Clinical Effectiveness of Tumor Markers in Squamous Cell Carcinoma of the Larynx

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Abstract. Background: The aim of this study was to examine and compare the value of the serum tumor markers CYFRA 21-1, TPA-M, SCCA and CEA in squamous cell carcinoma (SCC) of the larynx. Moreover, their possible clinical applications were studied. Materials and Methods: The serum levels of CYFRA 21-1, TPA-M, SCCA and CEA were measured in 79 patients with histologically-proven squamous cell carcinoma (SCC) of the larynx before and after treatment and in 77 healthy volunteers. The association of the results with the clinicopathological characteristics was investigated. Results: The study showed that none of the markers revealed a significant sensitivity; TPA-M indicated a positive correlation with the grade of differentiation. CEA indicated a positive correlation only with distant metastasis. Conclusion: Among the four markers, only TPA-M may play a role in monitoring the success of therapy and follow-up in patients with SCC of the larynx.

Laryngeal cancer represents 2.2\% and 0.4\% of all malignant tumors in males and females, respectively. The most frequent malignant tumor of the larynx is squamous cell carcinoma (SCC). Despite progress in oncological treatment, the prognosis of SCC of the larynx is still poor (1, 2). The American Cancer Society estimates that 9,880 new cases of laryngeal cancer (7,920 in men and 1,960 in women) will be diagnosed and 3,770 people (2,960 men and 810 women) will die from the disease in the United States in 2006. In approximately 25\% of the patients, the cancer is detected when metastasis is already present in the regional lymph nodes. Unfortunately, in 15\% of the patients distant metastases exist at the time of diagnosis (3).

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The identification of a non-invasive diagnostic tool to indicate the development of primary cancer and/or the presence of distant metastases would be very helpful. Several serum tumor markers have been evaluated in an attempt to improve diagnosis and follow-up, with controversial results. The value of serum tumor markers is still questionable (4, 5). However, recently there has been a remarkable improvement in commercial kits for serum tumor marker quantification and an evolution in the technical aspects of the methods of measurement, supporting a revival of interest.

The aim of the present study was to compare the sensitivities of tissue polypeptide antigen (TPA-M), cytokeratin 19 fragments (CYFRA 21-1), squamous cell carcinoma antigen (SCCA) and carcinoembryonic antigen (CEA) in laryngeal SCC and to evaluate the possible clinical applications of these markers. A cohort of healthy persons was included in the study to determine the potential usefulness of these markers as a screening tool.

Materials and Methods

Patients. Seventy-nine patients with an initial diagnosis of laryngeal SCC were enrolled in this study. The diagnosis was histologically confirmed from biopsies. The tumor extent, nodal involvement and distant metastases were assessed by a detailed physical examination and imaging investigation including CT scanning or MRI of the head and neck, chest X-ray and abdominal ultrasound. All the patients were staged according to the International Union against Cancer (UICC) TNM classification system (American Joint Committee on Cancer (AJCC), Staging Manual, Sixth Edition, 2002). The evaluation of the differentiation of the tumor (Grade) was also part of the staging process.

Eighty healthy subjects, without evidence of neoplasm, comprised the reference group. The healthy subjects were matched for age, gender and smoking habits (57 men, 23 women, 56 smokers, 24 non-smokers, 39 under 60 years, 17 between 60 and 70 years and 24 up to 70 years old). All the investigations were carried out with the informed consent of the patients, according to a protocol of the Ethical Committee of the University of Athens, Greece.
Methods. Blood samples were collected from each patient before any treatment and from each healthy control subject. Blood was also drawn from each patient 15 days after surgical intervention, 40 days after radiotherapy or after the end of chemotherapy. Radiotherapy (50-70 Gy) was provided to 36 patients while twelve of them received simultaneous chemotherapy (cisplatin). All the samples were centrifuged at 3000xg for 15 min, frozen and stored at –80°C until analysis.

Following the manufacturer’s instructions, the serum level of CYFRA 21-1 was measured by an electrochemiluminescent immunoassay (ECLIA) using the Elecsys reagent kit (Roche Diagnostics). The cut-off value was determined to be 3.3 ng/ml, also based on the manufacturer’s results. TPA-M and CEA were measured with quantitative chemiluminescent immunoassay kits (LIAISON TPA-M and LIAISON CEA), respectively. The cut-off value was 75 U/l for TPA-M and 2.5 ng/ml for CEA. SCCA was measured with a microparticle enzyme immunoassay kit (Abbott Diagnostics). The cut-off value was 1.6 ng/ml. All the cut-off values were based on the manufacturer’s results.

The serum levels of the four tumor markers were compared between normal, healthy volunteers and laryngeal SCC patients using the t-test and ANOVA test. The same tests were also used to determine whether there was a significant difference in the levels of tumor markers among different categorical variables including clinical stage, T stage, N stage, M stage, histological degree of differentiation and tumor sites.

Table I. The positive values for CEA, CYFRA 21-1, TPA-M and SCCA according to various clinical aspects of the disease.

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<td>2 66.7</td>
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</table>
Results

All the healthy subjects presented with levels less than the cut-off values for all the four tumor markers, which was also true for all the patients after treatment. Statistical analysis indicated that no significant correlation existed among the four markers used. Table I presents the positive values for CEA, CYFRA 21-1, TPA-M and SCCA according to various clinical aspects of the disease. Table II indicates that none of the markers was correlated with age, gender or cancer stage (p>0.05), using a relative statistical tool (ANOVA or t-test). Table III exhibits the sensitivity and specificity of each marker. ANOVA analysis indicated a positive correlation between the G status and TPA-M (p=0.004) (Figure 1) and, to a lesser extent, between the same factor and CEA (p=0.018). Similarly, ANOVA analysis shows a positive correlation between the N status and TPA-M (p=0.005) (Figure 2) and between the T status and TPA-M (p=0.022). A relatively strong correlation appeared to exist between distant metastasis and the CEA marker (p=0.009) (Figure 3), as revealed by the t-test. Finally, the correlation between the site of cancer and the CEA and TPA-M markers (p=0.008 and 0.023, respectively) was not significant, despite the relatively low p-values.

Discussion

In the current literature, the value of tumor markers in the diagnosis and prognosis of SCC of the larynx in particular has been contradictory (6-8). It was shown that none of the
available markers was able to detect this type of cancer at an early stage, as supported by the present study. Nevertheless, it is remarkable that the tumor marker levels of all patients were decreased to lower than the cut-off values after treatment. This is important in the evaluation of the therapy and, at the same time, excludes the possibility of there being a second primary tumor.

The positive correlation between the N stage and TPA-M could be of great clinical importance in cases of nodal invading metastases that recur after primary treatment. Very often the affected area is covered by a reconstructive flap or has been irradiated. The region becomes rigid and distorted and the clinical detection of nodal metastases is difficult. Thus, an accurate and sensitive method for early diagnosis of nodal metastasis is of paramount significance. Similar results have not been reported in other studies.

In the present study, TPA-M accurately presented the highest sensitivity of all four tumor markers. TPA-M measures the levels of three different cytokeratins (CK8,18,19), but cannot distinguish them. The results cannot be directly compared with the current literature, since there is a lack of similar work.

Some authors studied polyclonal TPA, but found it lacking specificity (9, 10). TPS (which detects the M3 epitope of cytokeratin 18) has been studied, but this marker only assesses one epitope of cytokeratin 18 (11), while CYFRA 8/18 detects one epitope from cytokeratin 8 and one from cytokeratin 18 (12). Although many researchers have reported a significantly high sensitivity of CYFRA 21-1 (13, 14) and of TPS (15), these results were not backed up by the present study. Pradier et al. (16) also demonstrated a reduced sensitivity for the CYFRA 21-1 marker.

Some authors have identified a relationship between the SCCA and CYFRA 21-1 levels and tumor stage (17, 18). Our study did not find any correlation with tumor site or with other parameters such as age, gender and site, which is in accordance with the study of Bongers et al. (19). The correlation between the M status and CEA was clear in this study. Ogava et al. (20) reached the same conclusion, though the relatively small number of cases studied should be noted. In the present study, the TPA-M levels were higher in patients with poorly-differentiated SCC in comparison to those whose tumors were moderately- or well-differentiated, as Dowek et al. (21) found for CYFRA 21-1. These facts, unfortunately, are not clinically significant because the main research interest is the early detection of cancer. Nevertheless, the use of cytokeratins as tumor markers is a reliable tool for detecting distant or local metastases, even at first diagnosis or in follow-up.

The serum levels of cytokeratins 8, 18 and 19 in patients with SSC of the head and neck(particularly of the larynx) are generally lower compared to the levels of patients with lung cancer (22). This may be explained by the relatively lower expression of these cytokeratins in the epithelium of the upper respiratory tract as compared to the lung (23). Another explanation could be the cell type heterogeneity in different sites (24). Since the proposed cut-off values for the evaluation of lung cancer are higher, it may be acceptable to use lower cut-off values when evaluating SCC of head and neck, in accordance with the above discussion, and as supported by other studies (25-27). The significance of the results if lower cut-offs are selected may be uncertain, since lower cut-off values would probably minimize the false-negative results but, at the same time, would also increase the false-positive results.

This study was prospective. The follow-up of these patients is to be continued so that the prognostic significance of these serum tumor markers for the detection of recurrence or distant metastasis can be evaluated. In addition, it would be interesting to monitor the patients who had initially exhibited high levels of TPA-M in relation to possible relapse. Currently, the value of the tested serum tumor markers in laryngeal SCC is still questionable.

References


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