Original Article

Prognostic Impact of Pretreatment Serum CYFRA Status in 1047 Patients with Esophageal Squamous Cell Carcinoma Who Underwent Radical Resection: A Japan Esophageal Society Promotion Research

Nobuki Ishioka, MD,[1] Takashi Suzuki, MD, PhD,[2] Satoshi Yajima, MD, PhD,[2]
Kentaro Murakami, MD, PhD,[3] Yu Ohkura, MD, PhD,[4] Takashi Fukuda, MD,[5]
Koichi Yagi, MD, PhD,[6] Akihiko Okamura, MD, PhD,[7] Isamu Hoshino, MD, PhD,[8]
Chikara Kunisaki, MD, PhD,[9] Yasuaki Nakajima, MD, PhD,[10] Kosuke Narumiya, MD, PhD,[11]
Ryo Ogawa, MD, PhD,[12] and Hideaki Shimada, MD, PhD[1,2]

Purpose: The prognostic significance of pretreatment serum C-terminus of cytokeratin 19 (CYFRA21-1, CYFRA) status was evaluated in the patients with surgically treated esophageal squamous cell carcinoma.
Methods: A total of 1047 patients with surgically treated esophageal cancer were enrolled in a multi-institutional study promoted by the Japanese Esophageal Society. This study included an up-front surgery group (n = 412), a neoadjuvant chemotherapy (NAC) group (n = 486), and a neoadjuvant chemoradiation/radiation therapy (NACRT/RT) group (n = 149). The pretreatment CYFRA status was analyzed to assess prognostic significance using multivariate analysis according to treatment modalities.
Results: The CYFRA-positive group was significantly associated with deep tumor. Univariate analysis showed that the overall survival of the CYFRA-positive group was significantly worse than that of the CYFRA-negative group, but the difference was not significant in the multivariate analysis. CYFRA was an independent risk factor for poor prognosis just in the NACRT/RT group.
Conclusions: The CYFRA-positive group was associated with deep tumor and poor survival. Pretreatment CYFRA was not an independent risk factor for poor prognosis in the up-front surgery group or NAC group. It was an independent risk factor for poor prognosis just in the NACRT/RT group.

Keywords: CYFRA21-1, esophageal cancer, neoadjuvant chemoradiation therapy, prognosis

[1]Department of Clinical Oncology, Toho University Graduate School of Medicine, Tokyo, Japan
[2]Department of Surgery, School of Medicine, Toho University, Tokyo, Japan
[3]Department of Frontier Surgery, Graduate School of Medicine, Chiba University, Chiba, Chiba, Japan
[4]Department of Gastroenterological Surgery, Toranomon Hospital, Tokyo, Japan
[5]Department of Gastroenterological Surgery, Saitama Prefectural Cancer Center, Saitama, Saitama, Japan
[6]Department of Gastrointestinal Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[7]Department of Gastroenterological Surgery, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo, Japan
[8]Division of Gastroenterological Surgery, Chiba Cancer Center, Chiba, Chiba, Japan
[9]Department of Surgery, Gastroenterological Center, Yokohama City University Medical Center, Yokohama, Kanagawa, Japan
[10]Department of Gastrointestinal Surgery, Tokyo Medical and Dental University, Tokyo, Japan
[11]Department of Surgery, Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo, Japan
[12]Department of Gastroenterological Surgery, Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan

Introduction

Various serum biomarkers—such as SCC-Ag, serum p53 antibodies, NY-ESO-1, and PD-L1—have been developed for esophageal cancer clinical practice.[1–4] The C-terminus of cytokeratin 19 (CYFRA21-1, CYFRA) is a soluble cytokeratin fragment and is used as a tumor marker in epithelial tumors. It is particularly useful for detecting non-small cell lung cancer,[5–7] and it has been reported to be associated with prognosis.[8] Several studies have also reported the clinicopathological significance of CYFRA in patients with esophageal squamous cell carcinoma (SCC).[9–18]

Several previous studies showed the prognostic impact of CYFRA in patients with surgically treated esophageal cancer.[14–18] Among these reports, two showed that the pretreatment CYFRA status was a poor prognostic factor,[14,15] while the others showed that it was not an independent prognostic factor.[16–18] No reports showed the prognostic impact of the pretreatment CYFRA status in patients receiving neoadjuvant treatment followed by surgery.

Therefore, the prognostic significance of the pretreatment CYFRA status was evaluated in 1047 patients with surgically treated esophageal cancer, based on the treatment modalities described in a multi-institutional study promoted by the Japanese Esophageal Society. Each treatment group—up-front surgery group, neoadjuvant chemotherapy (NAC) group, and neoadjuvant chemoradiation/radiation therapy (NACRT/RT) group—was analyzed separately, in order to assess the impact of the pretreatment CYFRA status on the overall survival of patients.

Materials and Methods

Patients

The retrospective multi-institutional study enrolled a total of 1156 patients with primary esophageal SCC who underwent radical surgery at eleven Japanese hospitals between 2008 and 2015. A flow chart describing patient selection is shown in **Supplemental Figure** (available Online). Among 1156 esophageal SCC patients, 70 no-curative patients and 39 patients treated by endoscopic resection were excluded. A total of 1047 patients, up-front surgery (n = 412) and preoperative treatment group (n = 635), were enrolled in this study. The up-front surgery group consisted of 325 men (78.9%) and 87 women (21.1%), with a median age of 66 years (range, 39–83 years). After surgery, all patients were classified by TNM stage according to the *Union for International Cancer Control (Esophageal Cancer)*, 8th Edition. Out of 412 patients

in the up-front surgery group, the pathological stages of 158 were pStage I, 106 were pStage II, and 148 were pStage III. The clinical stages of NAC group and NACRT/RT group were assessed before neoadjuvant treatment. The NAC group (n = 486) and the NACRT/RT group (n = 149) were analyzed separately to evaluate the clinical impact of CYFRA on the overall survival of patients. This study was approved by the ethics committee of Toho University, Faculty of Medicine (A18112_A17044_A16037).

Measurement of the serum CYFRA levels

Serum CYFRA levels were measured using blood samples taken before treatment and CYFRA levels were measured using the electrochemiluminescence immunoassay kit or the chemiluminescence immunoassay kit (LUMIPULSE [Fujirebio Diagnostics, Inc. Malvern, PA, USA], ECULOSIS [Roche Diagnostics K.K., Tokyo, Japan], or ARCHITECT [Abbott Japan LLC, Tokyo, Japan]). Measurements were taken at each facility. A quantity of 3.50 U/ml was used as the cutoff point to classify patients into the positive (3.50 U/ml and above) or negative (below 3.50 U/ml) groups.

Statistical analyses

Fisher's exact test and logistic regression analyses were used to compare the clinicopathological factors of the CYFRA-positive and negative groups. The probability of overall survival from surgery was calculated using the Kaplan–Meier estimator method, and the difference between groups was evaluated by the log-rank test. Clinicopathological factors associated with overall survival were assessed by univariate analysis followed by multivariate analysis, using the Cox proportional hazards models. All statistical analyses were performed using EZR statistical software.[19] A bilateral P value <0.05 was considered statistically significant.

Results

Clinicopathological characteristics of patients undergoing up-front surgery

The overall positive rate of the up-front surgery group was 10.4% (43 of 412). Among these patients, 158 (38.3%) were classified as pStage I, 106 (25.7%) as pStage II, and 148 (36.0%) as pStage III. The positive rate gradually increased with tumor stage as follows: pStage I, 5.1%; pStage II, 9.4%; and pStage III, 16.9% (**Fig. 1A**). **Table 1** shows that the CYFRA-positive status was significantly correlated with deep tumor (P <0.001).
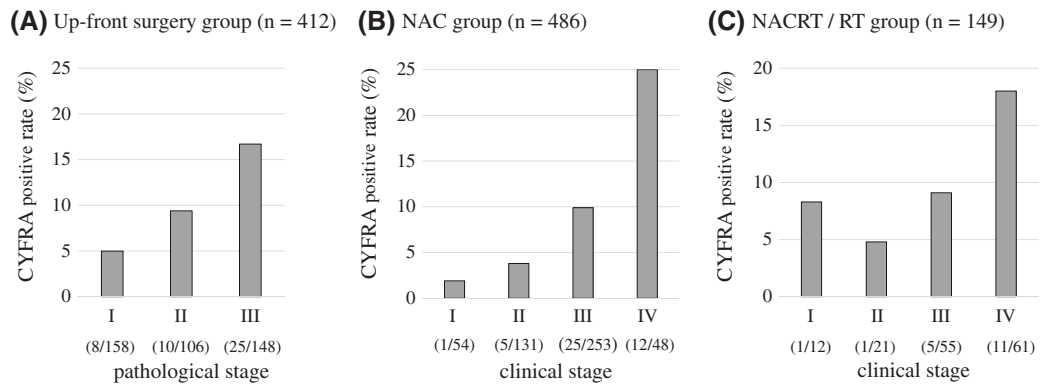
Fig. 1 Positive rates of CYFRA21-1 according to the tumor stages. (**A**) Up-front surgery group (n = 412), (**B**) NAC group (n = 486), and (**C**) NACRT/RT (n = 149). NAC: neoadjuvant chemotherapy; NACRT/RT: neoadjuvant chemoradiation/radiation therapy

Table 1 Comparisons of the positive rates of CYFRA21-1 according to clinicopathological factors in 412 patients with esophageal SCC who underwent up-front surgery

Variables	Number of patients (total = 412)	<3.5 ng/ml 369 (89.6%)	≥3.5 ng/ml 43 (10.4%)	Univariate P value[a]	Hazards ratio	95% CI[b]	Multivariate P value[c]
Gender							
Male	325	293 (90.2%)	32 (9.8%)	0.434	0.868	0.715–2.920	0.715
Female	87	76 (87.4%)	11 (12.6%)				
Age (years)							
≥65	253	223 (88.1%)	30 (11.9%)	0.252	1.450	0.018–0.131	0.305
<65	159	146 (91.8%)	13 (8.2%)				
Tumor depth							
pT3/T4	158	129 (81.6%)	29 (18.4%)	<0.001*	3.570	1.740–7.340	<0.001*
pT1/T2	254	240 (94.5%)	14 (5.5%)				
Nodal status							
pN (+)	211	184 (87.2%)	27 (12.8%)	0.146	1.160	0.567–2.380	0.681
pN (–)	201	185 (92.0%)	16 (8.0%)				

[a]Fischer's exact probability test. [b]Adjusted 95% CI. [c]Logistic regression analysis. *P <0.05 statistical significance. SCC: squamous cell carcinoma; CI: confidence interval

Clinicopathological characteristics of the NAC and NACRT/RT groups

The overall positive rate of the NAC group was 8.8% (43 of 486 patients). The rate gradually increased with the clinical stage of the tumor as follows: stage I = 1.9%, stage II = 3.8%, stage III = 9.9%, and stage IV = 25% (**Fig. 1B**). The overall positive rate of the NACRT/RT group was 12.1% (18 of 149 patients). Similarly, the positive rate increased with the clinical stage as follows: stage I = 8.3%, stage II = 4.8%, stage III = 9.1%, and stage IV = 18% (**Fig. 1C**). **Table 2** shows that the CYFRA status was significantly correlated with gender (P = 0.032), tumor depth (P <0.001), and nodal status (P = 0.021). In multivariate analysis, only tumor depth was independently correlated with CYFRA (P = 0.007).

Univariate and multivariate analyses of overall survival to evaluate the prognostic impact of the CYFRA status in each treatment modality group

In the up-front surgery group, the CYFRA-positive group showed a significantly worse overall survival compared to the CYFRA-negative group (P = 0.031) (**Fig. 2A**). However, multivariate analysis indicated that the CYFRA-positive status was not an independent risk factor for poor survival (P = 0.487) (**Table 3**). In the NAC group, there was no difference in overall survival between the CYFRA-positive and CYFRA-negative groups. (P = 0.254) (**Fig. 2B** and **Table 4**). In the NACRT/RT group, the CYFRA-positive group showed a significantly worse overall survival compared to the CYFRA-negative group (P = 0.034) (**Fig. 2C**). Multivariate analysis indicated that the CYFRA-positive

Table 2 Comparisons of the positive rates of CYFRA21-1 according to clinicopathological factors in 635 patients with esophageal SCC who underwent preoperative treatment followed by esophagectomy

Variables	Number of patients (total = 635)	<3.5 ng/ml 574 (90.4%)	≥3.5 ng/ml 61 (9.6%)	Univariate P value[a]	Hazards ratio	95% CI[b]	Multivariate P value[c]
Gender							
Male	547	489 (89.4%)	58 (10.6%)	0.032*	3.260	0.991–10.70	0.052
Female	88	85 (96.6%)	3 (3.4%)				
Age (years)							
≥65	351	318 (90.6%)	33 (9.4%)	0.893	0.920	0.537–1.570	0.760
<65	284	256 (90.1%)	28 (8.9%)				
Tumor depth							
cT3/T4	450	396 (88.0%)	54 (12.0%)	<0.001*	3.120	1.370–7.090	0.007*
cT1/T2	185	178 (96.2%)	7 (3.8%)				
Nodal status							
cN (+)	500	445 (89.0%)	55 (11.0%)	0.021*	1.890	0.779–4.590	0.159
cN (-)	135	129 (95.6%)	6 (4.4%)				

[a]Fischer's exact probability test. [b]Adjusted 95% CI. [c]Logistic regression analysis. *P <0.05 statistical significance. SCC: squamous cell carcinoma; CI: confidence interval
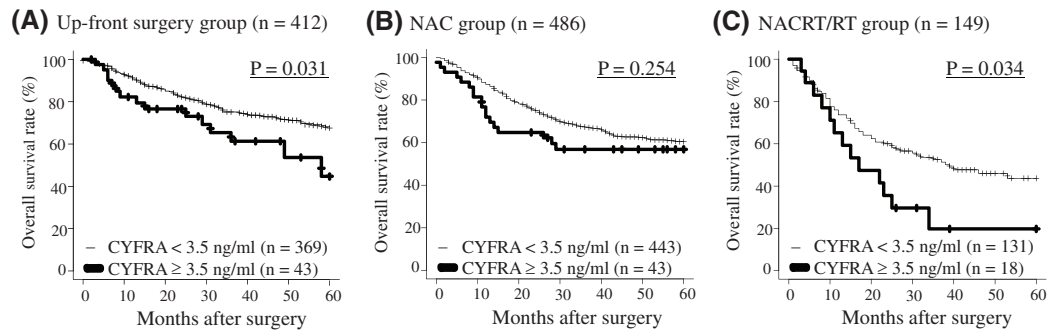

Fig. 2 Overall survival rates according to the status of pretreatment CYFRA21-1. (**A**) Up-front surgery group (n = 412), (**B**) NAC group (n = 486), and (**C**) NACRT/RT group (n = 149). NAC: neoadjuvant chemotherapy; NACRT/RT: neoadjuvant chemoradiation/radiation therapy

status was an independent risk factor for poor survival (P = 0.036) (**Table 5**).

Discussion

Based on the multi-institutional study promoted by the Japanese Esophageal Society, the present study evaluated the prognostic impact of the pretreatment serum CYFRA status in 1047 patients with esophageal SCC. Treatment modalities were assessed separately for the up-front surgery group (n = 412), NAC group (n = 486), and NACRT/RT group (n = 149). The overall positive rates were 10.4% (43/412), 8.8% (43/486), and 12.1% (18/149), respectively. There was a significant difference in overall survival between CYFRA-positive and CYFRA-negative patients in the up-front surgery and NACRT/RT groups.

In regards to the pathological significance of CYFRA status, the findings of this study were similar to those previously reported[11,14]; CYFRA status has been reported to correlate with tumor depth and size.[9,11,12,14] Similarly, the present study found a strong correlation with tumor depth.

In regards to the prognostic significance of CYFRA status, five reports have been produced on the prognosis of up-front surgery groups with esophageal SCC (**Table 6**). Two reports showed that the CYFRA-positive status was an independent poor prognostic factor,[14,15] while three other reports showed that it was not.[16–18] In the present study, the CYFRA-positive group showed a significantly worse prognosis than the CYFRA-negative group in univariate analysis. However, the CYFRA-positive status was not an independent poor prognostic factor in multivariate analysis. In fact, the up-front surgery group

Table 3 Univariate and multivariate analyses of risk factors for overall survival in 412 patients with esophageal SCC who underwent up-front surgery

Variables	Univariate P value[a]	Hazards ratio	95% CI[b]	Multivariate P value[c]
Gender				
Male	0.007*	2.373	1.322–4.259	0.004*
Female				
Age (years)				
≥65	0.211	1.332	0.903–1.967	0.148
<65				
Tumor depth				
pT3/pT4	<0.001*	2.574	1.719–3.852	<0.001*
pT1/pT2				
Nodal status				
pN (+)	<0.001*	1.580	1.046–2.387	0.030*
pN (–)				
CYFRA21-1				
≥3.5 ng/ml	0.031*	1.220	0.697–2.136	0.487
<3.5 ng/ml				

[a]Log-rank test. [b]Adjusted 95% CI. [c]Cox proportional hazards model. *P <0.05 statistical significance. SCC: squamous cell carcinoma; CI: confidence interval

Table 4 Univariate and multivariate analyses of risk factors for overall survival in 486 patients with esophageal SCC who underwent NAC followed by esophagectomy

Variables	Univariate P value[a]	Hazards ratio	95% CI[b]	Multivariate P value[c]
Gender				
Male	0.007*	2.373	1.322–4.259	0.004*
Female				
Age (years)				
≥65	0.211	1.332	0.903–1.967	0.148
<65				
Tumor depth				
pT3/pT4	<0.001*	2.574	1.719–3.852	<0.001*
pT1/pT2				
Nodal status				
pN (+)	<0.001*	1.580	1.046–2.387	0.030*
pN (–)				
CYFRA21-1				
≥3.5 ng/ml	0.031*	1.220	0.697–2.136	0.487
<3.5 ng/ml				

[a]Log-rank test. [b]Adjusted 95% CI. [c]Cox proportional hazards model. *P <0.05 statistical significance. SCC: squamous cell carcinoma; NAC: neoadjuvant chemotherapy; CI: confidence interval

included 99 patients who received postoperative adjuvant chemotherapy, and the effects of the postoperative chemotherapy may be the reason why the CYFRA-positive status has not been identified as an independent prognostic factor in the multivariate analysis.

In the NAC group, there was no significant difference in prognosis between the CYFRA-positive and CYFRA-negative groups in univariate analysis. This study was the first report to evaluate the significance of CYFRA status in a NAC group; further validation should be required to assess the effects of NAC on the CYFRA-positive group.

In the NACRT/RT group, the CYFRA-positive status was found to be an independent risk factor in multivariate analysis. Although no reports analyzing the significance of CYFRA status in the NACRT/RT group are

Table 5 Univariate and multivariate analyses of risk factors for overall survival in 149 patients with esophageal SCC who underwent NACRT/RT followed by esophagectomy

Variables	Univariate P value[a]	Hazards ratio	95% CI[b]	Multivariate P value[c]
Gender				
Male	0.044*	2.014	0.957–4.235	0.065
Female				
Age				
≥65 years	0.524	0.740	0.470–1.163	0.191
<65 years				
Tumor depth				
cT3/cT4	0.608	0.978	0.524–1.825	0.943
cT1/cT2				
Nodal status				
cN (+)	0.017*	2.179	1.123–4.231	0.021*
cN (−)				
CYFRA21-1				
≥3.5 ng/ml	0.034*	1.913	1.044–3.507	0.036*
<3.5 ng/ml				

[a]Log-rank test. [b]Adjusted 95% confidence interval. [c]Cox proportional hazards model.
*P <0.05 statistical significance. SCC: squamous cell carcinoma; NACRT/RT: neoadjuvant chemoradiation/radiation therapy; CI: confidence interval

Table 6 Summary of recent reports assessing the prognostic impacts of CYFRA in the patients with esophageal SCC who were treated with radical surgery

Reference	Year	Number of patients	Study design	Reported NAC	Reported NACRT	5-year survival (CYFRA+/-)	pTNM correlation	Prognostic impact of CYFRA
Shimada et al.	2003	157	Single institutional	-	-	Not applicable	Tumor depth, nodal status	Independent prognostic factor
Cao et al.	2012	379	Single institutional	-	-	33.0% vs. 85.0%	Not applicable	Independent prognostic factor
Yang et al.	2019	416	Single institutional	-	-	Not applicable	Nodal status, stage	Not independent prognostic factor
Qiao et al.	2019	315	Single institutional	-	-	32.5% vs. 42.7%	Tumor depth, nodal status, stage	Not independent prognostic factor
Yin et al.	2020	267	Single institutional	-	-	28.7% vs. 40.5%	Not applicable	Not independent prognostic factor
Our study	2021	412	Multi-institutional	+	+	44.7% vs. 68.2%	Tumor depth	Not independent prognostic factor

SCC: squamous cell carcinoma; NAC: neoadjuvant chemotherapy; NACRT: neoadjuvant chemoradiation therapy

available, it has been observed that CYFRA is an independent poor prognostic factor and is treatment resistant in the cases that received definitive chemoradiation therapy.[13,20] Therefore, in the NACRT/RT group, the therapeutic response of the CYFRA-positive group might be limited and the efficacy of NACRT/RT in improving prognosis might be small. Because the difference in the impact of the CYFRA-positive status between NAC and NACRT/RT groups may have been influenced by the chemotherapy dose, postoperative adjuvant chemotherapy may be required for the NACRT/RT group. Actually, the frequency of postoperative adjuvant chemotherapy was more in the NAC group than in the NACRT/RT group (22% vs 19%, respectively); the difference was not statistically significant (data not shown). Because the data of actual chemotherapy dose for those patients were not available, we could not assess the difference to confirm our hypothesis.

Our study has some limitations. (i) As no data on definitive CRT cases were available, it was not possible to verify existing reports. (ii) The characteristics of recurrence in the CYFRA-positive group were unknown, due to the lack of detailed data on recurrence types. (iii) As this is multi-institutional study, it was not possible to analyze the relationship between changes in CYFRA status and prognosis or recurrence because CYFRA monitoring data after surgery were not collected. Previous reports suggested that CYFRA monitoring may be useful in screening for recurrence.[9,21,22] The variation of CYFRA levels may assist in the prediction of recurrence and prognosis. (iv) As this multi-institutional study did not intend to analyze its association with other tumor markers, the interrelationships between other tumor markers were not assessed. Previous reports showed that combination analysis of CYFR21-1 and SCC-Ag could predict patients' prognosis.[17,18] (v) The changes in the CYFRA level between the pretreatment period and post-neoadjuvant treatment (preoperative) period is another very interesting issue. Further study will be required to assess this research question.

In conclusion, CYFRA-positive group was associated with deep tumor and poor survival. Pretreatment CYFRA was not an independent risk factor for poor prognosis in the up-front surgery group or NAC group. It was an independent risk factor for poor prognosis just in the NACRT/RT group. As the future plan, we would like to assess the perioperative changings in CYFRA. Moreover, a prospective observation study to compare the modality of neoadjuvant therapy, NAC versus NACRT, focusing on CYFRA-positive cases is required.

Ethical Approval

The ethical statement of this retrospective study was approved by the ethics committee of the Toho University Faculty of Medicine, Tokyo, Japan (A18112_A17044_A16037).

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Disclosure Statement

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