Labeling index in squamous cell carcinoma of the larynx.

LABELING INDEX IN SQUAMOUS CELL CARCINOMA OF THE LARYNX

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Two cell kinetic parameters, the ^{3}H-thymidine labeling index (TLI) and the mitotic index (MI), were studied in vitro on fragments of squamous cell carcinoma tissue of the larynx. They were evaluated to identify those elements able to characterize the growth of these solid tumors. The values of these parameters were analyzed as a function of the clinical stage and the involvement of the regional lymph nodes. Results showed a statistically significant increase in the TLI from stage T1 to T3. No statistically significant differences in the TLI values were observed between the patients with positive and negative lymph nodes. **HEAD & NECK 1991;13:344–348**

As yet, no reliable histomorphologic or biochemical parameters have been determined which, based on tumor growth and its correlation to clinical data, can be used safely in therapeutic planning to improve prognosis.

The proliferative rate of tumors is subject to a marked variability due to intrinsic characteristics, such as cell cycle time, growth fraction, cell loss, and degree of differentiation. Tumor growth is further influenced by the microenvironment and by the immunologic response of the patient. Therefore, the evolution of a malignant neoplasia can show much variability depending on factors linked to the disease, to the patient, and to treatment and its effectiveness in controlling tumor growth.

Even with these limitations, evaluation of the number of cells that are duplicating DNA at the time of surgical removal gives a direct indication of the growth capacity of the tumor. Several studies, performed on a large number of patients and with an adequate follow-up, demonstrate that the ^{3}H-thymidine labeling index (TLI) value at the time of surgery is a valuable prognostic instrument for neoplasias of the breast, ovaries, head, and neck.[1–6]

The aim of the present study was to analyze the correlations between cell kinetic parameters and clinical stage, involvement of regional lymph nodes before treatment, rate of relapse, metastasis, and survival when long-term follow-up data are available.

MATERIALS AND METHODS

Thirty one consecutive patients (27 men, 4 women), between 47 and 72 years of age (median 62 years), were examined in this study. All had tumors of the larynx, localized in the supraglottic region, and classified as squamous cell carcinomas.

Clinical staging, according to the TNM classi-

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fication (UICC 1978), is reported in Table 1. After histopathologic examination of the dissected lymph nodes, N was modified (N−, no lymph node metastasis; N+, presence of lymph node metastasis), and this pathologic N, reported in Table 1, was used in the analysis of our data.

Histologic degree of differentiation, according to Broder's grading, was performed. The incidence of well-, moderately, and poorly differentiated tumors was respectively 16.2% (5 of 31), 58% (18 of 31), and 25.8% (8 of 31).

A total laryngectomy was performed on 22 of the above patients. The remaining 9 patients underwent supraglottic horizontal laryngectomy. Mono- or bilateral radical neck dissection was performed on all patients.

Labeling Index (TLI) Determination. Tumor samples were obtained at the time of surgery. Immediately after removal 12 small fragments (volume 1–3 mm^3), were incubated separately in 1 mL of Mc Coy's 5A medium (70%), fetal calf serum (20%), and ^{3}H-thymidine (10%, 0.37 MBq, specific activity 740 GBq/mM) for 1 hour at 37°C in 5% CO_2 under continuous stirring. The fragments were then washed with cold NaCl 0.9%, fixed in Carnoy's, and embedded in polystyrene.[7]

Three-micron sections were processed by autoradiography using the dipping film technique (Ilford K5 emulsion). After a 10-day period of exposure, the autoradiographies were developed and the samples were stained with hematoxylin & eosin.

Tumor histology was examined in the sections where TLI and MI were determined. The total number of tumor cells, labeled tumor cells, and mitoses were counted in each section, using the optical microscope (magnification 1,000×). Because ^{3}H-thymidine has a limited capability of diffusion, counting was performed either in the outer peripheral zone (100 μm) or in the entire section if the distribution of the labeled cells was homogenous.

To evaluate the variability of tumor growth for each case, the TLI was obtained by counting at least 3 fragments cut from different areas of the tumor. Moreover 2 or more sections were counted from each fragment. Only 1 of 5 sections present on each slide was counted to avoid repeating the cell count twice. Usually more than 10,000 cells were recorded for each case. The TLI determined using this method was therefore a good indication of the proliferative condition of the whole tumor.

Table 2 reports an example of cell growth variability in the tumor. Reported are the total cell number (TCN), the TLI, and the MI in each section of the different fragments of tumor.

For each case, the average TLI and MI values obtained from the different sections and fragments were calculated. Because the TLI log showed normal distribution, the results of the individual group of patients were expressed as the geometric mean. The 95% confidence interval was calculated, and the Wilcoxon rank sum test for 2 independent univariate samples was used for statistical analysis.

RESULTS

The geometric mean of the TLI in the 31 cases of squamous cell carcinoma of the supraglottic larynx was 13.21% with a 95% confidence interval from 11.70 to 14.92 and a range between 6.88% and 30.33%.

The MI in these cases was 0.402% with a confidence interval from 0.297 to 0.545 and a range between 0.023% and 1.210%. Of these patients, only 2, with a TLI of 27.98% (T3N0−) and

Table 1. Clinical and histopathologic staging.

	T1	T2	T3	T4
Clinical staging				
N0	5	2	7	6
N1	1	1	2	4
N2	1	—	1	1
Lymph node histopathologic staging				
N−	4	2	7	4
N+	3	1	3	7

Table 2. Results of case 17.

	TCN	LI %	MI %
Fragment 1	4,511	17.07	0.665
	3,848	17.13	0.962
Total per fragment	8,359	17.09	0.802
Fragment 2	3,823	16.56	1.256
	2,773	14.50	1.262
Total per fragment	6,596	15.69	1.258
Fragment 3	4,287	13.83	0.653
	3,500	14.74	0.800
Total per fragment	7,787	14.24	0.719
Total	22,742	15.71	0.906

TCN, total cell number; LI, labeling index; MI, mitotic index.
Man, age 65, T3N0M0.

Table 3. LI and MI related to histologic grading.

Histologic grade	No. of cases	Labeling index			Mitotic index		
		Geometric mean	95% confidence interval	Range	Geometric mean	95% confidence interval	Range
Well differentiated	5	12.97	10.91–15.41	10.58–15.58	0.425	—	0.120–0.915
Moderately differentiated	18	13.32	11.29–15.71	7.30–30.33	0.428	0.267–0.686	0.023–1.210
Poorly differentiated	8	13.09	9.09–18.85	6.88–27.98	0.337	0.203–0.559	0.120–0.671

Table 4. LI and MI related to clinical staging of lymph nodes.

Clinical N stage	No. of cases	Labeling index			Mitotic index		
		Geometric mean	95% confidence interval	Range	Geometric mean	95% confidence interval	Range
N0	20	12.70	10.83–14.89	6.88–27.98	0.318	0.209–0.484	0.023–0.932
N1, N2	11	14.17	11.42–17.58	9.31–30.33	0.614	0.442–0.853	0.215–1.210

Table 5. LI and MI related to pathologic staging of lymph nodes.

Pathologic N stage	No. of Cases	Labeling index			Mitotic index		
		Geometric mean	95% confidence interval	Range	Geometric mean	95% confidence interval	Range
N–	17	12.98	10.94–15.40	6.88–27.98	0.339	0.207–0.555	0.023–0.932
N+	14	13.48	11.10–16.36	7.50–30.33	0.495	0.348–0.705	0.120–1.210

See Table 2 for abbreviations.

Table 6. LI and MI related to T stage.

T stage	No. of Cases	Labeling index			Mitotic index		
		Geometric mean	95% confidence interval	Range	Geometric mean	95% confidence interval	Range
T1	7	11.49	8.82–14.97	7.30–15.24	0.395	0.191–0.817	0.215–1.210
T2	3	12.34	—	7.53–15.92	0.540	—	0.453–0.671
T3	10	15.73	13.31–18.58	12.58–27.98	0.396	0.179–0.879	0.023–1.086
T4	11	12.54	9.70–16.21	6.88–30.33	0.383	0.235–0.624	0.120–0.915

30.33% (T4N1+) respectively, showed MI values greater than 1%. There was little probability of observing cells in mitosis, since this phase is brief compared to the S phase. This fact determined great fluctuactions in MI within the same histologic section and between fragments of the same tumor.

The TLI and MI are reported in Table 3 according to histologic grade of differentiation. The differences between poorly, moderately, and well-differentiated groups were not statistically significant.

When these 2 parameters were compared to the clinical involvement of the lymph nodes (Table 4), the values of the N0 cases were lower than the other cases, but only the differences in the MI appeared to be statistically significant ($p(z) = 0.01$).

Analysis of the cases based on the histopathologic involvement of lymph nodes showed that both TLI and MI values in N– patients were lower than in N+ patients, but the differences were not statistically significant (Table 5).

As reported in Table 6, when related to clini-

Table 7. LI and MI related to T and lymph node involvement.

		LI geometric mean	LI Range	MI geometric mean	MI Range
T1N+	(3)	10.21	7.30–15.24	0.442	0.215–1.210
T1N−	(4)	12.56	9.13–15.10	0.376	0.128–0.932
T2N+	(1)	15.65		0.671	
T2N−	(2)	10.95	7.53–15.92	0.484	0.453–0.518
T3N+	(3)	14.88	14.20–16.06	0.673	0.478–1.086
T3N−	(7)	16.10	12.58–27.98	0.318	0.023–0.906
T4N+	(7)	14.25	9.43–30.33	0.437	0.120–0.763
T4N−	(4)	10.02	6.88–13.21	0.295	0.120–0.915

See Table 2 for abbreviations.

cal staging, the TLI increased progressively with the tumor size and the differences between T1 and T3 were statistically significant ($p(z) = 0.025$). In the T4 cases, the TLI values were lower than the T3 values, but this difference was not statistically significant. The wide range of TLI observed in the T4 group confirmed the high variability of the T4 tumor growth.

The MI did not show significant differences in the different stages.

Analysis of the TLI according to stage and lymph node involvement did not demonstrate a homogenous pattern, probably due to the limited number of cases for each group (Table 7). However, the MI in the N− cases was lower than in the N+ cases. Further analysis of T4 cases showed that in the N− patients (both N0 and N1), the TLI was lower than in the N+ patients.

DISCUSSION

Analysis of the cell kinetic parameters can contribute to a better characterization of neoplastic growth, and the possible prognostic significance of pretreatment values cell kinetic parameters in human tumors has been studied in recent years.[1–5] In some cases these parameters can also be used to monitor the response to therapy.[6,8]

Previous studies show that in the larynx the geometric mean of the TLI in the normal epithelium is 6.30% (range 4.9%–7.6%).[9] In squamous cell papilloma, the TLI is 4.70% (range 1.8%–16.6%), in inflammatory tissues the geometric mean is 7.9% (range 3.0%–15.3%), whereas in squamous cell carcinoma it is 5.0% (range 1.0%–7.3%).[9] More recently, other authors give a median value, respectively, of 11.2% (range 3.2%–16.4%),[6] 8.5% (range 3.0%–15.1%),[10] and 11% (range 2.3%–22.4%).[5] The results of the present and previous studies[5,6,11] demonstrate that the carcinomas of the larynx have mean TLI values that are noticeable higher than those of the breast,[1,4] bladder,[12] thyroid, and colon–rectum.[13]

Usually the studies refer to the entire group of head and neck tumors; this can explain the wide range of TLI values, because tumors with totally different localizations and prognosis are included. The present study tried to eliminate a part of the variables by analyzing only the squamous cell carcinomas of the supraglottic region of the larynx.

The variability of the results obtained by the various authors is rather high and demonstrates the need to standardize the handling of tissue fragments and, above all, of the counting method used.

We feel that our results are validified by the large sample examined: in fact, many thousands of cells in different fragment levels and many tumor fragments were counted. The current method is long and time-consuming, but other alternative techniques for the study of the fraction of S phase cells, such as flow cytometry, are not yet completely satisfying. Moreover, autoradiographic techniques allow the study of the tumor structure and its relationship with the normal surrounding tissue.

The present study evidenced some correlations between the cell kinetic parameters and tumor staging. The TLI and MI in the N0 cases were lower than in N1 and N2 cases, although the differences were not statistically significant.

The TLI increased as the T increased, and statistically significant differences were demonstrated between T1 and T3, demonstrating a higher proliferative activity in larger tumors. T4 cases showed low TLI values, except in 1 patient whose TLI value was 30.33%. In general, the N− cases had a lower TLI than the N+ ones.

This study did not confirm the previously described observations concerning correlation be-

tween histologic grading and TLI. Above all, cell kinetic parameters do not appear to be related to the presence of micrometastases in the lymph nodes.[10] However, the number of cases studied to date is too limited to allow definite conclusions.

Given the short follow-up period, it was not possible to correlate cell kinetic parameters with the survival rate and the incidence of relapses and metastases or to verify the eventual use of TLI to predict prognosis.

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