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Serum Prognostic Markers in Head and Neck Cancer

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Abstract

Purpose: Recognized prognostic factors do not adequately predict outcomes of head and neck cancer (HNC) patients after their initial treatment. We identified from the literature nine potential serum prognostic markers and assessed whether they improve outcome prediction.

Experimental Design: A pretreatment serum sample was obtained from 527 of the 540 HNC patients who participated in a randomized controlled trial. During follow-up, 115 had a HNC recurrence, 110 had a second primary cancer (SPC), and 216 died. We measured nine potential serum prognostic markers: prolactin, soluble interleukin-2 (IL-2) receptor-α, vascular endothelial growth factor, IL-6, squamous cell carcinoma antigen, free β-human choriogonadotropin, insulin-like growth factor-I, insulin-like growth factor binding protein-3, and soluble epidermal growth factor receptor. Cox regression was used to identify a reference predictive model for (*a*) HNC recurrence, (*b*) SPC incidence, and (*c*) overall mortality. Each serum marker was added in turn to these reference models to determine by the likelihood ratio test whether it significantly improved outcome prediction. We controlled for the false discovery rate that results from multiple testing.

Results: IL-6 was the only serum marker that significantly improved outcome prediction. Higher levels of IL-6 were associated with a higher SPC incidence. The hazard ratio comparing the uppermost quartile to the lowest quartile of IL-6 was 2.68 (95% confidence interval, 1.49-4.08). IL-6 was also associated with SPC-specific mortality but not with mortality due to other causes. No marker improved outcome prediction for cancer recurrence or overall mortality.

Conclusions: IL-6 significantly improves outcome prediction for SPC in HNC patients. *Clin Cancer Res; 16(3); 1008–15. ©2010 AACR.*

Prognostic factors are variables that can account for some of the heterogeneity associated with the expected course and outcome of a disease (1). Established prognostic factors related to the tumor, the patient, or the environment are of primary importance in the management of cancer patients (2). There is a need to improve cancer outcome prediction beyond what is currently achieved by recognized clinical prognostic factors. Cancer prognostic marker studies abound in the medical literature, yet few markers have proved to be clinically useful (3). Statisticians have warned researchers about the pitfalls threatening the validity of prognostic marker studies (4–7). Potential prognostic markers that can be measured in the serum are particularly interesting because repeated measurements over the course of the disease can help additionally with treatment monitoring.

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Squamous cell carcinoma of the head and neck represents ~3% of all new cancers and 2% of all cancer deaths in the United States and Canada (8). Overall 5-year relative survival of patients with head and neck cancer (HNC) is just over 60% but depends on site and stage (8). Although patients diagnosed at stage I or II disease have a relatively good prognosis, ~5% will develop second primary cancers (SPC) annually mainly because of field cancerization (9). Potential prognostic serum markers have been investigated with the aim of improving outcome prediction for patients newly diagnosed with HNC. Studies have been conducted to assess whether pretreatment serum levels of tumor-associated antigen (10), proangiogenic (11–13) or proinflammatory (14–16) cytokines, growth factors (17), or hormones (18, 19) could help predict cancer recurrence, occurrence of SPC, or overall survival among HNC patients. These studies have yielded inconsistent results. Validation studies on independent data are typically missing.

In the present study, we systematically assessed the prognostic significance of nine previously identified serum markers in a large cohort of HNC patients. Using a standardized statistical approach, we tested whether these markers improved prediction beyond what is achieved by established prognostic factors for cancer recurrence, SPC incidence, or overall mortality.

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Translational Relevance

New prognostic markers of cancer outcome should improve outcome prediction beyond what is currently achieved by recognized predictors. In a large mature prospective cohort of patients treated for head and neck cancer, we followed a rigorous statistical approach to test the hypothesis that any of nine potential serum markers previously identified could improve outcome prediction for recurrence, second primary cancer, and overall survival. For eight potential prognostic markers, the study failed to confirm any of the associations previously reported with cancer outcomes. For pretreatment serum levels of interleukin-6 (IL-6), statistically significant associations were observed with both incidence and mortality from second primary cancers. This provides supportive evidence to the role of IL-6 in cancer outcome. As new molecules have been developed to target IL-6 and its receptors, our results could have practical applications to the practice of medicine because they will contribute to improve outcome prediction and management for cancer patients.

Materials and Methods

Study population. Between October 1, 1994 and June 6, 2000, 540 patients with stage I or II HNC were recruited in five radiation therapy centers in the province of Quebec, Canada, to participate in a randomized controlled trial (20). The institutional review board of each participating center approved the study protocol. All patients gave written informed consent before randomization. Patients were randomly assigned to receive a daily supplementation consisting of vitamin E (one capsule of 400 IU DL-α-tocopherol) and β-carotene (one capsule of 30 mg) or placebos during radiation therapy and for 3 years after radiation therapy ended. Concerns about adverse effects of β-carotene supplementation prompted the investigators to halt the use of β-carotene after the first 156 patients had been enrolled. The trial was continued with α-tocopherol alone. The main results of the trial concerning the supplementation were unexpected, showing an increased SPC incidence during the supplementation (20), a moderate reduction of acute adverse effects of radiation therapy (21), a tendency for a higher recurrence rate (21), and a decreased overall survival (22). Although the adverse effects of the supplementation on SPC and mortality were unanticipated at the time the trial was started, they are perfectly in line with what we presently know about β-carotene and α-tocopherol supplementation (23, 24).

Biochemical analysis of potential serum prognostic markers. Blood samples were collected at the time of randomization from 527 (97.6%) of the 540 trial participants. After processing, serum specimens were frozen and stored at −80°C. Based on a review of published studies reporting associations between serum markers and HNC outcomes, the following markers were selected for analysis: proclactin (18), soluble interleukin-2 (IL-2) receptor-α (s-IL-2R; ref. 14), vascular endothelial growth factor (VEGF; ref. 11), IL-6 (16), squamous cell carcinoma antigen (SCC Ag; ref. 10), free human choriogonadotropin β subunit (free β-hCG; ref. 19), insulin-like growth factor-I (IGF-I; ref. 17), and IGF-binding protein-3 (IGFBP-3; ref. 17). In addition, serum soluble epidermal growth factor receptor (EGFR) was also considered because both EGFR overexpression by head and neck tumors and EGFR-targeted therapy are associated with HNC outcome (25, 26).

All serologic experiments used thawed serum samples. Recommendations by the respective manufacturers were followed unless specified below with appropriate internal controls to assess the precision of the assays and maintain consistencies between batches. IGF-I, IGFBP-3, s-IL-2R, IL-6, prolactin, and free β-hCG were measured on an Immulite analyzer with chemiluminescent immunometric assays (Siemens Diagnostics). The s-IL-2R assay detects the soluble form of the IL-2 receptor (α-chain), which is about 10 kb smaller than the membrane-bound cell receptor. Release of the soluble form parallels the expression of the receptor on the cell surface. The free β-hCG method was modified by adding manually a sample of 80 μL in addition to the 20 μL sampling by the instrument. Results were thus divided by five to compensate for the increased volume. This modification increased sensitivity 5-fold and allowed detection of free β-hCG in most patients tested. The analytic sensitivity improved to 0.008 μg/L without affecting the linearity of the assay for the concentration range of interest. This kit does not react with whole hCG dimers.

VEGF and EGFR were assayed in duplicate by manual enzyme-linked immunoassays. Discrepancies over 15% triggered a reassay of the particular sample. For VEGF, the antibodies react with VEGF165, the most prominent isoform of VEGF found in the circulation that contains 165 amino acids (Biosource International). However, the same antibodies also react equally with VEGF121 so that nearly all circulating forms are measured. The EGFR kit (Siemens Diagnostics) was used to detect the soluble extracellular domain of the EGFR (also known as HER-1). The circulating domain of EGFR is produced by proteolytic cleavage of the receptor or by alternative transcription of primary RNAs. SCC Ag measurements were done on an Abbott IMx automated analyzer with an immunofluorescent methodology (Abbott Laboratories). For three patients, the amount of serum available was insufficient to test all markers. For four participants, plasma was collected instead of serum, which prevented the measurement of IGF-I but not of any other marker. The interassay variability of the control samples had a coefficient of variation of below 6% for all assays except for VEGF (coefficient of variation, 8.6%) and free β-hCG (coefficient of variation, 12.2%).

Follow-up. Follow-up information was obtained by the collaborating radiation oncologists and the study nurses every 6 mo during the 3 y after the end of radiation

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therapy and then once a year until the end of the study. At each follow-up visit, the radiation oncologist assessed the recurrence of the initial tumor and the occurrence of any SPC. Active clinical follow-up ended on June 30, 2003. We used recognized criteria to distinguish SPCs from local or distant recurrences of the initial HNC (20). Record linkage with the Quebec mortality files was done using the unique Quebec health insurance identifier from enrollment until December 31, 2006, for all but 10 participants that did not consent to this record linkage. All death certificates were obtained from the Institut de la Statistique du Québec.

Statistical analyses. The objective of the study was to determine whether any of the nine potential serum prognostic markers significantly improved outcome prediction beyond what was accomplished by recognized clinical prognostic factors. To this aim, we followed the recommendations for the use of appropriate statistical methods in the analysis of potential prognostic markers (4, 7). Three separate outcomes were studied: cancer recurrence, SPC incidence, and overall mortality. For each of the two clinically documented events, HNC recurrence and SPC, follow-up time was calculated from the randomization until the event (cancer recurrence or SPC), death, or the date of last visit before June 30, 2003. For overall mortality, follow-up was counted from randomization until the date of last visit (for the 10 participants mentioned previously), death, or December 31, 2006. Multivariate regression models for time to event data were used to establish reference models for cancer recurrence, SPC incidence, or overall mortality, including as covariables all known predictors that were independently associated with these respective outcomes with P value of < 0.05 (27). All regression models were stratified according to randomization arm (i.e., supplements or placebos).

For each outcome, expanded models were built by adding in turn each of the nine serum markers to the reference model. Firstly, the functional form of the serum variable was determined using the cumulative martingale residuals. These residuals determined whether the original continuous variable was appropriate for analysis; otherwise, indicator variables for each of the higher quartiles were compared with the lowest quartile. We adopted this strategy to avoid selecting an optimal functional form or cutpoints after looking at the data (4, 5). Thus, for different outcome analyses, the functional form of various variables in the final analyses could be either continuous or using quartile-based indicators.

Secondly, the proportionality assumption of the model was examined by standardized Schoenfeld residuals and formally tested (28). Thirdly, the overall adequacy of the model was assessed by examining deviance residuals (29). Serum IGF-I and IGFBP-3 were included together in the regression models, and their interaction was tested. The interaction term was removed from the model when nonsignificant ($P \geq 0.05$). This strategy for the analysis of the IGF axis variables was selected because it allows more flexibility than the ratio IGF-I/IGFBP-3. Hazard ratios (HR) and their 95% confidence intervals (95% CI) obtained in the expanded models were used to describe the relationship between the marker and the outcome. For continuous variables, HRs were calculated for 1 SD increment. Fourthly, for each serum variable, the partial likelihood ratio test (with 1 degree of freedom for continuous variables and 3 degrees of freedom for indicator variables based on the quartiles) was used to determine whether adding this variable to the reference model significantly improved outcome prediction (4). Fifthly, to control the overall false discovery rate to 0.05, the method of Benjamini and Hochberg was used (30). Because for each outcome nine markers were studied, the lowest P value observed should be <0.0056 to be considered statistically significant.

In addition, multivariate exploratory analyses were also conducted to examine the relationships between the serum markers significantly associated with an outcome in the main analyses and cause-specific mortality (death either from the initial HNC, from second primaries, or from noncancer causes). Exploratory analyses were also conducted using similar multivariate regression models to examine the association between the nine markers and the three outcomes separately in each arm of the trial. All statistical tests were two-sided. The analyses were conducted using SAS 9.1 (SAS Institute) and R software (R Development Core Team; ref. 31).

Results

The baseline personal and clinical characteristics of study participants are listed in Table 1. Descriptive statistics for the nine serum markers are presented in Table 2. After a median follow-up of 4.3 years, a recurrence of the initial cancer was observed among 115 participants. The median duration of follow-up for SPCs was 4.4 years, and 110 SPC were diagnosed. A total of 216 deaths occurred during a median follow-up of 8.1 years. The established predictor variables independently associated ($P < 0.05$) with each outcome were included in the respective reference models (Table 3). These variables were cancer stage and site for recurrence; age, smoking, and body mass index (BMI) for SPC; and age, cancer stage and site, smoking, BMI, alcohol intake, Karnofsky performance status, and Charlson comorbidity index for overall mortality. Treatment arm in the randomized trial was always controlled in the models by stratification.

None of the nine markers was associated ($P < 0.05$) with recurrence of the initial cancer (Table 4). IL-6 was the only marker associated ($P < 0.05$) with the incidence of SPCs (Table 5). Compared with patients with IL-6 in the lowest quartile, those in the uppermost quartile had a HR of 2.68 (95% CI, 1.49-4.08), whereas the middle quartiles had intermediary HRs of 1.58 (95% CI, 0.86-2.91) and 1.46 (95% CI, 0.78-2.73). The P value associated with the partial likelihood ratio test for 3 degrees of freedom was 0.0050, which remained statistically significant after controlling for the false-positive rate to 0.05 by the method of Benjamini and Hochberg. The mean baseline serum IL-6 level was 3.9 ng/L among the 407 patients who remained

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Table 1. Baseline personal and clinical characteristics of the 527 trial participants with pretreatment serum samples

Age, y [mean (SD)]	62.4 (9.8)
Male sex [*n* (%)]	415 (79)
Schooling: primary school only [*n* (%)]	225 (43)
Married [*n* (%)]	390 (74)
No. drinks per day over previous 10 y [mean (SD)]	2.11 (3.6)
Smoking during previous year [*n* (%)]	334 (63)
Family income <30,000$/y [*n* (%)]	327 (62)
Body mass index, kg/m^2 [mean (SD)]	26.1 (4.7)
Stage II HNC [*n* (%)]	202 (38)
Laryngeal cancer [*n* (%)]	440 (83)
Randomized to supplement arm of trial [*n* (%)]	268 (51)
Karnofsky performance status [mean (SD)]	96.5 (7.4)
Charlson comorbidity index [mean (SD)]	0.61 (0.95)

free of SPC during the follow-up, whereas it was, respectively, 7.4, 4.5, and 3.9 ng/L for those diagnosed with SPC during the first 2 years, between 2 and 4 years, and after 4 years of follow-up.

IL-6 was also associated ($P < 0.05$) with overall mortality (Table 6). Compared with patients with IL-6 in the lowest quartile, those in the uppermost quartile had a HR of 1.98 (95% CI, 1.29-3.04). This association was no longer considered statistically significant after controlling for the false discovery rate because the *P* value from the partial likelihood ratio test ($P = 0.0067$) was greater than the cutoff (0.0056). VEGF was also associated with overall mortality ($P = 0.05$, HR, 1.13; 95% CI, 1.00-1.28) but failed to reach statistical significance level after controlling for the false discovery rate.

We further explored the relationships between pretreatment serum IL-6 and HNC outcomes by considering cause-specific mortality. During the follow-up period, 61, 79, and 76 of the 527 patients died, respectively, from the initial HNC, from a second malignancy, and from noncancer causes. IL-6 was not associated with death from the initial cancer ($P = 0.36$) nor with death from noncancer causes ($P = 0.49$). In contrast, there was a strong association ($P < 0.0001$) with death from second cancers. Compared with patients with IL-6 in the lowest quartile, those in the uppermost quartile had a HR of 4.55 (95% CI, 2.01-10.29).

The associations between serum markers and HNC outcomes were very similar in the two arms of the trial. In particular for IL-6, there was no association with cancer recurrence in either arm whereas positive associations were observed with SPC and mortality in both arms. Compared with patients with IL-6 in the lowest quartile, those in the uppermost quartile had HRs for SPC, respectively, of 2.41 (95% CI, 1.22-4.73) in the supplement arm and of 4.40 (95% CI, 1.24-15.62) in the placebo arm. Similarly, compared with patients with IL-6 in the lowest quartile, those in the uppermost quartile had HRs for overall mortality, respectively, of 1.67 (95% CI, 0.96-2.94) in the supplement arm and of 2.52 (95% CI, 1.23-5.16) in the placebo arm.

Discussion

There are two main results in the present study. First, pretreatment serum levels of IL-6 are associated with both SPC incidence and mortality from SPC. Second, the study failed to confirm any of the associations previously reported between serum levels of the other potential prognostic markers and disease outcome among HNC patients. Although we examined markers that had been previously studied among HNC patients by other investigators, we did not exactly replicate their selection and analysis procedures. Our study cannot therefore be judged as an exact validation study. Although our results should be considered exploratory until confirmation occurs from an independent study using the same statistical approach on a similar patient population, our study results do suggest that these other eight markers are not robust predictors across different disease stages or sites.

There was an excellent consistency of the associations within the study population. The positive associations between serum IL-6 and SPC and mortality were even stronger in the placebo arm than in the supplement arm. Patients assigned to receive placebo in the trial are comparable with an observational cohort, which can be validly compared with other prospective cohorts of patients. Although everyone was treated with radiation, we cannot assume that the associations are related only to radiation sensitivity. They could also reflect general prognostic markers.

Several factors could explain why the previously reported associations were not observed in our study. False positive associations could have resulted from not taking into account multiple comparisons or from selecting optimal cutoffs (4, 5). The objective of our study, to test

Table 2. Baseline serum levels of the nine potential prognostic markers among the trial participants

Marker	Unit	*n**	Mean	SD	Q1	Median	Q3
EGFR	μg/L	525	54.3	7.8	49	54	59
Free β-hCG	μg/L	526	0.013	0.019	0.004	0.008	0.014
IGF-I	μg/L	520	152.6	55.8	112	143	190
IGFBP-3	mg/L	527	4.01	1.09	3.2	4.1	4.7
s-IL-2R	ku/L	527	554	239	394	511	643
IL-6	ng/L	527	4.10	5.09	2.2	3.1	4.4
Prolactin	μg/L	527	6.63	5.84	4.0	5.6	7.6
SCC Ag	μg/L	526	1.06	0.85	0.7	0.9	1.2
VEGF	ng/L	524	474	314	253	395	608

Abbreviations: Q1, first quartile; Q3, third quartile.
*Missing data for a few (0-7) patients depending on the marker.

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Table 3. Adjusted HRs and 95% CI associated in the respective reference models with significant known clinical predictors of (A) HNC recurrence, (B) SPC incidence, and (C) overall mortality. Trial arm assignment was controlled in the models by stratification

	P	Functional form	Comparison for HR	HR (95% CI)
(A) Cancer recurrence				
Cancer stage	<0.0001	Dichotomous	II versus I	2.45 (1.65–3.64)
Cancer site	0.047	Dichotomous	Larynx versus others	0.65 (0.42–0.99)
(B) SPC incidence				
Age	<0.0001	Quartiles	Quartile 2 versus quartile 1	3.68 (1.81–7.49)
			Quartile 3 versus quartile 1	4.27 (1.06–8.84)
			Quartile 4 versus quartile 1	5.37 (2.64–10.94)
Smoking*	0.040	Dichotomous	Yes versus no	1.57 (1.02–2.40)
BMI†	0.0033	Continuous	1 kg/m^2 increment	0.93 (0.89–0.98)
(C) Overall mortality				
Age	<0.0001	Continuous	1 year increment	1.07 (1.05–1.09)
Cancer stage	0.0025	Dichotomous	II versus I	1.57 (1.17–2.11)
Cancer site	0.0048	Dichotomous	Larynx versus others	0.61 (0.44–0.86)
Smoking*	0.0004	Dichotomous	Yes versus no	1.75 (1.29–2.38)
Alcohol†	0.0086	Continuous	1 drink/day increment	1.04 (1.01–1.08)
BMI	0.029	Continuous	1 kg/m^2 increment	0.97 (0.94–1.00)
Karnofsky score	0.0035	Continuous	1 unit increment	0.98 (0.96–0.99)
Charlson index	0.036	Continuous	1 unit increment	1.14 (1.01–1.30)

*Smoking during the year preceding radiation therapy.
†Number of alcoholic drinks per day during the 10 y preceding radiation therapy.

whether the potential prognostic markers significantly improve prediction beyond what is accomplished by recognized outcome predictors, was more stringent that those of previous studies which assessed whether the marker was associated with HNC outcome in survival analyses either by comparing crude Kaplan-Meier curves or by Cox multivariate models controlling for confounding. Most of the previously published studies have been conducted on small cohorts of patients where chance is more likely to contribute to spurious results. For example, four of the five studies (11, 12, 15, 32, 33) that investigated the relationship between VEGF and HNC outcomes and three

Table 4. Adjusted HRs and 95% CI for HNC recurrence associated with the nine potential prognostic markers

	P	Functional form	Comparison for HR	HR	95% CI
EGFR	0.98	Continuous	1 SD increment	1.00	0.83–1.21
Free β-hCG	0.10	Continuous	1 SD increment	0.75	0.54–1.06
IGF-I*	0.43	Continuous	1 SD increment	0.84	0.65–1.08
IGFBP-3*	0.81	Continuous	1 SD increment	1.16	0.91–1.46
s-IL-2R	0.17	Continuous	1 SD increment	0.86	0.70–1.07
IL-6	0.53	Continuous	1 SD increment	0.93	0.74–1.16
Prolactin	0.96	Continuous	1 SD increment	1.01	0.83–1.22
SCC Ag	0.11	Quartiles	Quartile 2 versus quartile 1	0.72	0.42–1.24
			Quartile 3 versus quartile 1	1.45	0.88–2.38
			Quartile 4 versus quartile 1	1.23	0.76–2.00
VEGF	0.65	Continuous	1 SD increment	0.96	0.79–1.16

*The statistical interaction between IGF-I and IGFBP-3 was not significant ($P = 0.69$). Both IGF-I and IGFBP-3 were included in the model.

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Table 5. Adjusted HRs and 95% CI for SPC associated with the nine potential prognostic markers

	P	Functional form	Comparison for HR	HR (95% CI)
EGFR	0.30	Continuous	1 SD increment	0.89 (0.72–1.11)
Free β-hCG	0.13	Continuous	1 SD increment	1.14 (0.96–1.36)
IGF-I*	0.29	Continuous	1 SD increment	0.96 (0.73–1.25)
IGFBP-3*	0.24	Continuous	1 SD increment	0.90 (0.68–1.19)
s-IL-2R	0.98	Continuous	1 SD increment	1.00 (0.81–1.23)
IL-6	0.0050	Quartiles	Quartile 2 versus quartile 1	1.58 (0.86–2.91)
			Quartile 3 versus quartile 1	1.46 (0.78–2.73)
			Quartile 4 versus quartile 1	2.68 (1.49–4.08)
Prolactin	0.070	Quartiles	Quartile 2 versus quartile 1	0.91 (0.50–1.69)
			Quartile 3 versus quartile 1	1.71 (1.00–2.90)
			Quartile 4 versus quartile 1	1.47 (0.84–2.58)
SCC Ag	0.11	Continuous	1 SD increment	1.12 (0.98–1.29)
VEGF	0.07	Continuous	1 SD increment	1.16 (0.99–1.35)

*The statistical interaction between IGF-I and IGFBP-3 was not significant ($P = 0.92$). Both IGF-I and IGFBP-3 were included in the model.

of the five studies (12, 14–16, 32) that examined IL-6 had fewer than 35 patients. The associations between serum markers and HNC outcomes could be specific to certain cancer sites not well represented in our study population. For example, the association between serum prolactin and survival was reported for tongue cancer (18, 34) but not for other HNC sites (35). The associations between serum markers and HNC outcomes could be stronger or only present among patients with advanced stages. In fact, the great majority of the previous investigations have been conducted on study populations made of either exclusively (11, 12, 18) or predominantly (13, 15, 16) advanced stage disease. Our study population is comparable with that of Wu et al. (17, 36), because both studies comprised only patients with stage I or II cancers and predominantly of the larynx. Such populations are ideally designed for chemoprevention trials and for the assessment of risk factors for SPC (20, 36). Wu et al. reported that elevated levels of IGF-I and both low and high serum concentrations of IGFBP-3 were associated with significantly higher odds ratios for SPC. In these analyses, optimal cutoff values were used after a recursive partitioning procedure on the same data set (5). In our study, neither IGF-I nor IGFBP-3 was associated with SPC incidence.

Our results comparing pretreatment serum levels of IL-6 with HNC outcomes deserve further consideration. In our study, the mean IL-6 concentration was 4.1 ng/L, which is

Table 6. Adjusted HRs and 95% CI for overall mortality associated with the nine potential prognostic markers

	P	Functional form	Comparison for HR	HR (95% CI)
EGFR	0.28	Quartiles	Quartile 2 versus quartile 1	0.79 (0.56–1.13)
			Quartile 3 versus quartile 1	0.70 (0.47–1.04)
			Quartile 4 versus quartile 1	0.76 (0.50–1.15)
Free β-hCG	0.073	Continuous	1 SD increment	1.12 (0.99–1.27)
IGF-I*	0.73	Continuous	1 SD increment	0.93 (0.77–1.13)
IGFBP-3*	0.95	Continuous	1 SD increment	1.07 (0.88–1.30)
s-IL-2R	0.36	Continuous	1 SD increment	1.07 (0.93–1.23)
IL-6	0.0067	Quartiles	Quartile 2 versus quartile 1	1.38 (0.89–2.13)
			Quartile 3 versus quartile 1	1.20 (0.77–1.89)
			Quartile 4 versus quartile 1	1.98 (1.29–3.04)
Prolactin	0.73	Continuous	1 SD increment	1.02 (0.91–1.14)
SCC Ag	0.74	Continuous	1 SD increment	0.98 (0.86–1.12)
VEGF	0.05	Continuous	1 SD increment	1.13 (1.00–1.28)

*The statistical interaction between IGF-I and IGFBP-3 was not significant ($P = 0.13$). Both IGF-I and IGFBP-3 were included in the model.

lower than levels observed in the two largest previous studies (14, 16). However, the reference range suggested by the kit manufacturer is 0 to 3.4 ng/L, and 40.6% of patients in our study had values exceeding 3.4 ng/L. In the study by Tartour et al. (14), a cutoff of 20 ng/L was selected to dichotomize the continuous IL-6 original variable, whereas in the study by Duffy et al. (16), a log transformation was used. These differences, along with differences in HNC site and stage distribution, render a direct comparison of our results with those from these two studies difficult. In the Tartour et al. study (14), both locoregional control and 24-month overall survival were lower among patients with higher IL-6 levels but the differences were not statistically significant. Duffy et al. followed for a median duration of 2.14 years a cohort of 444 HNC patients and specifically assessed the relationships between pretreatment IL-6 and recurrence and overall survival (16). The patients in the Duffy study had poorer outcomes than those in our trial because 85% of their study participants had stage III or IV whereas our trial was restricted to stage I or II. Laryngeal cancer, which has a relatively good prognosis compared with other HNC sites, affected >80% of the patients enrolled in our trial and only 26% of the patients in the Duffy study. These differences in site and stage between the two study populations could explain some of the differences in the results. In multivariate Cox models evaluating a log-transformed IL-6 variable, higher IL-6 levels were positively associated with recurrence ($P = 0.002$) and death ($P = 0.03$) in the Duffy study. In our study, we also observed a positive association between higher IL-6 levels and overall mortality. The associated P value ($P = 0.0067$) was lower than in the previous study, but after controlling for the false positive rate associated with the simultaneous assessment of nine markers, the association was no longer considered as statistically significant. Furthermore, the exploratory analyses indicated that the association of IL-6 with overall mortality was mainly attributable to deaths from SPCs. This confirms the very strong association observed between pretreatment IL-6 and SPC incidence in our study. We cannot compare our results concerning the association between IL-6 and SPC with those from the Duffy study because they did not report this outcome.

This is to our knowledge the first report of an association between serum IL-6 levels and SPC incidence among HNC patients. SPCs occurring among patients treated for HNC develop primarily in the lungs and in the head and neck sites (20, 36). Most SPCs are smoking-associated cancers, and the high incidence of SPC among HNC patients is attributed to field cancerization, which places them at a much higher risk of new cancers than the general population (9). A recent systematic review identified 36 studies investigating the association between circulating levels of IL-6 and HNC or lung cancer (37). All but one compared circulating levels between patients with prevalent cancer and controls. In this setting, high IL-6 levels could as well be a cause or a consequence of the tumor. Only one cohort study assessed prospectively the relationship between pre-diagnostic circulating IL-6 and lung cancer incidence (38). A higher IL-6 concentration was associated with a higher but not statistically significant risk of lung cancer. In contrast, we found that IL-6 is associated with SPC incidence even after adjusting for multiple confounders. In our study, baseline serum IL-6 level was particularly elevated for patients who were diagnosed with SPC within the first 2 years of follow-up. This elevation could reflect the inflammatory process associated with either the later stages of carcinogenesis or the presence of undetected cancers (39, 40). Elevated pretreatment IL-6 levels may reflect yet undetected secondary smoking-related lung cancer, its precursor lesions, or the effect of field cancerization. In our study population, IL-6 was on average slightly higher among smokers (4.3 ng/L) than among nonsmokers (3.8 ng/L).

IL-6 is a multifunctional cytokine playing an important role in immune response and inflammation. IL-6 is a regulator of the acute-phase response by which the organism responds to homeostasis disturbance caused by infection, injury, neoplastic growth, or immunologic disorders (41, 42). It has been suggested that IL-6 is involved in the host inflammatory reactions associated with cancer growth (39, 40). There is suggestive evidence that IL-6 could play a role in tumorigenesis, but a clear understanding of the mechanisms of action is currently lacking (43). Beyond its circulating levels, there is presently an enormous scientific interest in the genes, receptors, and biological pathways involved with IL-6 (44). New molecules have been developed to target IL-6 and its receptors, which may in the future help in the treatment of cancer patients.

In conclusion, our study indicates that pretreatment serum IL-6 levels among patients with localized HNC can improve outcome prediction for the incidence of SPCs. This result adds to the current evidence that IL-6 could provide a valuable prognostic marker for HNC patients. Additional studies are warranted to confirm the prognostic significance of IL-6 to improve our understanding of the biological mechanisms involved and to assess the clinical utility of IL-6 measurement at the time of diagnosis. In the long term, our results could have clinical and prognostic relevance for the management of patients with HNC and the early detection of new cancers.

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No potential conflicts of interest were disclosed.

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