

## CASE REPORT

# Primary neuroendocrine small cell carcinoma in larynx: case report and literature review

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## ABSTRACT

**BACKGROUND.** Neuroendocrine tumors of the larynx represent a rare group of neoplasms characterized by pathological and biological heterogeneity. The histological and immunohistochemical diagnosis is the most important step in the appropriate management of these tumors and the prognosis varies according to histological types. Conventional anatomical and functional imaging can be complementary for diagnosis, staging and monitoring of treatment response.

**MATERIAL AND METHODS.** Here we report on a case of a laryngeal neuroendocrine small cell carcinoma occurring in a 67-year-old man who was referred to our clinic for clinical reevaluation, diagnosis and treatment. The clinical presentation, the histopathological and immunohistochemical examination and management of this kind of tumor are highlighted.

**CONCLUSION.** Small cell neuroendocrine carcinomas are very aggressive neoplasms. Patients could benefit from surgery, but radiotherapy and chemotherapy remain the treatment of choice. Very low incidence of neuroendocrine tumors in the larynx and specifically very poor prognosis of neuroendocrine small cell carcinoma encouraged an extensive literature review.

**KEYWORDS:** small cell carcinoma, laryngeal, neuroendocrine, prognosis.

## INTRODUCTION

Neuroendocrine neoplasms of the larynx are a heterogeneous group of tumors and the most common nonsquamous types arising in this region. The origin of these tumors has not been clearly identified but pathologists believe that precursor cells belong to the diffuse neuroendocrine system<sup>1</sup>. The recent World Health Organization book simplified former classifications dividing neuroendocrine tumors in 3 subtypes: well-differentiated neuroendocrine carcinoma (typical carcinoid, neuroendocrine carcinoma grade I), moderately-differentiated neuroendocrine carcinoma (atypical carcinoid tumor, neuroendocrine carcinoma grade II) and poorly-differentiated neuroendocrine carcinoma (small cell neuroendocrine carcinoma and large cell neuroendocrine carcinoma)<sup>1</sup>. The most frequent type is the atypical carcinoid, while the typical carcinoid is a very rare tumor<sup>2,3</sup>. Laryngeal tumors with neuroendocrine morphology are a rare and diverse

group of neoplasms, which share specific pathological and immunohistochemical features, with prognosis dependent on the tumor type. They have received increasing attention in recent years because of a controversial terminology that was in need of standardization<sup>4</sup>.

Small cell carcinoma neuroendocrine type (SCC-NET) is a rare tumor (2.5-4% of all small cell carcinomas) and, according to one study, during the past 30 years only 160 cases of primary small cell carcinoma of the larynx have been published worldwide<sup>2</sup>. Most of the patients are between 60 to 70 years of age, with the male population being more commonly affected<sup>5</sup>. Heavy smoking is the most important risk factor involved in tumor etiology<sup>6</sup>. Clinical presentation is non-specific, sharing similar features to other types of laryngeal carcinomas. In consequence, diagnosing small cell carcinoma of the larynx relies strictly on histopathological examination and immunohistochemical testing<sup>7</sup>. Primary small cell carcinoma of the larynx

continues to challenge oncologists in treatment options. This is one of the most lethal of neoplasms associated with early widespread metastases and poor prognosis. Some studies reported a two- and five-year survival of 16% and 5% respectively<sup>8</sup>. Presence of metastases at the initial clinical presentation and treatment options are probably the most important features to consider for patient survival.

## CASE REPORT

A 67-year-old male patient was referred to our center with dysphonia and inspiratory dyspnea, with a progressive aggravating evolution for over 7 months. The patient had a 40 pack-year history of cigarette smoking with complete cessation 3 months prior to admission and reported daily alcohol consumption. The patient reported working in a toxic environment for over 10 years.

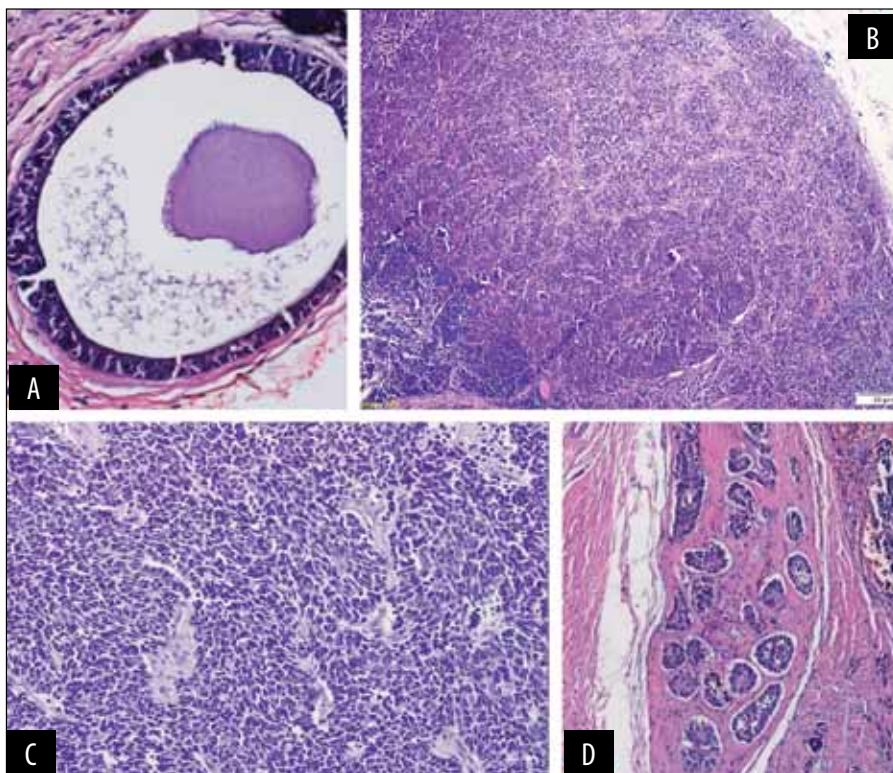
Past medical records outlined a prior admission in another ENT center for similar symptoms 2 months before he was referred to our clinic. Endoscopic biopsies with subsequent histopathological examination performed there suggested well-differentiated invasive squamous cell carcinoma.

On admission, we performed a full clinical workup including a chest X-ray that excluded other lesions, a

cardiology consult that revealed grade I/II arterial hypertension and a short PR interval on the electrocardiogram and an additional upper gastrointestinal series (barium meal) that showed no lesions.

In addition to a complete ENT examination, we performed an indirect laryngoscopy and nasopharyngolaryngeal fibroscopy that showed an ulcerated exophytic mass that infiltrated the right hemilarynx with extension to the anterior commissure and into one third of the vocal cord. Imagistic studies such as computer tomography and chest X-ray confirmed our results and excluded other lesions in other organs. Total laryngectomy with bilateral neck dissection was performed.

Gross examination showed a 2.5/2/1.5 cm exophytic, ulcerated glottic lesion which comprised both commissures and vocal cords and extended in the subglottic space. Specimen samples were fixed with 10% buffered formalin and were processed by conventional histopathological methods using paraffin embedding, sectioning and Hematoxylin–Eosin (HE) staining. Microscopic examination highlighted a tumor which grew in nest, sheets or trabeculae, with diffuse, cribriform, ribbon-like, pseudoglandular and rosette-like pattern (Figure 1A,B,C), composed of small to spindle-shaped cells with hyperchromatic granular (or “salt and pepper”) chromatin, scant cytoplasm, inconspicuous nucleoli, nuclear molding, high mitotic activ-



**Figure 1** A. 20x: Small cell carcinoma, neuroendocrine type: tumor showing a pseudoglandular/acinar pattern. B. 4x: tumor composed of sheets or ribbons of closely packed cells. C. 20x: tumor composed of closely packed cells with inconspicuous cytoplasm and oval/spindle nuclei with dense chromatin and absent nucleoli. Mitoses and apoptosis are present. D. 10x: tumor displaying a rosette architectural pattern.

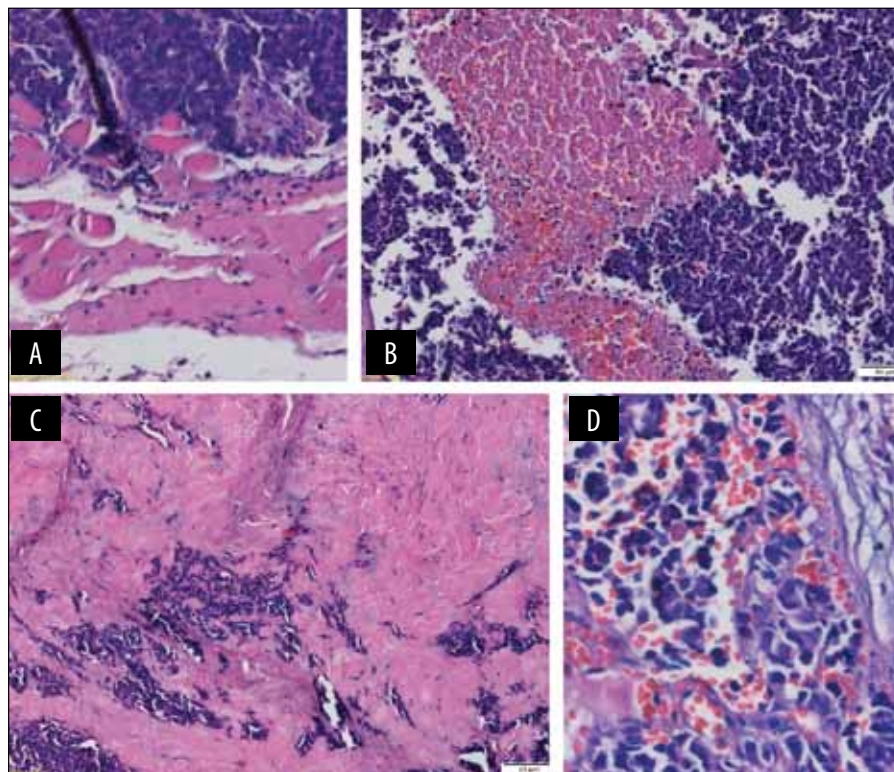
ity (more than 10 mitoses per 2 mm<sup>2</sup> or 10 high-power fields) and apoptosis (Figure 1D); the tumor infiltrated both the surface epithelium and laryngeal skeletal muscle fibers (Figure 2A); extensive areas of tumor necrosis (Figure 2B), neurotropism and angioinvasion (Figure 2D) were present. The tumor stroma showed extensive hyalinization (Figure 2C).

We have also performed immunohistochemical tests. The paraffin blocks acquired by histopathological processing were sliced at a microtome resulting in sections of 3- $\mu$ m thickness that were mounted on slides covered with poly-Lysine. After that, the sections were deparaffinised in toluene and alcohol successive baths, one hour (15 minutes by bath), rehydrated (three successive alcohol baths with decreased concentration: 96%, 80% and 70% (10 minutes in each bath) and followed by a bath with distilled water, where the sections were hold for 10 minutes. Washing in PBS (phosphate-buffered saline), incubation with normal serum, for 20 minutes, incubation with primary antibody over-night, Dako LSAB kit, washing in carbonate buffer and development in 3,3'-diaminobenzidine hydrochloride/ hydrogen peroxide and nuclear counterstain with Mayer's Hematoxylin were performed. We used the following antibodies from Leica: TTF1 antibody (clone: SPT24, dilution 1:100); immunologic: AE1/AE3 antibody (clone: AE1/AE3,

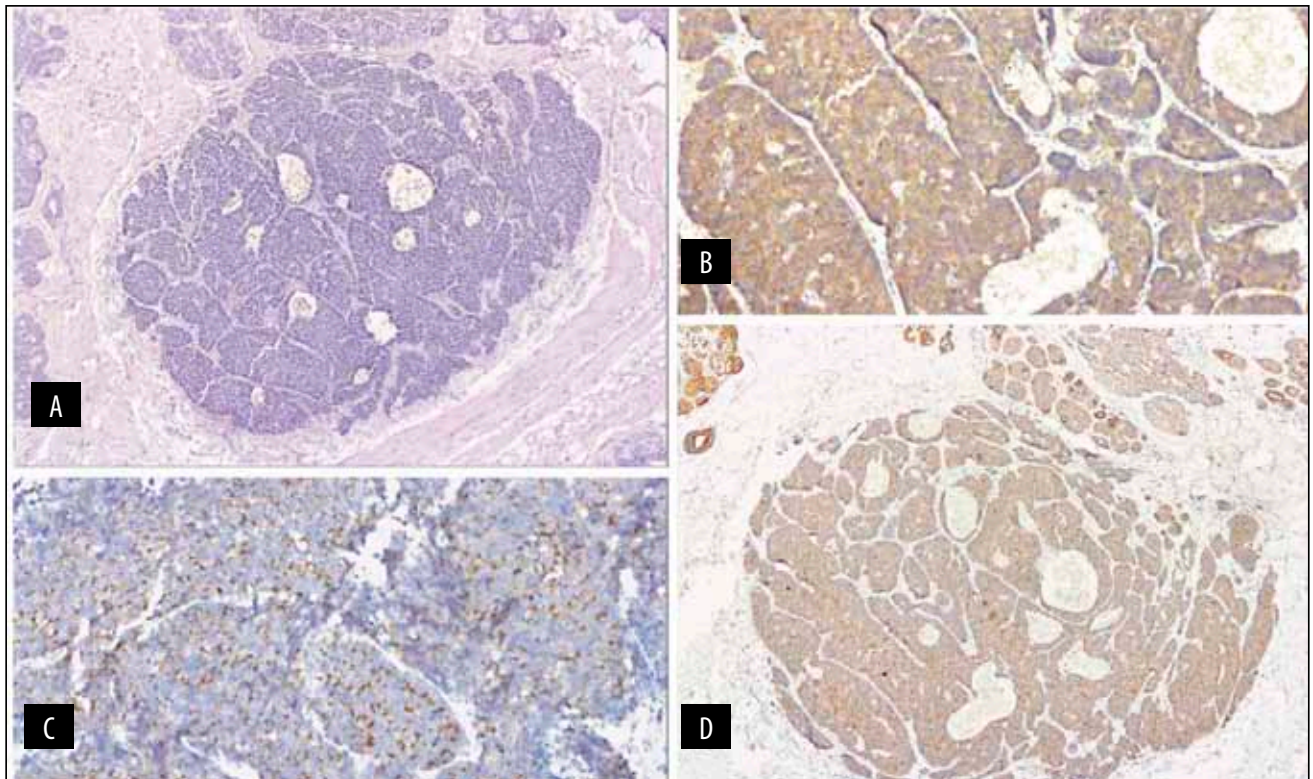
dilution 1:200); Cell Marque: Synaptophysin antibody (clone: MRQ-40, dilution 1:500); Cell Marque: Chromogranin A (clone: LK 2H10, dilution in 1:100); Ventana: p16 Cintec ready-to-use. We chose to perform immunohistochemistry testing on paraffin blocks that contained various tumor architectural patterns. Initial hematoxylin-eosin examination showed solid, insular and pseudoglandular patterns (Figure 3A). Synaptophysin markers (Figure 3B), chromogranin (Figure 3C) and AE1-AE3 (Figure 3D) were positive in tumour cells in a cytoplasmic granular pattern; Ki67 was positive in 60-65% of tumor cells. We also performed TTF1 marker (Figure 4A,B) which showed diffuse nuclear staining in tumor cells and p16 marker (Figure 4C,D) which showed similar intense nuclear staining. Metastatic carcinoma was present in 4 out of 13 lymph nodes (the largest 1.5 cm). Final histopathological TNM classification was pT3N2c.

## DISCUSSIONS

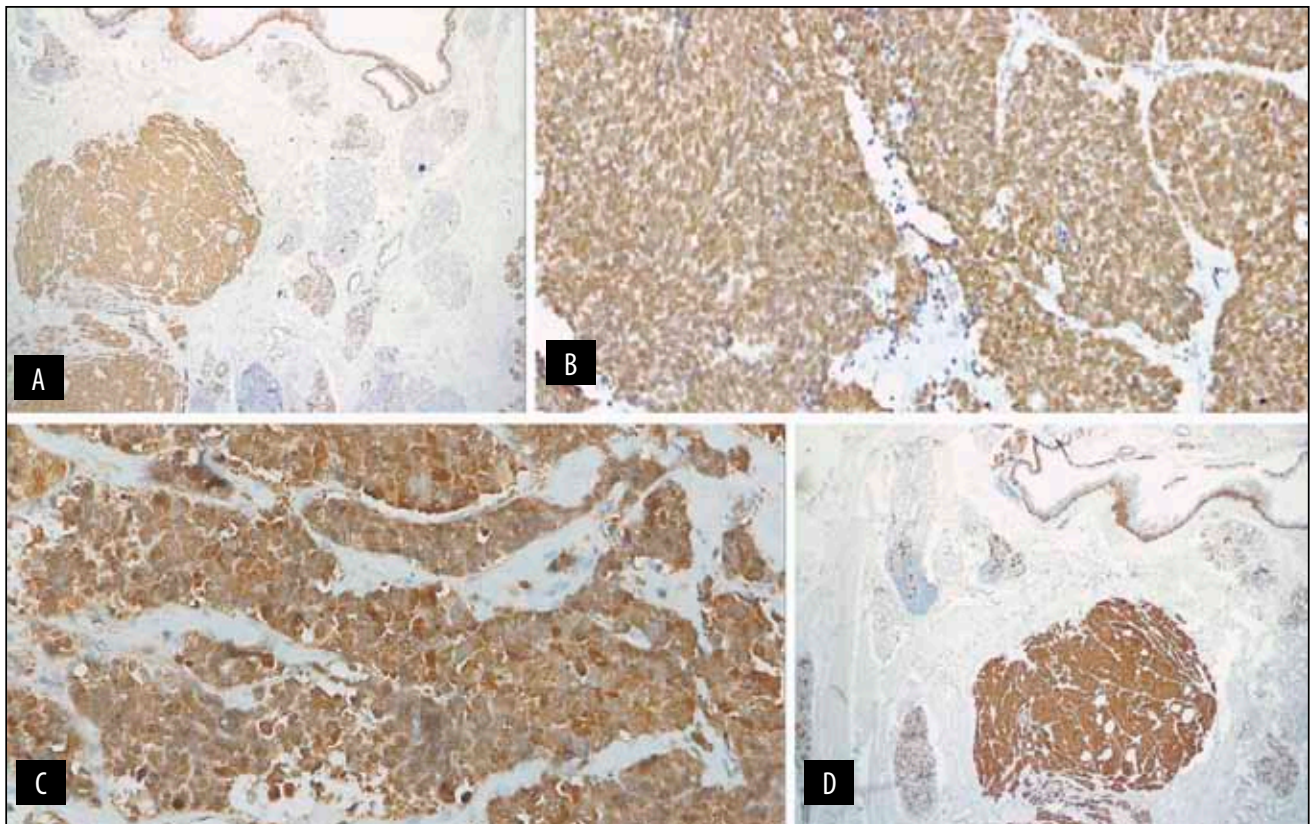
Neuroendocrine tumors of the larynx represent a rare group of neoplasms characterized by pathological and biological heterogeneity. The histological diagnosis is the most important step in the appropriate management of these tumors and sets guidelines regard-



**Figure 2** Small cell carcinoma, neuroendocrine type: A. 20x: Tumour infiltrating skeletal muscle fibers. B. 20x: Tumour showing foci of tumor necrosis. C. 10x: Extensive hyalinization surrounding tumor cells. D. 40x: Angiolymphatic invasion present.



**Figure 3** Small cell carcinoma neuroendocrine type: **A.** 5x: Tumor displaying solid, insular and pseudoglandular architectural patterns. **B.** 10x: Synaptophysin: positive staining in a granular cytoplasmic pattern. **C.** 20x: Chromogranin A: positive staining in a granular cytoplasmic pattern. **D.** 5x: AE1/AE3: positive staining in a granular cytoplasmic pattern.



**Figure 4** **A., B.** 2,5x, 20x: TTF1 intense nuclear staining in tumor cells and normal epithelium. **C., D.** 2,5x, 20x: p16 intense nuclear and cytoplasmic staining in tumor cells and normal epithelium.

ing prognosis and survival according to histological types. The present study reports a rare case of laryngeal small cell neuroendocrine carcinoma (SCCNET), the second most common subtype after atypical carcinoid, an unusual neoplasm accounting for only 0.5% of all laryngeal carcinomas<sup>1</sup>.

Neuroendocrine small cell carcinomas are usually seen in males (3/1 male/female ratio) with a median age at diagnosis ranging from 60 to 70 years<sup>9,10</sup>. An overwhelming percentage of patients have a history of heavy tobacco smoking<sup>1,11</sup>. Other research reports a female patient, 40 years old, with a history of consuming chewable forms of tobacco<sup>11</sup>. In our case, in addition to smoking, our patient had cumulative risk factors including heavy alcohol consumption and environmental exposure to toxic substances. Frequent presenting symptoms include dysphonia, dysphagia, or in some cases a neck mass<sup>12</sup>. Paraneoplastic processes such as Eaton-Lambert syndrome and syndrome of inappropriate secretion of antidiuretic hormone may occur in association with laryngeal neuroendocrine small cell carcinoma that can follow the clinical history and thus be useful for monitoring its evolution<sup>13</sup>. Although this rare tumor has been reported at various locations in the larynx, the supraglottic region is the most frequently involved site that was also present in our case<sup>10</sup>.

Conventional imagistic procedures such as computer tomography or indirect laryngoscopy are non-specific when it comes to tumor clinical behaviour and long-term prognosis. One study suggested that small cell neuroendocrine carcinoma of the larynx should be included in the diagnostic considerations when the CT-scan shows a laryngeal mass accompanied by large cervical lymph nodes without necrosis<sup>7</sup>. Grossly, the tumors usually arise submucosally, have a fleshy, ulcerated aspect and may vary in size from 0.5 cm to a size of 4 to 5 cm. Histologically, small cell neuroendocrine carcinoma displays diverse architectural patterns including nests, sheets, trabeculae with occasional nuclear palisading or rosette-like structures. This tumor is highly infiltrative, with frequent perineural and lymphovascular invasion. Tumor cells are small to medium-sized cells with hyperchromatic nuclei, finely granular chromatin and indistinct nucleoli with scant cytoplasm. Some of the classical features described are a high mitotic rate, necrosis, apoptosis, prominent crush artifact, the Azzopardi phenomenon and nuclear moulding<sup>1</sup>. In our patients' situation, computed tomography and indirect laryngoscopy did not show any particular feature that could point to a specific type of tumor. Additional chest X-ray excluded other lesions. Based on the morphology of this tumor, we established that the histopathological subtype of this poorly differentiated neuroendocrine carcinoma was small cell neuroendocrine carcinoma.

Small cell carcinoma may be immunoreactive for cytokeratins (particularly low-molecular-weight cytokeratins) and for general neuroendocrine markers, including chromogranin, CD56 and synaptophysin. In addition, small cell neuroendocrine carcinoma may be positive for thyroid transcription factor-1<sup>3,4</sup>. Our case showed a specific immunohistochemical pattern with a positive cytoplasmic granular staining for synaptophysin and chromogranin A, positivity for a cytokeratin, AE1/AE3, and a high proliferation index (Ki67) that confirmed the histopathological diagnosis. Considering other research studies for this kind of tumor, we also decided to perform immunohistochemistry for TTF1, which was undoubtedly positive in a nuclear pattern both in the tumor and in normal epithelium.

Human Papilloma Virus (HPV) is frequently involved in the development of high-grade neuroendocrine carcinomas in the anatomical sites in which this viral infection has a main oncogenic role<sup>14</sup>. High-grade neuroendocrine carcinomas of the head and neck, particularly of the larynx, have recently been associated with HPV infection<sup>1</sup>, but there is no sufficient scientific data regarding prognosis or survival. However, this kind of tumor has shown strong, diffuse positive p16 immunostaining. Recent research attributed this fact to specific immunological mechanisms, particularly Rb pathway dysregulation. Awareness of this immunohistochemical pattern of expression may avoid a possible diagnostic difficulty with HPV-associated non-keratinizing squamous cell carcinomas, which have a better prognosis and different treatment<sup>14</sup>. In light of this research, we decided to use p16 marker and, to our surprise, it showed intense nuclear and cytoplasmic staining in the tumor and normal epithelium. Although this finding can be attributed to the mechanism described earlier, we found it unusual and at the same time useful for ongoing research on different proteins involved in cell cycle regulation such as p16.

Differential diagnosis is essential when confronted with the possibility of a primary small cell neuroendocrine carcinoma. Initial systemic work-up is essential in order to evaluate whether extrapulmonary SCCNET of interest is primary or metastatic from the lung. There are a number of reports showing that SCCNET, which had been considered to originate primarily in the head and neck, turned out to be a metastasis from the lung<sup>15</sup>. TTF1 positivity is an additional confusing element as it is both positive in primary small cell carcinoma and in the metastatic one. In our case, however, we confirmed the larynx to be the primary location, using extensive imagistic studies.

Following the discharge from the hospital, we referred our patient for oncological evaluation and treatment. Unfortunately, we have no further information on the patient's follow-up or survival. Definitive

diagnosis before beginning treatment is of the utmost importance in order to administer targeted therapy to laryngeal SCCNET. Principally, other neuroendocrine neoplasms of the larynx such as typical and atypical carcinoid or large cell neuroendocrine carcinoma should be ruled out as they benefit from different prognosis<sup>16</sup>. Surgical procedures such as total laryngectomy, which cause a crucial decline in quality of life, are not advisable as a first treatment of choice, because laryngeal SCCNET is a lethal disease with a severely poor prognosis<sup>17</sup>.

General oncological consensus provides as treatment of choice for this type of tumor radiotherapy in combination with adjuvant or concurrent chemotherapy. Systemic chemotherapy is not recommended for laryngeal SCCNET even at early stage, because there is always a risk of occult metastases<sup>18</sup>. Patient survival has been associated with disease extent, which has proven to be a precise prognostic characteristic<sup>19</sup>.

Small cell neuroendocrine carcinoma of the larynx displays poor prognosis and materializes in a catastrophic clinical course. It is considered the most lethal malignancy of the larynx. More than 90% of patients have an unfavourable disease progression with extensive metastasis. Cervical lymph nodes, liver, lung, bones and bone marrow are considered the most predisposed sites for metastatic disease. Some researchers believe that small cell neuroendocrine carcinoma of the larynx, much like its lung counterpart, should be viewed as a systemic disease<sup>20</sup>. According to a 2008 publication, using the National Cancer Institute's Surveillance and End Results database, researchers outlined that 5-year survival for small cell carcinoma for specific sites such as glottis and supraglottis was 15% and 24.1%, respectively<sup>21</sup>.

## CONCLUSIONS

Primary SCCNET of the larynx is equally an unusual and lethal neuroendocrine neoplasm with an established etiopathogenesis that incriminates, among others, smoking and alcohol consumption as risk factors. Clinical presentation is rather nonspecific because it shares similar features with other types of laryngeal tumors. Histopathological and immunohistochemical studies are essential for diagnosis and patient care management. Differential diagnosis is mandatory and requires correlation with clinical and imaging information in order to exclude tumors from other sites. Considering the estimated survival for this malignancy, first treatment options should be focused on radiotherapy and chemotherapy, especially on cervical lymph nodes, in order to secure local control. Surgery should be viewed as an alternative option regarding patient management in view of severe de-

crease in quality of life and no improvement in clinical survival.

**Conflict of interest:** The authors have no conflict of interest.

**Contribution of authors:** All authors have equally contributed to this work.

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