

# p53 overexpression on the resection margins as a marker of local recurrence in glottic T1a carcinoma

## *L'espressione dell'oncoproteina p53 nei margini di resezione come marker di recidiva locale nel carcinoma glottico T1a*

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### Key words

Larynx carcinoma • Glottic carcinoma • p53 oncoprotein • Prognosis

### Parole chiave

Tumori della laringe • Carcinoma glottico • Oncoproteina p53 • Prognosi

### Summary

Glottic carcinoma, in the early stage, may benefit, with excellent results “quoad vitam atque quoad valitudinem”, from various modes of treatment – radiotherapy, laser microsurgery and cordectomy being the most common – in definitive cure of the disease. Nevertheless, recurrence, in relation to treatment, oscillates between 4.3-24.1% with laser CO<sub>2</sub> surgery, 5.5-32.4% for cordectomy and 5.3-34% for radiotherapy. Prognostic biological markers of recurrence remain to be elucidated, mainly due to the clinical differences in the subjects examined. The present study focused on patients with glottic T1a carcinoma treated with CO<sub>2</sub> laser surgery in which correlation between histo-pathological aspects and expression of p53 protein on resection borders were confirmed by onset of local recurrence. Study population comprised 39 patients treated with CO<sub>2</sub> laser surgery (January 1985 – December 1991) in Clinical Division of Otorhinolaryngology, University of Catania. Survival rate, free from recurrence, at 3 and 5 years for this patient group was 87.1% (34/39) and 82% (32/39), respectively. Disease-free survival at 3 and 5 years, was 86.6% in patients with positive resection margins for carcinoma and 87.5% and 79.1%, respectively, for patients with negative resection margins. Survival rate, free from local recurrence, in p53 positive patients, at 3 and 5 years was 78.9% and 68.4%, respectively. In p53 negative patients, survival, free from local recurrence, at 3 and 5 years, was 94.7%. Presence of overexpression of oncoprotein p53 on borders of resection with aspects of dysplasia of various degrees seems, therefore, a marker of high risk of tumour progression and recurrence.

### Riassunto

Il carcinoma glottico in stadio precoce può beneficiare di diverse modalità di trattamento con risultati eccellenti “quoad vitam atque quoad valitudinem”. La radioterapia, la microchirurgia laser o la cordectomia rappresentano i presidi terapeutici più comuni in grado di debellare in maniera definitiva la malattia. Tuttavia la comparsa di recidiva, in relazione al trattamento oscilla tra il 4,3 e il 24,1% per la chirurgia al laser CO<sub>2</sub>, tra il 5,5 e il 32,4% per la cordectomia e tra il 5,3 e il 34% per la radioterapia. Gli studi condotti fin ora, al fine di individuare sicuri markers biologici prognostici di recidiva non sono giunti a conclusioni. Ciò è da imputare soprattutto alla disomogeneità dei soggetti presi in esame. In questo studio abbiamo ritenuto interessante prendere in considerazione un gruppo di pazienti affetti da carcinoma glottico T1a trattati con chirurgia laser CO<sub>2</sub> su cui abbiamo verificato la correlazione tra aspetto istopatologico ed espressione della proteina p53 dei margini di resezione e comparsa di recidiva locale. Sono stati presi in considerazione 39 pazienti trattati con chirurgia laser CO<sub>2</sub> dal gennaio 1985 al dicembre 1991 presso la Divisione Clinicizzata di Otorinolaringoiatria dell'Università di Catania. La sopravvivenza libera da recidiva locale a 3 e 5 anni, per l'intero gruppo di pazienti, era di 87,1% (34/39) e 82% (32/39) rispettivamente. La sopravvivenza libera da malattia a 3 e 5 anni, era di 86,6% nei pazienti con margini di resezione positivi per carcinoma e di 87,5% e 79,1% rispettivamente per i pazienti con margini di resezione indenni. La sopravvivenza libera da recidiva locale a 3 e 5 anni nei pazienti p53 positivi era del 78,9% e 68,4% rispettivamente mentre nei pazienti p53 negativi la sopravvivenza libera da recidiva a 3 e 5 anni era del 94,7%. La presenza di una iperespressione della oncoproteina p53 nei margini di resezione con aspetti di displasia di vario grado sembra quindi rappresentare un marker di alto rischio di progressione tumorale e di recidiva.

### Introduction

Early glottic cancer may benefit from various methods of treatment with excellent results “quoad vitam

atque quoad valitudinem”. Radiotherapy, laser microsurgery and cordectomy are the most common forms of treatment, permanently eliminating the disease.

Nevertheless, recurrence and the relationship with treatment oscillates between 4.3 and 24.1% for laser CO<sub>2</sub> surgery<sup>1-5</sup>, between 5.5 and 32.4% for cordectomy<sup>6,7</sup> and between 5.3% and 34% for radiotherapy<sup>8-14</sup>. This finding re-opens the old theory concerning the biological characterization of head and neck tumours that are influenced, in their evolution, by the site of origin but it may also have its properties, intrinsic to the tumoural mass that modulate the aggressiveness and capacity of the metastasis.

Investigations, so far, aimed at defining biological prognostic markers of recurrence, have not reached univocal conclusions, due primarily to the heterogeneity of the individuals enrolled in the various studies.

We considered it of particular interest to study a homogeneous group of patients presenting T1a glottic carcinoma, treated with CO<sub>2</sub> laser surgery. Attention was, therefore, focused on the correlation between the histo-pathological aspects and p53 protein expression on resection borders and the onset of local recurrence.

It is well known that alterations of the p53 tumour suppressor gene, due to mutation or allelic loss, is a common early event in laryngeal carcinoma<sup>13-19</sup> and shows a close relationship with the carcinogenetic multistep process. The protein codified by this anomalous gene not used is accumulated in the cells and may be detected with histochemical techniques<sup>19</sup>. Its role as a prognostic marker of local recurrence remains to be clarified<sup>20-22</sup>.

## Patients and Methods

A total of 45 patients with T1a glottic carcinoma and treated with CO<sub>2</sub> surgical laser from January 1985 to December 1991, at the ENT Clinic, University of Catania, were taken into consideration. Six patients in whom at least one of the resection margins were not evaluable, were excluded from the study. Mean age of the remaining 39 patients (37 male, 2 female) was 58.2 years (range 37-71). Of these patients, 31 were smokers (23 smoked 20 cigarettes/day, 8 only 8 cigarettes/day), while 8 were non-smokers.

Histological diagnosis revealed a well-differentiated squamous cell carcinoma (SCC) in 11 cases, moderately differentiated in 20 and poorly differentiated in 8. Clinical statistics are outlined in Table I. All patients were examined every 3 months, for the first five years and, thereafter, every 6 months for the next five years. Every 3 months, patients also underwent fibroendoscopic evaluation. Every 6 months, patients were submitted to echographic examination of the neck and chest X-ray. Mean follow-up was 74.3 months (range 3-123 months).

Table I. Clinical characteristics.

Clinical parameters	No. patients (%)	p value
Sex		
Males	37 (94.8)	<0.05
Females	2 (5.2)	
Smokers		
Yes	31 (79.4)	<0.05
No	8 (29.6)	
Grade		
1	11 (25.7)	<0.05
2	20 (51.3)	
3	8 (23.0)	

## Treatment

Micro laryngoscopic surgery was carried out in all patients using a CO<sub>2</sub> laser with a microscope equipped with a 400 mm lens. General anaesthetic was performed with high frequency ventilation. Intubation of the trachea was effected using no. 10-16 charter tubes. Tubes were protected, in the distal portion, with gauze soaked in a physiological solution. High frequency ventilation did not allow stagnation of smoke produced in the surgical area and allowed penetration of blood into the bronchial tube. Eyes were protected to avoid corneal lesions. A laryngoscope was used (Microfrans) to allow good visualization of the glottis and, in particular, the anterior commissure.

With a clear view of the larynx, vocal cords were exposed and a type IV cordectomy was performed by means of CO<sub>2</sub> laser<sup>5</sup>.

Emission at 15 W power was used in laser surgery. Histopathological studies of the excised tissue were performed on serial sections to allow easier and more precise examination of the resection margins.

In the case of positive margins, further treatment included radiotherapy.

## HISTOPATHOLOGICAL ANALYSIS OF RESECTION MARGINS

Histopathological examination of the excised specimen was made according to the surgeon's indications and conclusions were advanced on the superior, inferior and deep or lateral parts of the resection margins. Samples were stained with haematoxylin-eosin.

## IMMUNOHISTOCHEMICAL DETERMINATION OF P53 PROTEIN

Surgical specimens, after being fixed in formalin embedded in 10% paraffin, were placed on new slides to detect the resection margins, as previously described. These preparations were carefully examined with

primary monoclonal antibodies for the native and mutated p53 protein (Pab1801 Oncogene, DO-1 Delta Biological, isopath pred.).

The prepared items were boiled in a microwave (MW650C, 4 cycles of 5 min in tampons citrate pH 6/citrate buffer pH 5).

Positivity was assessed using the anti-peroxidase system (Dako, LSAB 2), DAB as a chromogen and counterstain with Meyer emallume.

Positivity was expressed using the semiquantitative scale evaluating the intensity of the nuclear stain, as follows: (+) 1-10% positive cells; (++) 10-50% positive cells; (+++) >50% positive cells. A sample free from primary antibodies was used as a negative control.

### STATISTICAL ANALYSIS

Disease-free survival and overall survival at 5 years was calculated according to the Kaplan and Meyer<sup>23</sup> method. Statistical difference was assessed using a log rank test. Chi square test was used to evaluate the statistical significance of the clinical features of the patients.

### Results

Survival, free from local recurrence, at 3 and 5 years, for the entire group of patients was 87.1% (34/39) and 82% (32/39), respectively.

Patients presenting recurrence within the first 3 years were treated with total laryngectomy in 3 cases and with radiotherapy in 2 cases. Another 2 cases, in which local recurrence appeared within 5 years of follow-up, underwent total laryngectomy.

During the 10 years, distance metastasis occurred in 3 patients (2 lung, 1 liver). Of those patients presenting recurrence, 3 died from progressive disease and 1 for other reasons.

### RESECTION MARGINS: HISTOPATHOLOGICAL ANALYSIS

Histopathological analysis of the resection margins revealed that 38.45% of patients (15/39) showed at least one of the resection margins with considerable in situ or invasive carcinoma ( $p = 0.2$ ). As far as concerns the margins involved, these were: only the profound (lateral) margins in 6 cases, only the inferior in 5, only the in 3 whilst in 1 case, both the inferior and profound margins were affected. Considering all the resection margins examined, evidence of light dysplasia was observed in 6.8% of cases (8/117), moderate dysplasia in 7.6% (9/117) and severe dysplasia in 2.5% (3/117) while 69.2% (81/117) of those examined presented normal morphology. As far as concerns the patients presenting recurrence, resection margins were positive for carcinoma in 2 and negative in 5.

Disease-free survival, at 3 and 5 years, was 86.6% in patients with positive resection margins for carcinoma and 87.5% and 79.1%, respectively, for the patients with negative resection margins. All 15 patients with positive resection margins for carcinoma were submitted to radiotherapy.

The number of patients with p53 positive margins was: 6/15 (40%) in those with histopathologically positive margins and 13/24 (54.1%) in negative cases, the difference not being statistically significant ( $p=0.6$ ).

The statistical analysis curve of survival, free from local recurrence, at 5 years in the 2 groups of patients was not significant at the log rank test ( $p=0.57$ ) (Fig. 1).

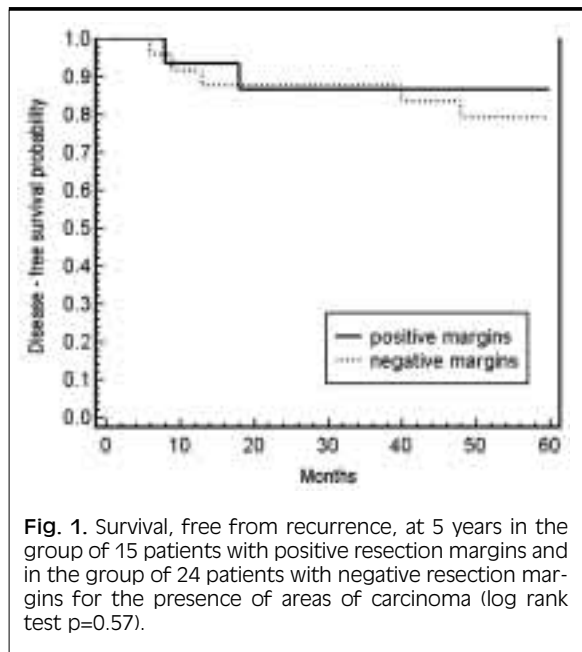


Fig. 1. Survival, free from recurrence, at 5 years in the group of 15 patients with positive resection margins and in the group of 24 patients with negative resection margins for the presence of areas of carcinoma (log rank test  $p=0.57$ ).

### DETERMINATION OF p53 ONCOPROTEIN IN RESECTION MARGINS

Overexpression of p53 protein was found in 48.7% (19/39) of the patients ( $p=0.1$ ).

Analysing the positivity and the characteristics of the resection margins, 31 out of 81 (38.2%) of the histopathologically normal (prevalently low) margins resulted p53 positive. Margins with slight dysplasia showed an overexpression of p53 in 37.5% (3/8), margins with moderate dysplasia, an overexpression of p53 protein in 55.5% (5/9) and margins with severe dysplasia, overexpression, reached 75% (2/3).

The resection borders with evidence of invasive carcinoma were p53 positive in 40% (6/15).

As far as concerns the patients presenting recurrence, 5 had resection margins with p53 positive light or

moderate dysplasia, 1 had p53 negative invasive carcinoma and 1 had p53 positive invasive carcinoma. The patients in those occurred a recurrence were 5 with borders of resection with light or moderate dysplasia p53 positive, 1 with invasive carcinoma p53 negative and 1 with invasive carcinoma p53 positive. Disease-free survival, at 3 and 5 years, in the p53 positive patients, was 78.9% and 68.4%, respectively, and in the p53 negative patients was 94.7%.

Radiotherapy was carried out in 6/19 (45%) patients in the p53 positive group and in 9/20 (31.5%) in the p53 negative group, the difference not being statistically significant ( $p=0.57$ ).

Statistical analysis of the survival curves in the two groups of patients shows a statistically significant difference (log rank test,  $p=0.03$ ) (Fig. 2).

## Discussion

Survival, free from local recurrence, at 5 years, in patients with positive resection margins was found to be higher compared to that in patients with negative margins resulting from in situ or invasive carcinoma (86.6% vs 79.1%), the difference, however, was not statistically significant.

Nevertheless, it should be pointed out that the post-operative radiotherapy to which patients with positive margins for carcinoma were submitted, could invalidate the interpretation of the prognostic meaning of the resection margins<sup>6,24-26</sup>.

The data emerging from the investigation concerning overexpression of the p53 oncoprotein in the resection margins seem to be the most relevant.

In patients with resection margins showing overexpression of the p53 oncoprotein, survival, free from recurrence, at 5 years, was less favourable, in comparison to that in patients with negative resection margins, the difference being statistically significant (68.4% vs 94.7%). This finding would appear to suggest that p53 oncoprotein plays an important role in tumour progression in those cases presenting resection borders with a dysplastic aspect and p53 overexpression.

In fact, as is well known<sup>13-19</sup>, p53 overexpression increases with progress of the dysplastic lesions.

The presence of p53 overexpression on the resection margins showing various degrees of dysplasia appear to represent a marker of high risk of tumour progres-

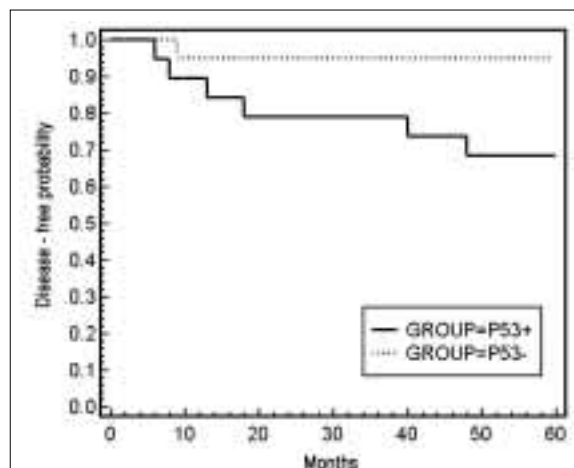


Fig. 2. Survival, free from local recurrence, at 5 years in the group of 20 patients with resection margins negative for p53 oncoprotein overexpression and in the group of 19 patients that expressed p53 oncoprotein in the resection margins (log rank test  $p<0.05$ ).

sion and recurrence. Radiotherapy, in these cases, would be able to prevent neoplastic progression.

According to some Authors<sup>27,28</sup>, in cells containing the normal p53 gene, following damage to DNA from ionised radiation, an arrest of the G2M and G1 phase occurs leading to protection from the damage, caused by radiations, and therefore, to radio resistance, or to apoptosis.

On the other hand, tumoural cells with impaired p53 are no longer able to stop the cell cycle in response to the damage caused by radiation and to mediate apoptosis, for which overexpression of the p53 oncoprotein, due to mutation, should be associated with radioresensitivity<sup>29,30</sup>.

Some studies on the predictive role of the p53 oncoprotein in response to radiotherapy have not found any significant difference between p53 positive and p53 negative patients<sup>30-33</sup>.

We can, therefore, hypothesize that the cellular mechanisms regulating the response to radiotherapy do not necessarily or exclusively play a significant role of the gene tumour suppressor p53, while the role of the functional alteration of this gene in promoting tumour progression seems evident.

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