

Cyfra 21-1 as a Serum Tumor Marker for Follow-up of Patients with Laryngeal and Hypopharyngeal Squamous Cell Carcinoma

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Abstract. *Aim: To evaluate the importance and potential of Cyfra21-1 as a tumor marker (TM) for follow-up of patients with squamous cell carcinoma (SCC) in laryngeal and hypopharyngeal cancer. Patients and Methods: Cyfra21-1 serum levels of 50 patients with laryngeal and hypopharyngeal SCC were evaluated by ECLIA assay. Statistical analysis was performed using Kruskal-Wallis and Jonckheere-Terpstra tests. Results: There was no significant correlation between Cyfra21-1 levels at the time of initial diagnosis and the clinicopathological parameters. The clinical performance of Cyfra 21-1 as an individual tumor marker for follow-up of patients was good. This is shown by the area under the curve (0.873) of their receiver operating characteristic curves. The sensitivity and specificity of Cyfra 21-1 at a cut-off 3.3 ng/ml were 61.1% and 96.9% respectively. Conclusion: Cyfra 21-1 is not suitable for early diagnosis of SCC of the larynx and hypopharynx. An abrupt increase of Cyfra 21-1 in serial measurements indicates impending disease progression in individual patients.*

Head and neck squamous cell carcinomas (HNSCC) are rapidly proliferating tumors. Following the treatment of early-stage disease, the most frequent disease-related event is the development of a second primary tumor. In advanced disease, local or distant recurrence is common and represents the most common cause of death (45%), followed by comorbidity (21%), treatment-related complications (15%), and second primary tumors (9%) (1).

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Despite improvements in diagnosis and management, the long-term survival rates of patients with HNSCC are among the lowest compared with the major types of cancer. For the last 30 years the rates have almost been constant (2), although a certain improvement by radiochemotherapy protocols in comparison to isolated radiotherapy has been observed. Nonetheless, effective treatment planning would be enhanced by the identification of new prognostic indicators that more accurately reflect the biological behavior of a particular tumor in relation to its host (3). The diagnostic application of keratins, although already established in tumor pathology, will be further refined and extended. Examples of very recent new developments in tumor classification are the recognition of the basal-like subtype of breast cancer on the basis of the expression of K5 and the prognostic relevance of K19 in endocrine pancreatic tumors. At present, the panel of keratins introduced into routine diagnosis as histopathological tumor markers is distinctly small, essentially consisting of K5, K7, K8/K18, K19 and K20 (4).

It has been observed that when malignant cells disintegrate, partially degraded cytokeratin (CK) fragments are released into the circulation and can be quantified using various commercially available specific serological assays. The levels of serum markers reflect the tumor burden but are not sensitive enough to be used for screening and early diagnosis of primary cancer. By contrast, the role of serum tumor markers is established in the diagnosis of recurrent disease and in the evaluation of response to treatment (5).

Cytokeratin fraction 21-1 (Cyfra 21-1) is a well-accepted tumor marker with high sensitivity and specificity in non-small cell lung cancer, especially squamous cell carcinoma (independent prognostic factor) (6). In HNSCC, the clinical value of Cyfra 21-1 as a tumor marker has been debated inconclusively, probably due to difficulties in finding the appropriate cut-off level (7).

The aim of this study was to evaluate the importance of Cyfra 21-1 at the time of initial diagnosis and its potential as a tumor marker for follow-up of patients with SCC in two major sub-sites of the head and neck (laryngeal and hypopharyngeal tumors), without determination of a specific cut-off level.

Instead, repeated testing of Cyfra 21-1 during management and comparison of Cyfra 21-1 levels at the time of initial diagnosis with subsequent levels (post-therapy, follow-up) was performed to detect any abrupt rise in the serum levels.

Patients and Methods

A total of 50 patients with primary diagnosis of laryngeal and hypopharyngeal SCC, treated between 2003 and 2007, were included in this evaluation. The diagnosis was confirmed by histological biopsy findings. Tumor extent, nodal involvement, and distant metastases were assessed by a detailed physical examination, endoscopic examination and imaging investigations (e.g. B-mode ultrasonography of the neck, chest computed tomography (CT), neck CT, bone scan, liver scan). All patients were staged according to the International Union against Cancer (UICC) and TNM classification system (8). The patients were followed up in the Oncological Clinic of the Department.

Venous blood samples (6 ml) were collected after informed consent was obtained from patients for treatment of their laryngeal or hypopharyngeal SCC. The samples were allowed to clot, then centrifuged at room temperature and stored at -80°C until processing.

The serum level Cyfra 21-1 of 50 patients with laryngeal and hypopharyngeal 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; the 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.

The serum levels of Cyfra 21-1 were determined for each individual patient at the time of primary diagnosis, 6-8 weeks post-therapy (either surgery, chemoradiotherapy or combined), and at least one follow-up in a period extending from 6 months to 1.5 years (available data). The serum levels of Cyfra 21-1 obtained for each individual patient were plotted against time of collection as it is easier to detect changes in serum levels at an early stage. Since only the change over time of the Cyfra 21-1 serum level in individual patients was tested for correlation with the individual's clinical course, this study was independent of any reference values in, for example, healthy individuals.

Statistical analysis. Statistical analysis was performed using SPSS 15.0 for windows. Nonparametric tests (Kruskal-Wallis and Jonckheere-Terpstra tests) were used for the significance estimate. A *p*-value of less than 0.05 was considered statistically significant. Box and whisker plots were also used, as they are very useful when two or more datasets are being compared. Receiver operating characteristic (ROC) curve was used to evaluate the efficacy of Cyfra 21-1 as a tumor marker for follow-up at different cut-off points.

Results

Fifty patients with laryngeal or hypopharyngeal SCC were included in this evaluation. The patients ranged in age from 40 to 82 years. Forty-two patients were men and 8 women. The anatomical sites of HNSCC were the larynx (n=26) and hypopharynx (n=24). Five patients had stage I disease, 8 stage II, 8 stage III; and 29 stage IV disease, according to

the International Union against Cancer (UICC) and TNM staging system (8).

According to the clinical course of the patients, a division into two major groups was performed: The first group (n=32) consisted of all patients who had complete remission during the first year's follow-up. The second group (n=18) included those patients with local residual disease, recurrence and/or distant metastasis during the first year's follow-up. The organ distribution of the metastases was as follows: local residual disease (n=7), lung metastases (n=4), local residual disease with lung metastases (n=1), local residual disease with bone metastases (n=1), local residual disease with lung and brain metastases (n=1), local residual disease with nasopharynx metastases (n=1), lung and liver metastases (n=1), bone and skin metastases (n=1), suprarenal gland metastases (n=1).

A wide range of serum Cyfra 21-1 levels at the time of initial diagnosis were obtained, ranging from 0.32-13 ng/ml (mean=1.95 ng/ml, median=1.4 ng/ml). Using the Jonckheere-Terpstra test, no significant correlation existed between the serum concentration of Cyfra 21-1 at time of initial diagnosis and the clinicopathological parameters primary tumor (*p*=0.916), N-status (*p*=0.424), and histological grade (*p*=0.462), nor with the clinical stage (*p*=0.504).

The clinical performance of Cyfra 21-1 as a tumor marker for follow-up of the analyzed patient population for a range of decision levels to separate those patients with local residual disease and/or distant metastasis from those patients without this condition (complete remission) is shown by the area under the curve (AUC) (0.873) of their ROC curves, which indicates the good discriminatory ability of the test (Figure 1).

Cyfra 21-1 sensitivity and specificity to differentiate between patients with complete remission and the other group of patients with local residual disease, recurrence and/or distant metastasis during follow-up at a cut-off level of 3.3 ng/ml were 61.1% and 96.9% respectively.

For the patients with local residual disease and/or distant metastasis (n=18), the Cyfra 21-1-concentration was higher for the last observed value during follow-up than at the time of diagnosis. Median values for Cyfra 21-1 at the time of diagnosis were 1.4 ng/ml, while that for the follow-up was 2.7 ng/ml. The increase of the interquartile range was even stronger for follow-up values of Cyfra 21-1: at the time of diagnosis: 2.69 ng/ml, for the last observed value: 7.68 ng/ml (Figure 2). Figures 3-6 demonstrate representative examples of the change of the Cyfra 21-1 serum levels in individual patients during the clinical course.

Discussion

Patients with head and neck tumors have a high risk of early locoregional relapse that is difficult to diagnose. Despite recent advances in surgery and multimodal treatment regimens, the prognosis HNSCC remains poor. Improvement

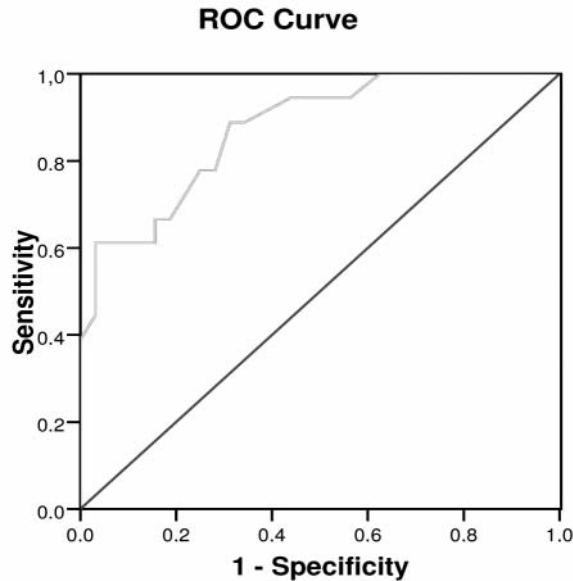


Figure 1. Receiver operating characteristic (ROC) curve for Cyfra 21-1 to detect local residual disease, recurrence and/or distant metastasis during follow-up of patients with laryngeal and hypopharyngeal squamous cell carcinoma. Area under curve (AUC)=0.873.

in survival for head and neck cancer relies partly on the ability to predict the risk of recurrence after initial treatment and early diagnosis of local recurrence (9).

A few studies have shown that Cyfra 21-1 is a highly sensitive and specific marker, providing a valuable prognostic indicator for the detection of recurrent disease and also for the evaluation of response to treatment (10, 11). However, their use in early diagnosis of disease is restricted because increase in cytokeratin levels in sera of patients with HNSCC is based on tumor burden rather than on the stage of the disease (10). Doweck (12) reported that Cyfra 21-1 can be used in HNSCC region at a sensitivity of 60%, with a good correlation with tumor stage and an inverse correlation with the grade of tumor differentiation. Their further studies showed that measurements of Cyfra 21-1 levels in blood provides a simple, non-invasive test to the head and neck oncologist as a prognostic tool and an additional monitoring system for early recognition of progression of the disease (12).

Niemann *et al.* (6) demonstrated a clear correlation between tumor growth, lymph node metastasis, and Cyfra 21-1 serum levels. Cyfra 21-1 was found to be a helpful serological marker in the follow-up of patients and it has also been proposed as a marker for monitoring head and neck cancer. According to Deng *et al.* (13), serum Cyfra 21-1 may be appropriate for clinical use as a reliable tumor marker for HNSCC. Maass *et al.* (14) were the first to show the potential role of Cyfra 21-1 as a serological marker for the detection of distant metastases (sites: pulmonary, liver, osseous, cutaneous, mediastinal), or local and neck recurrences in HNSCC.

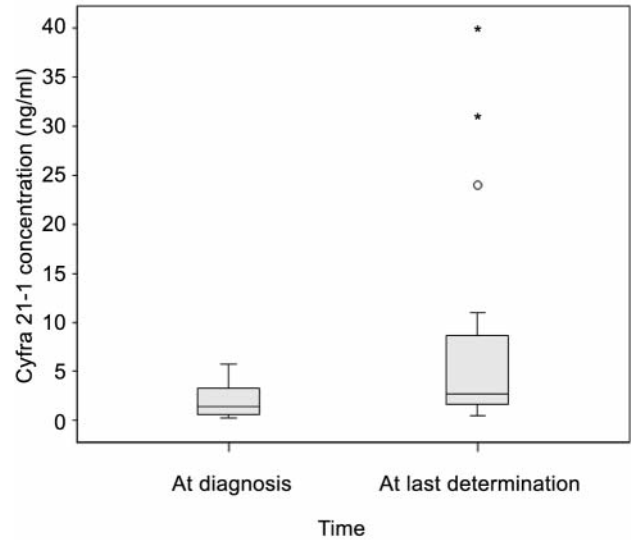


Figure 2. Comparison of Cyfra 21-1 concentration in serum between the time of initial diagnosis and follow-up in patients with local recurrence, residual disease and/or distant metastases.

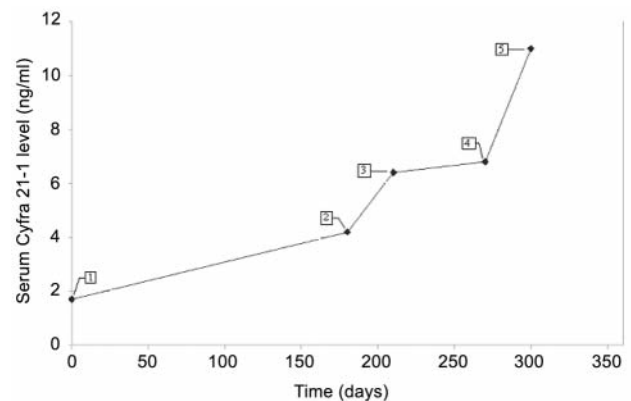


Figure 3. Serial measurements of the serum Cyfra 21-1 levels during the clinical course of a patient with a T3N0M0 laryngeal (supraglottic) squamous cell carcinoma. An abrupt rise in the serum Cyfra 21-1 level is seen at follow-up, which is correlated with the clinical diagnosis of distant metastasis. 1: Primary diagnosis, 2: post-therapy, 3: before the clinical appearance of distant metastasis, 4: diagnosis of bone metastasis, 5: diagnosis of skin metastasis.

Although, Hoffmann-Fazel *et al.* (15) found a low sensitivity of Cyfra 21-1 for detection of primary tumor, they found it to be a good screening marker for distant metastasis (sites: lung, liver, brain, skin, mediastinum), second primary tumor, and locoregional recurrence of the tumor, respectively, in head and neck cancer. In contrast, Pradier *et al.* (16) did not find Cyfra 21-1 to be an appropriate parameter in identifying patients with head and neck cancer at risk of either residual disease after treatment, or recurrent or progressive disease. Wollenberg

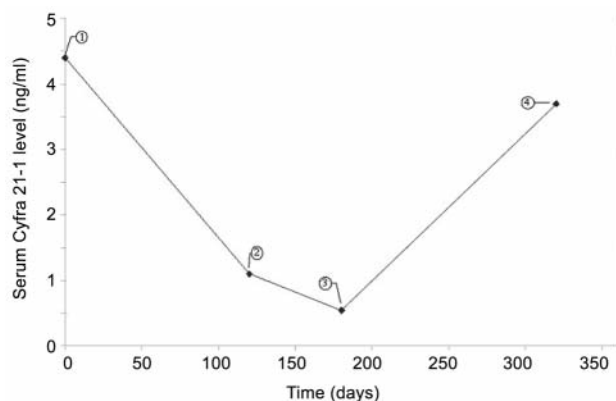


Figure 4. Serial measurements of the serum Cyfra 21-1 levels during the clinical course of a patient with a T4N3M0 hypopharyngeal squamous cell carcinoma. An abrupt rise in the serum level of Cyfra 21-1 on follow-up correlated with the diagnosis of local residual disease and lung metastasis. 1: Primary diagnosis, 2: post-therapy, 3: follow-up, 4: diagnosis of local recurrent disease and distant metastasis (lung).

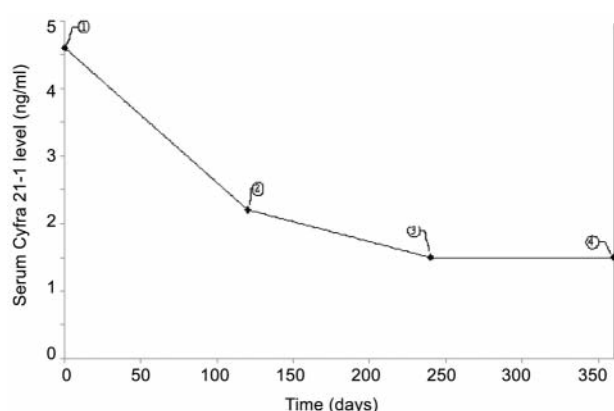


Figure 5. Serial measurements of the serum Cyfra 21-1 levels during the clinical course of a patient with a T3N2AM0 hypopharyngeal squamous cell carcinoma. An absence of any abrupt rise in the serum level of Cyfra 21-1 on follow-up correlated with the clinical course of the disease (complete remission). 1: Primary diagnosis, 2: post-therapy, 3: follow-up, 4: follow-up (complete remission).

et al. (17) also did not find any superiority of Cyfra 21-1 as compared to squamous cell carcinoma antigen (SCCAg) and carcinoembryonic antigen (CEA) with regard to their sensitivity at the time of first diagnosis of relapse. Deng *et al.* (13) reported that HNSCC patients who showed local recurrence or distant metastasis within 1-6 months after surgery remained Cyfra 21-1-positive even after treatment.

From the above studies, it is apparent that there is contradictory information emerging from different laboratories. This may be because the head and neck region has been evaluated as a whole without consideration for sub-sites. Recent expression profiling studies show that each sub-site in the head and neck region has a unique molecular signature, thereby necessitating the evaluation of the levels of cytokeratin fragments in serum for each of these sites individually with larger numbers of patients (18-20).

In this evaluation this concept was applied, hence the serum level of Cyfra 21-1 was evaluated in two major sub-sites of the head and neck, namely in laryngeal and hypopharyngeal tumors, as they share many similarities with a relevant number of patients (n=50) without determination of a specific cut-off level for thresholds between normal and pathological values. However, the sensitivity and specificity of a test are dependent on the cut-off value that is used. Doweck *et al.* (10) reported that at a cut-off of 1.3 ng/ml, the sensitivity of Cyfra 21-1 as a tumor marker for HNSCC was 60% with a specificity of 94%. Niemann *et al.* (7) determined the cut-off level of Cyfra 21-1 for patients with HNSCC to be 2.2 ng/ml. Many other authors suggest a cut-off level as low as 1 ng/ml (21).

The ideal cut-off level for patients with HNSCC is still a matter of controversy, as when a higher cut-off level is selected, the false-positive fraction will decrease with increased specificity but on the other hand, the true-positive fraction and sensitivity

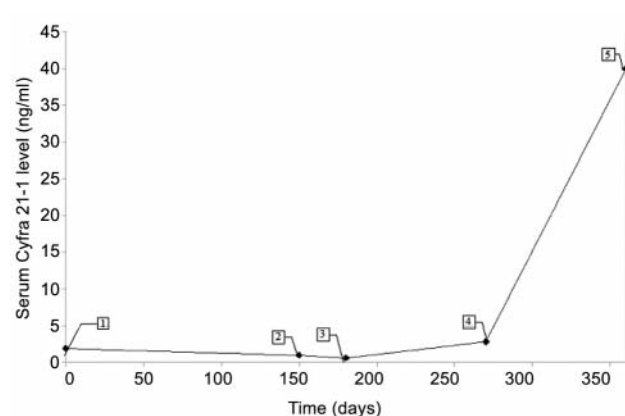


Figure 6. Serial measurements of Cyfra 21-1 in a patient with T3N1M0 hypopharyngeal squamous cell carcinoma show an abrupt rise in serum concentration (40 ng/ml) during the first year of follow-up, which correlated with clinical diagnosis of distant lung metastasis. 1: Primary diagnosis, 2: post-therapy, 3, 4: follow-up, 5: distant lung metastasis.

will decrease; when a lower cut-off value is selected, then the true-positive fraction and the sensitivity will increase and therefore the true-negative fraction and specificity will decrease. To avoid this matter of controversy, we tried in this evaluation to look for the rising level of Cyfra 21-1 during follow-up of patients rather than to concentrate on absolute values only.

In this evaluation, 64% of our patients had complete remission, while 36% had local residual disease and/or distant metastasis. The most common site for distant metastasis was the lung (63%), but it is clear that any organ can be affected by distant metastasis (*e.g.* liver, bone, skin, suprarenal gland).

In this study, Cyfra 21-1 serum concentration was not a suitable tumor marker for early diagnosis of SCC of the

larynx or hypopharynx as a wide range of serum Cyfra 21-1 levels at the time of diagnosis were obtained without any significant correlation between it and the clinicopathological parameters.

The clinical performance of Cyfra 21-1 as a tumor marker for follow-up of patients to separate those patients with local residual disease and distant metastasis from those patients without this condition was good. This is shown by the AUC (0.873) of their ROC curves and the sensitivity and specificity for Cyfra 21-1. The increase in the median value of Cyfra 21-1 (2-fold) at time of follow-up with a progressive increase of the interquartile range (2.9-fold) was indication of local residual disease and metastasis in comparison to the values at time of initial diagnosis in the same group of patients; while in the group of patients with complete remission, the median value for Cyfra 21-1 during follow-up (1.4 ng/ml) was smaller than the value at time of initial diagnosis (1.1 ng/ml).

An abrupt increase of Cyfra 21-1 in serial measurements during follow-up would seem to indicate impending disease progression and provides early prognostic information, particularly on tumor progression and metastatic formation in the individual patient. Therefore, the Cyfra 21-1 serum concentration is a good marker for follow-up in patients with SCC of larynx and hypopharynx to detect residual or recurrent disease and distant metastasis early, and in the case of an abrupt rise of serum Cyfra 21-1, staging procedures are recommended. Finally, it is important to note that this study was retrospective. Further prospective trials with serial measurements of Cyfra 21-1 at regular intervals are encouraged to establish its routine use in clinical practice.

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