CYFRA 21-1 Serum Analysis in Patients with Esophageal Cancer

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ABSTRACT
This study was conducted to determine a potential use of CYFRA 21-1 in patients suffering from carcinoma of the esophagus. CYFRA 21-1 serum concentrations of 50 patients with histologically proven malignant lesion of the esophagus were compared with 50 healthy persons, 50 patients with benign esophageal disease, and 50 patients with benign lung disease. Additional analysis of serum carcinoembryonic antigen, CA 72-4, and squamous cell carcinoma-antigen serum concentrations were performed. The patients with esophageal carcinoma underwent follow-up tumor marker examinations every three months for 1 year. Analysis to detect statistically significant differences was conducted to estimate a cutoff and to evaluate tumor entity, tumor stage, survival, and tumor-free survival.

CYFRA 21-1 at a cutoff of 1.40 ng/ml showed an overall sensitivity to esophageal carcinoma of 36% (45.5% to squamous cell carcinoma, 17.6% to adenocarcinoma) at a specificity of 97.3%. CYFRA 21-1 concentrations showed a tendency to higher serum levels depending on local tumor burden. A correlation of CYFRA 21-1 with various N- or M-stage disease was not observed. Postoperative development in terms of survival and tumor-free survival showed significant correlation to preoperative CYFRA 21-1 concentrations. Clinical tumor recurrence was preceded by CYFRA 21-1 elevation by 3.4 months. For prognosis and follow-up, this marker is justified for additional analysis in a larger series of patients suffering from carcinoma of the esophagus.

INTRODUCTION
Esophageal carcinoma still has a poor prognosis due to late diagnosis, rapid growth and spread, and high rate of recurrence. Most patients present with advanced disease at the time of diagnosis (1). In comparison to other malignancies of the gastrointestinal tract, there are no suitable biomarkers for esophageal carcinoma. SCC-antigen2 and CEA have been used as tumor markers for esophageal carcinoma, but their sensitivity has not proven satisfactory (2, 3).

A recently established monoclonal antibody, CYFRA 21-1, has been shown to react exclusively with cytokeratin 19 (4). Cytokeratin 19 with an isoelectric pH of 5.2 and a molecular weight of 40,000 Da is found in normal cells as well as in their malignant counterparts. It is expressed in the unstratified or pseudounstratified epithelium of the bronchial tree and is used as the most sensitive tumor marker for lung carcinomas, except for small-cell carcinomas of the lung (5, 6). It is a member of the intermediate filament group of proteins, and its physiological function is unknown (7). As it is released after cell death, fragments of intermediate filaments are soluble in serum and are therefore detectable with monoclonal antibodies (6). Besides in lung cancer, CYFRA 21-1 proved valuable for uterine carcinomas (8, 9), head and neck carcinomas (10), gastric cancer (11), and recently for SCC of the esophagus in vitro and in vivo (12). Studies on CYFRA 21-1 for gastrointestinal cancer reported limited clinical use because of low sensitivity (6). Nevertheless, for gastric cancer, CYFRA 21-1 showed significant increases for stage IV disease (11). Esophageal epithelium contains cytoskeleton-4, -5, and -13 within its cytoskeleton (13), whereas esophageal SCC contains cytokeratin-19 (14). A possible use of CYFRA 21-1 in SCC of the esophagus was shown by Yamamoto et al. (12). Immunocytochemical staining revealed that some SCC cells had cytokeratin-19 fragments in their cytoplasm. In addition, cells expressing cytokeratin-19 in bone marrow have been shown to be a sensitive marker for the prediction of recurrence in various SCCs (15). Because most of the esophageal carcinomas are SCC, this study was carried out to examine CYFRA 21-1 in esophageal carcinoma patients with respect to sensitivity, specificity, correlation with local tumor burden, spread of disease, tumor recurrence, and the course of the disease.

PATIENTS AND METHODS
During the period of November 1995 to August 1996, 50 consecutive patients admitted for surgery suffering from a histologically proven malignant lesion of the esophagus were enrolled for this clinical, prospective study. Serum analysis of the tumor markers CYFRA 21-1, CEA, CA 72-4 and SCC-antigen were performed preoperatively and during the routine follow-up at 3, 6, 9, and 12 months postoperatively. To determine a cutoff for CYFRA 21-1, 50 healthy persons, 50 patients suffering from benign esophageal disease (reflux esophagitis, esophageal varicose, benign esophageal stenosis), and 50 patients with benign pulmonary disease (chronic obstructive lung disease, pulmonary fibrosis, chronic bronchitis, sarcoidosis) were examined for serum CYFRA 21-1 concentrations.

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2 The abbreviations used are: SCC-antigen, squamous cell carcinoma antigen; CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma.
Evaluation of CYFRA 21-1 was completed in a two-step sandwich immunoradiometric assay using the Centocor CYFRA 21-1 (CIS-Diagnostik, Dreieich, Germany). This kit includes the two monoclonal antibodies, MAB KS 19-1 and MAB BM 19-21, which detect the soluble cytokeratin-19 fragment. In addition, analyses of CEA and CA 72-4 tumor antigens were performed by using extinction measurements (ELISA; Abbot-IMX, commercial kits). Biomarkers and tumor stage were correlated after pathohistomorphological examination of the resected specimens.

**Statistical Assessment.** Nonparametric approximation was applied to estimate the cutoff. The nonparametric Mann-Whitney test was used for comparison of the different groups and various tumor stages. The log-rank test was used for comparison of survival rates. Results of $P < 0.05$ were regarded as significant. The software used for statistical analysis was GraphPad Prism (GraphPad; San Diego, CA).

**RESULTS**

**Evaluation of CYFRA 21-1 for Esophageal Carcinoma.**

The group of healthy probands ($n = 50$; male:female, 1.2:1; mean age: 48.4 years) with a CYFRA 21-1 median of 0.58 ng/ml did not differ significantly from patients with benign lung disease ($n = 50$; male:female, 1:1.3; mean age: 64.5 years) with a median of 0.68 ng/ml and patients with benign esophageal disease ($n = 50$; male:female, 1.4:1; mean age: 53.7 years) with a median CYFRA 21-1 of 0.68 ng/ml respectively (Fig. 1). The comparison of CYFRA 21-1 serum concentration for male and female did not show any significant difference. CYFRA 21-1 serum concentrations in patients suffering from esophageal carcinoma ($n = 50$; male:female, 4:1; mean age: 58.9 years) with a median of 1.15 ng/ml were increased significantly in comparison with all other groups. Comparing the group with esophageal carcinoma with the healthy probands, $P = 0.0004$, with the group with benign lung disease, $P = 0.0011$, and with the group...
with benign esophageal disease, \( P = 0.0044 \). The combination of healthy persons and patients with benign diseases \( (n = 150) \) showed a median CYFRA 21-1 of 0.65 ng/ml. Comparing those with patients suffering from esophageal carcinoma, the \( P \) was <0.0001.

The nonparametric approximation and univariate analysis showed a cutoff of 1.38 ng/ml for the 95% confidence interval. Raising the cutoff to a more practical level of 1.40 ng/ml, CYFRA 21-1 revealed a sensitivity of 36% for esophageal carcinoma at a specificity of 97.3%. ROC analysis showed almost equal areas under the curves, particularly for comparison of benign with malignant esophageal disease.

Analyzing esophageal carcinomas with respect to their cellular origin, a significant difference was found. This study included a total of 33 patients suffering from scc of the esophagus and 17 patients with an esophageal adenocarcinoma (Fig. 2). The median for CYFRA 21-1 in scc of the esophagus was 1.26 ng/ml. For patients with esophageal adenocarcinoma, the median amounted to 0.83 ng/ml. Thus an increase in sensitivity was observed in patients suffering from scc of the esophagus up to 45.5%; on the other hand, the sensitivity fell to 17.6% in cases of esophageal adenocarcinomas. Statistical analysis showed significantly higher concentrations in scc compared with adenocarcinoma of the esophagus \( (P = 0.0391) \). scc showed significant difference \( (P < 0.0001) \) compared with all groups, whereas adenocarcinoma did not. Comparison of survival curves in patients with a preoperative CYFRA 21-1 concentration above or below the cutoff showed significant difference \( (P < 0.0001) \) in survival and tumor-free survival. Median survival of patients (Fig. 3) with a preoperative CYFRA 21-1 concentration \( >1.40 \) ng/ml was 270 days versus an indefinable median survival \( (>95\% \text{-year survival}) \) of those with a preoperative CYFRA 21-1 level below the cutoff. Median tumor-free survival (Fig. 4) was 210 days in patients with elevated CYFRA 21-1 levels and 415 days in patients with preoperative CYFRA 21-1 concentrations \( <1.40 \) ng/ml.

Larger local tumor burden showed a rise in serum CYFRA 21-1 concentration for esophageal carcinoma. For esophageal scc, CYFRA 21-1 showed a median of 0.92 ng/ml for stage T1 \( (n = 5) \), 1.28 ng/ml for stage T2 \( (n = 9) \), 1.43 ng/ml for stage T3 \( (n = 17) \), and 4.33 ng/ml for stage T4 \( (n = 2) \). The adenocarcinomas of the esophagus showed median levels exceeding the cutoff only in T4-stage disease. No significant difference could be observed by comparing the single adenocarcinoma tumor stages, but comparison of T1 versus T3+4 showed a significant difference \( (P < 0.0001) \).

No correlation was found comparing CYFRA 21-1 serum concentrations with positive and negative lymphonodular tumor findings. A clear distinction between M0 and M1 tumor stages could not be made because too few M1 stages were enrolled, but the analysis implied at least a tendency to higher CYFRA 21-1 levels in connection with metastatic disease.

The preoperative CYFRA 21-1 values showed a correlation with the resectability. Three patients enrolled in this study proved unresectable, their CYFRA 21-1 levels being 1.08, 5.40, and 8.50 ng/ml, adding up to a median of 5.40 ng/ml.

No discrimination was possible for R0 \( (n = 38; \text{median}, 0.97 \text{ng/ml}) \) and R1 \( (n = 9; \text{median}, 1.91 \text{ng/ml}) \) operations. The development of the 47 patients operated on was monitored either for 1 year or until the death of the patient. In cases with uneventful development \( (n = 23) \), the CYFRA 21-1 levels dropped significantly \( (P = 0.0198) \) from a preoperative median of 1.15 ng/ml by almost a half \( (0.69 \text{ng/ml}) \) at first reexamination 3 months postoperatively. In cases of local or metastatic tumor recurrence, CYFRA 21-1 concentrations showed an increase in serum concentrations during the time of readmission to a median of 2.67 ng/ml. All but eight patients with a preoperative CYFRA 21-1 value above the cutoff showed a decrease of CYFRA 21-1 below the cutoff at 3 months postoperatively.

**Fig. 3** Survival of patients in relation to their preoperative CYFRA 21-1 concentration (above and below the estimated cutoff value of 1.40 ng/ml).

**Fig. 4** Tumor-free survival of patients in relation to their preoperative CYFRA 21-1 concentration (above and below the estimated cutoff value of 1.40 ng/ml).

**Fig. 5** Comparison of different tumor markers in patients with esophageal carcinoma (adenocarcinoma and scc).
indicating a reduction of tumor mass. After supportive chemotherapy and/or radiation therapy in cases of advanced or recurrent disease, marked low CYFRA 21-1 levels were observed. In cases without tumor recurrence, CYFRA 21-1 concentrations did not alter. In patients with eventful developments a significant rise of CYFRA 21-1 was observed. This rise advanced clinical proof by an average of 3.4 months (range 1–5 months).

**Serum Analysis of CEA in Esophageal Carcinoma.** CEA analyses in patients with carcinoma of the esophagus indicated a low sensitivity of 14%. The comparison of scc with esophageal adenocarcinoma showed significant difference ($P = 0.0312$). There was no correlation of CEA concentrations with T-, N-, or M-stage disease, nor with the extent of resection.

**Serum Analysis of CA 72-4 in Esophageal Carcinoma.** CA 72-4 revealed a low sensitivity of 16%. For this tumor marker, higher concentrations were found in esophageal adenocarcinoma. Discrimination of different local, lymphonodular, or metastatic tumor stages were not observed in CA 72-4 examinations.

**Serum SCC-antigen Analysis in Esophageal Carcinoma.** There was a zero sensitivity in esophageal adenocarcinomas and, in scc, the sensitivity amounted to 8.3%. Nor was there any significant difference found comparing scc with adenocarcinoma.

Fig. 5 demonstrates the different diagnostic sensitivities of the four tested tumor markers for esophageal carcinoma.

**DISCUSSION**

Tumor markers are supportive for establishing diagnosis, estimating prognosis, monitoring treatment, detecting recurrence, and screening. CYFRA 21-1, developed for lung cancer (6), showed a sensitivity ranging from 57.0 to 73.1% to this particular disease (5, 6, 15). Yamamoto et al. (12) showed a 47.9% sensitivity at a specificity of 100% for higher CYFRA 21-1 concentrations in patients suffering from scc. Furthermore, they could demonstrate a correlation between CYFRA 21-1 concentration and tumor size, tumor depth, pTNM stage, resectability, and curability. These results were superior to sensitivities of CEA (39%), CA 50 (41%), and CA 19-9 in scc of the esophageal carcinoma reported by Munk-Wiklund et al. (2). Even the SCC-antigen in human scc showed less sensitivity to esophageal scc, with 42.7% (3).

Using a kit for immunoradiometric assay of CYFRA 21-1 in serum (CIS Diagnostik, Dreieich, Germany), a different cut-off value in comparison to the other reported cutoff for CYFRA 21-1 determined with a two-step sandwich ELISA kit (Enzymun-Test CYFRA 21-1, Boehringer-Mannheim GmbH, Mannheim, Germany) may be plausible, especially inasmuch as we could demonstrate significant differences in comparison with healthy persons and patients suffering from benign diseases of the esophagus and the lung. Our findings showed a higher sensitivity (45.5%) to esophageal scc. We were also able to demonstrate the correlation of CYFRA 21-1 serum concentrations and local tumor burden, which was increased in advanced T-stages, reduced after surgery, and with markedly lower levels during postoperative chemotherapy and/or radiation therapy. Regarding prognosis, this study showed a significant difference in survival and tumor-free survival. Besides, the clinical potential of CYFRA 21-1 during the follow-up of esophageal carcinoma disease was demonstrated. Because this marker proved to be parallel to the clinical course in the case of esophageal squamous cell carcinoma, CYFRA 21-1 is justified for additional analysis in a larger series of patients. For screening purposes CYFRA 21-1 seems to lack sensitivity.

**REFERENCES**


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