# Immunohistochemical Localization of Interleukin-10 in Human Oral and Pharyngeal Carcinomas

Stephen W. Chandler, MD; Christopher H. Rassekh, MD; Susan M. Rodman, EdD; Barbara S. Ducatman, MD

Objectives/Hypothesis: Interleukin-10 (IL-10) is an immunosuppressive cytokine with numerous, welldescribed effects on the human cellular and humoral immune response. The oncogenic potential of IL-10 has been previously investigated in bronchogenic carcinoma, nasopharyngeal carcinoma, Waldeyer's ring carcinoma, and serum supernatants of patients with squamous cell carcinoma of the head and neck (SCCHN). The purpose of the study was to determine the prevalence and cellular localization of IL-10 in human SC-CHN. Study Design: Immunohistochemistry of archival tissues. Methods: Paraffin-embedded archival tissues were retrospectively obtained from 98 patients with oral and pharyngeal squamous cell carcinoma. Using a standard immunohistochemical technique, these specimens were stained with a polyclonal antibody to IL-10. Results: Using these methods, we found specific localization of antigenic IL-10 to individual tumor cells in 65% of tumors studied. Intensity of staining was significantly, but inversely, related to tumor grade and N stage; there also existed a significant staining predisposition for oral cavity lesions when samples from this site were compared with tissues derived from elsewhere in the pharynx. Furthermore, IL-10 was not localized to normal epithelial keratinocytes or inflammatory cells at the level of sensitivity achieved by the immunohistochemical methods used in the study. Conclusions: The findings demonstrate that IL-10 can be specifically localized to human oral and pharyngeal cancer cells. These data also suggest an inverse association for both tumor grade and N stage with specific tumor marker staining. Future studies should investigate the role of this cytokine in the pathogenesis of human SCCHN. Key

Laryngoscope 112: May 2002

Words: Interleukin-10, cytokine, Waldeyer's ring, squamous cell cancer, head and neck malignancy, immunohistochemistry, oral cavity, oropharynx, nasopharynx. Laryngoscope, 112:808–815, 2002

### INTRODUCTION

Human squamous cell carcinoma of the head and neck (SCCHN) displays a variable clinical course. Oral and pharyngeal cancers represent a significant subset of these malignancies with more than 30,000 new cases diagnosed per year resulting in nearly 8000 deaths in the United States.<sup>1</sup> Variable tumor behavior may reflect individual tumor attributes such as the ability to avoid containment or detection by host immune responses. Studies indicate that several factors may lead to a relatively immunosuppressed state in patients with SCCHN.<sup>2</sup> Such immunosuppression may exert its impact at either the local (tissue) level or systemically. Soluble factors such as cytokines may play a role in this phenomenon.

One immunomodulatory agent that has received attention in oncology research recently is the cytokine interleukin-10 (IL-10). This molecule was initially isolated by Mosmann et al.<sup>3</sup> in 1986 and Fiorentino et al.<sup>4</sup> in 1989. Since then, it has been shown to have immunosuppressive bioactivity against several aspects of the cellular immune system. It was originally identified as a product of CD4<sup>+</sup> T-lymphocytes. It is also thought to be secreted by a number of different cells including keratinocytes.<sup>5</sup>

We became interested in such a cytokine after the discovery that a subset of patients with aggressive nasopharyngeal carcinoma had dual infection with Epstein-Barr virus (EBV) and human papillomavirus (HPV).<sup>6</sup> These "dual" virus cases were associated with different histological and molecular changes than in the cases with EBV alone, and some of the HPV subtypes that were detected were unusual. We postulated that the EBV might be producing a factor that facilitated HPV infection. This theory led us to IL-10 because EBV produces a viral IL-10 with a high degree of homology to the human IL-10.<sup>7</sup> Nasopharyngeal carcinoma speci-

Presented at the Meeting of the Southern Section of the Triological Society, Marco Island, Florida, January 11, 2001.

 $<sup>\</sup>mbox{Dr.}$  Chandler is the recipient of the Lester Brown Award for Resident Research.

From the Departments of Otolaryngology—Head and Neck Surgery (s.w.c., c.h.R., s.M.R.) and Pathology (b.s.d.), West Virginia University, Morgantown, West Virginia, U.S.A.

Editor's Note: This Manuscript was accepted for publication December 7, 2001.

Send Correspondence to Christopher H. Rassekh, MD, Post Office Box 9200, Morgantown, WV 26506-9200, U.S.A. E-mail: crassekh@hsc.wvu.edu

mens were difficult to obtain in the United States; therefore, we chose to study Waldeyer's ring because these tumors are the most closely related anatomically and histologically and share some clinical features with nasopharyngeal carcinoma.

The current study was undertaken to confirm that human IL-10 is present in cancers of the oral cavity and pharynx and also to determine the cellular localization of the IL-10. In addition, we sought to determine whether immunohistochemical study alone would be an adequate methodology. Also, we hoped to determine whether expression of IL-10 was associated with any clinical, histological, or other molecular features of the tumor.

### MATERIALS AND METHODS

A retrospective review of charts and pathological information was performed from 1985 to 1999. This identified 98 patients with squamous cell carcinoma of the oral cavity and pharynx whose archival tissue from the primary site could be obtained. During this period, 146 patients were seen with oral and pharyngeal cancer at our institution. Patients in whom squamous cell carcinoma could not be confirmed were excluded as were patients with mixed histological findings. Control tissues included normal epithelium from the cancer patients and tissue from Waldeyer's ring that was not malignant (discarded tonsillectomy specimens). All human subject investigations were performed in accordance with the West Virginia University Institutional Review Board policies and were approved before the project. A review of medical records was performed to collect data regarding age, sex, disease stage (T and N) and grade (grade 1 = well differentiated, grade II = moderately differentiated, grade III = poorly differentiated), and site of the tumor. Polyclonal anti-human IL-10-specific antiserum was produced by immunization of rats with human recombinant IL-10 (subclone JES3-9D7, Pharmingen, San Diego, CA). The specificity of the antibodies had previously been confirmed by Western blot analysis by the manufacturer. Paraffin-embedded archival tissues were processed for immunohistochemical staining using standardized techniques. Briefly, 5-µm tissue sections were cut, dewaxed with xylene, and rehydrated through graded ethanol concentrations. Tissue nonspecific binding sites were blocked with bovine serum following microwave antigen retrieval. Sections were washed and incubated with optimal concentrations of rat polyclonal (subclone JES3-9D7, Pharmingen) antibodies to human IL-10 or the same subclass rat immunoglobulin G (IgG) (rat IgG1, Pharmingen). Tissues were washed and incubated for 60 minutes with secondary goat anti-rat biotinylated antibodies (Biogenex, San Ramon, CA), washed twice in TRIS buffered saline, and then stained. Diaminobenzidine (DAB) was used for chromogenic localization of the IL-10. Slides were counterstained with hematoxylin and photographed with the use of an Olympus, (Tokyo, Japan) BX40 photomicroscope. Immunohistochemistry

TABLE I. Grading of the Immunohistochemistry Results (intensity).
Grade 0+ = No immunoperoxidase staining noted
Grade 1+ = Light brown blush without specific intracytoplasmi granular staining
Grade 2+ = Granular Intracytoplasmic staining present
Crade 2 - Internet doop brown grapular staining of the coll

Grade 3+ = Intense, deep brown granular staining of the cell cytoplasm



Fig. 1. Low-power view (original magnification  $\times$ 10) of a poorly differentiated (grade III) carcinoma with intense interleukin-10 (IL-10) staining (grade 3+) of the tumor cells in the subepithelial layer. The noncancerous epithelium and lymphocytes adjacent to the tumor do not stain.

results were graded using standard pathology protocols for reporting of stain intensity and an estimate of the percentage of cells stained. Table I illustrates the grading system. Statistical analysis of the data was performed using logistic regression procedures within SAS (Cary, NC), version 6.12, for personal computers. The values obtained from this analysis represent likelihood ratios. Data were considered statistically significant if P values were less than .05.

### RESULTS

Tumor staging was as follows: 21.4%, T1; 22.4%, T2; 18.4%, T3; and 37.8%, T4. Of the patients, 51.5% had N0 necks, and of those with N-positive necks, the breakdown was as follows: 14.4%, N1; 28.9%, N2; and 5.2%, N3. Primary site sources of the tissue specimens for the patients were Waldeyer's ring (n = 46), oral cavity (n = 41), or elsewhere in the pharynx (n = 11). Grades were determined to be as follows: 37.8%, grade I; 38.8%, grade II; and 23.5%, grade III. Histological diagnosis of squamous cell carcinoma was confirmed in all 98 patients. Specimens



Fig. 2. High-power view (original magnification  $\times$ 40) of a carcinoma (moderately differentiated, grade II) demonstrating the stain in the tumor cells (grade 2+).

### Laryngoscope 112: May 2002

Chandler et al.: Oral and Pharyngeal Carcinomas

TABLE II.					
List of Specific Tumor Sites.					

Site	No. of Patients
Anterior ⅔ of tongue	7
Anterior FOM	6
Base of tongue	22
Border of tongue	3
Cheek mucosa	4
FOM NOS	6
Hard palate	3
Hypopharynx NOS	1
Lateral wall of oropharynx	2
Lower gum	2
Mouth NOS	1
Nasopharynx NOS	1
Overlapping lesion of hypopharynx	1
Overlapping lesion of nasopharynx	2
Overlapping lesion of oropharynx	1
Overlapping lesion of palate	1
Posterior wall nasopharynx	3
Posterior wall of oropharynx	3
Retromolar trigone	5
Soft palate	1
Tongue NOS	2
Tonsil NOS	19
Upper gum	1

FOM = floor of mouth; NOS = not otherwise specified.

from one patient were inadequate for staining in that insufficient malignant tumor for analysis was present in the immunoperoxidase reactions. Among the 97 evaluable patients, staining for IL-10 was detected in 65%. Definite staining was seen in the cytoplasm of tumor cells only. Most important, no cells other than tumor cells localized for Il-10 (Figs. 1 and 2). Normal tonsil did not stain with our technique, and noncancerous tissues from the cancer patients (adjacent to the tumor) also did not stain in any case. We graded the staining intensity as shown in Figure 3 and Table I. We found that staining was inversely proportional to differentiation; tumors that were less welldifferentiated stained less commonly (Fig. 4). Also, although the numbers of specimens are small and the tissues are archival, the study suggests that N0 necks are more likely to stain (Fig. 5). In addition, our study indicated that oral cavity (Fig. 6) is more likely to produce Il-10 than tumors of the pharynx (including Waldeyer's ring).

In the current series, 45 of the patients had a minimum follow-up of 2 years. There was a small difference in 2-year disease-free survival between the patients who had positive IL-10 staining (19 of 27 [70%]) and those who had negative IL-10 staining (10 of 18 [55%]); however, this did not achieve statistical significance (z = 1.0104, P = not significant). Only 15 patients had a minimum follow-up of 5 years, precluding analysis of that subset.

### DISCUSSION

The current study shows that IL-10 can be localized to the squamous cells in oral or pharyngeal carcinoma. It also demonstrates that immunohistochemical study is effective in demonstrating this in 65% of cases. These findings suggest that IL-10 may be associated with carcinogenesis. Prior studies have indicated that IL-10 may be important in several cancers.<sup>8-10</sup> Other studies have evaluated the possibility that IL-10, specifically, viral IL-10, may be important in nasopharyngeal carcinoma. For example, Yao et al.<sup>11</sup> performed a study using in situ hybridization, polymerase chain reaction, and immunohistochemical analysis evaluating the association of EBV and IL-10. They found that 29 of 41 cases expressed EBER-1 RNA by in situ hybridization. Of 29 cases that were EBV-positive, 19 expressed IL-10 in the tumor cells. Of 12 cases that were EBV-negative, 9 expressed IL-10 in the tumor cells. The researchers also found that the number of cytotoxic cells increased with EBV expression and decreased with IL-10 expression. Their investigation was not able to determine whether IL-10 was actually induced by EBV.

Another study suggested that IL-10 may play a significant role in bronchogenic carcinoma.<sup>12</sup> In head and neck cancer, there is a gap in the literature regarding localization of IL-10 in archival tissue. However, there are several interesting studies that should be mentioned. In 1997, Avradopoulos et al.<sup>13</sup> produced evidence suggesting that the mechanism of immunosuppression in patients with head and neck cancer was probably IL-10. This was accomplished using a phytohemagglutinin stimulation assay. The immunosuppressive effect was blocked by antibody to IL-10, but not by antibody to transforming growth factor beta  $(TGF-\beta)$ . Young et al.<sup>14</sup> examined SCCHN tissues and analyzed for immune mediators and the immune infiltrate. Their investigation found that TGF- $\beta$ , prostaglandin E-2, and IL-10 were all associated with reduced CD8<sup>+</sup> and CD4<sup>+</sup> cells, that granulocyte-macrophage colony-stimulating factor (GM-CSF) was associated with CD34<sup>+</sup> cells. and that metastatic cancers released higher levels of these mediators.



Fig. 3. Breakdown of number of patients by intensity of staining for IL-10 in their specimens.

#### Chandler et al.: Oral and Pharyngeal Carcinomas

opyright © The American Laryngological. Rhinological and Otological Society. Inc. Unauthorized reproduction of this article is prohibited

Patient         Last         Group         Site	List of Patients With Site, Stage, and IL-10 Staining Results as well as Outcome.								
1         3+         20         AWD         OC         Border of tangue         T.3         N0         M0           3         1+         10         DWD         HP         Posterior wall nasopharynx         T.4         N0         M0           4         2+         40         DWD         OC         Hard Palate         T.4         N0         M0           5         3+         10         DWD         VC         FOM NOS         T.4         N2         M0           6         2+         10         DWD         VC         FOM NOS         T.4         N0         M0           7         0         0         DWD         VC         Posterior wall of orpharynx         T.3         N0         M0           10         2+         10         DWD         W         Tonsil NOS         T.1         N.2         M0           11         2+         40         DWD         W         Tonsil NOS         T.1         N.2         M0           12         2+         40         DWD         WC         Anterior 56 of tongue         T.4         N3         M0           13         14         0         DWD         CC         Anter	Patient No.	Characteristic	Percent	Last Status	Group	Site	T Stage	N Stage	M Stage
2     0     0     NED     OC     Check mucosia     T.2     N0     M0       4     2+     40     DWD     OC     Hard Palate     T.4     N0     M0       5     3+     10     DND     W     Base of tongue     T.4     N.2     M0       6     2+     10     DWD     OC     FOM NOS     T.4     N.2     M0       7     0     0     DWD     OC     FOM NOS     T.4     N.2     M0       9     1+     20     DND     OC     Robistrow and orophanyms     T.3     N0     M0       10     2+     10     DWD     W     Tonsil NOS     T.4     N.2     M0       11     2+     40     DWD     W     Tonsil NOS     T.4     N.2     M0       12     2+     40     DWD     W     Tonsil NOS     T.4     N.2     M0       14     0     0     DWD     W     Corraphanyms NOS     T.1     N.2     M0       14     0     DWD     OC     Anterior 74 of tongue     T.4     N.3     M0       15     0     DWD     OC     Anterior 74 of tongue     T.4     N.3     M0 <td< td=""><td>1</td><td>3+</td><td>20</td><td>AWD</td><td>OC</td><td>Border of tongue</td><td>Т 3</td><td>N0</td><td>M 0</td></td<>	1	3+	20	AWD	OC	Border of tongue	Т 3	N0	M 0
3       1+       10       DWD       HP       Posterior wall nasopharynx       T.4       N.0       M.0         5       3+       10       DND       W       Base of tongue       T.2       N.1       M.0         6       2+       10       DWD       OC       FOM NOS       T.2       N.1       M.0         6       2+       10       DWD       OC       FOM NOS       T.3       N.0       M.0         7       0       0       DWD       OP       Posterior wall of oropharynx       T.3       N.0       M.0         9       1+       20       DWD       OP       Posterior wall of oropharynx       T.3       N.0       M.0         11       2+       10       DWD       W       Tonsit NOS       T.4       N.0       M.0         12       2+       40       DWD       W       Tonsit NOS       T.4       N.1       M.0         13       2+       40       DWD       W       Consit NOS       T.4       N.1       M.0         14       0       0       DWD       CC       Anteior 76 of tongue       T.1       N.2       M.0         15       0       0 <td>2</td> <td>0</td> <td>0</td> <td>NED</td> <td>OC</td> <td>Cheek mucosa</td> <td>T 2</td> <td>N0</td> <td>M 0</td>	2	0	0	NED	OC	Cheek mucosa	T 2	N0	M 0
4     2+     40     DWD     OC     Hard Palate     T.4     N.0     M.0       5     3+     10     DND     W     Base of tongue     T.2     N.1     M.0       6     2+     10     DWD     OC     FOM NOS     T.4     N.2     M.0       7     0     0     DWD     OP     Posterior valid of orpharynx     T.3     N.0     M.0       9     1+     20     DND     OC     Retronolar trigone     T.4     N.0     M.0       10     2+     10     DWD     W     Tonsil NOS     T.4     N.0     M.0       11     2+     40     DWD     W     Tonsil NOS     T.4     N.2     M.0       13     2+     40     DWD     W     Tonsil NOS     T.4     N.2     M.1       14     0     0     DWD     OC     Anterior % of tongue     T.4     N.2     M.1       15     0     0     DWD     OC     Anterior % of tongue     T.1     N.3     M.0       17     0     0     DWD     OC     Anterior % of tongue     T.1     N.2     M.0       16     1+     20     DWD     OC     Upper gum     T.4	3	1+	10	DWD	HP	Posterior wall nasopharynx	Т4	NO	M 0
5     3+     10     DND     W     Base of tongue     T.2     N 1     M 0       6     2+     10     DWD     CC     FOM NOS     T.4     N 2     M 0       7     0     0     DWD     OP     Posterior wall of oropharynx     T.3     N 0     M 0       8     0     0     DWD     OP     Posterior wall of oropharynx     T.3     N 0     M 0       10     2+     10     DWD     W     Tonsi NOS     T.4     N 0     M 0       11     2+     10     DWD     W     Tonsi NOS     T.4     N 0     M 0       12     2+     40     DWD     W     Tonsi NOS     T.4     N 0     M 0       13     2+     40     DWD     W     Ontarior 7 5 of tongue     T.4     N 2     M 1       14     0     0     DWD     CC     Anterior 7 5 of tongue     T.4     N 1     M 0       15     0     0     DWD     CC     Anterior 7 5 of tongue     T.1     N 2     M 0       18     2+     50     DWD     CC     Anterior 7 5 of tongue     T.1     N 0     M 0       22     3+     80     DWD     CC     An	4	2+	40	DWD	OC	Hard Palate	Τ4	NO	M 0
6     2+     10     DWD     OC     FOM NOS     T4     N 2     M 0       7     0     0     DWD     OP     Posterior wall of oropharynx     T3     N 0     M 0       9     1+     20     DND     OC     Retromolar trigone     T4     N 0     M 0       10     2+     10     DWD     W     Tonsil NOS     T3     N 0     M 0       11     2+     10     DWD     W     Tonsil NOS     T4     N 0     M 0       12     2+     40     DWD     W     Tonsil NOS     T1     N 2     M 0       13     2+     40     DWD     W     Tonsil NOS     T4     N 3     M 1       14     0     0     DWD     OC     Anterior % of tongue     T4     N 3     M 1       15     0     0     DWD     OC     Anterior % of tongue     T4     N 3     M 1       16     1+     20     DWD     OC     Anterior % of tongue     T1     N 6     M 0       10     0     DWD     OC     Bape of tongue     T4     N 3     M 0       21     0     0     DWD     OC     Anterior % of tongue     T1     N 6	5	3+	10	DND	W	Base of tongue	T 2	N 1	M 0
7         0         0         DWD         HP         Overlapping lesion of hypopharynx         T.3         N.2         M.0           8         0         0         DWD         OP         Posterior wall of oropharynx         T.3         N.0         M.0           9         1+         10         DWD         W         Tonsil NOS         T.4         N.0         M.0           11         2+         10         DWD         W         Tonsil NOS         T.4         N.0         M.0           12         2+         40         DWD         W         Tonsil NOS         T.1         N.2         M.0           13         2+         40         DWD         W         Coverlapping lesion of nasopharynx         T.1         N.3         M.0           14         0         0         DWD         CC         Anterior % of tongue         T.4         N.0         M.0           16         1+         20         DWD         CC         Border of tongue         T.1         N.0         M.0           17         0         0         DWD         CC         Border of tongue         T.1         N.0         M.0           18         2+         0	6	2+	10	DWD	OC	FOM NOS	Т4	N 2	M 0
8         0         0         DWD         OP         Posterior wall of oropharym.         T.3         N0         M.0           9         1+         20         DND         OC         Retromolar trigone         T.4         N0         M0           11         2+         10         DWD         W         Tonsil NOS         T.3         N0         M0           12         2+         40         DWD         W         Tonsil NOS         T.1         N.2         M1           13         2+         40         DWD         W         Tonsil NOS         T.1         N.2         M1           14         0         0         DWD         W         Overlapping lesion of nasopharym.         T.1         N.3         M1           15         0         0         DWD         W         Tonsil NOS         T.1         N.2         M0           16         1+         20         DWD         OC         Anterior 5/or fongue         T.1         N.2         M0           17         0         0         DWD         OC         Base of tongue         T.1         N.0         M0           21         0         ND         DWD         CC	7	0	0	DWD	HP	Overlapping lesion of hypopharynx	Т 3	N 2	M 0
9         1+         20         DND         OC         Retromolar trigone         T 4         N0         M 0           10         2+         10         DWD         W         Tonsil NOS         T 4         N0         M 0           11         2+         10         DWD         W         Tonsil NOS         T 4         N0         M 0           12         2+         40         DWD         W         Tonsil NOS         T 1         N 2         M 1           13         2+         40         DWD         W         Tonsil NOS         T 1         N 2         M 0           14         0         0         DWD         W         Coverlapping lesin of nasopharynx         T 1         N 3         M 0           16         1+         20         DWD         OC         Anteior 7% of tongue         T 1         N 2         M 0           17         0         0         DWD         OC         Anteior 7% of tongue         T 1         N 3         M 1           18         2+         50         DWD         OC         Anteior 7% of tongue         T 3         N 0         M 0           22         3+         80         DWD	8	0	0	DWD	OP	Posterior wall of oropharynx	Т 3	NO	M 0
10         2+         10         DWD         W         Tonsil NOS         T3         N0         M 0           11         2+         10         DWD         W         Tonsil NOS         T4         N0         M 0           13         2+         40         DWD         W         Base of tongue         T2         N 1         M 0           14         0         0         DWD         VC         Anterior % of tongue         T4         N 2         M 1           15         0         0         DWD         VC         Anterior % of tongue         T4         N 3         M 0           16         1+         20         DWD         VC         Anterior % of tongue         T4         N 3         M 1           17         0         0         DWD         OC         Upper gum         T4         N 0         M 0           18         2+         20         NED         OC         Anterior % of tongue         T1         N 0         M 0           21         0         0         DWD         OC         Anterior % of tongue         T3         N 0         M 0           22         3+         80         DWD         OC	9	1+	20	DND	OC	Retromolar trigone	Т4	NO	M 0
11       2+       10       DWD       W       Tonsil NOS       T 4       N0       M 0         12       2+       40       DWD       W       Base of tongue       T 2       N 1       M 0         14       0       0       DWD       W       Verdapring lesion of nasopharynx       T 4       N 2       M 1         15       0       0       DWD       VC       Anterior % of tongue       T 4       N 1       M 0         16       1+       20       DWD       CC       Anterior % of tongue       T 4       N 1       M 0         17       0       0       DWD       OC       Anterior % of tongue       T 1       N 0       M 0         19       3+       10       DWD       OC       Border of tongue       T 1       N 0       M 0         21       0       DWD       OP       Posterior wall of oropharynx       T 3       N 0       M 0         22       3+       80       DWD       V       Tonsil NOS       T 4       N 2       M 0         24       3+       40       DWD       CC       Lower gum       T 4       N 0       M 0         25       2+	10	2+	10	DWD	W	Tonsil NOS	Т 3	NO	M 0
12         2+         40         DWD         W         Base of tongue         T2         N         M         M           13         2+         40         DWD         C         Anterior % of tongue         T4         N         2         M           14         0         0         DWD         C         Anterior % of tongue         T4         N         2         M           15         0         0         DWD         W         Overlapping lesion of nasopharynx         T1         N         3         M         0           16         1+         20         DWD         C         Anterior Fond         T3         N         M         M           17         0         0         DWD         C         Upper gum         T4         N         M         M           18         2+         50         DWD         C         Border of tongue         T1         N         M	11	2+	10	DWD	W	Tonsil NOS	Т4	NO	M 0
13         2+         40         DWD         HP         Hypopharynx NOS         T1         N.2         M.0           14         0         0         DWD         OC         Anterior % of tongue         T.4         N.2         M.1           15         0         0         DWD         OC         Anterior FOM         T.4         N.1         M.0           16         1+         20         DWD         OC         Anterior FOM         T.4         N.1         M.0           17         0         0         DWD         W         Tonsil NOS         T.4         N.0         M.0           18         2+         50         DWD         OC         Border of tongue         T.1         N.2         M.0           20         2+         20         NED         OC         Anterior % of tongue         T.1         N.0         M.0           21         0         0         DWD         W         Tonsil NOS         T.3         N.0         M.0           22         3+         80         DWD         W         Base of tongue         T.4         N.0         M.0           24         3+         40         DWD         W         <	12	2+	40	DWD	W	Base of tongue	Τ2	N 1	MO
14         0         0         DWD         OC         Anterior % of fongue         T.4         N.2         M.1           15         0         0         DWD         W         Overlapping lesion of nasopharynx         T.1         N.3         M.0           16         1+         20         DWD         OC         Anterior FOM         T.4         N.1         M.0           17         0         0         DWD         W         Tonsil NOS         T.4         N.0         M.0           18         2+         50         DWD         OC         Anterior FOM         T.4         N.0         M.0           20         2+         20         NED         OC         Anterior % of tongue         T.1         N.0         M.0           21         0         0         DWD         VP         Posterior wall of oropharynx         T.3         N.0         M.0           22         3+         80         DWD         W         Tonsil NOS         T.3         N.0         M.0           23         1+         30         DWD         WC         Lower gum         T.4         N.2         M.0           24         3+         00         DWD	13	2+	40		HP	Hypopharynx NOS	T 1	N 2	MO
15         0         0         DWD         W         Overlap ping lesion of nasopharynx         T         1         N         M           16         1+         20         DWD         OC         Anterior FOM         T         4         N         M         M           17         0         0         DWD         OC         Upper gum         T         4         N         M         M           18         2+         50         DWD         OC         Upper gum         T         N         M         M           20         2+         20         NED         OC         Anterior % of tongue         T         N         M	14	0	0	סעים	00	Anterior $\frac{2}{2}$ of tongue	ТД	N 2	M 1
16         1+         20         DWD         VC         Anterior FOM         T4         N1         M0           17         0         0         DWD         W         Tonsil NOS         T4         N3         M1           18         2+         50         DWD         OC         Upper gum         T4         N3         M1           18         2+         50         DWD         OC         Upper gum         T4         N3         M1           19         3+         10         DWD         OC         Border of tongue         T1         N0         M0           21         0         0         DWD         OP         Posterior wall of oropharynx         T3         N0         M0           22         3+         80         DWD         W         Tonsil NOS         T3         N0         M0           23         1+         30         NED         OC         Retronolar trigone         T4         N1         N2         M0           24         3+         40         DWD         OC         Lower gum         T4         N1         M0           25         2+         30         DWD         PO         Deste	15	0	0	סעים	W	Overlapping lesion of pasopharypx	т 1	N 3	MO
17         0         0         DWD         VC         Finite NOW         Totali NOS         T4         N1         M1           18         2+         50         DWD         OC         Upper gum         T4         N0         M0           19         3+         10         DWD         OC         Border of tongue         T1         N2         M0           20         2+         20         NED         OC         Anterior % of tongue         T1         N0         M0           21         0         0         DWD         OP         Posterior wall of oropharynx         T3         N0         M0           22         3+         80         DWD         W         Tonsil NOS         T3         N0         M0           23         1+         30         NED         OC         Retromolar trigone         T3         N0         M0           24         3+         40         DWD         W         Base of tongue         T4         N1         M0           26         3+         60         DWD         HP         Posterior wall nasopharynx         T3         N0         M0           29         2+         30         DWD	16	1+	20	חשם	00	Anterior FOM	T 4	N 1	MO
In         Instant No.         InstantNo.         Instant No.         Ins	17	0	20		W		т л	NS	M 1
10         2+1         30         DWD         OC         Opparigned         14         No         Mo           19         3+         10         DWD         OC         Anterior % of tongue         T1         N0         M0           20         2+         20         NED         OC         Anterior % of tongue         T1         N0         M0           21         0         0         DWD         OP         Posterior wall of oropharynx         T3         N0         M0           22         3+         80         DWD         W         Tonsil NOS         T3         N0         M0           23         1+         30         NED         OC         Retromolar trigone         T4         N2         M0           24         3+         40         DWD         WD         Base of tongue         T4         N0         M0           25         2+         30         DWD         W         Base of tongue         T4         N1         M0           26         3+         60         DWD         WD         Posterior wall nasopharynx         T3         N0         M0           30         3+         50         DND         OC<	10	2+	50		00		т 4 Т 4	NO	MO
15       3-1       10       DWD       OC       Borter of tongue       11       11       N2       M 0         20       24       20       NED       OC       Anterior % of tongue       T1       N0       M 0         21       0       0       DWD       VP       Posterior wall of oropharynx       T3       N0       M 0         22       3+       80       DWD       W       Tonsil NOS       T3       N0       M 0         23       1+       30       NED       OC       Retromolar trigone       T3       N0       M 0         24       3+       40       DWD       OC       Lower gum       T4       N 2       M 0         25       2+       30       DWD       W       Base of tongue       T4       N 1       M 0         26       3+       60       DWD       HP       Posterior wall nasopharynx       T3       N0       M 0         28       0       0       DWD       OC       FOM NOS       T2       N 2       M 0         31       0       0       DWD       OC       FOM NOS       T4       N 2       M 0         32       1+ <t< td=""><td>10</td><td>2+</td><td>10</td><td></td><td>00</td><td>Porder of tongue</td><td>14 T4</td><td>NU O</td><td>MO</td></t<>	10	2+	10		00	Porder of tongue	14 T4	NU O	MO
20         2.7         20         NED         OC         Antenior 3 of origination         11         No         M 0           21         0         0         DWD         OP         Posterior wall of oropharynx         T 3         N0         M 0           22         3+         80         DWD         W         Tonsil NOS         T 3         N0         M 0           23         1+         30         NED         OC         Retromolar trigone         T 4         N2         M 0           24         3+         40         DWD         OC         Lower gum         T 4         N0         M 0           25         2+         30         DWD         W         Base of tongue         T 4         N 1         M 0           26         3+         60         DWD         W         Base of tongue         T 4         N 1         M 0           27         1+         10         DND         W         Base of tongue         T 4         N 2         M 0           30         3+         50         DND         OC         FOM NOS         T 2         N 2         M 0           31         0         0         DWD         OC	19	3+	20		00	Aptorior <sup>2</sup> / <sub>2</sub> of tongue	т 1		MO
21         0         0         DWD         OF         Posterior wall of orpinarying         1.3         NO         MO           22         3+         80         DWD         W         Tonsil NOS         T.3         NO         MO           23         1+         30         NED         OC         Retromolar trigone         T.3         NO         MO           24         3+         40         DWD         OC         Lower gum         T.4         N.2         MO           25         2+         30         DWD         W         Base of tongue         T.4         N.1         MO           26         3+         60         DWD         HP         Posterior wall nasopharynx         T.3         NO         MO           27         1+         10         DND         W         Base of tongue         T.4         N.1         MO           29         2+         30         DWD         OC         FOM NOS         T.2         N.2         MO           31         0         0         DWD         OC         FOM NOS         T.4         N.2         MO           32         1+         10         DWD         W	20	2+	20			Antenor 73 of longue		NO	
22         3+         60         DWD         W         Totsin NOS         1.3         N0         M0           23         1+         30         NED         OC         Retromolar trigone         T3         N0         M0           24         3+         40         DWD         OC         Lower gum         T4         N2         M0           25         2+         30         DWD         W         Base of tongue         T4         N1         M0           26         3+         60         DWD         HP         Posterior wall nasopharynx         T2         N2         M0           27         1+         10         DND         W         Base of tongue         T4         N1         M0           28         0         0         DWD         OC         Cheek mucosa         T2         N0         M0           30         3+         50         DND         OC         FOM NOS         T4         N2         M0           31         0         0         DWD         V         Lateral wall of oropharynx         T4         N2         M0           33         2+         30         DND         V         Base of tong	21	0	0		UP W			NU NO	
231+30NEDOCHetrofinital rugorite13N0M0243+40DWDOCLower gumT4N 2M 0252+30DWDWBase of tongueT4N 1M 0263+60DWDHPPosterior wall nasopharynxT2N 2M 0271+10DNDWBase of tongueT4N 1M 02800DWDCCCheek mucosaT2N 2M 0303+50DNDOCFOM NOST4N 2M 03100DWDOCFOM NOST4N 2M 0321+10DWDWLateral wall of oropharynxT4N 2M 0332+30DNDWBase of tongueT4N 2M 0342+50DWDOPPosterior wall of oropharynxT2N 2M 03500DWDOCAnterior FOMT4N 2M 036020DWDOPSoft palateT4N 2M 03800DWDWBase of tongueT4N 2M 03900AWDWBase of tongueT4N 0M 04100DWDCCHard PalateT4N 0M 0423+40DWDWBase of ton	22	3+	80		VV OO	Tonsii NOS	13	NU NO	
24 $3+$ 40DWDOCLower gum14N 2M 025 $2+$ 30DWDWBase of tongueT 4N0M 026 $3+$ 60DWDHPPosterior wall nasopharynxT 2N 2M 0271+10DNDWBase of tongueT 4N 1M 02800DWDHPPosterior wall nasopharynxT 3N0M 0292+30DWDOCCheek mucosaT 2N 2M 0303+50DNDOCFOM NOST 4N 2M 03100DWDCCFOM NOST 4N 2M 0332+30DNDWBase of tongueT 4N 2M 0342+50DWDOCAnterior FOMT 4N 2M 03500DWDOCAnterior FOMT 4N 2M 0372+90DWDOPSoft palateT 4N 2M 03800DWDWBase of tongueT 1N 0M 03900AWDWBase of tongueT 4N 2M 0403+40DWDWBase of tongueT 4N 0M 04100DWDOCHaterior % of tongueT 4N 0M 0423+40DWDWBas	23	1+	30	NED	00	Retromolar trigone	13	NU NLO	
25       2+       30       DWD       W       Base of tongue       14       N0       M0         26       3+       60       DWD       HP       Posterior wall nasopharynx       T2       N2       M0         27       1+       10       DND       W       Base of tongue       T4       N1       M0         28       0       0       DWD       HP       Posterior wall nasopharynx       T3       N0       M0         29       2+       30       DWD       OC       Cheek mucosa       T2       N2       M0         30       3+       50       DND       OC       FOM NOS       T4       N2       M0         31       0       0       DWD       W       Lateral wall of oropharynx       T4       N2       M0         32       1+       10       DWD       W       Base of tongue       T4       N2       M0         33       2+       30       DND       W       Base of tongue       T4       N2       M0         36       0       0       DWD       OC       Anterior FOM       T4       N2       M0         37       2+       90       DWD <td>24</td> <td>3+</td> <td>40</td> <td>DWD</td> <td>00</td> <td>Lower gum</td> <td>14 T4</td> <td>N 2</td> <td>IVI U</td>	24	3+	40	DWD	00	Lower gum	14 T4	N 2	IVI U
26         3+         60         DWD         HP         Posterior wall nasopharynx         12         N 2         M 0           27         1+         10         DND         W         Base of tongue         T 4         N 1         M 0           28         0         0         DWD         HP         Posterior wall nasopharynx         T 3         N0         M 0           30         3+         50         DND         OC         Cheek mucosa         T 2         N 2         M 0           31         0         0         DWD         OC         FOM NOS         T 4         N 2         M 0           32         1+         10         DWD         W         Lateral wall of oropharynx         T 4         N 2         M 0           33         2+         30         DND         W         Base of tongue         T 4         N 2         M 0           34         2+         50         DWD         OP         Posterior wall of oropharynx         T 2         N 0         M 0           35         0         0         DWD         V         Base of tongue         T 4         N 0         M 0           36         0         0         D	25	2+	30	DWD	W	Base of tongue	14	NU	MO
271+10DNDWBase of tongue14N 1M 02800DWDHPPosterior wall nasopharynxT 3N0M 0292+30DWDOCCheek mucosaT 2N 2M 03100DWDOCFOM NOST 4N 2M 0321+10DWDWLateral wall of oropharynxT 4N 2M 0332+30DNDWBase of tongueT 4N 2M 0342+50DWDOPPosterior wall of oropharynxT 2N 0M 03500DWDOCAnterior FOMT 4N 2M 036020DWDOPSoft palateT 4N 2M 0372+90DWDOPSoft palateT 4N 2M 03800DWDWBase of tongueT 1N 0M 03900AWDWBase of tongueT 4N 0M 04100DWDOCHard PalateT 4N 0M 0423+40DWDWBase of tongueT 4N 0M 0432+80DWDOCHard PalateT 4N 0M 04400DWDWBase of tongueT 4N 0M 0452+10DWDOC	26	3+	60	DWD	HP	Posterior wall nasopharynx	12	N 2	MO
28000DWDHPPosterior wall nasopharynxT 3N0M 0292+30DWDOCCheek mucosaT 2N0M 0303+50DNDOCFOM NOST 4N 2M 03100DWDOCFOM NOST 4N 2M 0321+10DWDWLateral wall of oropharynxT 4N 2M 0332+30DNDWBase of tongueT 4N 2M 0342+50DWDOPPosterior wall of oropharynxT 2N 0M 03500DWDOCAnterior FOMT 4N 0M 036020DWDOPSoft palateT 4N 2M 0372+90DWDOPSoft palateT 4N 2M 03800DWDWBase of tongueT 1N 0M 0403+40DWDWBase of tongueT 4N 0M 04100DWDOCHard PalateT 4N 0M 0423+40DWDWBase of tongueT 4N 0M 0432+80DWDOCRetromolar trigoneT 4N 0M 04400DWDWBase of tongueT 4N 3M 0452+10DWD	27	1+	10	DND	W	Base of tongue	14	N 1	MO
292+30DWDOCCheek mucosaT 2N0M 0303+50DNDOCFOM NOST 2N 2M 03100DWDOCFOM NOST 4N 2M 0321+10DWDWLateral wall of oropharynxT 4N 2M 0332+30DNDWBase of tongueT 4N 2M 0342+50DWDOPPosterior wall of oropharynxT 2N 0M 03500DWDOCAnterior FOMT 4N 2M 036020DWDOPSoft palateT 4N 2M 0372+90DWDOPSoft palateT 4N 2M 03800DWDWBase of tongueT 1N 0M 03900AWDWBase of tongueT 4N 0M 04100DWDOCHard PalateT 4N 0M 0423+40DWDWBase of tongueT 4N 0M 0432+80DWDOCRetromolar trigoneT 4N 0M 04400DWDWBase of tongueT 4N 0M 0432+80DWDOCRetromolar trigoneT 4N 3M 04400DWDWCAnte	28	0	0	DWD	HP	Posterior wall nasopharynx	Т З	N0	M 0
30 $3+$ 50       DND       OC       FOM NOS       T 2       N 2       M 0         31       0       0       DWD       OC       FOM NOS       T 4       N 2       M 0         32       1+       10       DWD       W       Lateral wall of oropharynx       T 4       N 2       M 0         33       2+       30       DND       W       Base of tongue       T 4       N 2       M 0         34       2+       50       DWD       OP       Posterior wall of oropharynx       T 2       N 0       M 0         35       0       0       DWD       OC       Anterior FOM       T 4       N 2       M 0         36       0       20       DWD       VC       Anterior FOM       T 4       N 2       M 0         37       2+       90       DWD       OP       Soft palate       T 4       N 2       M 0         38       0       0       DWD       W       Base of tongue       T 1       N 2       M 0         40       3+       40       DWD       W       Base of tongue       T 4       N 0       M 0         41       0       0	29	2+	30	DWD	OC	Cheek mucosa	Т 2	N0	M 0
31         0         0         DWD         OC         FOM NOS         T 4         N 2         M 0           32         1+         10         DWD         W         Lateral wall of oropharynx         T 4         N 2         M 0           33         2+         30         DND         W         Base of tongue         T 4         N 2         M 0           34         2+         50         DWD         OP         Posterior wall of oropharynx         T 2         N0         M 0           35         0         0         DWD         OC         Anterior FOM         T 4         N 2         M 0           36         0         20         DWD         W         Overlapping lesion of nasopharynx         T 2         N 2         M 0           37         2+         90         DWD         OP         Soft palate         T 4         N 2         M 0           38         0         0         DWD         W         Base of tongue         T 1         N 2         M 0           40         3+         40         DWD         W         Base of tongue         T 4         N 0         M 0           41         0         0         DWD	30	3+	50	DND	OC	FOM NOS	T 2	N 2	M 0
$32$ 1+10DWDWLateral wall of oropharynxT 4N 2M 0 $33$ 2+30DNDWBase of tongueT 4N 2M 0 $34$ 2+50DWDOPPosterior wall of oropharynxT 2N 0M 0 $35$ 00DWDOCAnterior FOMT 4N 0M 0 $36$ 020DWDWOverlapping lesion of nasopharynxT 2N 2M 0 $37$ 2+90DWDOPSoft palateT 4N 2M 0 $38$ 00DWDWBase of tongueT 1N 0M 0 $39$ 00AWDWBase of tongueT 4N 2M 0 $41$ 00DWDWBase of tongueT 4N 0M 0 $42$ 3+40DWDWBase of tongueT 4N 0M 0 $43$ 2+80DWDOCHard PalateT 4N 0M 0 $41$ 00DWDWBase of tongueT 3N 0M 0 $43$ 2+80DWDOCHard PalateT 4N 3M 0 $44$ 00DWDWBase of tongueT 4N 3M 0 $45$ 2+10DWDOCAnterior $\frac{1}{3}$ of tongueT 4N 3M 0 $46$ 00DNDOCCheek mucosaT 1N 0M 0 <td>31</td> <td>0</td> <td>0</td> <td>DWD</td> <td>OC</td> <td>FOM NOS</td> <td>Τ4</td> <td>N 2</td> <td>M 0</td>	31	0	0	DWD	OC	FOM NOS	Τ4	N 2	M 0
33 $2+$ 30DNDWBase of tongueT 4N 2M 034 $2+$ 50DWDOPPosterior wall of oropharynxT 2N0M 03500DWDOCAnterior FOMT 4N0M 036020DWDWOverlapping lesion of nasopharynxT 2N 2M 037 $2+$ 90DWDOPSoft palateT 4N 2M 03800DWDWBase of tongueT 1N0M 03900AWDWBase of tongueT 4N 2M 04100DWDOCHard PalateT 4N0M 04100DWDWBase of tongueT 3N0M 043 $2+$ 80DWDOCHard PalateT 4N 0M 04400DWDWBase of tongueT 3N0M 045 $2+$ 10DWDOCAnterior $\frac{1}{2}$ of tongueT 3N0M 04600DNDOCCheek mucosaT 1N0M 047 $2+$ 30AWDOCTongue NOST 1N0M 04800NEDWTonsil NOST 4N 1M 0493+80DNDWTonsil NOST 4N 1M 0	32	1+	10	DWD	W	Lateral wall of oropharynx	Τ4	N 2	M 0
$34$ $2+$ $50$ DWD       OP       Posterior wall of oropharynx $T2$ N0       M 0 $35$ 0       0       DWD       OC       Anterior FOM       T4       N0       M 0 $36$ 0       20       DWD       W       Overlapping lesion of nasopharynx       T2       N 2       M 0 $37$ $2+$ 90       DWD       OP       Soft palate       T4       N 2       M 0 $38$ 0       0       DWD       W       Base of tongue       T1       N 2       M 0 $39$ 0       0       AWD       W       Base of tongue       T4       N 0       M 0 $41$ 0       0       DWD       W       Base of tongue       T4       N0       M 0 $42$ $3+$ 40       DWD       W       Base of tongue       T4       N0       M 0 $43$ $2+$ 80       DWD       OC       Retromolar trigone       T4       N3       M 0 $44$ 0       0       DWD       OC       Anterior $\frac{2}{3}$ of tongue       T3       N0       M 0 <t< td=""><td>33</td><td>2+</td><td>30</td><td>DND</td><td>W</td><td>Base of tongue</td><td>Т4</td><td>N 2</td><td>M 0</td></t<>	33	2+	30	DND	W	Base of tongue	Т4	N 2	M 0
35       0       0       DWD       OC       Anterior FOM       T 4       N0       M 0         36       0       20       DWD       W       Overlapping lesion of nasopharynx       T 2       N 2       M 0         37       2+       90       DWD       OP       Soft palate       T 4       N 2       M 0         38       0       0       DWD       W       Base of tongue       T 1       N 2       M 0         39       0       0       AWD       W       Base of tongue       T 4       N 0       M 0         40       3+       40       DWD       W       Base of tongue       T 4       N0       M 0         41       0       0       DWD       W       Base of tongue       T 4       N0       M 0         42       3+       40       DWD       W       Base of tongue       T 4       N0       M 0         43       2+       80       DWD       OC       Retromolar trigone       T 4       N0       M 0         44       0       0       DWD       OC       Anterior $\frac{2}{3}$ of tongue       T 3       N0       M 0         45       2+	34	2+	50	DWD	OP	Posterior wall of oropharynx	T 2	N0	M 0
$36$ 020DWDWOverlapping lesion of nasopharynxT 2N 2M 0 $37$ 2+90DWDOPSoft palateT 4N 2M 0 $38$ 00DWDWBase of tongueT 1N 0M 0 $39$ 00AWDWBase of tongueT 1N 2M 0 $40$ 3+40DWDWBase of tongueT 4N 0M 0 $41$ 00DWDOCHard PalateT 4N 0M 0 $42$ 3+40DWDWBase of tongueT 3N 0M 0 $43$ 2+80DWDOCRetromolar trigoneT 4N 3M 0 $44$ 00DWDWBase of tongueT 3N 0M 0 $45$ 2+10DWDOCAnterior $\frac{1}{2}$ of tongueT 3N 0M 0 $46$ 00DNDOCCheek mucosaT 1N 0M 0 $47$ 2+30AWDOCTongue NOST 1N 0M 0 $48$ 00NEDWTonsil NOST 4N 1M 0 $49$ 3+80DNDWTonsil NOST 4N 0M 0	35	0	0	DWD	OC	Anterior FOM	Τ4	N0	M 0
$37$ $2+$ $90$ DWDOPSoft palateT 4N 2M 0 $38$ 00DWDWBase of tongueT 1N0M 0 $39$ 00AWDWBase of tongueT 1N 2M 0 $40$ $3+$ 40DWDWBase of tongueT 4N0M 0 $41$ 00DWDOCHard PalateT 4N0M 0 $42$ $3+$ 40DWDWBase of tongueT 3N0M 0 $43$ $2+$ 80DWDOCRetromolar trigoneT 4N 3M 0 $44$ 00DWDWBase of tongueT 4N 3M 0 $45$ $2+$ 10DWDOCAnterior $\frac{1}{2}$ of tongueT 3N0M 0 $46$ 00DNDOCCheek mucosaT 1N0M 0 $47$ $2+$ 30AWDOCTongue NOST 1N0M 0 $48$ 00NEDWTonsil NOST 3N 2M 0 $49$ $3+$ 80DNDWTonsil NOST 4N 1M 0 $50$ 00DWDWTonsil NOST 4N 0M 0	36	0	20	DWD	W	Overlapping lesion of nasopharynx	T 2	N 2	M 0
3800DWDWBase of tongueT 1N0M 03900AWDWBase of tongueT 1N 2M 0403+40DWDWBase of tongueT 4N0M 04100DWDOCHard PalateT 4N0M 0423+40DWDWBase of tongueT 3N0M 0432+80DWDOCRetromolar trigoneT 4N 3M 04400DWDWBase of tongueT 4N 3M 0452+10DWDOCAnterior ½ of tongueT 3N0M 04600DNDOCCheek mucosaT 1N0M 0472+30AWDOCTongue NOST 1N0M 04800NEDWTonsil NOST 4N 1M 0493+80DNDWTonsil NOST 4N 1M 05000DWDWTonsil NOST 4N 0M 0	37	2+	90	DWD	OP	Soft palate	Τ4	N 2	M 0
$39$ 00AWDWBase of tongueT 1N 2M 0 $40$ $3+$ 40DWDWBase of tongueT 4N0M 0 $41$ 00DWDOCHard PalateT 4N0M 0 $42$ $3+$ 40DWDWBase of tongueT 3N0M 0 $43$ $2+$ 80DWDOCRetromolar trigoneT 4N 3M 0 $44$ 00DWDWBase of tongueT 4N 3M 0 $45$ $2+$ 10DWDOCAnterior $\frac{2}{3}$ of tongueT 3N0M 0 $46$ 00DNDOCCheek mucosaT 1N0M 0 $47$ $2+$ 30AWDOCTongue NOST 1N0M 0 $48$ 00NEDWTonsil NOST 4N 1M 0 $49$ $3+$ 80DNDWTonsil NOST 4N 1M 0 $50$ 00DWDWTonsil NOST 4N 0M 0	38	0	0	DWD	W	Base of tongue	T 1	N0	M 0
$40$ $3+$ $40$ DWDWBase of tongueT 4N0M 0 $41$ 00DWDOCHard PalateT 4N0M 0 $42$ $3+$ $40$ DWDWBase of tongueT 3N0M 0 $43$ $2+$ $80$ DWDOCRetromolar trigoneT 4N 0M 0 $44$ 00DWDWBase of tongueT 4N 3M 0 $45$ $2+$ 10DWDOCAnterior $\frac{2}{3}$ of tongueT 3N0M 0 $46$ 00DNDOCCheek mucosaT 1N0M 0 $47$ $2+$ $30$ AWDOCTongue NOST 1N0M 0 $48$ 00NEDWTonsil NOST 4N 1M 0 $49$ $3+$ $80$ DNDWTonsil NOST 4N 0M 0 $50$ 00DWDWTonsil NOST 4N 0M 0	39	0	0	AWD	W	Base of tongue	T 1	N 2	M 0
4100DWDOCHard PalateT 4N0M 0423+40DWDWBase of tongueT 3N0M 0432+80DWDOCRetromolar trigoneT 4N0M 04400DWDWBase of tongueT 4N 3M 0452+10DWDOCAnterior $\frac{2}{3}$ of tongueT 3N0M 04600DNDOCCheek mucosaT 1N0M 0472+30AWDOCTongue NOST 1N0M 04800NEDWTonsil NOST 4N 1M 0493+80DNDWTonsil NOST 4N 1M 05000DWDWTonsil NOST 4N 0M 0	40	3+	40	DWD	W	Base of tongue	Τ4	N0	M 0
423+40DWDWBase of tongueT 3N0M 0432+80DWDOCRetromolar trigoneT 4N0M 04400DWDWBase of tongueT 4N 3M 0452+10DWDOCAnterior % of tongueT 3N0M 04600DNDOCCheek mucosaT 1N0M 0472+30AWDOCTongue NOST 1N0M 04800NEDWTonsil NOST 3N 2M 0493+80DNDWTonsil NOST 4N 1M 05000DWDWTonsil NOST 4N0M 0	41	0	0	DWD	OC	Hard Palate	Τ4	N0	M 0
432+80DWDOCRetromolar trigoneT 4N0M 04400DWDWBase of tongueT 4N 3M 0452+10DWDOCAnterior % of tongueT 3N0M 04600DNDOCCheek mucosaT 1N0M 0472+30AWDOCTongue NOST 1N0M 04800NEDWTonsil NOST 3N 2M 0493+80DNDWTonsil NOST 4N 1M 05000DWDWTonsil NOST 4N0M 0	42	3+	40	DWD	W	Base of tongue	Т З	N0	M 0
44         0         0         DWD         W         Base of tongue         T 4         N 3         M 0           45         2+         10         DWD         OC         Anterior % of tongue         T 3         N0         M 0           46         0         0         DND         OC         Cheek mucosa         T 1         N0         M 0           47         2+         30         AWD         OC         Tongue NOS         T 1         N0         M 0           48         0         0         NED         W         Tonsil NOS         T 3         N 2         M 0           49         3+         80         DND         W         Tonsil NOS         T 4         N 1         M 0           50         0         0         DWD         W         Tonsil NOS         T 4         N0         M 0	43	2+	80	DWD	OC	Retromolar trigone	Τ4	N0	M 0
45         2+         10         DWD         OC         Anterior % of tongue         T 3         N0         M 0           46         0         0         DND         OC         Cheek mucosa         T 1         N0         M 0           47         2+         30         AWD         OC         Tongue NOS         T 1         N0         M 0           48         0         0         NED         W         Tonsil NOS         T 3         N 2         M 0           49         3+         80         DND         W         Tonsil NOS         T 4         N 1         M 0           50         0         0         DWD         W         Tonsil NOS         T 4         N0         M 0	44	0	0	DWD	W	Base of tongue	Τ4	N 3	M 0
46         0         0         DND         OC         Cheek mucosa         T 1         N0         M 0           47         2+         30         AWD         OC         Tongue NOS         T 1         N0         M 0           48         0         0         NED         W         Tonsil NOS         T 3         N 2         M 0           49         3+         80         DND         W         Tonsil NOS         T 4         N 1         M 0           50         0         0         DWD         W         Tonsil NOS         T 4         N0         M 0	45	2+	10	DWD	OC	Anterior 3/3 of tongue	Т 3	N0	M 0
47         2+         30         AWD         OC         Tongue NOS         T 1         N0         M 0           48         0         0         NED         W         Tonsil NOS         T 3         N 2         M 0           49         3+         80         DND         W         Tonsil NOS         T 4         N 1         M 0           50         0         0         DWD         W         Tonsil NOS         T 4         N 0         M 0	46	0	0	DND	OC	Cheek mucosa	T 1	N0	M 0
48         0         0         NED         W         Tonsil NOS         T 3         N 2         M 0           49         3+         80         DND         W         Tonsil NOS         T 4         N 1         M 0           50         0         0         DWD         W         Tonsil NOS         T 4         N 0         M 0	47	2+	30	AWD	OC	Tongue NOS	T 1	N0	M 0
49         3+         80         DND         W         Tonsil NOS         T 4         N 1         M 0           50         0         0         DWD         W         Tonsil NOS         T 4         N 0         M 0	48	0	0	NED	W	Tonsil NOS	Т 3	N 2	M 0
50 0 0 DWD W Tonsil NOS T 4 N0 M 0	49	3+	80	DND	W	Tonsil NOS	Т4	N 1	M 0
	50	0	0	DWD	W	Tonsil NOS	Т4	N0	M 0

## TABLE III.

### Laryngoscope 112: May 2002

### Chandler et al.: Oral and Pharyngeal Carcinomas

811

(continues)

Patient	Characteristic	Percent	Last Status	Group	Site	T Stage	N	M
		50			Hard Dalata		NO	MO
50	2+	50			Overlapping losion of palate	ТЛ	NU	MO
52	2+	20	NED	W/		T 3	N 2	MO
50	2+	30	NED	00	Anterior <sup>2</sup> / <sub>2</sub> of tongue	T 1	NO	MO
55	0	0	NED	W	Base of tongue	т 1 Т 2	N 3	MO
56	0	0		۷۷ ۱۸/	Nasophan/ny NOS	T 1	NB	MO
57	0	0		۷۷ ۱۸/	Base of tongue	ТЛ	N 2	MO
58	0	0		۷۷ ۱۸/		T 2	NO	MO
50	2+	40		۷۷ ۱۸/		T 3	N 2	MO
60	2+	10		۷۷ ۱۸/	Base of tongue	T 1	NO	MO
61	0	0		۷۷ ۱۸/	Base of tongue	т 1	N 2	MO
62	0	0	NED	VV \\/		ТЛ	NO	MO
62	2⊥	80		VV \\/	Rass of tonguo	T 2	NU 1	MO
64	3+ 2+	20		VV \\/	Base of tongue	T 2	NO	MO
65	0	20		VV \\\		1 Z T 4	NO	MO
66	0	50		00	Potromolar trigono	14 T 0	NU O	MO
67	2+	50		00	Anterior 2/ of tengue			MO
60	0	90		00 W/		1 Z T 1	NO	MO
60	0	10		00			NO	MO
70	2+	10		00	Anterior EQM	14 T 0	NO	MO
70	2+	20		00			NU NLO	
71	0	10		VV OD	Overlanding legion of graphenery	14 T4	N 3	
72	2+	10		UP W	Tanail NOS	14 T4	NU NLO	
73	2+	10		VV VV		14 T 2		
74	3+	50		VV VV	Tonsii NOS			
75	3+	40		VV VV	Base of tengue	14 T4		
70	1+	10		VV OC		14 T1	IN I	
70	2+	10		00	FOM NOS		NO	
70	0	0		00	FOM NOS	13	NO	
79	2+	30		00	Mouth NOS		NU NLO	
00	0	50		VV VV				
01	2+	50		VV VV		14 T 0		
02	1+	50		VV VV	Tonsii NOS		NO	
03	0	10		VV OC	Anterior FOM		NO	
04 05	2+	10		00		13		
00	1+	20		00	Anterior 2/ of tengue			
00	3+	30		00	Anterior 73 of longue		IN I	
0/	2+	40		00	Anterior FOM	14 T4	IN I	
88	0	0	NED	00	Retromolar trigone	14 T 1	NU NO	
89	2+	30	NED	00	Cheek mucosa		NU	
90	3+	10	NED	00	Border of tongue	11	NU	MU
91	2+	25		00	FOM NOS	12	NU	MO
92	0	0	NED	VV	Base of tongue	11	NU	MO
93	U	U	AWD	VV	Base of tongue	13	N 1	MÜ
94	2+	20	NED	W	Base of tongue	12	N 2	MO
95	2+	40	NED	UC		12	N 2	MO
96	2+	40	NED	W		12	N 1	MO
97	3+	40	NED	OC	Anterior 3/3 of tongue	Γ4	N0	M 0

TABLE III. (Continued) List of Patients With Site. Stage. and IL-10 Staining Results as well as Outcome

FOM = floor of mouth; NOS = not otherwise specified; AWD = alive with disease; DWD = dead with disease; NED = no evidence of disease, alive; DND = dead with no disease; OC = oral cavity; W = Waldeyer's ring; HP = hypopharynx; OP = oropharynx.

### Laryngoscope 112: May 2002

ppyright © The American Laryngological, Rhinological and Otological Society, Inc. Unauthorized reproduction of this article is prohibited



Fig. 4. Bar graph of percentage of patients with positive or negative IL-10 staining in their tumors versus grades.

Our findings indicate that lower grade of differentiation is associated with increased IL-10 expression. However, we advise caution in the interpretation of these findings. First, the archival tissues used in the present study are not uniformly controlled. Standardized handling of specimens will be mandatory if meaningful analysis of that type of data is to be performed. In addition, it is possible that even with proper standardization in tissue handling the results of molecular techniques and in vitro studies will be different than for immunohistochemical studies. A pilot study, which was presented at the Fourth International Conference on Head and Neck Cancer in Toronto (1996), using reverse transcriptase polymerase chain reaction (RT-PCR) by one of the authors (C.H.R.) indicated that viral IL-10 was present in significant amounts in a small subset of tumors arising in Waldeyer's ring, including tumors that did not involve the nasopharynx. In addition, human IL-10 was overexpressed in these cases. The current study was undertaken partly to validate the RT-PCR findings in a larger cohort of patients. In fact, although the previously mentioned pilot RT-PCR studies have suggested that IL-10 (both human and viral) is overexpressed in pharyngeal cancers, the correlation with grading is not consistent with the current study. This is probably a reflection of methodological differences between immunohistochemical study and polymerase chain reaction, as well as of the abovementioned concerns about archival tissue specimens. Fujieda et al.<sup>15</sup> have recently demonstrated that IL-10 expression is associated with expression of plateletderived endothelial growth factor. They found in their study that intense staining was associated with a poorer prognosis in oral and oropharyngeal carcinomas. Our immunohistochemical study does not support a prognostic correlation but confirms the presence and localiza-



Fig. 5. Bar graph of percentage of patients with positive or negative IL-10 staining in their tumors versus node stages.

### Laryngoscope 112: May 2002

Chandler et al.: Oral and Pharyngeal Carcinomas

Copyright © The American Laryngological, Rhinological and Otological Society, Inc. Unauthorized reproduction of this article is prohibited





tion of IL-10. In any case, the major finding of the current study is that the cytokine is localized to the cancer cells themselves and in the majority of cases it can be detected with immunohistochemical analysis at the level of sensitivity in our study. It is important to note that IL-10 staining was not found in noncancerous epithelium or inflammatory cells in our study. In the study of Fujieda et al.,<sup>15</sup> infiltrating mononuclear cells did pick up the stain and a higher overall percentage of specimens stained. It may be that antigen retrieval can be increased and a prognostic correlation found, but in our study, which has more patients (97 vs. 58) this appears to be unlikely. In our study, even normal tonsil did not stain. It is possible that this is because our antigen retrieval process was less effective or may be attributable to differences in primary antibody affinity. Antigen retrieval may be affected by the process in which the tissues are procured. Again, however, we would be cautious about interpreting such archival tis-



Fig. 7. Low-power (10x) view of normal tonsil stained with IL-10 demonstrating absence of any stain.

sue studies for prognostic significance. The finding that IL-10 is produced, and apparently in great quantities, in the cytoplasm of head and neck squamous cells is common to both studies. It is possible that the quality of the tumor specimens in the study of Fujieda et al.<sup>15</sup> was superior to ours and that the follow-up and treatment strategy were more consistent. The lack of staining in normal tonsil and infiltrating lymphocytes in our study does suggest that, at least, these cells have less IL-10 than tumor cells. Nevertheless, the prognostic and other correlations would not hold up to multivariate analysis because of small sample sizes in both studies. Examination of premalignant lesions for IL-10 staining would be of value in investigating IL-10 as truly carcinogenic; however, it appears to be associated with malignant cells in head and neck cancer. Much more investigation will be necessary to clarify whether this finding has importance in pathogenesis or other outcome measures. However, IL-10 and related genes may be future targets for gene discovery and possibly even therapeutic intervention. Mechanistic in vitro studies and additional human tissue studies will be the next logical step in this regard. Immunohistochemisty is a valuable tool because it allows the study of archival tissues and is less expensive and less time-consuming than molecular techniques such as RT-PCR and in situ hybridization. However, a combination of these research tools and other in vitro techniques will be needed to investigate this cytokine further.

### CONCLUSION

Interleukin-10 is present in human oral and pharyngeal carcinoma cells and can be detected by immunohistochemical study with a sensitivity that does not localize the cytokine to normal epithelium or lymphocytes. Further studies are needed to determine the importance of human and viral IL-10 in head and neck cancer, including its relationship to grade, stage, site, and outcome and

### Laryngoscope 112: May 2002

Chandler et al.: Oral and Pharyngeal Carcinomas

other clinical and histological parameters. Molecular studies are needed to determine whether this cytokine is involved, and at what level, in dual infection with EBV and HPV and whether these molecular events are associated with other molecular events such as oncogene and tumor suppressor gene expression. Interleukin-10 and associated mediators may be future targets for gene therapy research.

### Acknowledgments

The authors acknowledge Pat Turner, HT (ASCP), for her invaluable assistance in setting up the immunohistochemical staining protocol, and Beverly Clemmer for assistance with the final manuscript preparation and other assistance during the project.

### BIBLIOGRAPHY

- Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics 2000. CA Cancer J Clin 2000;50:7–33.
- Young MR, Wright MA, Lozano Y. Mechanisms of immune suppression in patients with head and neck cancer: influence on the immune infiltrate of the cancer. Int J Cancer 1996;67:333–338.
- Mosmann TR, Cherwinski H, Bond MW, Giedlin MA. Two types of murine T-cell clone. J Immunol 1986;136: 2348-2357.
- Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse helper T-cell. J Exp Med 1989;170:2081–2095.
- Rivas JM, Ullrich SE. Keratinocyte-derived IL-10. J Invest Dermatol 1992;98:578-581.

- Rassekh CH, Rady P, Arany I, et al. Combined EBV and HPV in nasopharyngeal carcinoma. *Laryngoscope* 1998;108: 362–367.
- Hus DH, De Waal Malefyt R, Fiorentino DF, Dang MN. Expression of interleukin-10 activity by Epstein-Barrvirus protein BCRF1. Science 1990;250:830-832.
- Benjamin D, Knobloch TJ, Dayton MA. Human B-cell interleukin-10. Blood 1992;80:1289-1294.
- Emile D, Touitou R, Raphael M, Peuchmaur M, Devergnee O. In vivo production of interleukin-10 by malignant cells in AIDS lymphomas. *Eur J Immunol* 1992;22:2937–2942.
- Yamamura M, Modlin RL, Ohmen JD. Local expression of anti-inflammatory cytokines in cancer. J Clin Invest 1993; 91:1005-1010.
- Yao M, Ohshima K, Suzumiya J, Kume T, Shiroshita T, Kikuchi M. Interleukin-10 expression and cytotoxic-T-cell response in Epstein-Barr-virus-associated nasopharyngeal carcinoma. *Int J Cancer* 1997;72:398-402.
- Smith DR, Kunkel SL, Burdick MD, et al. Production of interleukin-10 by human bronchogenic carcinoma. Am J Pathol 1994;145:18-25.
- Avradopoulos K, Mehta S, Blackinton D, Wanebo. Interleukin-10 as a possible mediator of immunosuppressive effect in patients with squamous cell carcinoma of the head and neck. Ann Surg Oncol 1997;4:184-190.
- Young MR, Wright MA, Lozano Y. Mechanisms of immune suppression in patients with head and neck cancer: influence on the immune infiltrate of the cancer. *Int J Cancer* 1996;67:333–338.
- Tsusuki H, Fan GK, Saito H. IL-10 Expression is associated with the expression of platelet-derived endothelial growth factor and prognosis in oral and oropharyngeal carcinoma. *Cancer Lett* 1999;136:1–9.