Concomitant Analysis of Salivary Tumor Markers—A New Diagnostic Tool for Oral Cancer

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Abstract

Purpose: Oral squamous cell carcinoma (OSCC) is a common human malignancy. Circulatory epithelial tumor markers were previously investigated in the serum of OSCC patients but almost never in their saliva, in spite of the fact that there is a direct contact between the saliva and the oral cancer lesion. The purpose of the current study was to examine tumor markers in the saliva of OSCC patients.

Experimental Design: We measured the concentrations of the six most studied epithelial serum circulatory tumor markers in the saliva of OSCC (tongue) patients.

Results: Significant increases (of 400%) in salivary concentrations of Cyfra 21-1, tissue polypeptide antigen, and CA125 were shown. Salivary concentrations of CA19-9, SCC, and carcinoembryonic antigen were increased without statistical significance. A concurrent analysis of the three significantly increased markers revealed sensitivity, specificity, and negative and positive predictive values of 71%, 75%, 71%, and 75%, respectively.

Conclusions: The increase reported in salivary tumor markers may be used as a diagnostic tool, especially when a concurrent analysis for significantly increased markers is done. Salivary testing is noninvasive, making it an attractive, effective alternative to serum testing, and the possibility of developing home testing kits would further facilitate it as a diagnostic aid, enabling patients to monitor their own health at home and is important for those who live far from their treatment centers and especially for those at risk of developing OSCC.

Oral squamous cell carcinoma (OSCC) is a common human malignancy, with an increasing incidence (especially in younger people) and a 5-year mortality rate of ~50% (1–4), which has not changed significantly in >50 years (5–11). Its location and treatment in the mouth/face/neck result in a relatively high rate of related morbidity, as the treatment frequently results in a significant mutilation and compromised functions. OSCC includes both mobile (oral) and base of tongue cancer lesions. Most often, an oral cancer lesion is located at the lateral border of the tongue, whereas one located at the base of tongue is considered especially lethal. Clinically, it is important to note that the therapeutic modality currently offered to patients is based on traditional stage-predicting indices (based mostly on the tumor-node-metastasis criteria) and on histologic grading. Unfortunately, these predictors are subjective and relatively unreliable, as often two tumors with identical staging and grading behave in totally different fashions, and although one responds to therapy, the other is lethal. Accordingly, there has been an ever-growing effort dedicated to the basic research of oral cancer, focusing on the identification of biological indicators for the diagnosis of its biological nature and aggressiveness. Circulatory tumor markers for OSCC were investigated in various studies (12–21) and showed relatively moderate sensitivity and specificity values with relation to diagnosis, prognosis predicting, or treatment monitoring. For example, Kurokawa et al. analyzed circulatory carcinoembryonic antigen (CEA), SCC, immunosuppressive acidic protein, and Cyfra concentrations in OSCC patients and found sensitivity and accuracy values of 81% and 77.8%, respectively, and when they analyzed CEA, SCC, and immunosuppressive acidic protein, the values were 69% and 90.3%, respectively (22, 23). Hoffmann et al. (21) and Krimmel et al. (20), who analyzed circulatory levels of SCC, CEA, CA19-9, and CA125, found correlation with the tumor burden for only the SCC antigen. They reported rather low sensitivity values for this antigen (except for patients with distant metastasis) and noted that the circulatory SCC antigen had not been routinely used previously, as its reported sensitivity was relatively low in other studies as well (15-40%), although its specificity was quite high (70-90%). Hellner et al. (16) reported that circulatory SCC sensitivity in oral cancer patients was only 24%, whereas it was much lower for CEA. Zoller et al. (17, 18) reported that, although CA19-9, CA125, and CA15-3 exhibited poor sensitivity, the sensitivity values for circulatory SCC and CEA in oral cancer patients were 33% and 43%,

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Received 11/5/05; revised 4/9/06; accepted 5/4/06.

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doi:10.1158/1078-0432.CCR-05-2412

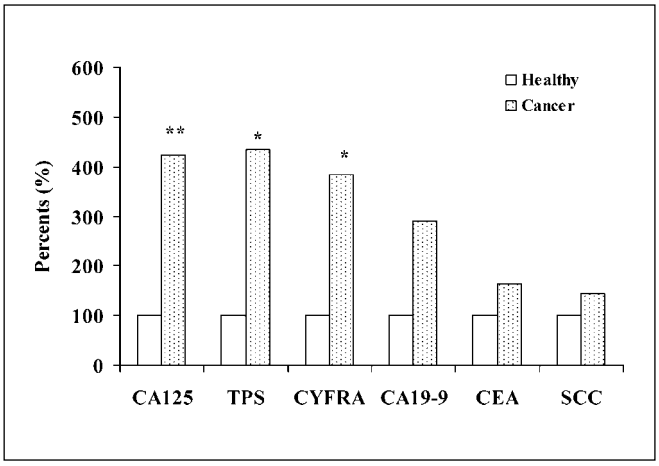

Fig. 1. Salivary concentrations of CA125, TPS, Cyfra 21-1, CA19-9, CEA, and SCC tumor markers in healthy (*empty columns*; $n = 16$) and OSCC patients (*dotted columns*; $n = 14$). The medians of the healthy controls and the OSCC patients were compared with the Wilcoxon rank-sum test (pairs of subgroups). *, $P \leq 0.05$; **, $P \leq 0.01$.

respectively. Such a wide range was also found for other circulatory markers, such as Cyfra 21-1 or tissue polypeptide antigen (TPS), which were in the range of 25% to 96% and 65% to 75%, respectively (12–15). One method suggested, to improve the sensitivity and accuracy of such an analysis, was to examine various circulatory markers concurrently (to do a "combination assay"; refs. 22, 23).

Surprisingly, almost no studies examined tumor markers in the saliva of OSCC patients. Such an examination might be of great benefit because of the direct contact between the oral cancer lesion and saliva, particularly because salivary analysis is a useful diagnostic tool for other distant malignancies, such as breast carcinoma (24). Moreover, these scarce salivary reports focused only on one commonly analyzed tumor marker, the CEA (25, 26), which did not prove to be sensitive or specific enough.

The purpose of the current study was to examine the saliva of OSCC (tongue) patients for the six most often studied serum circulatory epithelial tumor markers: Cyfra 21-1, TPS,

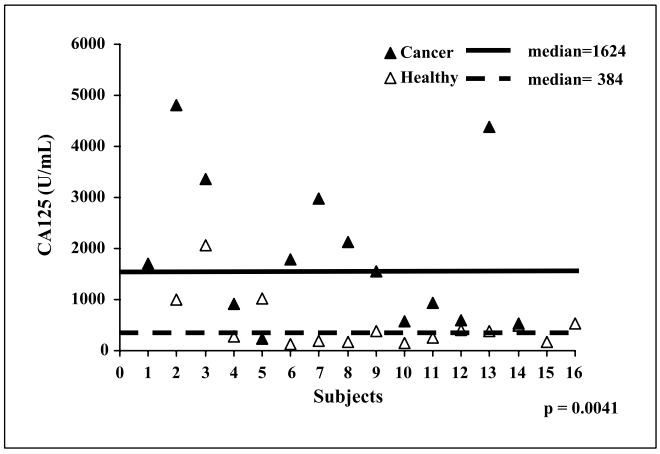

Fig. 2. Scatter plot of the salivary CA125 concentrations of examined cancer and healthy subjects.

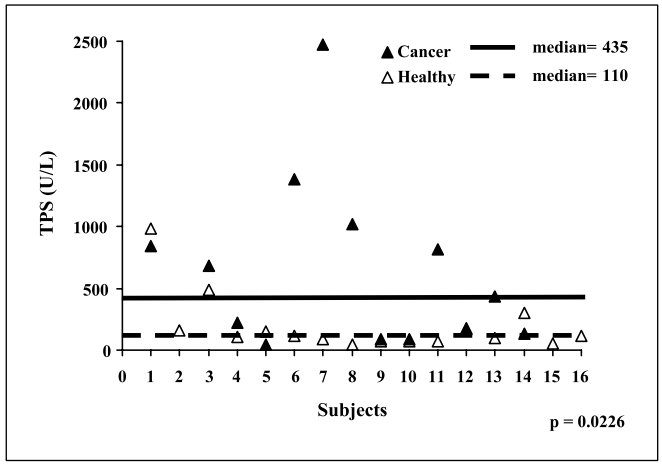

Fig. 3. Scatter plot of the salivary TPS concentrations of examined cancer and healthy subjects.

CEA, SCC, CA125, and CA19-9. The data obtained were then compared with other traditionally examined variables used for the diagnosis and evaluation of the severity of the disease.

Materials and Methods

Patients and study design. The data analyzed in the current study relate to 21 patients who received definitive treatment for tongue SCC and were monitored for up to 42 months. The mean age of the group was 68 ± 17 (range, 30-86) and included 12 females and 9 males. For 14 of these patients, salivary analysis was obtained as well, which was compared with a control group of 16 healthy individuals matched for age and sex. The data obtained included staging (according to the tumor-node-metastasis criteria), histologic grading, depth of the tumor, maximal tumor diameter, localization at the base versus mobile part of the tongue, and the patients' age and sex. Other data obtained were the salivary concentrations of the carbohydrate antigens CA125 and CA19-9, TPS, CEA, SCC, and Cyfra 21-1. They were measured shortly before the administration of the definitive curative treatment, which included surgical removal of the primary tongue tumor, neck dissection, and, often, postoperative adjuvant radiotherapy. These data were correlated with the patients' accumulative survival and disease-free survival (DFS) data.

Saliva collection. Whole saliva was collected shortly before the administration of definitive therapy under nonstimulatory conditions in a quiet room between 8 a.m. and noon, at least 1 hour after eating. Patients were asked to generate saliva and to spit into a wide test tube for 10 minutes as described previously (27). Following collection, the saliva was immediately centrifuged at 800 × *g* at 4°C for 10 minutes to remove squamous cells and cell debris. The resulting supernatant was used for the further biochemical analysis.

Assessment of salivary tumor markers. Salivary samples were stored at −70°C until analyzed, when all six markers were assayed. The TPS and Cyfra 21-1 were analyzed as described previously (12, 28). Briefly, TPS was assayed using monoclonal immunoradiometric assay (BEKI Diagnostics AB, Bromma, Sweden). The assay measures the M3 epitope soluble fragments of human cytokeratin 18. Cyfra 21-1 was evaluated using a kit (Elsa-Cyfra 21-1 immunoradiometric assay kit, CIS Bio International, Gif-Sur-Yvette, France). Cyfra 21-1 was developed using two monoclonal antibodies (BM 19-21 and KS 19-1) that react with different epitopes on cytokeratin 19 found in the samples. The first monoclonal antibody was immobilized in plastic tubes, whereas the second antibody was iodinated. When the

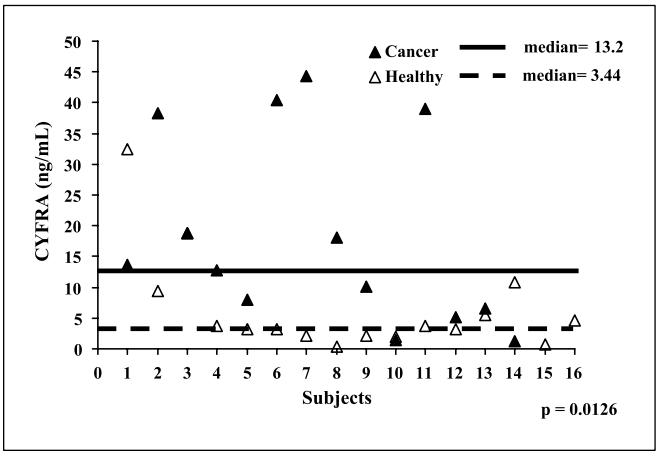

Fig. 4. Scatter plot of the salivary Cyfra 21-1 concentrations of examined cancer and healthy subjects.

sample contained cytokeratin 19 fragments, their epitopes cross-linked both antibodies, resulting in an increase in the radioactivity as measured by a gamma counter. SCC, CEA, CA19-9, and CA125 were determined with a microparticle enzyme-linked immunoassay distributed by Abbot (Tokyo, Japan) and done as described previously (29–31).

Statistical analysis. For categorical variables, frequencies, percentages, and distribution were calculated. For continuous variables, ranges, medians, means, and SE were calculated. Median values were calculated because of the large in-borne variability of variables in saliva (a common practice). Because we analyzed small (<30) groups, nonparametric statistical tests were used. Distributions of categorical variables were compared and analyzed with the Fisher-Irwin exact test. The medians between subgroups of patients were compared with the Wilcoxon rank-sum test (pairs of subgroups). A correlation matrix of estimators was used to analyze the correlation coefficients between the salivary markers. For classification analysis, cutoff values were calculated as mean plus SE of healthy controls. Sensitivity and specificity values were calculated as the fraction of observations, which were correctly classified. The cumulative incidence estimate was used to calculate the probability of survival and DFS rates as a function of time. The log-rank test was used to compare pairs of cumulative incidence estimators.

Results

Clinical data, staging, pathologic grading, dimensions, site, and extension of the tumors

The distribution of the 21 patients according to tumor size (T) revealed that 9 patients had T_1 tumor and 10 had T_2 tumor, whereas only 2 patients had T_3 and T_4 tumors (1 of each). That is, 90% of the patients had early (small to moderate) tumors. In 16 of 21 (76%) patients, there were no neck metastasis (N_0), whereas 4 patients were diagnosed with N_1 and 1 patient with N_2. None had distant metastasis (all patients were M_0). Accordingly, 71% of the patients were diagnosed with early-stage tumors (I and II), whereas only 29% of the patients were diagnosed with advanced stages (III and IV). Similarly, most of the patients (80%) were diagnosed with well-differentiated and moderately differentiated tumors (7 and 12 patients with grades 1 and 2, respectively) and only 2 patients were diagnosed with poorly differentiated lesions.

The mean tumor diameter was 2.5 ± 1.3 cm (range, 0.8-6.0 cm), and the mean depth was 8.5 ± 6.4 mm (range, 1-26 mm). The correlation rates between the diameter and T and the diameter and N were 0.82 and 0.25, respectively, whereas the correlation rates between the depth and T and the depth and N were 0.40 and 0.34, respectively.

Only 21% of the patients smoked (3 of the 14 for whom this information was available). The rate of smokers in the control group was not significantly different (4 of 16). About 20% of the patients had other or previous malignancies (4 of 20 for whom these data were available) but not in the head and neck region, and none had previously been treated with radiotherapy. None of the controls was treated with radiotherapy or had previous head and neck cancer. In 17 (85%) patients, the tumor was located in the oral (mobile) tongue (oral cancer; 13 in the anterior/middle portion and 4 tumors located posterior laterally), whereas in 3 (15%) patients the tumor was located at the base of tongue (oropharyngeal cancer). In 26% of the patients (5 of 19 available), the tumor extended beyond the lingual region and expanded locally toward neighboring regions, as the floor of the mouth.

Salivary tumor markers

Individual analysis. Salivary tumor marker analysis was available in 14 cancer patients and in 16 healthy controls. The salivary concentrations in healthy control patients of CA125, TPS, Cyfra 21-1, CA19-9, CEA, and SCC were 384 units/mL, 110 units/L, 3.44 ng/mL, 27.1 units/mL, 197.6 ng/mL, and 140 ng/mL, respectively (Figs. 1-4). The salivary concentrations of all six tumor markers were higher in cancer patients compared with controls. The salivary levels in cancer patients of CA125, TPS, Cyfra 21-1, CA19-9, CEA, and SCC were higher by 4.2 ($P = 0.0041$), 3.9 ($P = 0.0026$), 3.8 ($P = 0.0126$), 2.9 (not significant), 1.6 (not significant), and 1.4 (not significant) times, respectively, compared with controls (Figs. 1-4). According to the matrix correlation analysis done, a cross-talking among a few of these salivary markers was noted (i.e., a simultaneous increase of different markers in same patients). The correlation rate for Cyfra 21-1 and CA125 was 0.60 and for Cyfra 21-1 and TPS it was 0.90, whereas the correlation rate for Cyfra 21-1 and CEA was 0.48 and for Cyfra 21-1 and SCC it was 0.45. The correlation rate for TPS and CEA was 0.70 and for TPS and CA125 it was 0.50.

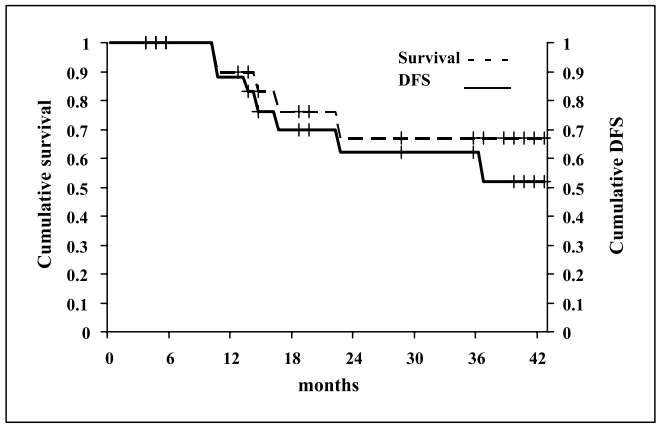

Fig. 5. Overall cumulative survival and DFS probabilities for the 21 tongue SCC patients.

Table 1. DFS probability rates by the pathologic grading (P = 0.05, log-rank test)

DFS grade	Patients (n = 21)	Range (mo)	Died patients	Recurrence patients	DFS (24 mo)	DFS (42 mo)
1	7	14-43		2	1.00	0.75
2	12	3-43	5		0.43	0.43
3	2	3-13		1	0.0	0.0

Concurrent analysis. The three salivary tumor markers that were found to be most substantially and significantly increased in the cancer patients were Cyfra 21-1, TPS, and CA125, which were all increased by ~400%. Therefore, we did an analysis in which all patients in whom any of these three markers was equal or above cutoff levels were defined as patients with disease and vice versa (^ disease, CA125 $\geq$ 1,823 and/or Cyfra $\geq$ 8.7 and/or TPS $\geq$ 253; ^ no disease, CA125 < 1,823 and Cyfra < 8.7 and TPS < 253). We used the analysis to define the various predictive values and found the followings values: (*a*) sensitivity factor [(10 / 14) × 100 = 71%], (*b*) specificity factor [(12 / 16) × 100 = 75%], (*c*) positive predictive value [(10 / 14) × 100 = 71%], (*d*) negative predictive value [(12 / 16) × 100 = 75%], (*e*) false-negative value [(4 / 14) × 100 = 28%], and (*f*) false-positive value [(4 / 16) × 100 = 25%].

Survival and DFS

Cumulative survival. The cumulative survival rate of all patients (n = 21) both at 24 and 36 months (2 and 3 years) was 67%. The cumulative survival rate of all patients (n = 21) at 42 months (3.5 years) was 67% (Fig. 5).

Cumulative DFS survival. The cumulative DFS rate of all patients (n = 21) at both 24 and 36 months (2 and 3 years) was 63%. The cumulative DFS rate of all patients (n = 21) at 42 months (3.5 years) was 52% (Fig. 5).

Cumulative DFS by grade. The cumulative DFS rate (75%) of patients with grade 1 at 42 months was higher than in patients with grade 2 (43%). The latter in turn was higher than in patients with grade 3 (0%). This difference was significant (P = 0.05; Table 1).

Cumulative DFS by stage. The cumulative DFS rate (78%) of patients with stages I and II at 42 months was significantly higher than in patients with stages III and IV (17%; P = 0.01; Tables 2 and 3).

Cumulative DFS by N. The cumulative DFS rate (73%) of patients with N = 0 at 42 months was higher than in patients with N = 1 (25%). This difference did not reach statistical significance (P = 0.14).

Cumulative DFS by site. The cumulative DFS rate (55%) of patients with base of tongue tumors (n = 3) at 42 months was higher than in patients with mobile tongue tumors (33%; n = 18). This difference did not reach statistical significance (P = 0.25).

Cumulative DFS by depth. The cumulative DFS rate (75%) of patients with depth ≤5 mm was higher than in patients with depth >5 mm (23%). This differences did not reach statistical significance (P = 0.13).

Cumulative DFS by diameter. The cumulative DFS rate of patients with diameter <2 cm at 42 months was found higher than in patients with diameter ≥2 cm (33%). This difference did not reach statistical significance (P = 0.12).

Cumulative DFS by sex, age, smoking, other malignancies, extensiveness, or salivary markers. The cumulative DFS rate (72%) of female patients at 42 months was found significantly higher than in male patients (23%; P = 0.04).

No significant correlations were found between the cumulative DFS values and any of the following variables: age, smoking habits, other malignancies, or an extension of the tumor beyond lingual margins. Positive correlations were not found between the cumulative DFS and any of the measured salivary marker levels.

Discussion

The most important result found is that several salivary tumor markers were found to be significantly increased (by 400%) in the saliva of oral (tongue) cancer patients. That is important with respect to both clinical- and pathogenesis-related aspects of oral cancer, and the various characteristics of this cancer show that, indeed, a representative group of tongue cancer patients was analyzed in the current study. Both the total and the DFS survival probabilities were found to be similar to those found in other studies, as was the important predictive roles that tumor staging, grading, N, and depth values have (6, 32). The fact that only a minority of the patients were smokers and that most of them were diagnosed at early stages is typical of previous Israeli tongue cancer series (32).

The increase, shown in salivary tumor markers of the cancer patients, may be used as a diagnostic tool especially when a concurrent analysis is done for several salivary markers. That suggests that this new diagnostic tool is of special importance for patient monitoring, as it is often very difficult to distinguish

Table 2. DFS probability rates by staging: I to IV (P = 0.0273, log-rank test)

DFS stage	Patients (n = 21)	Range (mo)	Died patients	Recurrence patients	DFS (24 mo)	DFS (42 mo)
I	7	5-43	1	1	0.75	0.75
II	8	3-43		1	0.83	0.83
III	4	10-36	3	1	0.25	0.0
IV	2	14-41	1		0.5	0.5

Table 3. DFS probability rates by staging: I and II (early) to III and IV (advanced; $P = 0.01$, log-rank test)

DFS stage	Patients ($n = 21$)	Range (mo)	Died patients	Recurrence patients	DFS (24 mo)	DFS (42 mo)
I-II	15	3-43	1	2	0.78	0.78
III-IV	6	10-41	4	1	0.33	0.17

clinically between a postoperative and/or irradiated scarred oral mucosa and a recurring cancer lesion. Accordingly, such an analysis might turn into a valuable diagnostic tool as it might save many unnecessary biopsies and hospital/outpatient clinical visits. Three of the markers analyzed (Cyfra 21-1, TPS, and CA125) were significantly increased (by 400%; $P \leq 0.01$), whereas the increase of the other three did not reach statistical significance, probably resulting from a relatively large variation of the increase in these salivary tumor markers. Such a variation probably explains why the increase found in salivary CEA (160%) did not reach statistical significance, an increase which is similar to that found by Negri et al., who reported of a 145% increase in the salivary CEA levels in OSCC patients (25, 33, 34). It is also important to note that the known increasing effect that smoking has on CEA levels could not account for the difference between the cancer patients and the controls, as the numbers of smokers among these two groups were similar. We further improved the value of the salivary analysis by doing a combined analysis of various markers as previously suggested for circulatory markers (22, 23). Indeed, it was shown that, when a concurrent analysis of the three significantly increased markers was done, the sensitivity, specificity, and negative and positive predictive values were in the range of 72% to75%, comparable with those obtained when circulatory markers were measured in the serum of OSCC patients.

In summary, the significant increase in salivary tumor markers (~4-fold) is encouraging in light of the many advantages of saliva measurement in comparison with serum analysis. The definitive diagnosis of OSCC is obviously based on a harvested biopsy, but it would be highly desirable and beneficial if salivary tumor marker analysis could be done on a routine basis between biopsies. The increase in salivary tumor markers may be used as a diagnostic tool, especially when a concurrent analysis for significantly increased markers is done. That is because salivary harvesting is noninvasive (35), which may make it an attractive, effective alternative to serum testing, and the possibility of developing home testing kits for such markers further facilitates it as a diagnostic aid, enabling patients to monitor their own health at home. That is especially important for people who live far from treatment centers and especially for those at high risk for developing oral cancer (such as patients with previous OSCC or with premalignant lesions). However, caution should be taken and note made of the fact that this suggested salivary analysis may be regarded as an aid and not as a replacement for other well-established diagnostic tools available for OSCC.

Acknowledgments

We thank Mrs. Shlomit Gan for her assistance in the preparation of this article.

References

1. Kantola S, Parikka M, Jokinen K, et al. Prognostic factors in tongue cancer—relative importance of demographic, clinical, and histopathological factors. Br J Cancer 2000;83:614–9.
2. Myers JN, Elkins T, Roberts D, Byers RM. Squamous cell carcinoma of the tongue in young adults: increasing incidence and factors that predict treatment outcomes. Otolaryngol Head Neck Surg 2000;122:44–51.
3. Alho OP, Kantola S, Pirkola U, Laara E, Jokinen K, Pukkala E. Cancer of the mobile tongue in Finland—increasing incidence, but improved survival. Acta Oncol 1999;38:1021.
4. Esser D, Anke S, Roessner A, Freigang B. Second carcinomas in cancers of the mouth cavity, pharynx, and larynx. Clinical, histopathologic, and cell kinetic findings. Laryngorhinootologie 2000;79:478–82.
5. Nagler RM, Braun J, Daitzman M, Laufer D. Spiral CT angiography—an alternative vascular evaluation technique for head and neck microvascular reconstruction: a preliminary experience. Plast Reconstr Surg 1997;100:1697–703.
6. Hinerman RW, Mendenhall WM, Morris CG, Amdur RJ, Werning JW, Villaret DB. Postoperative irradiation for squamous cell carcinoma of the oral cavity: 35-year experience. Head Neck 2004;26:984–94.
7. Yeole BB, Sankaranarayanan R, Sunny ML, Swaminathan R, Parkin DM. Survival from head and neck cancer in Mumbai (Bombay), India. Cancer 2000;89:437–44.
8. Ribeiro KC, Kowalski LP, Latorre MR. Impact of comorbidity, symptoms, and patients' characteristics on the prognosis of oral carcinomas. Arch Otolaryngol Head Neck Surg 2000;126:1079–85.
9. Sparano A, Weinstein G, Chalian A, Yodul M, Weber R. Multivariate predictors of occult neck metastasis in early oral tongue cancer. Otolaryngol Head Neck Surg 2004;131:472–6.
10. Yuen PW, Lam KY, Chan AC, Wei WI, Lam LK. Clinicopathological analysis of local spread of carcinoma of the tongue. Am J Surg 1998;175:242–4.
11. Muir C, Weiland L. Upper aerodigestive tract cancers. Cancer 1995;75:147–53.
12. Nagler RM, Barak M, Ben-Aryeh H, Peled M, Filatov M, Laufer D. Early diagnostic and treatment monitoring role of Cyfra 21-1 and TPS in oral squamous cell carcinoma. Cancer 1999;35:1018–25.
13. Bhatavdekar JM, Patel DD, Vora HH, Balar DB. Circulating markers and growth factors as prognosticators in men with advanced tongue cancer. Tumour Biol 1993;14:55–8.
14. Bhatavdekar JM, Patel DD, Vora HH, Balar DB. Circulating prolactin and TPS in monitoring the clinical course of male patients with metastatic tongue cancer: a preliminary study. Anticancer Res 1993;13:237–40.
15. Yen TC, Lin WY, Kao CH, Cheng KY, Wang SJ. A study of a new tumour marker, CYFRA 21-1, in squamous cell carcinoma of the head and neck, and comparison with squamous cell carcinoma antigen. Clin Otolaryngol 1998;23:82–6.
16. Hellner D, Klapdor R, Gundlach KH, Schmelze R. Results with the use of the SCC antigen in squamous cell carcinomas of the oral cavity [in German]. Dtsch Z Mund Kiefer Gesichtschir 1989;13:291–5.
17. Zoller J, Fiehn W, Mende U, Hotz G. The diagnostic value of the tumor markers CEA, "Ca 19-9," "Ca 125," "Ca15-3," and "SCC" for the detection of recurrent tumors in patients with tumors of the head and neck. Dtsch Z Mund Kiefer Gesichtschir 1990;14:254–9.
18. Zoller J. The value of "tumor markers" in the therapy and aftercare of carcinoma of the oral mucosa [review; in German]. Dtsch Zahn Mund Kieferheilkd Zentralbl 1992;80:351–7.
19. Kuo WR, Lee KW, Ho KY, Tsai SM, Chiang FY, Juan KH. Tissue polypeptide antigen, carcinoembryonic antigen, carbohydrate antigen, and CA125 levels as tumor markers in squamous cell carcinoma of the head and neck. Kaohsiung J Med Sci 1999;15:152–8.
20. Krimmel M, Hoffmann J, Krimmel C, Cornelius CP, Schwenzer N. Relevance of SCC-Ag, CEA, CA 19.9, and CA 125 for diagnosis and follow-up in oral cancer. J Craniomaxillofac Surg 1998;26:243–8.
21. Hoffmann J, Munz A, Krimmel M, Alfter G. Intraoperative and postoperative kinetics of serum tumor markers in patients with oral carcinoma. J Oral Maxillofac Surg 1998;56:1390–3.
22. Kurokawa H, Tsuru S, Okada M, Nakamura T, Kajiyama M. Evaluation of tumor markers in patients with squamous cell carcinoma in the oral cavity. Int J Oral Maxillofac Surg 1993;22:35–8.

23. Kurokawa H, Yamashita Y, Tokudome S, Kajiyama M. Combination assay for tumor markers in oral squamous cell carcinoma. J Oral Maxillofac Surg 1997;55:964–6.

24. Bigler LR, Streckfus CF, Copeland L, et al. The potential use of saliva to detect recurrence of disease in women with breast carcinoma. J Oral Pathol Med 2002;31:421–31.

25. Negri L, Pacchioni D, Calabrese F, Giacomasso S, Mastromatteo V, Fazio M. Serum and salivary CEA and GICA levels in oral cavity tumours. Int J Biol Markers 1988;3:107–12.

26. Airoldi M, Negri L, Pedani F, Gerbino G, Camoletto D, Piantino P. Serum and salivary CEA in benign and malignant lesions of the oral cavity. Boll Soc Ital Biol Sper 1984;60:865–70.

27. Hasnis E, Reznick AZ, Pollack S, Klein Y, Nagler RM. Synergistic effect of cigarette smoke and saliva on lymphocytes—the mediatory role of volatile aldehydes and redox active iron and the possible implications for oral cancer. Int J Biochem Cell Biol 2004;36:826–39.

28. Rydlander L, Ziegler E, Bergman T, et al. Molecular characterization of a tissue-polypeptide-specific-antigen epitope and its relationship to human cytokeratin 18. Eur J Biochem 1996;241:309–14.

29. Beretta E, Malesci A, Zerbi A, et al. Serum CA 19-9 in the postsurgical follow-up of patients with pancreatic cancer. Cancer 1987;60:2428–31.

30. Bast RC, Jr., Klug TL, St John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 1983;309:883–7.

31. Staab HJ, Anderer FA, Brummendorf T, Fischer R. Prognostic significance of preoperative carcinoembryonic antigen in stomach and colorectal cancer. Cancer Detect Prev 1983;6:149–53.

32. Nagler RM, Kerner H, Laufer D, Ben-Eliezer S, Minkov I, Ben-Itzhak O. Squamous cell carcinoma of the tongue: the prevalence and prognostic roles of p53, Bcl-2, c-erbB-2, and apoptotic rate as related to clinical and pathological characteristics in a retrospective study. Cancer Lett 2002;186:137–50.

33. Clasen B, Pere P, Senekowitsch R, Menz E. Squamous cell carcinoma-associated antigen (SCC) as a tumor marker in the initial diagnosis of carcinomas of the head and neck region. Results of a prospective study after 24 months [in German]. Laryngorhinootologie 1990;69:275–80.

34. Screm MC, Grandis S, Cartei G, Cattaruzzi E. Detection of five circulating antigens in patients with head and neck squamous cell carcinoma. Int J Biol Markers 1989;4:35–9.

35. Nagler RM, Hershkovich O, Lischinsky S, Diamond E, Reznick AZ. Saliva analysis in the clinical setting: revisiting an underused diagnostic tool. J Investig Med 2002;50:214–25.