These three factors were correlated with prognosis in univariate analysis. However, only the degree of lymphatic metastasis was correlated with prognosis. That may be because the contribution of the number of lymph node metastasis and involved lymphatic regions is attributable to the degree of lymphatic metastasis. The rate of lymphatic metastasis is reported correlated with the depth of infiltration. Rice et al. (1998) conclude that for patients with esophageal carcinoma, T is an important predictor of N. The percentage of patients with N1 disease is 0% for Tis, 11% for T1, 43% for T2, 77% for T3, and 67% for T4 ($p < 0.001$). In the present study, we used T and N stage to stratify the patients. Univariate and multivariate analyses showed that both T and N stage were independent prognostic factor of

ESCC, which was consistent with the above reports. The study suggested that the prognosis of patients with deeper infiltrations and more involved regional lymph node was worse.

Performance status and tumor length were generally prognostic factor of ESCC. Dirk et al. (2005) evaluate prognostic factors in patients with Stage II/III esophageal carcinoma with nonsurgical treatment. Tumor length was found to maintain significance for overall survival (OS), distant metastasis (DM), and local failure (LF), performance status lost for OS. In Mitsuhiko's report, overall survival was more significantly affected by Karnofsky Performance Status than by the patient's age. The influence of performance status on cumulative survival for stage I and II disease was more pronounced in patients in their 80s. For patients with early stage disease (I, ΠA and ΠB), the overall survival rate of the octogenarians was significantly affected by the KPS (P = 0.009), while the KPS did not affect the survival of younger patients (P = 0.958). In contrast, for the advanced stages (Ш and IV), the overall survival of the patients younger than 80 years was affected significantly by the KPS (P =0.048), whereas it was not in the octogenarians (P =0.963) (Kawashima M, 1998).

In conclusion, we proposed that, among pretreatment clinicopathologic characteristics and biomarkers of patients with ESCC treated with definitive CRT, T stage, N stage, and serum CYFRA21-1, hemoglobin and CEA levels before CRT were independent prognostic factors. Among biomarkers, CYFRA21-1 and hemoglobin showed a better predictive significance than CEA for long-term outcomes.

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