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Pathology in Focus

Laryngeal leiomyosarcoma

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Abstract
We report one case of leiomyosarcoma (LMS) of the larynx occurring in a patient with a history of immunosuppressive therapy, and offer a critical review of the literature. Epstein-Barr virus (EBV) genome was not identified in the neoplastic cells. The patient was treated with endoscopic resection and post-operative radiotherapy. Lung metastasis and thyroid infiltration became evident 14 months following treatment despite the absence of laryngeal recurrence. Progressive decline occurred and the patient died 15 months after diagnosis.

Key words: Laryngeal neoplasms; Leiomyosarcoma; Immunohistochemistry; Herpes virus 4, human; Lymph node excision

Introduction

Laryngeal leiomyosarcoma (LMS) is a malignant tumour of smooth muscle origin and it is rarely encountered in the larynx. The first case of laryngeal LMS was described by Frank in 1941.[1] Since then, to the best of our knowledge, in the literature there have been approximately 40 reported cases of laryngeal LMS.[2–4]

Considering that it is difficult to distinguish a leiomyosarcoma from a spindle-cell carcinoma without immunohistochemical investigation, it is possible that some cases described as leiomyosarcoma were in fact spindle-cell carcinomas.

We present a case of LMS of the larynx and discuss the diagnostic problems, and therapy associated with this rare tumour.

Case report

In May 1995, a 41-year-old white Italian clerk came to the Department of Otolaryngology – Head and Neck Surgery of the University of Udine, Italy, because of a nine-month history of progressive dysphonia. The patient's medical history included immunosuppressive therapy with steroids and azathioprine since 1978 for kidney transplantation due to chronic renal failure, followed by cyclosporin since 1989 for a second transplantation because of kidney rejection. At admission, physical examination revealed a 12 mm exophytic lesion on the inferior side of the anterior third of the right vocal fold, extending towards the anterior commissure. Vocal fold mobility was seen to be intact. No cervical lymph nodes were found. Microlaryngoscopy was performed and the mass was completely removed. Histopathological examination of the resected specimens revealed the presence of a leiomyosarcoma. Because of the reported correlation between LMS and EBV[5] in immunodeficient patients, we also used a polymerase chain-reaction (PCR) method for the detection of this virus in the neoplastic cells. The patient received 66 Gy delivered in daily two Gy fractions over the course of seven weeks. In July 1996, the patient was re-evaluated for the sudden appearance of a painful swelling of the thyroid gland and the presence of dyspnoea. Chest X-ray showed the presence of bilateral lung nodules with a wide mediastinal enlargement. A transbronchial lung biopsy was then performed and the lesion corresponded to a metastasis from LMS. The sonographic study of the thyroid revealed the presence of multiple nodular lesions. On cytological examination, the smear was characterized by single cells and clusters of cells with abundant eosinophilic cytoplasm and indistinct cell borders. The nuclei were cigar-shaped with finely granular chromatin and indistinct nucleoli. Progressive decline occurred and the patient died in August 1996 (15 months after diagnosis). Necropsy was not performed.

Methods

Immunohistochemistry

After the routine procedure for formalin fixing and paraffin embedding, tissue blocks were sectioned at 4 μm for haematoxylin and eosin (H & E) staining and immunohistochemistry. Sections were immunostained using the avidin-biotin-peroxidase complex (ABC) method[6] by using primary antibodies directed towards smooth-muscle actin (clone1A4), desmin (cloneD33), neuron-specific enolase (cloneH14), S-100 protein (polyclonal) and anti-human cytokeratin (clone 5D3) for cytokeratin proteins 8, 18 and 19 and clone AE_1/AE_3 for cytokeratin acid (AE_1) and basic (AE_3) subfamily. All

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TABLE I
ANTIBODIES USED FOR IMMUNOSTAINING

Antiserum	Clone	Source	Dilution	Immunostaining
Smooth-muscle actin	IA4	DAKO	1:200	Positive
Desmin	D33	DAKO	1:100	Negative
Neuron-specific enolase	H14	DAKO	1:2500	Negative
S-100 protein	Polyclonal	DAKO	1:1000	Negative
Cytokeratin	5D3	Biogenex	1:50	Negative

antibodies were purchased from DAKO Denmark except for clone 5D3 that was purchased from Biogenex (Table I).

Electron microscopy

Electron microscopy was performed on formalin-fixed paraffin-embedded tissue.

Polymerase chain-reaction (PCR) for EBV

Formalin-fixed paraffin-embedded tissues were cut into 10 μm-thick sections. DNA was extracted through digestion using 0.5 mg/ml proteinase K in 50 mM Tris, 1 mM EDTA-5% Tween 20 buffer and incubation at 37°C for two days. To determine if there was assessable DNA, the specimen was subjected to amplification with primers for the beta globin genomic sequence. For the Epstein-Barr viral genome, PCR was performed as described by Herbst[7] with primers 5'GCAGTAACAGGTAATCTCTGG3' and 5'ACCAGCAATAGCTGCAGGACC3'. A positive control for EBV consisted of Raji DNA and negative control consisted of human genomic DNA, known to be negative for EBV infection, while an assay without added DNA was used as a control. The PCR product was subjected to Southern blot hybridization using a ^{32}P-labelled oligonucleotide internal probe.

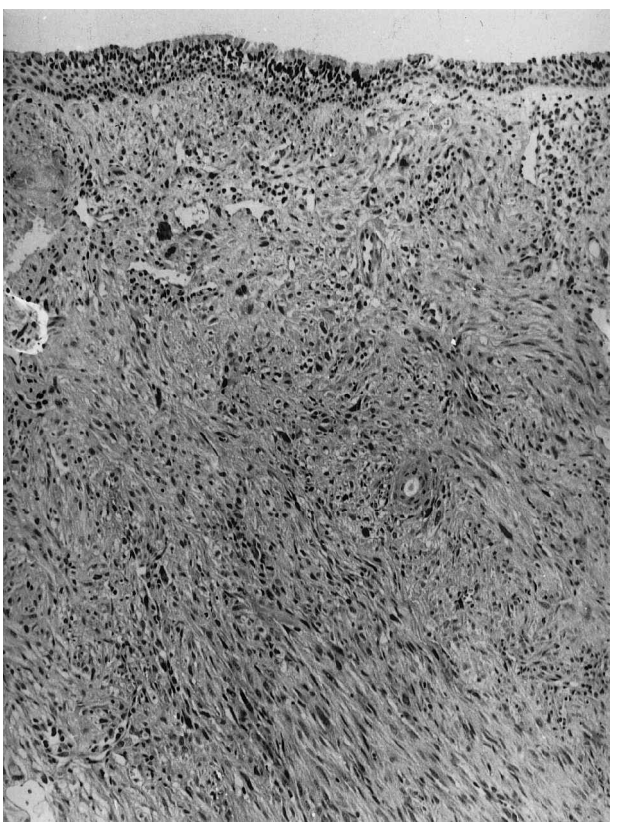

FIG. 1
A low-power view of tumour showing interlacing bundles of enlarged spindle cells (H & E; ×100).

Results

Pathological findings

On gross examination, the pathological specimen consisted of fragments of soft yellowish-pink masses with focal haemorrhage and necrosis. The tumour was composed of interlacing bundles of enlarged spindle cells with eosinophilic cytoplasm, and plump blunt-ended nuclei with stippled chromatin. There was great mitotic activity (20 mitoses/10 high-power fields), as well as cellular pleomorphism and necrosis. The tumour was partially covered by normal stratified squamous and respiratory epithelium. No dysplastic changes were seen in the overlying squamous epithelium (Figures 1 and 2). Immunohistochemistry revealed marked expression of smooth-muscle actin (Figure 3) while desmin, neuron-specific enolase, S-100 protein and cytokeratin 5D3 were negative (Table I). The tumour was then classified as laryngeal LMS.

The lung biopsy was composed of fragments of lung tissue, one of which was infiltrated with a proliferation of pleomorphic spindle cells positive for smooth-muscle actin (Figure 4). The cytological examination of the thyroid aspirate showed clusters of spindle cells similar to the ones in the lung tissue.

Ultrastructural finding

Tumour cells were round to spindle-shaped and separated by a thin band of electron-dense, sometimes interrupted, basal lamina; nuclei were often folded or showed invaginations. Numerous thin myofilaments were evident (Figure 5).

Molecular findings

The analysis of the DNA extracted from the blocks of the tumour examined using PCR and Southern blot excluded the presence of a band with a molecular weight size corresponding to EBV.

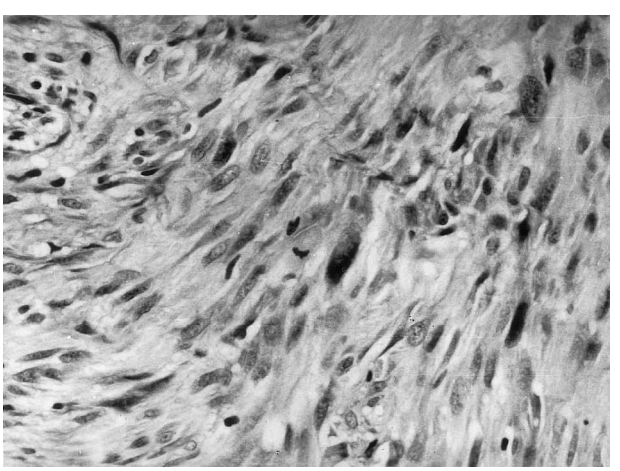

FIG. 2
Higher-power view showing pleomorphic spindle cells with plump blunt-ended nuclei. Mitotic figures are present (H & E; ×400).

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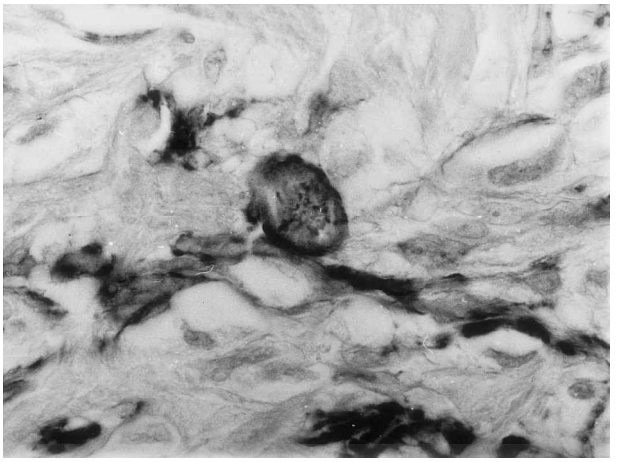

FIG. 3

Immunohistochemical stain for smooth-muscle actin is positive in the cytoplasms of the tumour cells. A mitotic figure is present (avidin-biotin-peroxidase; ×1000).

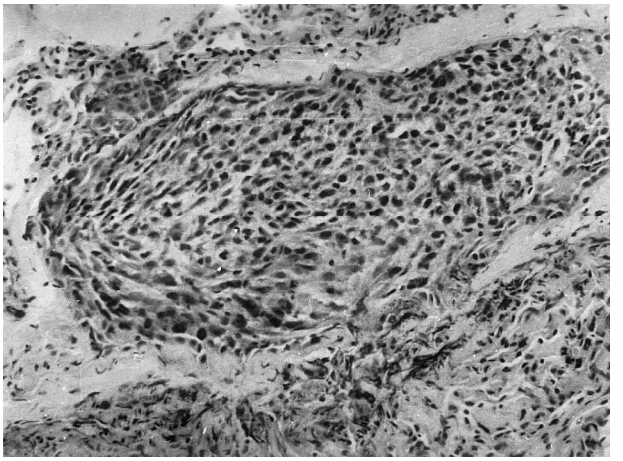

FIG. 4

Transbronchial lung biopsy showing a metastasis from laryngeal leiomyosarcoma (H & E; ×100).

Discussion

The majority of leiomyosarcomas of the head and neck arise in the oral cavity, superficial soft tissues such as the scalp, paranasal sinuses and jaws.

Smoking or alcohol consumption are not considered risk factors for sarcomas,[8] whereas a history of prior irradiation, the presence of one of various syndromes (tuberous sclerosis, von Recklinghausen's neurofibromatosis, Gardner's syndrome, retinoblastoma, Werner's syndrome, Turcot's syndrome, multiple basal cell carcinoma syndrome), or a history of immunosuppression seem to be correlated with a higher incidence of this neoplasm.[9-12] LMSs in immunosuppressed patients have been frequently found to be associated with EBV.[5]

Thirty of the approximately 40 cases of LMS of the larynx quoted by literature have sufficient clinicopathological information for evaluation. The remaining cases either lack details for analysis or are published in medical journals not available for our review. Considering also our case, LMS of the larynx occurred in 24 males (77.4 per cent) and seven females (22.6 per cent) averaging 49 years of age (median 46, range eight to 87 years). The tumours originated from or involved the following regions: supraglottis 10 cases (32.3 per cent), supraglottis-glottis two cases (6.5 per cent), glottis 15 cases (48.4 per cent), supraglottis-glottis-subglottis one case (3.2 per cent), glottis-subglottis one case (3.2 per cent), subglottis two cases (6.5 per cent). Hoarseness, stridor, dyspnoea, choking sensation and difficulty in swallowing are the most common complaints.[13,14]

The morphological diagnosis may be problematic on conventional light microscopy and should be supported by immunocytochemical and ultrastructural investigations. Histologically, it is characterized by prominent interlacing bundles and fascicles of elongated spindle cells with elongated 'cigar-shaped' blunt-ended nuclei, prominent nucleoli and abundant eosinophilic cytoplasm. Occasionally, large pleomorphic cells with irregular vesicular nuclei and multiple nucleoli can be seen. The mitotic rate is usually increased, and atypical mitotic figures are present. On immunostaining the LMS is positive for muscle-specific actin and negative for S-100 protein. Desmin is generally negative.

The differential diagnosis from leiomyoma, fibrosarcoma, spindle-cell carcinoma, malignant melanoma and inflammatory myofibroblastic tumour may be difficult, especially in small biopsy specimens. Immunohistochemical, immunocytochemical and ultrastructural investigations are necessary to distinguishing LMS from other spindle-cell tumours. In the past, leiomyosarcomas have been confused with other spindle-cell malignancies. It is likely that several so-called leiomyosarcomas with lymph node metastases were in fact spindle-cell carcinomas. Certainly the possibility that LMS have been inadequately evaluated needs to be considered.

LMS is not a lesion with high metastatic potential. Haematogenous metastases occur infrequently, usually involving the lungs, as in our case. Lymph nodes metastases are very rare and are seen late in the disease course.[14]

To our knowledge, 25 cases of leiomyosarcoma of the larynx have been described over the past three decades, nearly all of which were without early lymph node metastases. The only two cases of early lymph node metastases were described before 1970 and none of these reports provided histochemical, immunohistochemical or ultrastructural confirmation.

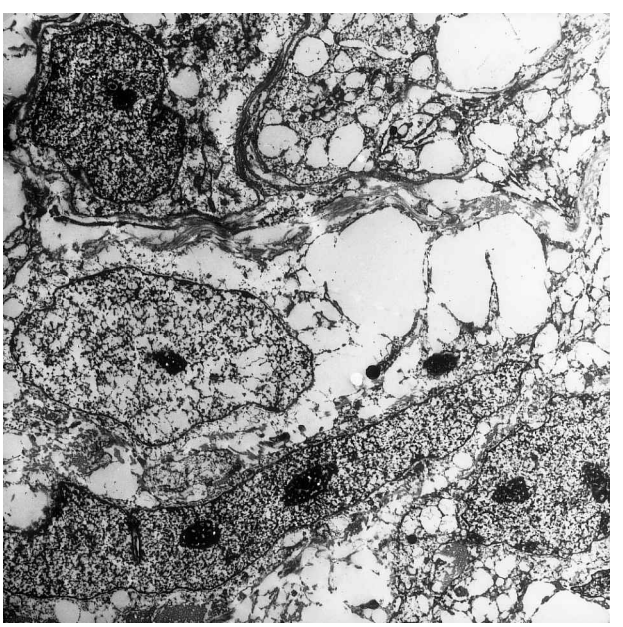

FIG. 5

Electron micrograph of the neoplasm. Cells are characterized by elongated shape, grooved nuclei and numerous thin filaments with dense bodies.

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Surgery is the treatment of choice for this tumour. Considering the absence of metastases to cervical lymph nodes in all cases of LMS in which the diagnosis was supported by immunocytochemical and ultrastructural investigations, neck dissection is not recommended. The tumour response to radiotherapy is relatively poor.

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