

## Histopathological prognostic and risk factors in patients with laryngeal neoplasms

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### Abstract

Head and neck cancer is a public health problem, accounting for the fifth place of all human cancers. Each year in the US, 3% of new cases of cancer develop in this sphere. Histopathology of the primary tumor and/or laterocervical adenopathy is essential to establish the therapeutic strategy. Histopathological findings reveal a diagnostic certainty and also provide prognostic information. *Patients and Methods:* We have prospectively studied a number of 95 patients with laryngeal neoplasms, which were treated in the ENT Clinic of Timisoara, Romania. The group of patients with neoplastic disease was subdivided into two groups: group 1 – patients with palpable laterocervical adenopathy and detected primary tumor (T+N+), and group 2 – patients without palpable laterocervical adenopathy, but with detected primary tumor (T+N0). Only patients with squamous cell carcinomas were included in this study. We assessed the following microscope parameters: neoplasm confirmation, histological type of neoplasm, the degree of tumor differentiation, intratumoral inflammatory reaction, perineural infiltration and the cell invasion in the small vessels. All patients underwent surgery. *Results:* N2 and N3 stage adenopathies were more common in patients with medium or low degree of cell differentiation; they were associated with weak inflammatory response of the primary tumor, within vascular invasion alone or associated with the perineural infiltration. N0 and N1 adenopathies were associated with average and good cell differentiation degree, with rich inflammatory infiltrate. *Conclusions:* Histopathological examination of the primary tumor and cervical adenopathy is essential in establishing the diagnosis and the therapeutic approach. Histopathological examination offers information on the prognosis. Cell differentiation degree, inflammatory reaction and the cell invasion in the small vessels are the main prognostic factors of laryngeal neoplasms.

**Keywords:** squamous-cell carcinoma, laryngeal and pharyngeal neoplasms, laterocervical lymph nodes metastasis.

### Introduction

Discovering a tumor at the level of the cervical region represents one of the frequent situations an ENT specialist finds himself in. It poses a real diagnostics problem because it can be the expression of a large diversity of diseases in need of an ample differential diagnostics. The positive diagnostics of the tumor is the result of a complete and competent investigation, which must take into account the anatomic and clinical particularities of the tumor, the occurrence circumstances and the characteristics of the lymphatic circulation, very complex in the cervical region. The neoplastic tumors located in the head and neck sphere are squamous-cell carcinoma although the carcinoma are developed on the surface of the mucous membrane but are extended in depth having a great metastasis capacity on the lymphatic path. This is why the evaluation of the primary tumor and the metastasis or possible metastasis is essential for their diagnosis and treatment. The histopathological examination of the primary tumor and/or the laterocervical adenopathy is essential for establishing the therapeutic strategy. Aside from the diagnostics certainty it establishes, following some histopathological parameters may offer information related to the prognostics of the patients with neoplastic tumors in the laryngeal and pharyngeal sphere. Although

its treatment has been established, an improvement in the survival rates is warranted. The main therapeutic dilemma occurs when a patient presents with an N0 neck that may potentially harbor an occult metastatic disease [1]. Many studies have focused on the research as prognostic markers in laryngeal cancer. The histological characteristics have been subjects of intensive research as prognostic markers. Management of N0 neck is advocated when the possibility of occult neck metastasis is greater than 20% [2]. Locoregional recurrence appears to be particularly important in laryngeal cancer. It can be seen in up to 21% of the patients [3] and was 4.7% in another study [4]. Histological characteristics such as the grade of tumor differentiation have also been reported as prognostic factors [5–7]. Other histological factors that have been implicated with poor prognosis are the extracapsular spread of the tumor in N+ neoplasms [8], the degree of keratinization of the carcinoma [9] and the perineural infiltration or the carcinoma and the cell invasion in the small vessels [10]. These factors must be the mandatory parameters of the histopathological examination.

The aim of the present study is to investigate histological characteristics of laryngeal squamous cell carcinoma and correlations with neck metastasis as prognostic factors.

## ☞ Patients and Methods

Between June 2002 and June 2005, 115 patients with laryngeal neoplasms were diagnosed and treated in our department. Among the 115 patients, 95 were included in the present study. The rest of the patients were excluded from the study because they were treated with radiotherapy, the size of the tumor did not allow the collection of an appropriate tissue specimen, inadequate follow up, or lack of histological proof for verification of diagnosis. During the operation, a tissue specimen from the tumor and neck nodes was collected from each patient. Clinical follow-up ranged from four to nine years. A consent form and patient approval were obtained prior to the operation in order to use the biological material for the study. We also obtained the approval for the study by the bioethics and deontology committee.

The patients with laryngeal neoplasms have been divided in two groups:

- group 1: patients with palpable laterocervical lymph nodes and distinctive primary tumor (T+N+) – 49 patients;
- group 2: patients without palpable laterocervical lymph nodes but with distinctive primary tumor (T+N0) – 49 patients.

The certainty histopathological diagnostics of the primary tumor was established on the tumor fragments sampled before the operation or from the operation part and for the adenopathy from the lymph nodes removed during the operation or the material obtained preoperative through fine-needle aspiration biopsy, drill biopsy or excisional biopsy (cervicotomy). Only the patients with squamous-cell carcinoma have been included in the study. The histopathological examination of the primary tumor investigated the following parameters: the confirmation of neoplasm diagnosis, type of neoplasm histopathology,

degree of tumor differentiation, intratumoral inflammatory reaction, perineural invasion, vascular invasion. For all sample specimens was used Hematoxylin–Eosin (HE) staining.

## ☞ Results

### Patients with distinctive primary tumor and palpable lymph nodes (T+N+)

Forty-nine patients have been studied, all (100%) patients presenting epithelial carcinoma (Table 1).

The grade of tumor differentiation (G) has been determined using the Broder's classification of the squamous-cell carcinoma:

- G1: well-differentiated carcinoma;
- G2: medium differentiated carcinoma;
- G3: poorly differentiated carcinoma;
- G4: non-differentiated or anaplastic carcinoma.

The correlation between the degree of cellular differentiation, the presence of adenopathy and neck lymph nodes recurrence was studied and presented in Table 2.

The inflammatory reaction in the primary tumor was studied on all the histological parts. This was classified in high, moderate and weak according to the incidence of the lymphocytic infiltrate and plasma cell elements (Table 3).

The distribution of patients with distinctive primary tumor and laterocervical palpable adenopathy according to the stage of the adenopathy and the degree of inflammatory reaction of the primary tumor and neck lymph node recurrence is presented in Table 4.

The vascular invasion and the perineural invasion were the last parameters followed when performing the histopathological examination of the primary tumor (Table 5).

**Table 1 – The distribution of patients with distinctive primary tumor and laterocervical palpable adenopathy according to the stage of the adenopathy and the grade of tumor differentiation of the primary tumor**

N / G	N1 (%)	N2 (%)	N3 (%)	Total (%)
G1 (%)	11 (22.44%)	2 (4.08%)	1 (2.04%)	14 (28.57%)
G2 (%)	9 (18.36%)	4 (8.16%)	5 (10.20%)	18 (36.73%)
G3 (%)	4 (8.16%)	6 (12.24%)	4 (8.16%)	14 (28.57%)
G4 (%)	1 (2.04%)	1 (2.04%)	1 (2.04%)	6 (6.12%)
Total (%)	25 (51%)	13 (26.52%)	11 (22.44%)	49 (100%)

**Table 2 – The distribution of patients with distinctive primary tumor and laterocervical palpable adenopathy according to the stage of the adenopathy and the grade of tumor differentiation of the primary tumor and neck lymph nodes recurrence**

N / G	N1 (%)	N2 (%)	N3 (%)	Total (%)
G1 (%)	1 (9.09%)	–	–	1 (9.09%)
G2 (%)	2 (18.18%)	1 (9.09%)	–	3 (27.27%)
G3 (%)	–	3 (27.27%)	4 (36.36%)	7 (63.63%)
Total (%)	3 (27.27%)	4 (36.36%)	4 (36.36%)	11 (100%)

**Table 3 – The distribution of patients with distinctive primary tumor and laterocervical palpable adenopathy according to the stage of the adenopathy and the inflammatory reaction of the primary tumor**

N / Inflammatory reaction	N1 (%)	N2 (%)	N3 (%)	Total (%)
Rich (%)	7 (14.28%)	3 (6.12%)	1 (2.04%)	11 (22.44%)
Moderate (%)	10 (20.40%)	3 (6.12%)	2 (4.08%)	15 (30.61%)
Weak (%)	8 (16.32%)	7 (14.28%)	8 (16.32%)	23 (46.93%)
Total (%)	25 (51.02%)	13 (26.53%)	11 (22.44%)	49 (100%)

**Table 4 – The distribution of patients with distinctive primary tumor and laterocervical palpable adenopathy according to the stage of the adenopathy and the degree of inflammatory reaction of the primary tumor and neck lymph node recurrence**

N / Inflammatory reaction	N1 (%)	N2 (%)	N3 (%)	Total (%)
Rich (%)	–	1 (9.09%)	–	1 (9.09%)
Moderate (%)	–	1 (9.09%)	1 (9.09%)	2 (18.18%)
Weak (%)	3 (27.27%)	2 (18.18%)	3 (27.27%)	8 (72.72%)
Total (%)	3 (27.27%)	4 (36.36%)	4 (36.36%)	11 (100%)

**Table 5 – The distribution of patients with distinctive primary tumor and laterocervical palpable adenopathy according to the perineural invasion and vascular invasion in the primary tumor and parameter N**

N / Invasion mode	N1 (%)	N2 (%)	N3 (%)	Total (%)
Perineural (%)	2 (13.33%)	–	–	2 (13.33%)
Vascular (%)	2 (13.33%)	2 (13.33%)	1 (6.66%)	5 (33.33%)
Both (%)	2 (13.33%)	3 (20%)	3 (20%)	8 (53.33%)
Total (%)	6 (39.99%)	5 (33.33%)	4 (26.66%)	15 (100%)

### Patients with distinctive primary tumor and without palpable clinical adenopathy (T+N0)

Forty-six patients with neoplastic tumors of the larynx were studied but the patients were not found with palpable clinical adenopathy (T+N0). The patients were divided in two groups: a group of 23 (50%) patients who suffered a classical surgery and selective node dissection and who had an investigation during the surgery of the neck lymph nodes and another group made of the same number of patients (23–50%) who experienced a laser CO<sub>2</sub> intervention where there was no dissection of the neck (the policy “wait and see” was applied). On all the 46 (100%) “N0” patients, the microscopic examination of the primary tumor was performed, discovering squamous-cell carcinoma. The same histopathological parameters were followed as in the case of “N+” group (Table 6).

**Table 6 – The distribution of the epidermoid carcinoma cases according to the grade of tumor differentiation (G)**

Degree of tumor differentiation	No. of patients treated through classic surgery (%)	No. of patients treated through laser CO <sub>2</sub> surgery (%)
G1	3 (13.63%)	4 (17.39%)
G2	15 (68.18%)	16 (69.56%)
G3	3 (13.63%)	3 (13.04%)
G4	1 (0.39%)	0
Total	22 (100%)	23 (100%)

The inflammatory reaction within the tumor was another parameter followed. It represents a defense reaction to the neoplastic process, the power of defense being directly proportional to its richness (Table 7).

**Table 7 – Correlations between classic and laser CO<sub>2</sub> surgery regarding inflammatory reaction**

Inflammatory reaction	Classic surgery	Laser CO <sub>2</sub> surgery
Weak	8 (34.78%)	3 (13.04%)
Moderate	8 (34.78%)	10 (43.47%)
Rich	7 (30.43%)	10 (43.47%)
Total	23 (100%)	23 (100%)

The perineural invasion and vascular invasion are two indicators of tumor aggressiveness and is rendered in the Table 8.

**Table 8 – The distribution of the cases according to the perineural/vascular invasion and therapeutic options**

Mode of invasion	Classic surgery	Laser CO <sub>2</sub> surgery
Perineural invasion	6 (26.08%)	6 (26.08%)
Vascular invasion	7 (30.43%)	2 (8.60%)

In order to have a more exact image of the two parameters, we have studied the combinations between them, meaning the division of the patients in (Table 9):

- Patients with tumors without perineural or vascular invasion;
- Patients with perineural invasion and without vascular invasion;
- Patients without perineural invasion and with vascular invasion;
- Patients with perineural and vascular invasion.

**Table 9 – Distribution of “N0” cases according to the combination of the tumor invasion methods and therapeutic option**

Mode of invasion	No. of patients treated through classic surgery	No. of patients treated through laser CO <sub>2</sub> surgery
Without perineural or vascular invasion	13 (56.62%)	17 (39.91%)
With perineural invasion and without vascular invasion	3 (13.04%)	4 (17.39%)
Without perineural invasion and with vascular invasion	5 (21.73%)	0
With perineural and vascular invasion	2 (8.69%)	2 (8.69%)
Total	23 (100%)	23 (100%)

Every case was analyzed separately from the point of view of the combinations of invasion methods, separately for each therapeutic option and was correlated with the possibility of relapse occurrence. The results were given in Table 10.

**Table 10 – Correlations between the combinations of invasion methods and the relapses occurred on clinic “N0” patients**

Mode of invasion	No. of patients with relapse treated through laser CO <sub>2</sub> surgery	No. of patients with relapse treated through classic surgery	Total (%)
Without perineural or vascular invasion	0	2 (22.22%)	2 (20%)
With perineural invasion and without vascular invasion	0	1 (11.11%)	1 (10%)
Without perineural invasion and with vascular invasion	0	3 (33.33%)	3 (30%)

Mode of invasion	No. of patients with relapse treated through laser CO <sub>2</sub> surgery	No. of patients with relapse treated through classic surgery	Total (%)
<i>With perineural and vascular invasion</i>	1 (100%)	3 (33.33%)	4 (40%)
Total relapses	1 (100%)	9 (100%)	10 (100%)

N1 cervical adenopathies are frequently associated with the medium or good degree of cellular differentiation, this association being present at 20 (80%) patients of the total of 25 patients with N1. The N2 and N3 were more frequent in patients with a moderate or weak degree of cell differentiation. In 13 patients with N2, on 10 (76.92%) patients corresponds a G2–G3 degree of differentiation and in 11 N3 patients, on nine (81.81%) patients corresponds a G2–G3 degree of differentiation (Table 1). It can be observed that of the 11 of the lymph nodes failure, seven (63.63%) were encountered in patients with a small degree of cellular differentiation and at 10 (90.9%) in patients with G2–G3 degree of cellular differentiation. If we think at the Broders classification of the squamous-cell carcinoma, where the range of classification of the grade of histological differentiation is rather large (25%), it can be concluded that the occurrence and development of lymph node metastasis from cancers with larynges localization are significantly correlated with the grade of histological differentiation. For patients with “N0”, the tumor grade of histological differentiation has not showed differences between the two groups, the great majority of the tumors presenting a moderate degree of differentiation (G2), encountered at 31 (67.39%) of the patients.

Twenty-three (46.93%) of the 49 patients presented weak inflammatory reaction in the primary tumor but almost 80% of the total patients had moderate to weak lymphocytic infiltrate and plasma cell. Also, as the stage of the adenopathy increases, the inflammatory reaction tends to decrease, thus, in N2 patients, the weak lymphocytic infiltrate and plasma cell is encountered at seven of the 13 patients (53.84%) (Figure 1), whereas in N3 patients, this appears at eight of the 11 patients (72.72%), which is a significant difference. The inflammatory reaction for patients without clinical adenopathy revealed a mild displacement to moderate-rich of the group subject to the laser CO<sub>2</sub> surgery (20 patients – 43.47%) in comparison to the classic surgery group (32.6%). It is mandatory to correlate the plasma cell infiltrated in the primary tumor (Figure 2) with the stage of the adenopathy and with the presence of the nodes relapses. Eight (72.72%) patients with tumors discovered with weak plasma cell infiltrate have developed node relapse and only one case (9.09%) with rich lymphocytic infiltrate and plasma cell has developed node relapse. Also, it is observed that in patients with N3 metastatic laterocervical adenopathy, plasma cell infiltrate was very poor represented in three of the four patients (75%) but also in three patients with N1 stage (Figure 3), although they had smaller laterocervical adenopathies, all three (100%) have developed lymph node metastasis. We can conclude based on this data that there is no linearity relation between the increase of volume of the lymph nodes and the defense

reaction expressed by the inflammatory reaction, the metastasis being encountered also on small size ganglions when the defense capacity of the body is over passed.

The perineural and vascular invasion was discovered in 15 patients of the group we studied. From these, perineural invasion was discovered in two (13.33%) patients, only vascular invasion in five (33.33%) patients, and eight (53.33%) patients presented both with perineural invasion and with perivascular invasion. Given the fact that singular perineural invasion was found only in two patients of the total of 15, it may be concluded that it does not have a significant statistic correlation with the occurrence of metastasis whereas singular vascular invasion found in five (33.33%) patients of the 15 patients seems to have a statistic significance if we take into consideration also its involvement to the eight (53.33%) patients with both types of invasions. Expressed mathematically, it can be observed that the probability of metastasis by perineural invasion (13.33% + 53.33% = 66.66%) is much lower than the one with vascular invasion (33.33% + 53.33% = 86.66%) which is closer to the absolute value.

Analyzing the two parameters for “N0” patients, it may be observed that there are no significant differences between the two groups of patients and, thus, it has no significant importance in the occurrence and development of local and/or nodal recurrence. We can make this statement taking into consideration the relapses recorded on the periodical examinations of the patients. In the group of the 23 patients operated through laser CO<sub>2</sub> during the study, only one local relapse (4.43% of the 23 patients and respectively, 2.17% of the total studied group of 46 patients) was recorded. In the case of patients treated with classic surgery, local and/or nodes relapses occurred on nine patients (39.13% of the group or 19.56% of the entire group of 46 patients). If we follow the two methods of invasion it may be observed that on the patients with classic surgery, the two parameters have similar values, the vascular invasion was present at seven (30.43%) of the 23 patients and the perineural invasion was present at six (26.08%) patients. For the patients of the second group, the perineural invasion was present at six (26.08%) patients whereas the vascular invasion was present only at two (8.6%) patients.

The perineural and vascular invasion was discovered in a number of 15 patients of the group studied. From these, only perineural invasion was discovered at two (13.33%) patients only vascular invasion at five (33.33%) patients and eight (53.33%) patients presented with both perineural invasion and perivascular invasion.

The interpretation of the data registered in this table offer very important information, that the relapses in the larynges cancers are associated mostly with vascular invasion. Seventy percent of the relapses registered in the patients who formed the entire group have presented tumors only with vascular invasion and/or perineural invasion. If we can take into account that the perineural invasion was involved singularly only on a small percentage of the total relapses, it means that within this combination with perivascular invasion, the primordial role in the occurrence and development of the relapses was held by the vascular invasion.



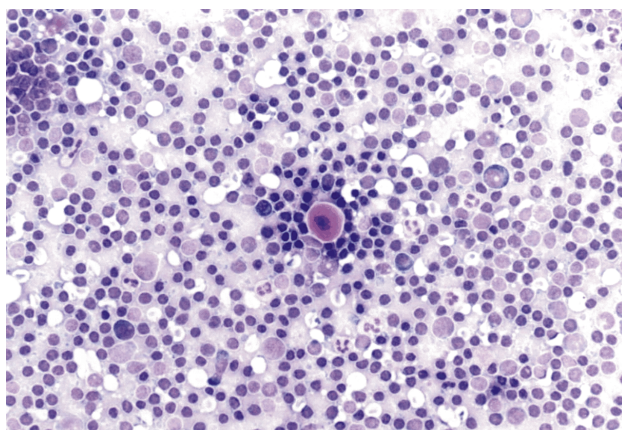


Figure 1 – Metastasis of squamous cell carcinoma (cytodiagnosis). BPT–Drăgan staining,  $\times 400$ .

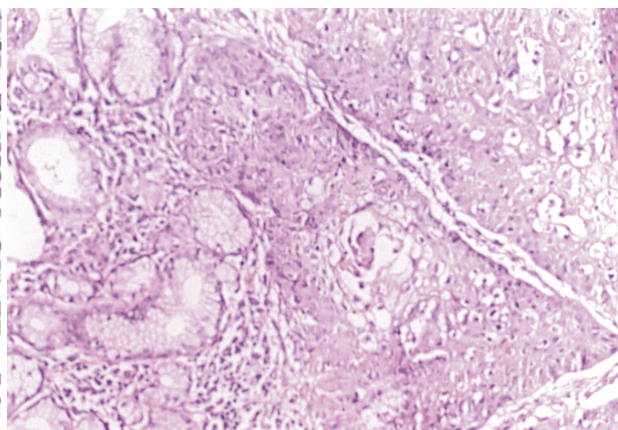


Figure 2 – Squamous cell carcinoma. HE staining,  $\times 200$ .

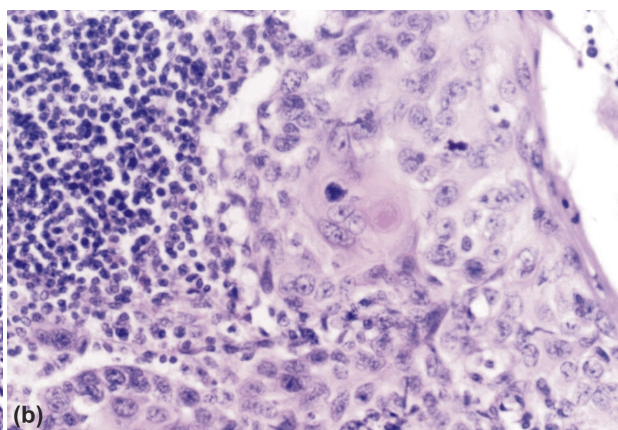
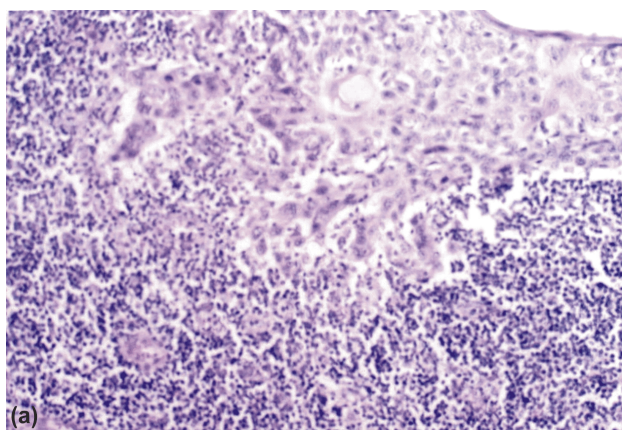


Figure 3 – Metastasis of squamous cell carcinoma. HE staining: (a)  $\times 200$ ; (b)  $\times 400$ .

## Discussion

The same results are obtained by Yilmaz T *et al.* [11] on a group of 94 patients with laryngeal neoplasm stating that vascular invasion would play an independent role in the development of nodes relapses, reducing also the time frame between the intervention and occurrence of neck node recurrence.

Obtaining a significant static correlation with perineural invasion by Magnano M *et al.* [12] who studied 207 patients with laryngeal neoplasm, the basis of the tongue and hypopharynx and by Hoşal AS *et al.* [13] on a group of 60 patients with tongue neoplasm.

Nakayama A *et al.* [14] have tried to determine the development mechanism of the cervical metastasis studying 23 patients with oral cavity cancer, following aside from the histological parameters of the tumor, also the diameter of the lymphatic vessels inside the tumor in comparison to the tissues not invaded by the tumor. They find a significant static correlation only by increasing the diameter of the intratumoral and peritumoral lymphatic vessels and the degree of cellular differentiation of the tumor, the other factors lacking static significance.

Laramore GE and Coltrera MD [15] mention that, globally, 20–25% of the cancer patients in the head and neck region develop cervical metastasis.

Vogl ES [16] quoting Millon RR and Cassisi N [17], state that the metastasis rate depends on the localization and size of the primary tumor (T), with the exception of rhinopharynx and pyriform sinus to which the metastasis rate is dependent on T.

Cerne C *et al.* [18] tried to discover the patients with elevated risk of developing local or nodal recurrences and define these factors, which would have the highest predictive value from the point of view of nodes reactivity. For this, they studied a group of 26 patients with squamous-cell cancer of the oral cavity and clinic “N0” who were subject to the surgery treatment for the primary tumor and selective neck dissection for the lymph nodes areas. The lymph nodes found and removed during the dissection were histopathological investigated. The dominant lymph node reactivity was defined as primarily lymphocytic with germination centers, normal, with sinus histiocytosis or with lymphocyte depletion. These parameters were compared in patients who did not have local or nodal recurrence neck signs at the periodical examinations to the patients who presented local or neck node recurrence. Following the study, it was showed at the patients without relapses that these parameters were present in a percentage of 73%, with the exception of the germination centers, which was 27% but which was present in percentage of 73% of the patients with local or neck node recurrence.

The grade of differentiation of the carcinoma affects the recurrence rate in our study. Vlachtsis K *et al.* [19] reports the grade of histological differentiation of the carcinoma did not affect significantly the recurrence rate or the survival. Some authors report the same conclusion regarding the grade of differentiation [20–22]. Other authors reports that the grade of differentiation has prognostic significance [6] and others say that routinely

used histological differentiation degree has limited application because of only partly correlation with cancerous process [23, 24]. In our study, inflammatory reaction represents an important prognostic factor in patient with laryngeal carcinoma. The same results report Takes RP *et al.* [25]. Bryne M *et al.* [9] and Almadori G *et al.* [26] considers that peritumor inflammatory response, and vascular and perineural invasion important prognostic factors.

## ✉ Conclusions

The accurate pathologic staging of the neck metastases in patients with upper aerodigestive tract squamous cell carcinoma is important for providing prognostic information and optimizing the treatment plan for the patient. Histopathological examination offers information on the prognosis. Grade of differentiation, inflammatory reaction and the tissue invasion are the main prognostic factors of laryngeal neoplasms.

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