

## CASE REPORT

# Large cell neuroendocrine carcinoma of the larynx: a case report and a review of the classification of this neoplasm

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*J Clin Pathol* 2005;58:658–661. doi: 10.1136/jcp.2004.019927

This report describes a case of large cell neuroendocrine carcinoma (LCNEC) of the larynx. A 74 year old man who presented with otalgia underwent direct laryngoscopy with biopsy, which revealed an invasive poorly differentiated carcinoma. Laryngectomy with bilateral neck dissections revealed invasion of the pre-epiglottic space by the tumour, with metastases to bilateral lymph nodes (AJCC T3N2c). The tumour was characterised by large cells with vesicular chromatin and prominent nucleoli. The cells were arranged in organoid and trabecular patterns with a background of extensive necrosis and numerous mitotic figures. Immunohistochemical and ultrastructural analyses confirmed the neuroendocrine nature of the tumour. Metastatic disease was present in the liver, and the patient died within weeks of surgery. LCNEC carcinoma is a rare tumour of the larynx. Recognition at this site is essential so that proper patient management can be initiated.

Pulmonary neuroendocrine tumours are relatively common neoplasms that were recently classified by the World Health Organisation (WHO).<sup>1</sup> Four different tumour types were recognised: typical carcinoid, atypical carcinoid, small cell carcinoma, and large cell neuroendocrine carcinoma (LCNEC).<sup>1</sup> Criteria for the diagnosis of these neuroendocrine tumours include mitoses, necrosis, and cell size. Specifically, LCNEC has neuroendocrine morphology, a high mitotic rate ( $> 10/2 \text{ mm}^2$ ), and necrosis.<sup>1</sup> The cells are large with moderate to abundant cytoplasm, vesicular or finely granular chromatin, and frequent nucleoli.<sup>1</sup> In addition, positive immunohistochemical staining for one or more neuroendocrine markers must be present and/or neurosecretory granules demonstrated by electron microscopy.<sup>1</sup> Recently, LCNEC has been reported in extrapulmonary sites such as the bladder,<sup>2</sup> gallbladder,<sup>4</sup> and the ampulla of Vater.<sup>3</sup>

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In the larynx, squamous cell carcinoma is the most common malignant tumour.<sup>6</sup> Although less common, many neuroendocrine tumours have been reported in the larynx, with the first case being reported in 1969.<sup>7</sup> Most cases have been reported as carcinoids, atypical carcinoids, malignant carcinoids, and neuroendocrine carcinomas, not otherwise specified.<sup>7</sup> Using the strict morphological criteria for pulmonary LCNEC set forth in the 2004 WHO classification, we report such a tumour arising in the larynx.



**Figure 1** Supraglottic mass involving the left false vocal cord with compression of the left ventricle.

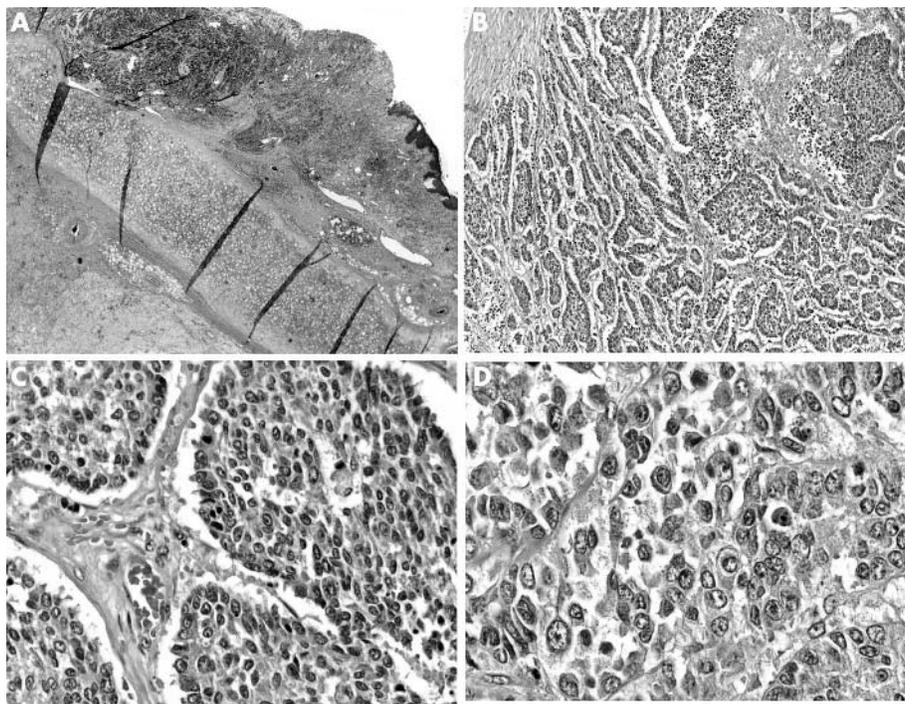
### CASE REPORT

A 74 year old man with a 65 year smoking history and a history of colon cancer in 1984 presented with otalgia. Direct laryngoscopy with biopsy revealed an invasive poorly differentiated carcinoma. Laryngectomy with bilateral modified neck dissections was performed. The tumour was supraglottic in location, and involved the left false vocal cord with compression of the left ventricle. It was exophytic and tan yellow to tan grey and friable (fig 1). The tumour invaded the pre-epiglottic space, with metastases to bilateral lymph nodes (AJCC T3N2c). Ultrasound biopsy of the liver revealed metastatic disease. The patient died within weeks of surgery. The cause of death was unknown. No postmortem examination was conducted.

### MATERIALS AND METHODS

Sections were examined under routine light microscopy using formalin fixed, paraffin wax embedded tissue stained routinely with haematoxylin and eosin. Immunohistochemistry with a panel of stains including antibodies to keratin AE1/AE3 (Chemicon International, Temecula, California, USA; 1/500 dilution), chromogranin (Hybritech, San Diego, California, USA; 1/2500 dilution), synaptophysin (Biogenex, San Ramon, California, USA; 1/400 dilution), and calcitonin (Dako, Carpinteria, California, USA; 1/300 dilution) was performed on the paraffin wax blocks. The immunohistochemical analysis of both the tumour and the positive and negative controls was performed on a Dako automated immunostainer with the Envision Plus detection system preceded by antigen retrieval in a citrate solution (DakoCytomation, Carpinteria, California, USA). A representative fragment of formalin fixed tumour was submitted in Karnovsky's fixative for electron microscopy. The tissue was minced and postfixed in 1% osmium tetroxide. The tissue was

**Abbreviations:** LCNEC, large cell neuroendocrine carcinoma; WHO, World Health Organisation



**Figure 2** Neoplastic cells infiltrating the epiglottic soft tissues with ulceration of the overlying squamous mucosa. Tumour cells are arranged in organoid and trabecular patterns with pseudorosette-like structures, abundant mitoses, and necrosis. The cells are large with eosinophilic cytoplasm, vesicular chromatin, and prominent nucleoli (haematoxylin and eosin stained; original magnification:  $\times 2$  (A),  $\times 10$  (B),  $\times 40$  (C), and  $\times 60$  (D)).

then dehydrated through a graded series of ethanols, infiltrated with Spurr's resin, and polymerised at  $65^{\circ}\text{C}$  overnight. Thin sections ( $1\ \mu\text{m}$  thick) were cut and appropriate areas selected for transmission electron microscopy. Ultrathin  $100\ \text{nm}$  sections were cut and placed on copper grids and contrasted with uranyl acetate and lead citrate. Representative sections were imaged using a JEOL 1210 (Peabody, Massachusetts, USA) electron microscope.

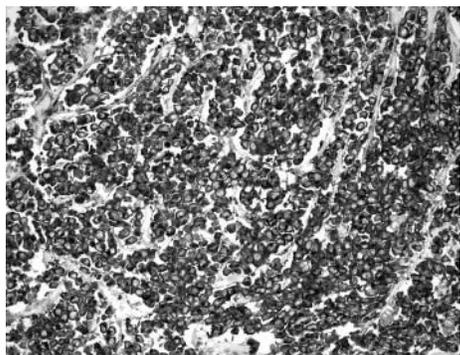
## RESULTS

Histology revealed an infiltrative mass with extension into the epiglottic soft tissue. The tumour extended to the surface epithelium with extensive ulceration. Focal lymphovascular invasion was identified. Perineural invasion was not present. The individual tumour cells were large with abundant eosinophilic cytoplasm, vesicular chromatin, and prominent nucleoli. The cells were arranged predominantly in organoid and trabecular patterns with scattered pseudorosette-like structures. There was extensive necrosis and numerous

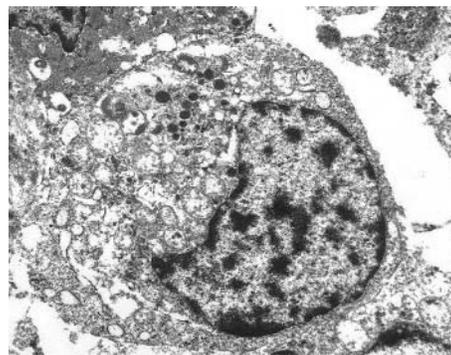
mitotic figures ( $> 10/10$  high power fields; fig 2A–D). In the presence of appropriate positive and negative controls, immunohistochemistry revealed strong and diffuse cytoplasmic staining for chromogranin and synaptophysin (fig 3). In addition, antikeratin AE1/AE3 showed strong cytoplasmic staining. Calcitonin was negative. Ultrastructural analysis revealed dense core neurosecretory granules (fig 4). The morphology and cytology of the tumour cells in the bilateral cervical lymph nodes and the liver were identical to that seen in the laryngeal tumour.

## DISCUSSION

The 1991 WHO classification of laryngeal tumours divided neuroendocrine neoplasms into carcinoid, atypical carcinoid, small cell carcinoma, and paraganglioma.<sup>8</sup> Included in the atypical carcinoid category are moderately differentiated neuroendocrine carcinoma, large cell neuroendocrine carcinoma, and medullary carcinoma-like carcinoid.<sup>9</sup> Atypical carcinoid is the most frequent of the neuroendocrine lesions,



**Figure 3** Cytoplasmic immunoreactivity with anti-synaptophysin (immunoperoxidase; original magnification,  $\times 20$ ).



**Figure 4** Electron microscopy: neurosecretory granules in the cytoplasm of the tumour cell.

with Barnes estimating that they are at least 15 times more common than typical carcinoid.<sup>9</sup> Histologically, the atypical carcinoid category is characterised by larger cells, prominent nucleoli, occasional mitoses, spotty necrosis, pleomorphism, and vascular and perineural invasion.<sup>9</sup> Clinically, atypical carcinoid has an aggressive course.<sup>9</sup>

Here, we report a large cell neuroendocrine carcinoma of the larynx based upon the criteria set forth by the WHO (2004) for pulmonary neuroendocrine neoplasms. Specifically, the cell morphology and diffuse neuroendocrine staining coupled with the abundant mitoses and necrosis support the diagnosis of this distinctive entity as a large cell neuroendocrine lesion, akin to that described in the lung. Furthermore, the abundant mitoses remove this tumour from the atypical carcinoid category as described by Barnes.<sup>9</sup>

Entities that enter the differential based upon both site and morphology include carcinoid, small cell carcinoma, and large cell non-keratinising squamous carcinoma. The abundant mitoses and necrosis exclude a diagnosis of carcinoid. Although small cell carcinoma shares both abundant mitoses and necrosis with large cell neuroendocrine carcinoma, the large cells with prominent nucleoli and abundant cytoplasm do not fit morphologically with a small cell carcinoma. Although a poorly differentiated squamous carcinoma must be considered, neither the cell morphology nor the diffuse and strong neuroendocrine positivity support this diagnosis. Furthermore, recognition of the typical morphology of LCNEC along with focal pseudorosette-like formation helps to rule out a poorly differentiated squamous carcinoma.

The terminology of neuroendocrine lesions of the larynx is confusing, largely because of the lack of consistency within the classification of these tumours. Hartley *et al* point out that early reports of carcinoid tumours of the larynx were divided into typical and atypical subtypes, with atypical tumours also being termed LCNEC to distinguish them from small cell neuroendocrine carcinoma of the larynx.<sup>10</sup> In addition, Wenig and Gnepp in their comprehensive review of laryngeal neuroendocrine tumours showed that this group of tumours (with the exception of the extremes of carcinoid and small cell carcinoma) have been “synonymously referred to as typical, aggressive, malignant or pleomorphic carcinoid, or large cell neuroendocrine carcinoma”.<sup>11</sup> Thus, although LCNEC of the larynx has been cited in the literature, it is unclear in many instances whether these published cases meet the criteria for pulmonary LCNEC set forth by the WHO in 2004. For example, Hartley *et al* described a case of LCNEC of the larynx that they synonymously refer to as “atypical carcinoid”.<sup>10</sup> The cells were described as hyperchromatic with inconspicuous nucleoli and scanty cytoplasm.<sup>10</sup> This cytology is not comparable with large cell neuroendocrine carcinoma. Furthermore, no mention was made of mitoses or necrosis.

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In 1985, Woodruff *et al* published a series of nine cases of LCNEC.<sup>12</sup> These cases were divided into two groups, with the first group of seven cases composed primarily of large polyhedral cells, and the second group of two cases composed of both large and small cells.<sup>12</sup> Morphologically, group 1 resembled LCNEC, and all seven cases stained for neuron specific enolase. Our present case is most comparable with group 1. However, within group 1, two cases had no mitoses and four cases showed a “high mitotic rate”.<sup>12</sup> Again, no mention was made of the specific number or the mitotic rate

of the seventh case.<sup>12</sup> The presence or absence of necrosis was not documented. Therefore, it is not clear whether the four mitotically active cases within group 1 qualify as LCNEC based on the WHO pulmonary criteria.<sup>1</sup> Interestingly, Woodruff *et al* found six of the seven cases within group 1 to be immunopositive for calcitonin.<sup>12</sup> Hence, distinction between medullary carcinoma of the thyroid and LCNEC at a metastatic focus, although essential to prognosis and management, would be a difficult task. Woodruff *et al* suggested that both thyroid C-cells and LCNEC elaborate calcitonin as a result of inductive influences by the same regional endoderm.<sup>12</sup> Our case was negative for calcitonin.

In 1991, Milroy *et al* described 41 cases of LCNEC of the larynx.<sup>13</sup> They placed LCNEC in a spectrum between well differentiated carcinoid tumour and poorly differentiated small cell carcinoma.<sup>13</sup> Histologically, the tumour cells were described as varied, with some exhibiting a large vesicular nucleus with eosinophilic cytoplasm but some with less cytoplasm and more basophilic nuclei.<sup>13</sup> Mitoses were present, but infrequent.<sup>13</sup> However, occasional tumours had a “high mitotic rate”, although this was not quantified.<sup>13</sup> In addition, the presence or absence of necrosis was not documented.

In 1989, Wenig *et al* proposed a classification of laryngeal neuroendocrine tumours as well differentiated (synonymous with carcinoid tumour), moderately differentiated (including those entities previously described as atypical, pleomorphic, malignant, or anaplastic carcinoid), and poorly differentiated (including small cell carcinoma, both oat cell and intermediate cell variants).<sup>11</sup> It is not clear whether entities labelled as LCNEC were placed within the moderately differentiated category. Their review of 54 cases of moderately differentiated neuroendocrine carcinoma identified mitotic figures in only eight of the cases.<sup>14</sup> No quantification of the mitoses was reported. Most of the cases contained either no or infrequent mitoses.<sup>14</sup> In addition, individual cell necrosis was not generally a feature, and when identified was associated with surface ulceration.<sup>14</sup>

Hence, it would appear that a review of the present classification of neuroendocrine tumours of the larynx may be worthy of investigation. This should be based upon a review of clinical material of a larger series. In this context, it is tempting to propose that laryngeal LCNEC should be classified in a similar way to the lung (WHO, 2004<sup>1</sup>). The term LCNEC could possibly be removed from the “atypical carcinoid”/moderately differentiated category of the larynx (WHO, 1991), and should be used only for those tumours that display necrosis, a high mitotic count, and large cell size similar to pulmonary LCNEC. Although previous reports of LCNEC of the larynx mention a high mitotic rate, it is unclear whether these tumours meet the current criteria of the 2004 WHO classification of lung tumours.<sup>1</sup> Thus, our present case

### Take home messages

- We report a rare case of large cell neuroendocrine carcinoma (LCNEC) of the larynx in a 74 year old man
- This case may be the first reported case of LCNEC of the larynx based on the 2004 World Health Organisation pulmonary criteria
- Recognition of LCNEC of the larynx and developing uniformity in the classification of this neoplasm are essential to gather more data on the biological behaviour of these tumours at this site, so that appropriate prognostic data and treatment modalities can be determined

may be the first reported case of LCNEC of the larynx based on the 2004 WHO pulmonary criteria.

In this respect, the recently proposed nomenclature for neuroectodermal neoplasms of the head and neck appears to be appropriate<sup>7</sup>: Mills divided these tumours into well differentiated, moderately differentiated, and poorly differentiated.<sup>7</sup> The terminology of atypical carcinoid is abandoned and included in the moderately differentiated category. Both small cell carcinoma and LCNEC are placed in the poorly differentiated category.<sup>7</sup> Because LCNEC is a rare tumour, its clinical behaviour in the larynx is unknown. In the lung, however, it has a similar prognosis after stratification for stage as small cell carcinoma.<sup>1</sup> Although the behaviour of LCNEC in the larynx cannot be extrapolated from its behaviour in the lung, it probably behaves in a similar manner to small cell carcinoma of the larynx. In the larynx, Barnes points out that the cumulative five and 10 year survival rates for atypical carcinoid are 48% and 33%, respectively, and for small cell carcinoma 16% and 5%, respectively.<sup>9</sup> We feel that placing this lesion in the moderately differentiated/atypical carcinoid category could possibly assume a better clinical outcome. Examination of a large series is essential, because meaningful clinical data cannot be drawn from a single case. Recognition of LCNEC of the larynx and developing uniformity in the classification of this neoplasm are essential to gather more data on the biological behaviour of these tumours at this site, so that appropriate prognostic data and treatment modalities can be determined.

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Accepted for publication 8 November 2004

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