

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Possible role for interleukins as biomarkers for mortality and recurrence in oral cancer.**

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1509031> since 2023-08-04T16:32:03Z

*Published version:*

DOI:10.5301/jbm.5000142

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This is the author's final version of the contribution published as:

Arduino PG;Menegatti E;Cappello N;Martina E;Gardino N;Tanteri C;Cavallo F;Scully C;Broccoletti R. Possible role for interleukins as biomarkers for mortality and recurrence in oral cancer.. THE INTERNATIONAL JOURNAL OF BIOLOGICAL MARKERS. 30 (2) pp: 262-266.  
DOI: 10.5301/jbm.5000142

The publisher's version is available at:

<http://www.biological-markers.com/attach/26A09154-61D2-44D5-86DB-E8C4CB3CF93F/50852821-225F-4A6A-ABF7-8B379C0A03DE>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/1509031>

# The International Journal of Biological Markers

## Possible role for interleukins in mortality and recurrence in oral cancer.

--Manuscript Draft--

<b>Manuscript Number:</b>	JBM-D-14-00064R1
<b>Full Title:</b>	Possible role for interleukins in mortality and recurrence in oral cancer.
<b>Short Title:</b>	IL-6 and IL-8 in oral cancer.
<b>Article Type:</b>	Short communication
<b>Section/Category:</b>	Cancer Epidemiology Biomarkers
<b>Keywords:</b>	Oral cancer, Prognostic markers, Interleukins, Recurrence, Survival.
<b>Corresponding Author:</b>	Paolo G. Arduino, DDS, MSc University of Turin Turin, ITALY
<b>Corresponding Author's Institution:</b>	University of Turin
<b>First Author:</b>	Paolo G. Arduino, DDS, MSc
<b>Order of Authors:</b>	Paolo G. Arduino, DDS, MSc Eugenio Martina Elisa Menegatti Nazario Cappello Nicolò Gardino Carlotta Tanteri Franco Cavallo Crispian Scully Roberto Broccoletti
<b>Manuscript Region of Origin:</b>	ITALY
<b>Abstract:</b>	<p><b>Objectives:</b> Salivary and serum levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) have previously been studied in oral cancer with conflicting results.</p> <p><b>Methods:</b> We designed a controlled study to assess the correlation between pre-treatment salivary and serum levels of IL-6 and IL-8, and all-cause survival and cancer recurrence in oral cancer patients.</p> <p><b>Results:</b> Fifty-two oral cancer patients and 52 healthy control cases were selected. In univariate analysis, salivary IL-6 and IL-8 seemed to be more expressed in cases (<math>p &lt; .001</math> and <math>p = .010</math> respectively). Multivariate analysis showed that higher pre-treatment saliva IL-6 levels were significantly associated with better survival (HR, 8.62; 95% CI, 1.21-62.50, <math>p = .031</math>).</p> <p><b>Conclusions:</b> To date, this is the largest prospective controlled study that analyses the pre-treatment salivary and serum levels of IL-6 and IL-8 in oral cancer patients, suggesting salivary IL-6 as possible prognostic biomarkers, but further validation on a larger sample is still necessary.</p>
<b>Additional Information:</b>	
<b>Question</b>	<b>Response</b>
Please supply the Corresponding Author's full contact information: First Name, Last Name, Institution, Address, Zip code, City, State, Country, Tel/fax/mobile numbers	"First Name: Paolo Last Name: Arduino Institution: University of Turin Address: Via Nizza 230 Zip code: 10100 City: Turin State: Country: Italy

	Tel/fax/mobile: +00390116331522
Please confirm that the manuscript has been seen and approved by all authors	Yes, I confirm on behalf of all authors that that the manuscript has been seen and approved by all authors
Please confirm that the manuscript is not under consideration for publication elsewhere in a similar form, in any language, except in abstract form	I confirm that the manuscript is not under consideration for publication elsewhere in a similar form, in any language, except in abstract form
Has your study received financial support?	No grants or funding have been received for this study
Financial/proprietary interest - Do you or your co-authors have any financial interest related to this study? Financial interest is defined as any financial gain or expectancy of financial gain brought to the Author or to his/her family; ownership of stock options in a manufacturing company; involvement in any for-profit or not-for-profit corporation where the Author or his/her family is a director or recipient of a grant, including consultant and travel aid.	None of the authors has financial interest related to this study to disclose
Meeting presentation. Have the data been presented at a scientific meeting?	This article was not previously presented at a meeting
INFORMED CONSENT: if the manuscript reports results of experimental investigation on human subjects, please confirm:	Informed consent was obtained by all participants
DECLARATION OF HELSINKI - if the manuscript reports results of experimental investigation on human subjects, please confirm:	The study was in adherence with the Declaration of Helsinki
IC -IRB Approval - In case of manuscripts reporting the results of experimental investigation on human subjects, human derived materials, or human medical records state that the study was performed with informed consent and following all the guidelines for experimental investigation in human subjects required by the Institutional Review Board (IRB)/Ethics Committee of which all authors are affiliated.	Institutional Committee or Institutional Review Board has approved the study
Please add the date of approval by IRB or IC as follow-up to "IC -IRB Approval - In case of manuscripts reporting the results of experimental investigation on human subjects, human derived materials, or human medical records state that the study was performed with informed consent and following all the guidelines for experimental investigation in human subjects required by the Institutional Review Board (IRB)/Ethics Committee of which all authors are affiliated." "	Sep 01, 2007

If the manuscript include animal experimentation, please state the adherence to the NIH Guide for the Care and Use of Laboratory Animals or equivalent	The manuscript does not report the results of an animal experimentation
Clinical Trials Registration. For articles reporting on a clinical trial the Journal requires its prospective registration.	This article does not report a Clinical Trial
We have used the following Reporting and Study design guidelines:	No guidelines available for the subject concerned in this manuscript
Are you submitting your paper on behalf of an Organization or Scientific Society?	No. This is an independent submission.
Please confirm that the response to reviewers includes the original comments, the authors' reply and it indicates where changes have been made in the manuscript (page and line number)	I confirm that Reviewers' comments have been added together with authors replies in the space provided
<b>Response to Reviewers:</b>	<p>Dear editor and reviewer,</p> <p>Ref.: Ms. No. JBM-D-14-00064 Possible role for interleukins in mortality and recurrence in oral cancer. The International Journal of Biological Markers</p> <p>Thank you for your decision and comments regarding the above manuscript.</p> <p>In order to expedite the processing of the revised manuscript, we have attempted to answer the reviewers' comment in a point-by-point manner. The attached highlights the changes in yellow.</p> <p>#Reviewer 1:</p> <ul style="list-style-type: none"> <li>- As required primary and secondary endpoints have been better described at the beginning of the M&amp;M section.</li> <li>- Some lines about the sample size have been added, as required, when discussing about statistical methods.</li> <li>- As suggested, in the Discussion section the weaknesses and strengths of the study have been discussed; moreover, the potential clinical applications of the achieved results have been better commented.</li> </ul> <p>We now hope that our revisions will be acceptable.</p> <p>Yours sincerely, Paolo Giacomo Arduino DDS, MSc (Oral Med) Department of Surgical Sciences University of Turin, Turin, Italy</p>

## **Possible role for interleukins in mortality and recurrence in oral cancer.**

Paolo G. Arduino<sup>1</sup>, Elisa Menegatti<sup>2</sup>, Nazario Cappello<sup>3</sup>, Eugenio Martina<sup>1</sup>, Nicolò Gardino<sup>1</sup>, Carlotta Tanteri<sup>1</sup>, Franco Cavallo<sup>3</sup>, Crispian Scully<sup>4</sup>, Roberto Broccoletti<sup>a</sup>.

<sup>1</sup>Department of Surgical Sciences, University of Turin, Turin, Italy;

<sup>2</sup>Department of Clinical and Biological Science, University of Turin, Turin, Italy;

<sup>3</sup>Department of Public Health and Paediatric Sciences, Medical Statistics Unit, University of Turin, Turin, Italy

<sup>4</sup>UCL, London, UK

Short title: IL-6 and IL-8 in oral cancer.

Computer system: Mac OS X, Version 10.8.4; Word-processor: Microsoft ®

Word 2011 for Mac, Version 14.4.5.

CORRESPONDING AUTHOR:

Dr. Paolo G. Arduino

Department of Surgical Sciences, CIR – Dental School

Via Nizza 230, 10126 Turin, Italy; phone: + 390116631522.

E-mail: paologiacomo.arduino@unito.it

**ABSTRACT**

**Objectives:** Salivary and serum levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) have previously been studied in oral cancer with conflicting results.

**Methods:** We designed a controlled study to assess the correlation between pre-treatment salivary and serum levels of IL-6 and IL-8, and all-cause survival and cancer recurrence in oral cancer patients.

**Results:** Fifty-two oral cancer patients and 52 healthy control cases were selected. In univariate analysis, salivary IL-6 and IL-8 seemed to be more expressed in cases ( $p<.001$  and  $p=.010$  respectively). Multivariate analysis showed that higher pre-treatment saliva IL-6 levels were significantly associated with better survival (HR, 8.62; 95% CI, 1.21-62.50,  $p=.031$ ).

**Conclusions:** To date, this is the largest prospective controlled study that analyses the pre-treatment salivary and serum levels of IL-6 and IL-8 in oral cancer patients, suggesting salivary IL-6 as possible prognostic biomarkers, but further validation on a larger sample is still necessary.

**Key words:** Oral cancer, Prognostic markers, Interleukins, Recurrence, and Survival.

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most frequent oral cancer, accounting for more than 90% of cases worldwide. Despite substantial improvements in diagnosis and therapy, OSCC still has a low estimated 5-year overall survival rate of 50% even in the United States and Western Europe (1, 2). Knowledge of specific prognostic and predictive factors could be therefore crucial in determining appropriate therapy.

Many different biological and molecular factors have been proposed as prognostic factors in OSCC, but they are far removed from a real impact on routine clinical care (3). Cytokines play an important role in the initiation and maintenance of inflammatory and immune responses as well as intercellular cross-talking (4). Interleukin-6 (IL-6) is a multifunctional cytokine synthesized in response to defined stimuli by a variety of different cells (5). Interleukin-8 (IL-8) belongs to a super family of chemokines that has chemotactic activity for neutrophils, eosinophils, basophils, monocytes, mast cells, dendritic cells, natural killer (NK) cells, and lymphocytes [6]. Salivary and serum levels of IL-6 and IL-8 have been studied in head and neck cancer but with conflicting results, inadequate follow-up and on different types of tumours (7-10).

Therefore, we designed a study to assess the correlation between pre-treatment salivary and serum levels of IL-6 and IL-8, and all-cause survival and cancer recurrence in OSCC patients.

## METHODS

This was a prospective cohort study: the primary explanatory variable was pre-treatment salivary and serum IL-6 and IL-8 levels; control variables were

age, sex, smoking, tumour site and stage, and different treatment modalities.

The secondary outcome variables were tumour recurrence and all-cause survival.

#### *Patients' selection and control variables*

Consecutive Caucasian patients, attending the Oral Medicine Section of the Department of Surgical Sciences, University of Turin, between January and September 2008, were enrolled. Local ethical committee approval was obtained before the trial started and all subjects gave written informed consent.

Newly diagnosed patients with biopsy proven OSCC were approached for participation in this study.

Excluded were those 1) <18 years of age, 2) pregnant or breast feeding, 3) or with mental health issues, 4) with diagnosis of oral lichen planus, 4) or with clinical diagnosis of periodontitis. A group of control subjects, unrelated to the cases, were recruited from the population attending the University Hospital of Turin. All controls presented with no oral lesions.

Demographic information, smoking, alcohol consumption, tumour site, T classification and neck nodes involvement, treatment, outcome and survival rate (at December 31<sup>st</sup>, 2012) were recorded. Tumour grade (well, moderately or poorly differentiated) (11), was given by two different pathologists. C-reactive protein was also assessed.

#### *Saliva and serum collection*

Subjects were asked to refrain from eating, drinking, or oral hygiene for at least one hour prior to unstimulated whole saliva collection; they rinsed out their mouths with tap-water and then waited at least 5 min before expectorating into a 50 cc Falcon tube. The tube was centrifuged at 3500 *rpm* (2600g) for 15 min at 4 °C (Hermle bench centrifuge Z300, Labnet International, Woodbridge, NJ 07095); the supernatant obtained was immediately transferred to a new tube and frozen at -80 °C. Serum samples were collected at the same time, by centrifuging whole venous blood at 3000 *rpm* (1000g) for 10 minutes at 15°C (Hermle bench centrifuge Z300, Labnet International, Woodbridge, NJ 07095). The aliquots were stored at -80°C.

### *Interleukins*

Samples were tested using ThermoScientific/Pierce Biothecnology (Rockford, IL, USA) ELISA kits for recombinant human IL-6 and IL-8. Cytokine protein levels were determined by solid phase quantitative sandwich immunoassay technique. The minimum detectable doses were 1.0 pg/mL for IL-6 and 2.0 pg/mL for IL-8. All testing was performed in duplicate per manufacturer instructions and means were used for data reporting.

### *Statistical analysis*

A study with 52 case patients and 52 control subjects has been planned, in order to detect at least a true difference in the mean response of experimental and control subjects of  $\pm 0.52$  standard deviation with an 80% power. The Type I error probability associated with this test of the null hypothesis that the population means of the experimental and control groups are equal is 0.05. If

we suppose a Bonferroni setting, the detection of true difference in the mean response is  $\pm 1.71$  standard deviation.

Means and frequency distributions were examined for all variables. Describing general information, data was reported as means and standard deviation ( $\pm$  SD). Associations between salivary and serum interleukins and control variables were assessed by using analysis of variance. For univariate and multivariate analyses, interleukins were treated as a continuous variable after log transformation [ $\ln(\text{IL} + 2)$ ]. Univariate and multivariate Cox proportional hazards models were used to study the relation between salivary and serum IL-6 and IL-8, control variables, and time to recurrence and death. Time to recurrence and death was measured from the pre-treatment blood draw date. Crude and adjusted ORs were computed by using logistic regression analyses to assess the independent effects of control and pre-treatment cases' variables. Statistical analyses were performed using the IBM-SPSS statistics software version 20.

## RESULTS

Cases and controls were comparable with regards to age, gender and risk factors.

Fifty-two cases of OSCC were selected, of whom 32 were male and 20 female (mean age  $66.19 \pm 14.97$  years). The follow-up period ranged from 12 to 60 months (median of 39.4 months). During the period considered, 10 (19.2%) patients died because of the original OSCC. There was no recurrence in 29 (55.8%) patients, whilst 13 (25%) developed a recurrence. No second primary tumours were detected.

*Control variables and association with pre-treatment levels of interleukins*

Table 1 shows the differences between cases and controls in interleukin expression. In univariate analysis, salivary IL-6 and IL-8 seemed to be more expressed in cases ( $p < .001$  and  $p = .010$  respectively) than in controls; similar results were also present in the multivariate analysis, also adding a statistical larger expression of serum IL-8 ( $p = .006$ ) for pre-treatment cases.

Older persons had higher salivary and serum IL-6 levels than did younger persons, the converse from salivary and serum IL-8 expression, but neither data had statistical significance. Smokers were more likely to have higher salivary IL-6 levels than non-smokers. Those with larger cancers had higher levels of salivary IL-6 ( $p = .011$ ). There was no association between salivary and serum interleukins and gender, cancer site, node association, serological inflammation data grading or treatment type; there was only a larger expression of salivary IL-6 and salivary IL-8 and lymph node involvement but again without statistical significance.

*IL-6, IL-8 and control variables as independent predictors of recurrence and survival*

The results of the univariate and multivariate Cox proportional hazards regression models for recurrence and survival are detailed in Table 2. Serum IL-6 was not included in the final model, because the levels were too low to perform the analysis.

Univariate and multivariate analyses did not show that higher pre-treatment salivary or serum levels of interleukins were associated with recurrence.

Analyses showed that patients who underwent radiotherapy remained significantly susceptible to recurrence, even after controlling for the other variables.

Univariate analysis showed that larger cancers, nodal involvement and chemotherapy were significantly negatively associated with survival. Both analyses showed that higher pre-treatment saliva IL-6 levels were significantly associated with survival ( $p < .05$ ). Older age, nodal involvement, cancer site and having chemotherapy were also independently associated with poorer survival in the multivariate analysis, whereas gender was not associated with survival.

## **DISCUSSION**

OSCC is one of the most complex malignancies to control, and only a small improvement has been seen in the survival rate over the last decades; the problem of metastasis, recurrences and difficulties in reconstruction comprise the main obstacles in treatment; therefore, early detection would be a powerful approach in improving quality of life, and outcome and survival rate (12).

The use of saliva as a diagnostic bio-fluid has long been recognised, and it has many advantages over other specimens like blood, exfoliated cells and urine (13). To date, some studies based on salivary and serum cytokine expression profile have endeavoured to provide information relevant to survival rate and recurrence of OSCC, but several critical issues still need to be addressed before these findings can be fully utilized in patient care. One of the biggest problems is that samples have included not only oral cancer but

also oropharyngeal carcinomas - which generally have a different aetiopathogenesis (human papillomavirus) and a better prognosis.

In the present study, we set out to identify whether IL-6 and IL-8 could potentially be useful as biomarkers for *oral* cancers. Interleukin 6 was detected at higher concentrations in the saliva of OSCC cases compared to controls, and IL-8 was detected at higher concentrations both in the saliva and in the serum of patients with OSCC. Moreover, salivary IL-6 levels have been demonstrated to be useful also as independent prognostic factors for OSCC survival rate.

Data confirmed the hypothesis that saliva should be preferred to blood in this type of research, having the potential to serve as non-invasive, widely accessible screening tools that do not rely on the localization of a lesion for diagnosis; collection is also inexpensive and can be performed in any setting. If becoming a routine, saliva, as a diagnostic tool for OSCC, would also comprise a suitable instrument for population screening, monitoring of patients at high risk of recurrences, and subsequently for improving survival rates (12).

Serum measurements of cytokines may reflect the systemic response to tumour development and progression, and may not be simply indicative of local biochemical events at the tumour site. Moreover, several studies have outlined the importance of autocrine IL-6 signalling in different type of carcinoma - such as lung, breast, skin, and in head and neck squamous cell carcinoma cell lines, where findings indicate that endogenous IL-6 could play an important role in the growth of tumour and exert its action by an autocrine growth mechanism. Therefore, it seems that variation of salivary levels of IL-6

and IL-8 could represent disease markers that could possibly reflect accurately tumour status (13-15).

Higher levels of the salivary cytokines (above all IL-6) may have significant usefulness as surrogate biomarkers in the efficiency of chemoprevention therapy (7). Although, the present data is from a relatively small sample size (even if the biggest in oral cancer ever reported), the results are significant enough to merit further investigation.

Determination of the exact mechanisms leading to increased IL-6 and IL-8 secretion in recurrent and advanced disease might also lead to new therapeutic interventions or monitoring.

To the best of our knowledge, this is the largest prospective control study that analyses the pre-treatment salivary and serum levels of IL-6 and IL-8 in OSCC patients, confirming earlier results [16], that have suggested salivary IL-6 and IL-8 as diagnostic biomarkers for OSSC, but further validation on a larger sample is still necessary in a multicentre population study.

**Conflict of interest**

The authors declare that they have no conflict of interest.

## References

1. Moore SR, Johnson NW, Pierce AM, Wilson DF. The epidemiology of mouth cancer: a review of global incidence. *Oral Dis* 2000; 6: 65-74.
2. Bagan J, Sarrion G, Jimenez Y. Oral cancer: clinical features. *Oral Oncol* 2010; 46: 414-7.
3. Arduino PG, Carrozzo M, Chiecchio A, et al. Clinical and histopathologic independent prognostic factors in oral squamous cell carcinoma: a retrospective study of 334 cases. *J Oral Maxillofac Surg* 2008; 66: 1570-9.
4. Nibali L, Fedele S, D'Aiuto F, Donos N. Interleukin-6 in oral diseases: a review. *Oral Dis* 2012; 18: 236-43.
5. Kishimoto T, Akira S, Narazaki M, Taga T. Interleukin-6 family of cytokines and Gp130. *Blood* 1995; 86: 1243-54.
6. Yuan A, Chen JJ, Yao PL, Yang PC. The role of interleukin-8 in cancer cells and microenvironment interaction. *Front Biosci* 2006; 10: 853-65.
7. Rhodus NL, Ho V, Miller CS, Myers S, Ondrey F. NF-kappaB dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. *Cancer Detect Prev* 2005; 29: 42-5.
8. Sato J, Goto J, Murata T, Kitamori S, Yamazaki Y, Satoh A, Kitagawa Y. Changes in saliva interleukin-6 levels in patients with oral squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 110: 330-6.

9. Duffy SA, Taylor JM, Terrell JE, et al. Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *Cancer* 2008; 113: 750-7.
10. Jablonska E, Piotrowski L, Grabowska Z. Serum Levels of IL-1b, IL-6, TNF-a, sTNF-RI and CRP in Patients with Oral Cavity Cancer. *Pathol Oncol Res* 1997; 3: 126-9.
11. Pindborg JJ, Reichart PA, Smith CJ, van der Waal I: World Health Organisation histological typing of cancer and precancer of the oral mucosa (ed 2). New York, Springer, 1997.
12. Osman TA, Costea DE, Johannessen AC. The use of salivary cytokines as a screening tool for oral squamous cell carcinoma: a review of the literature. *J Oral Maxillofac Pathol* 2012; 16: 256-61.
13. Gao SP, Mark KG, Leslie K, et al. Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. *J Clin Invest* 2007; 117: 3846-56.
14. Lederle W, Depner S, Schnur S, et al. IL-6 promotes malignant growth of skin SCCs by regulating a network of autocrine and paracrine cytokines. *Int J Cancer* 2011; 128: 2803-14.
15. Bran G, Götte K, Riedel K, Hörmann K, Riedel F. IL-6 antisense-mediated growth inhibition in a head and neck squamous cell carcinoma cell line. *In Vivo* 2011; 25: 579-84.
16. Osman TA, Costea DE, Johannessen AC. The use of salivary cytokines as a screening tool for oral squamous cell carcinoma: a review of the literature. *J Oral Maxillofac Pathol* 2012; 16: 256-61

**TABLE I - UNIVARIATE AND MULTIVARIATE ANALYSES OF CONTROL AND PRE-TREATMENT CASES' VARIABLES**

	<i>Variable</i>	$\beta$	<i>SE</i>	<i>OR</i>	<i>CI</i>	<i>P</i>
<u><i>Univariate</i></u>						
	Saliva IL6	1.83	.483	6.25	2.42-16.11	<b>&lt;.001</b>
	Saliva IL8	1.17	.455	3.24	1.33-7.91	<b>.010</b>
	Serum IL8	0.77	.445	2.16	0.90-5.17	.083
	Gender	.10	.452	1.11	0.45-2.68	.821
	Smoking	.71	.495	2.04	0.77-5.37	.151
	Age	.69	.450	2.00	0.83-4.83	.123
<u><i>Multivariate</i></u>						
	Saliva IL6	1.76	.641	5.83	1.66-20.47	<b>.006</b>
	Saliva IL8	1.37	.640	3.95	1.12-13.84	<b>.032</b>
	Serum IL8	1.88	.681	6.56	1.73-24.93	<b>.006</b>
	Gender	.64	.602	1.89	0.58-6.16	.288
	Smoking	.32	.628	1.38	0.40-4.74	.604
	Age	.54	.606	1.71	0.52-5.62	.375

Legend:

SE: Standard Error

OR: Odds ratio

CI: 95% Confidence Interval

**TABLE II - UNIVARIATE AND MULTIVARIATE RATIOS OF IL6 AND IL8 FROM COX PROPORTIONAL HAZARD REGRESSION MODELS FOR RECURRENCE AND DEATH EVENTS**

**RECURRENCE EVENTS**

Variable Name	U.M.R. <sup>§</sup>			M.M.R. <sup>§</sup>		
	Hazard Ratio	95% CI	P	Hazard Ratio	95% CI	P
Log IL6_saliva*	1.77	0.52-5.99	.355	3.94	0.48-32.07	.200
Log IL8_serum*	1.66	0.49-5.55	.412	2.54	0.68-14.70	.162
Log IL8_saliva*	1.98	0.57-6.85	.278	1.52	0.25-5.88	.648
Age (>68 vs ≤68)	2.42	0.70-8.33	.160	3.77	0.96-14.70	.056
Gender (female vs male)	1.10	0.33-3.66	.869	1.19	0.23-6.10	.827
pT (pT1 vs pT2 plus pT4)	2.79	0.82-9.51	.101	1.63	0.19-13.69	.650
N (N0 vs N1 plus N2)	1.36	0.41-4.54	.618	1.73	0.44-2.13	.434
G (G2 plus G3 vs G1)	1.51	0.40-5.75	.541	1.60	0.25-10.28	.620
Site (others vs tongue plus floor)	1.63	0.47-5.63	.440	1.39	0.33-5.89	.650
Surgery (yes vs no)	1.46	0.18-11.49	.718	1.71	0.04-76.92	.783
Chemiotherapy (yes vs no)	3.14	0.87-11.23	0.78	6.06	0.43-10.75	.349
Radiotherapy (yes vs no)	4.83	1.21-19.23	<b>.026</b>	5.61	1.33-23.81	<b>.018</b>

Variable Name	U.M.R. <sup>§</sup>			M.M.R. <sup>§</sup>		
	Hazard Ratio	95% CI	P	Hazard Ratio	95% CI	P
Log IL6_saliva*	4.83	1.29-29.31	<b>.026</b>	8.62	1.21-62.50	<b>.031</b>
Log IL8_serum*	1.03	0.26-4.12	.968	1.69	0-13-2.08	.687
Log IL8_saliva*	1.74	0.42-7.30	.448	1.16	0.09-14.70	.909
Age (>68 vs ≤ 68)	1.09	0.69-1.69	.130	12.34	1.23-125	<b>.033</b>
Gender (male vs female)	1.86	0.37-9.24	.446	2.58	0.41-16.02	.307
pT (pT2 plus pT4 vs pT1)	1.03	1.26-83.33	<b>.029</b>	1.602	0.02-90.68	.819
N (N1 plus N2 vs N0)	5.31	1.07-26.31	<b>.041</b>	20.05	1.63-245.89	<b>.019</b>
G (G2 plus G3 vs G1)	3.31	0.40-27.03	.263	3.14	0.17-57.17	.440
Site (others vs tongue plus floor)	2.61	0.52-12.94	.240	6.95	1.05-45.93	<b>.044</b>
Surgery (no vs yes)	1.94	0.30-9.65	0.416	1.85	0.17-20.09	.612
Chemiotherapy (yes vs no)	5.81	1.38-24.39	<b>.016</b>	8.13	1.38-47.6	<b>.020</b>
Radiotherapy (yes vs no)	4.83	0.97-2.38	.054	2.17	0.08-58.82	.642

**DEATH EVENTS**

§U.M.R.=univariate model results

§M.M.R.=multivariate model results

\* over the mediana vs under the mediana

**This document is signed by the Corresponding author on behalf of all co-authors.**

The Author who signs this Agreement has full right, power and authority to enter into this Agreement on behalf of all co-Authors. All the listed Authors have agreed to be listed Authors, and have granted the signing Author the Authority to enter into this agreement on their behalf.

<b>Corresponding Author Date</b>	

<b>Manuscript title</b>	

**Transfer of copyright**

In consideration of the action of the Publisher in editing, publishing in print and hosting the final version on a dedicated server the present submission (manuscript, tables, and figures) after acceptance, the Author(s) transfer to **Wichtig Editore Srl** the full copyright of the Manuscript as follows:

- a) to publish, reproduce, distribute, display and store the manuscript in all forms, formats and media whether now known or developed (including print, digital and electronic forms) throughout the world,
- b) to translate the manuscript into other languages, create adaptations, summaries or extracts or other derivative works based on the manuscript in print, digital or electronic formats
- c) to license others to do any or all of the above.

**Retained rights**

Provided the source is fully quoted at all times, Authors are hereby granted the right to:

- a) reproduce the manuscript in whole or in part in any printed book or thesis of which they are the author(s).
- b) They and any academic institution where they work at the time may reproduce the Manuscript in a reasonable number of copies for the purpose of course teaching. This does not apply if a commercial charge is made for the training course.
- c) To post a copy of the Manuscript as accepted for publication after peer review (in Word or Text format) on the Authors' website provided that they also link to the article to the Journal's web site.
- d) To reuse figures or tables created by them and contained in the Manuscript in other works created by them.

**Compliance with funding bodies**

Authors retain the right to provide a copy of the final peer-reviewed manuscript to the NIH or other funding agencies upon acceptance for publication, in accordance to the organization's policy. It is the Authors' responsibility to take the necessary actions to achieve compliance.

**Authorship responsibility**

Each author certifies that he/she has participated sufficiently in the preparation of this work to take public responsibility for it.

**Each Author warrants and certifies that:**

- a) this Work does not violate any trademark registrations nor the right of privacy of any person, contains no libelous, obscene, or other unlawful matter, and does not infringe upon the statutory or common law copyright or any other right of any person or party.
- b) Each Author warrants that this Manuscript is original, has not been published elsewhere in any form, electronic or in print – even in part – and is not being considered for publication elsewhere while under consideration for this publication. The Authors certify also that the data on which the manuscript is based will be supplied upon request of the reviewers if required.
- c) Each author further warrants that he or she has obtained, prior to submission, written releases from patients whose names or photographs are submitted as part of the Work and that they will be supplied upon request.
- d) Nothing in the Contribution is obscene, defamatory, libelous, violates any right of privacy or infringes any intellectual property rights (including copyright, patent or trademark) or any other human, personal or other rights of any person or entity or is otherwise unlawful.
- e) Nothing in the Contribution infringes any duty of confidentiality which any of the Authors may owe to anyone else or violates any contract, express or implied, of any of the Authors, and all of the institutions in which work recorded in the Contribution was carried out have authorised its publication.
- f) If the Contribution includes materials of others excerpts (text, figures, tables, or illustrations), the Authors have obtained written permission from the copyright holder prior to submission to enable them to grant the rights contained herein. Copies of all such permissions are attached to the submission and credit to the original publication has been properly acknowledged in the manuscript.
- g) Authors hereby consent to the inclusion of electronic links from the Contribution to third-party material wherever it may be located.

If the manuscript will for any reason not be published, the Publisher will give prompt notice to the Author and this agreement shall terminate. Neither the Author(s) nor the Publisher shall be under any further liability or obligation



**Signed for and on behalf of the Authors:** Paolo G. Arduino

Dear editors,

We are proud to submit to your prestigious journal this brief report detailing salivary and serum levels of interleukin-6 (IL-6) and interleukin-8 (IL-8) in patients with “pure” oral cancer. We designed a prospective controlled study to assess the correlation between pre-treatment salivary and serum levels of IL-6 and IL-8, and all-cause survival and cancer recurrence in oral cancer patients.

Fifty-two oral cancer patients and 52 healthy control cases were selected. In univariate analysis, salivary IL-6 and IL-8 seemed to be more expressed in cases ( $p < .001$  and  $p = .010$  respectively). Multivariate analysis showed that higher pre-treatment saliva IL-6 levels were significantly associated with better survival (HR, 8.62; 95% CI, 1.21-62.50,  $p = .031$ ). To date, this is the largest prospective controlled study that analyses the pre-treatment salivary and serum levels of IL-6 and IL-8 in oral cancer patients, suggesting salivary IL-6 as possible prognostic biomarkers, but further validation on a larger sample is still necessary.

To the best of our knowledge, similar findings have never been documented.

Regards

Dr. Paolo Giacomo Arduino and co-authors.