Cyfra 21-1 as a Tumor Marker for Follow-up of Patients with Squamous Cell Carcinoma of the Oropharnyx

KHALED ALKOTYFAN[1], SUSANNE WIEGAND[1], HANS-HELGE MÜLLER[2], JOCHEN P. WINDFUHR[3], JOCHEN A. WERNER[1] and ANDREAS M. SESTERHENN[1]

[1]*Department of Otolaryngology, Head and Neck Surgery, University-Hospital Giessen and Marburg, Campus Marburg, Germany;*
[2]*Institute of Medical Biometry and Epidemiology, Philipps-University of Marburg, Marburg, Germany;*
[3]*Department of Otolaryngology, Maria Hilf Hospital, Moenchengladbach, Germany*

Abstract. *The aim of this study was to evaluate the importance and potential of Cyfra 21-1 as a tumor marker (TM) for follow-up of patients with squamous cell carcinoma (SCC) of oropharynx. Patients and Methods: Cyfra 21-1 serum levels of 50 patients with oropharyngeal SCC were evaluated by ECLIA assay. Statistical analysis was performed using the Jonckheere-Terpstra test. Results: There was no significant correlation between the Cyfra 21-1 level at the time of initial diagnosis, nor with the clinical and pathological parameters: T-stage (p=0.5), lymph node metastasis (p=0.73), and histological grade (p=0.35). The sensitivity and specificity of Cyfra 21-1 as a follow-up tumor marker was 45% and 93.3%, respectively. In cases of local tumor recurrence or distant metastasis, a higher concentration of Cyfra 21-1 during follow-up was observed than at the time of initial diagnosis. Conclusion: Cyfra 21-1 serum concentration is not suitable for use in early diagnosis of SCC of the oropharynx. An abrupt increase of Cyfra 21-1 during follow-up indicates disease progression or distant metastasis in the individual patient, independently from the cut-off value.*

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy and is a major cause of cancer worldwide (1, 2). The incidence of head and neck cancers is three-fold higher among men than women (3). Smoking and alcohol are by far the most common etiological factors. Heavy tobacco users have a 5- to 25-fold higher risk of developing head and neck cancer than do nonsmokers. More recently, the incidence of oropharyngeal cancer in younger populations has increased and is associated with exposure to the human papilloma virus (HPV) (3-5). The detection of high-risk HPV DNA in oral exfoliated cells and HPV-specific antibodies in serum can be considered as clinically relevant surrogate markers for the presence of HPV-associated head and neck cancer, with high sensitivity (83%) and specificity (88%) (6). Patients with oropharyngeal cancer have a high risk of early locoregional relapse, particularly within the first two years after initial diagnosis (7, 8). The early recognition of disease progression and particularly of distant metastasis would lead to a substantial improvement of the survival rate for those patients. Suitable tumor markers can be helpful for diagnosis and early detection of local recurrence and distant metastasis. The measurement of Cyfra 21-1 in patients with SCC of the head and neck is an established tumor marker, which has been described in previous studies (9-17). Cyfra 21-1 is the serum soluble fragment of cytokeratin 19 and was first described in the mid 1990s (13, 14). Cytokeratin 19 is expressed by normal and benign epithelial cells and by various carcinomas, particularly lung cancer. Cyfra 21-1 serum levels in patients with HNSCC are generally lower than in lung cancer patients. This is due to the expression of cytokeratin 19 in the epithelium of the upper aerodigestive tract being lower than that of the deeper respiratory tract (17). On the other hand, Cyfra 21-1 serum levels are significantly higher in patients with HNSCC compared to a healthy or control group (14). For SCC of the lung, Cyfra 21-1 had a better sensitivity than carcinoembryonic antigen (CEA) for SCC (18). Increased concentration of Cyfra 21-1 in patients with lung cancer was shown to be associated with poorer prognosis in comparison to patients with a normal concentration (19, 20). Cyfra 21-1 serum concentration is not a suitable tumor marker for diagnosis of HNSCC, but an increase of Cyfra 21-1 indicates impending disease progression in the individual patient (9, 17). Sawant *et al*. (1) reported a high sensitivity and specificity

Correspondence to: Andreas M. Sesterhenn, MD, Department of Otolaryngology, Head and Neck Surgery, University-Hospital Giessen and Marburg, Campus Marburg, Deutschhausstrasse 3, D-35037 Marburg, Germany. Tel: +49 64215866808, Fax: +49 6421 5866367, email: sesterhe@med.uni-marburg.de

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of Cyfra 21-1 in patients with oropharyngeal cancer (84% and 93%, respectively). They measured a significant reduction of the serum marker after surgical therapy of the primary tumor. The aim of this study was to evaluate the importance of Cyfra 21-1 as a tumor marker in patients with oropharyngeal SCC at the time of initial diagnosis in correlation with tumor size, histologic grading, and lymph node metastasis. In addition, the sensitivity and specificity of Cyfra 21-1 as a follow-up marker in cases of distant metastasis and local recurrence needed to be verified. The serum concentration of Cyfra 21-1 was evaluated at the time of diagnosis, at 4-6 weeks after primary therapy, one year after initial diagnosis, and after further follow-up examinations.

Patients and Methods

A total of 50 patients with primary diagnosis of oropharyngeal SCC, treated between 2001 and 2007, were included in this retrospective evaluation. The diagnosis was confirmed by histological biopsy findings. Tumor extent, nodal involvement, and distant metastasis were assessed by a detailed physical examination, panendoscopy, and imaging studies (*e.g.* B-mode sonography, PET scan, scintigraphy of the skeleton, CT scan and MRI). All patients were staged according to the TNM (tumor, node, distant metastasis) classification of the International Union against Cancer (UICC) (21) (Table I). The patients were followed up in the Oncologic Clinic of the Department. Venous blood samples (6 ml) were collected after written informed consent was obtained from patients for treatment of oropharyngeal SCC. Samples were allowed to clot, then centrifuged at room temperature and stored at –80˚C until processing. The serum level of Cyfra 21-1 of 50 patients with oropharyngeal SCC was evaluated by ECLIA, Elecsys® 2010 analyser [Boehringer Mannheim (BM), Mannheim, Germany]. Cytokeratin 19 fragments were detected by the monoclonal antibodies Ks 19-1 and BM 19-21; these antibodies are specific for two different epitopes of cytokeratin 19. The calculated concentration of Cyfra 21-1 was expressed in ng/ml and the cut-off level of 3.3 ng/ml was used, according to the manufacturer's instructions. Cyfra 21-1 serum levels were determined for each patient at the time of initial diagnosis, 6-8 weeks post-therapy (either surgery, chemoradiotherapy or combined), and one year after initial diagnosis, as well as in the further course of the disease.

Statistical analysis. Statistical analysis was performed using SPSS 15.0 for Microsoft Windows XP. The nonparametric Jonckheere-Terpstra test was used for estimating significance. A *p*-value of less than 0.05 was considered statistically significant. Box and whisker plots were used to compare two or more data sets. The efficacy of Cyfra 21-1 as a tumor marker for follow-up at different cut-off points was evaluated with the aid of receiver operating characteristics (ROC) curve.

Results

Fifty patients (40 male and 10 female patients with an average age of 55 years, range: 43-71 years, standard deviation: 7.28 years) with oropharyngeal SCC, were treated between January 2001 and January 2007 and included in the present evaluation. Most patients were over 50 years of age (76%, n=38), and between the ages of 50 and 65 years old. Primary treatment varied depending on tumor stage, histology and the resectability. In this study, 60% of the patients received primary radiochemotherapy, and 38% received primarily surgical therapy. After conclusion of surgical therapy, 18% of the patients received adjuvant radiochemotherapy. The extent of neck dissection (selective, SND; modified radical, MRND; radical neck dissection, RND) was determined depending on the lymph node status. Eight weeks after conclusion of primary radiochemotherapy, salvage neck dissection was performed in 26 patients.

Table I. *Distribution of oropharyngeal SCC according to the TNM/UICC stages and histological grading.*

		N	Percentage (%)
Tumor size (T)	T1	5	10
	T2	17	34
	T3	12	24
	T4	16	32
Cervical lymph node status (N)	N0	12	24
	N1	3	6
	N2A	4	8
	N2B	16	32
	N2C	11	22
	N3	4	8
Distant metastasis (M)	M0	49	98
	M1	1	2
Tumor stage (UICC)	I	2	4
	II	3	6
	III	5	10
	IV	40	80
	G1	4	8
Histological grading	G2	41	82
	G3	5	10

Correlation between Cyfra 21-1 serum concentration at initial diagnosis and T stage. Box-whisker plots where used for the graphical demonstration of Cyfra 21-1 serum concentration distribution according to T stage. The median Cyfra 21-1 serum concentration for T1 and T2 tumors was 1.1 ng/ml. The median value for T3 and T4 tumors at the time of diagnosis was at 1.4 ng/ml (Figure 1). However, no significant correlation was found between the size of the primary tumor and the value of Cyfra 21-1 at the time of initial diagnosis (Jonckheere Terpstra test p=0.5).

Correlation between Cyfra 21-1 serum concentration at initial diagnosis and N stage. The highest Cyfra 21-1 serum concentration of 2.7 ng/ml was observed in the lymph node stage N2a (Figure 2). All other N categories had significantly lower concentrations (N2b: 1.1 ng/ml; N2c: 1.6 ng/ml and N3: 1.2 ng/ml). Therefore, no significant correlation between the Cyfra 21-1 serum concentration and lymph node

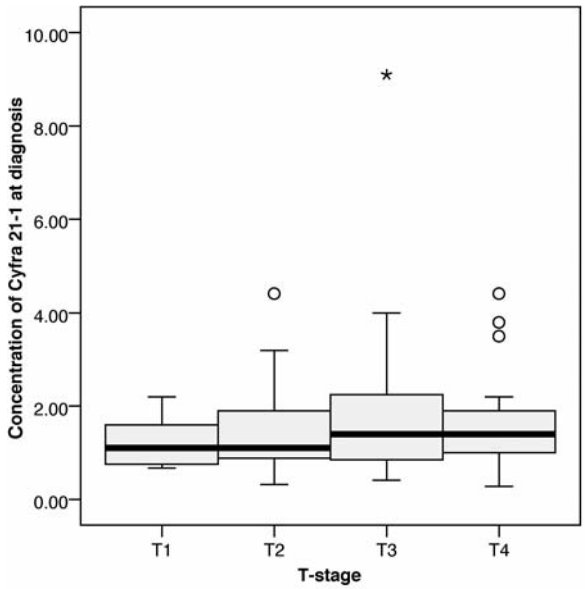

Figure 1. *Concentration of Cyfra 21-1 at diagnosis as a function of stage.*

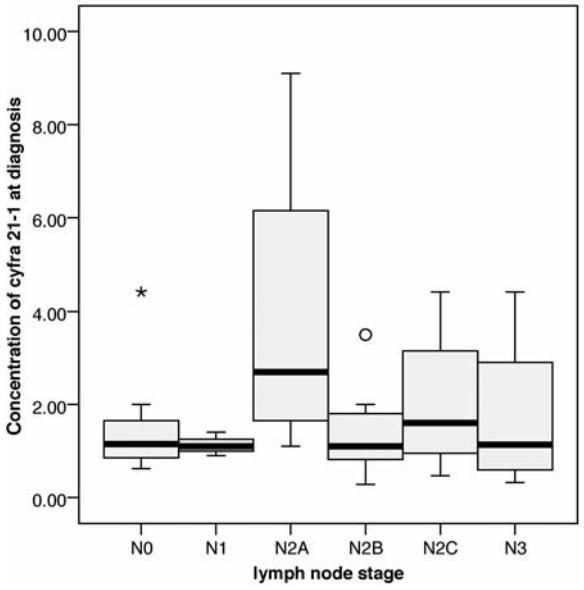

Figure 2. *Concentration of Cyfra 21-1 at diagnosis as a function of N stage.*

metastasis at the time of initial diagnosis (Jonckheere Terpstra test p=0.73) was observed.

Correlation between the Cyfra 21-1 serum concentration at initial diagnosis and histological grading. The median Cyfra 21-1 concentration was 1.4 ng/ml, 1.6 ng/ml and 0.93 ng/ml for G1, G2 and G3 tumor grading, accordingly (Figure 3). No statistically significant correlation was found between Cyfra 21-1 concentration and tumor grading (Jonckheere-Terpstra-test p=0.35).

Specificity and sensitivity in the use of the Cyfra 21-1 serum concentration as a follow-up marker for oropharyngeal SCC. Local tumor recurrences or distant metastases were found in 20 out of 50 patients (40%), whereas in the other 30 patients (60%) there was complete remission. Subsequently, the sensitivity and specificity of the Cyfra 21-1 was measured for one year after the diagnosis, taking the usual cut-off value 3.3 ng/ml as a basis. Eleven patients had a Cyfra 21-1 concentration higher than 3.3 ng/ml. Of the 20 patients with local recurrence, lymph node or distant metastasis, 9 patients (45%) had a Cyfra 21-1 concentration of at least 3.3 ng/ml. This corresponds to a sensitivity of 45%. Complete remission was found in 30 patients: 28 of these patients had a Cyfra 21-1 concentration of ≤3.3 ng/ml in the follow-up one year after diagnosis. Thus, the specificity was 93.3%. To examine the suitability of the Cyfra 21-1 serum concentration as a predictor at the time of primary diagnosis for the occurrence of distant metastasis and local recurrence, an ROC curve was

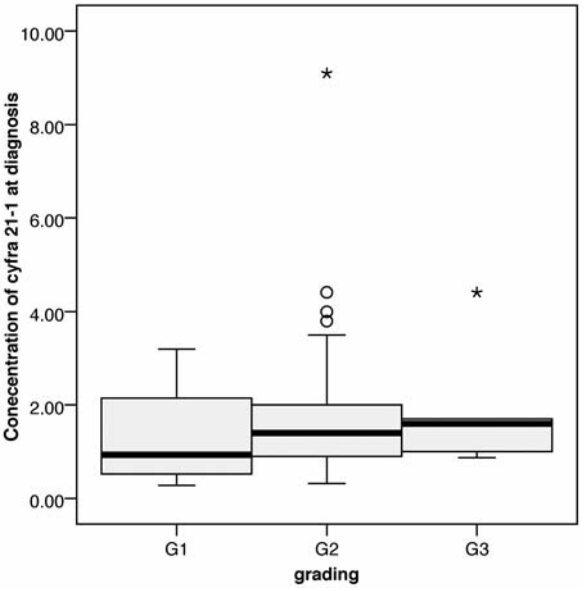

Figure 3. *Concentration of Cyfra 21-1 at diagnosis as a function of tumor grading.*

used. The diagnostic performance of a test to discriminate diseased cases from normal cases is evaluated using ROC curve analysis. ROC curves can also be used to compare the diagnostic performance of two or more laboratory or diagnostic tests. Accuracy is measured by the area under the ROC curve (AUC). An area of 1 represents a perfect test; an

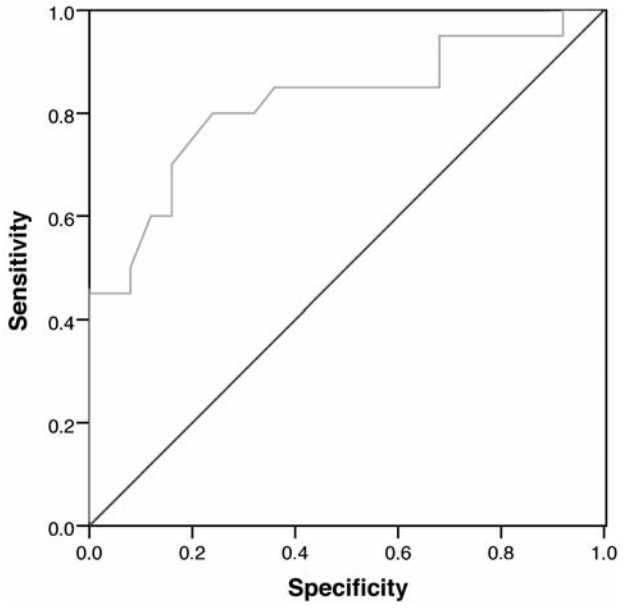

Figure 4. *Receiver operating characteristic (ROC) curve for Cyfra 21-1 to detect local residual disease, recurrence and/ or distant metastases during follow-up of patients with oropharyngeal SCC. Area under curve (AUC)=0.787.*

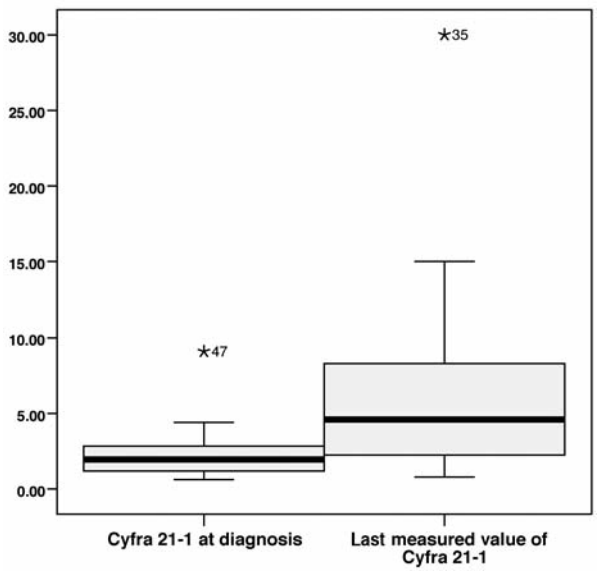

Figure 5. *Comparison of Cyfra 21-1 (ng/ml) concentration in serum between the time of initial diagnosis and follow-up in patients with local recurrence, residual disease and/ or distant metastases.*

area of 0.5 means that the diagnostic test is not better than the coincidental assignments "ill" or "healthy". The clinical use of Cyfra 21-1 as a tumor marker is shown by the AUC of 0.79 of the ROC curve with all patients and shows a good discriminative ability (Figure 4). In cases of local tumor recurrence or distant metastasis, a higher concentration of Cyfra 21-1 was measured than at the time of initial diagnosis. The median value for Cyfra 21-1 at the time of diagnosis was 1.95 ng/ml and at the last measured value was 4.6 ng/ml (Figure 5). The median value for Cyfra 21-1 without this condition was approximately the same during follow-up and at the time of initial diagnosis (1.1 ng/ml).

Discussion

In this study, the clinical relevance of Cyfra 21-1 as a prognostic marker for oropharyngeal cancer was examined retrospectively. Cyfra 21-1 serum concentration was shown not to be a suitable tumor marker for early diagnosis of SCC of the oropharynx. At the time of initial diagnosis, no significant correlation between the Cyfra 21-1 and clinicopathological parameters (T stage, N stage, and histologic grading) was found. The clinical performance of Cyfra 21-1 in the follow-up as a tumor marker to separate patients with local tumor recurrence and distant metastases from patients without evidence of recurrence or progression was good. This is shown by the AUC (0.78) of the ROC curve. In cases of tumor relapse or distant metastasis, a higher concentration of Cyfra 21-1 (2.35-fold) during follow-up was measured than at the time of initial diagnosis, while the median values of Cyfra 21-1 in patients without this condition at the time of initial diagnosis and during follow-up was approximately the same. The specificity of Cyfra 21-1 as a follow-up tumor marker was 93.3%; while the sensitivity was 45%. In preliminary work, the cut-off value for Cyfra 21-1 was set to 3.3 ng/ml (12, 17, 22). Niemann *et al.* (14) reported that Cyfra 21-1 serum levels were significantly lower in a reference group with benign diseases of the head and neck compared to a group with benign lung disease (cut-off value 2.2 ng/ml). The cut-off value determination of Cyfra 21-1 for HNSCC is controversially discussed in the literature. Cyfra 21-1 serum levels in patients with HNSCC are generally lower than in lung cancer patients; thus the clinical use of Cyfra 21-1 for HNSCC with a cut-off value of 3.3 ng/ml is uncertain and may be too high (11, 14, 17, 19, 20). The low sensitivity of Cyfra 21-1 as a follow-up marker could be explained by the fact that there was no simultaneous increase in the concentration of Cyfra 21-1 and tumor growth. Doweck *et al.* (20) reported that the concentration of Cyfra 21-1 (cut-off value 1.5 ng/ml) in the follow-up was elevated before or during clinical detection of recurrence. In another study, Niemann *et al.* (14) reported that patients with distant metastases showed increased Cyfra 21-1 levels at a cut-off value of 2.2 ng/ml. Furthermore, a significant correlation of tumor markers and recurrence has been reported by Zhong *et al.* (15). Patients with local recurrence presented higher Cyfra

21-1 concentrations compared to those in complete remission. Céruse *et al*. (19) showed that the sensitivity of Cyfra 21-1 was 72% at the time of diagnosis while the specificity was 94%. The cut-off level for Cyfra 21-1 was defined as 1 ng/ml. The dilemma in patients with head and neck cancer is the high risk of early locoregional relapse and distant metastasis. The timely detection of distant metastases is often difficult, since most do not provoke any clinical symptoms. Despite recent advances in surgery and multimodal treatment regimens, the prognosis of HNSCC remains poor (23, 24). Improvement in survival for head and neck cancer patients relies partly on the ability to predict the risk of recurrence after initial treatment and early diagnosis of local recurrence (11, 20). Only a few studies have shown that Cyfra 21-1 is a highly sensitive and specific marker, providing a valuable prognostic impact for the detection of recurrent disease and also for the evaluation of response to treatment (25). Doweck *et al*. (11, 20) reported that Cyfra 21-1 levels in HNSCC were in good correlation with tumor stage and an inverse correlation with histologic grade. At a cut-off level of 1.3 ng/ml, the sensitivity of Cyfra 21-1 was 60% and the specificity was 94%. Other studies demonstrated a clear correlation between tumor size, lymph node metastasis, and Cyfra 21-1 serum levels (13, 19). Further studies showed that measurements of Cyfra 21-1 levels in blood provide a simple, non-invasive test as a prognostic tool and an additional monitoring system for early recognition of disease progression (25, 26). Maass *et al*. (26) described the role of Cyfra 21-1 as a serological marker for the detection of distant metastases or local and neck recurrences in HNSCC. Hoffmann-Fazel *et al*. (22) found a low sensitivity of Cyfra 21-1 in detecting primary tumors, but found elevated Cyfra 21-1 serum levels correlating with the development of distant metastasis, secondary tumor and locoregional recurrence. In contrast, Wollenberg *et al*. (27) did not find any superiority of Cyfra 21-1 as compared to squamous cell carcinoma antigen (SCCAg) and carcinoembryonic antigen (CEA) with regards to their sensitivity at the time of the first diagnosis of relapse. Büntzel *et al*. (28) was unable to show any significat relationship between the lymph node metastasis and the elevation of tumor markers (SCCAg, CEA, Cyfra 21-1) in patients with advanced head and neck cancer. In conclusion, measurement of Cyfra 21-1 in oropharyngeal cancer at the early stage of diagnosis does not provide any enhancement for diagnosis or screening. The increase of Cyfra-21-1 levels in HNSCC provides additional indication for detection of local recurrences and particularly of distant metastasis in the individual patient independently from the cut-off value. When an abrupt increase in the Cyfra 21-1 levels is observed, staging procedures are strongly recommended. Additionally, it should be taken into account that Cyfra 21-1 concentration may be affected by the total protein concentration, as well as the hematocrit level.

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