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Case report

Metastasizing neuroendocrine carcinoma of the larynx with calcitonin and somatostatin secretion and CEA production, resembling medullary thyroid carcinoma

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Summary. A 55-year-old man presented with a metastasizing moderately differentiated neuroendocrine carcinoma of the larynx (atypical carcinoid). Immunocytochemical demonstration of neuroendocrine markers (neuron-specific enolase and chromogranin-A) and presence of membrane-bound neurosecretory granules in the cells established the neuroendocrine nature of the tumour. In addition, the tumour was found to produce calcitonin, somatostatin and carcino-embryonic antigen (CEA). Calcitonin and somatostatin were also secreted. On the basis of this particular marker constellation the tumour closely resembles medullary thyroid carcinoma. Review of the recent literature on carcinoids of the larynx reveals immunoreactivity for calcitonin and CEA in a high percentage of cases.

Key words: Laryngeal neuroendocrine carcinoma – Carcinoid – Calcitonin – Somatostatin – Carcino-embryonic antigen

Introduction

Neuroendocrine tumours of the larynx, although uncommon, have received increasing attention in recent years. Since Goldman et al. reported the first carcinoid of the larynx, in 1969, several case reports (Grupe Larsen and Krag Jacobsen 1989) and two large studies including 67 patients have been published (Woodruff et al. 1985; Wenig et al. 1988). From these articles it has emerged that the spectrum of neuroendocrine tumours of the larynx is comparable to that of the lung. Wenig et al. (1988)

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Table 1. Source and dilution of the applied antibodies

Antisera	Dilution	Source
Cytokeratin CAM 5.2[a]	1/10	Becton Dickinson, (Mountain View, Calif.)
Neuron-specific enolase (NSE)[b]	1/200	Dako (Santa Barbara, Calif.)
Chromogranin-A[a]	1/5000	Hybritech (San Diego, Calif.)
S100[b]	1/200	Dako (Santa Barbara, Calif.)
Carcino-embryonic antigen (CEA)[a]	1/3	Behring (Marburg, FRG), BMA 130c
Calcitonin[b]	1/1500	Ortho, Janssen Pharmaceuticals (Beerse, Belgium)
Somatostatin (SRIF)[b]	1/20000	Gift of Dr. J. De Mey (Janssen, Beerse, Belgium)
Serotonin[b]	1/400	Gift of Dr. Verhofstad (University of Nijmegen, The Netherlands)
Adrenocorticotropin (ACTH)[b]	1/750	Biogenex (Dublin, Calif.)
Met-enkephalin[b]	1/128	Milab (Malmo, Sweden)
Bombesin[b]	1/2000	Immunonuclear Co (Minnesota, Minn.)
Neurotensin[b]	1/5000	Milab (Malmo, Sweden)
Insulin[a]	1/10	Biogenex (Dublin, Calif.)
Glucagon/glicentin[b]	1/1024	Milab (Malmo, Sweden)
Bovine pancreatic polypeptide (BPP)[b]	1/20000	Gift of Dr. R.E. Chance (Lilly, Indianapolis, Ind.)
Neurofilaments[a]		Boehringer (Mannheim, FRG)
(MW 68,000)	1/10	
(MW 160,000)	1/10	
(MW 200,000)	1/10	

[a] Monoclonal; [b] polyclonal

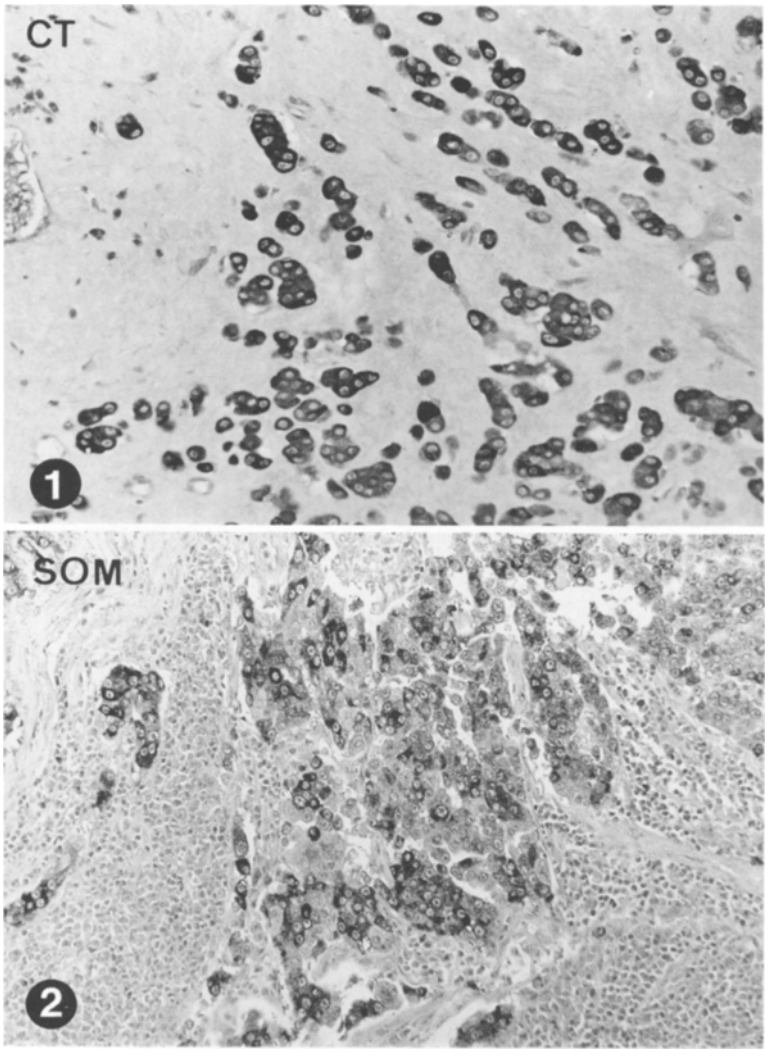

Fig. 1. Primary larynx tumour with positive immunostaining for calcitonin (CT). ×200

Fig. 2. Lymph node metastasis with positive immunostaining for somatostatin (SOM). ×200

therefore adapted Gould's classification of neuroendocrine lung neoplasms (Gould et al. 1983) to laryngeal neuroendocrine carcinomas, and divided these tumours into well-differentiated neuroendocrine carcinoma (carcinoid) (Blok et al. 1985), moderately differentiated neuroendocrine carcinoma (atypical carcinoid) (Johnson et al. 1986), and poorly differentiated neuroendocrine carcinoma (small-cell undifferentiated carcinoma) (Gnepp et al. 1983; Kyriakos et al. 1978; Googe et al. 1988). Despite these structural similarities, the neuroendocrine tumours of the lung and larynx produce different marker hormones. While the tumours in the lung most commonly express bombesin and serotonin (Gould et al. 1988), a great deal of the neuroendocrine carcinomas of the larynx elaborate calcitonin. As calcitonin is the marker of medullary thyroid carcinoma (Schröder et al. 1988), it has been suggested that calcitonin-secreting laryngeal neuroendocrine tumours represent ectopic thyroid medullary carcinoma (Sweeny et al. 1981).

Here we report on another neuroendocrine tumour of the larynx that shares many similarities with a medullary thyroid carcinoma. In particular, it is apparently the second reported case with an elevated serum calcitonin level.

Case report

A 55-year-old man was admitted to our hospital because of hoarseness and dysphagia. Laryngoscopy revealed a tumour on the laryngeal surface of the epiglottis. A lymph node in the left submandibular triangle was palpable. Chest radiographs showed no abnormalities. Three submandibular lymph nodes were removed and a biopsy taken from the laryngeal tumour. Histological examination of these specimens revealed a solid tumour. After combined radio- and chemotherapy, the primary lesion at the epiglottis disappeared but the lymph node remained the same size. Subsequently, a left neck dissection was performed followed by a second cycle of radiotherapy as well as chemotherapy. The tumour recurred at the initial site 18 months later. The patient was treated by total laryngectomy. One year later the patient developed two skin metastases and a cerebral metastasis; these were removed. A few months later the skin metastases recurred. Although combination chemotherapy was started, the patient detoriated and finally died of metastatic disease within 3 years of diagnosis.

The patient's serum calcitonin level was 3790 pg/l (normal: <100 pg/ml) and rose to 6378 pg/ml 5 min after pentagastrin administration. The levels of somatostatin (80 pg/ml; normal <25 pg/ml) and neuron-specific enolase (NSE 23 ng/ml; normal <12.5 ng/ml) were also found to be increased. Normal serum levels were obtained for thyroxine (T_3 and T_4), thyroid stimulating hormone, thyroxin binding globulin, cortisol; alpha-fetoprotein, parathyroid hormone, gastrin, insulin, C-peptide, glucagon, carcino-

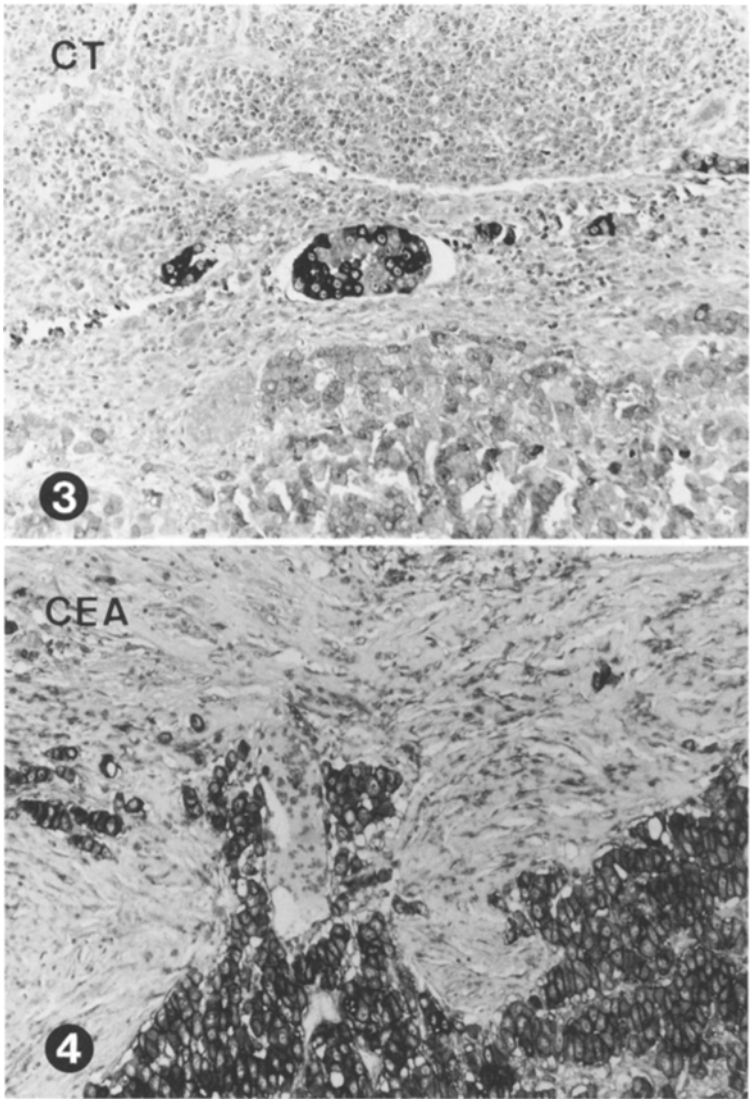

Fig. 3. Skin metastasis with positive immunostaining for calcitonin (CT). ×200

Fig. 4. Cerebral metastasis with positive immunostaining for carcino-embryonic antigen (CEA). ×200

embryonic antigen (CEA), carbohydrate antigen 125 (CA 125) and adrenocorticotropin.

Materials and methods

Samples from the primary tumour, its recurrence and the metastases were fixed in Bouin's solution and embedded in paraffin. Routine sections were stained with haematoxylin and eosin, the Grimelius and the Fontana technique, PAS (with or without diastase digestion), Alcian blue and Congo red.

Immunocytochemical staining was performed according to the unlabelled peroxidase anti-peroxidase method of Sternberger et al. (1970) or to the avidin-biotin-complex method (Guesdon et al. 1979). The antisera used are listed in Table 1. The percentage of positive cells was semi-quantitatively graded on a scale of 0–4 (positivity score: 1: <25%; 2:25–50%; 3:50–75%; 4: >75%). Positive and negative controls were systematically used.

For electron microscopy, tissue pieces were fixed in 2.5% glutaraldehyde (0.1 M cacodylate, pH 7.4), post-fixed in 1% osmium tetroxide and embedded in Spurr. The ultra-thin sections were contrasted with uranyl acetate and lead citrate and examined ultrastructurally with a Zeiss EM 9S.

Results

The two pre-operative biopsies from the primary tumour, the lymph node metastasis, the recurrent tumour and the metastases of the skin and the cerebrum all showed a similar microscopical pattern. The cells were mainly arranged in solid clusters. Small glands and/or trabecular structures were only occasionally formed. The cell nuclei were large and hyperchromatic and were surrounded by a well-developed cytoplasm. In the lymph node metastasis and the skin and cerebral metastases, a purely solid pattern was present. Mitoses were rare. The stroma contained no amyloid. In the first series of tumour fragments (before therapy) a few cells stained with Alcian blue and PAS after diastase digestion. After radiation the number of strongly PAS- and Alcian-blue-positive cells was increased. With the Grimelius technique, the majority of the cells were argyrophilic but negative by the argentaffin Fontana technique. Immunocytochemically (Figs. 1–4) the tumour was positive for calcitonin, somatostatin, NSE, CEA, cytokeratin (CAM

Table 2. Immunocytochemical findings

Antiserum	Primary tumour		Metastases		
			Lymph node	Skin	Brain
	a	b	a		
Cytokeratin CAM 5.2	3	4	4	4	4
NSE	3[c]	1[c]	2[c]		
Chromogranin-A	4	4	4		
S100	–	–			
CEA	4	–	–	4	4
Calcitonin	3	4	3	–	3
Somatostatin (SRIF)	3	4	3	3	3
Serotonin	–	–			
ACTH	–	–	–	–	–
Met-enkephalin	–	–	–	–	–
Bombesin	–	–	–	–	–
Neurotensin	–	–	–	–	–
Insulin	–	–	–	–	–
Glucagon/glicentin	–	–	–	–	–
BPP	–	–	–	–	–
Neurofilaments					
68000	–	–	–	–	–
160000	+	+	–	+	–
200000	+	+	–	–	–

Positivity score: (1) <25%; (2) 25–50%; (3) 50–75%; (4) >75%

[a] Before radiation and chemotherapy
[b] After radiation and chemotherapy
[c] Weakly positive
CEA = carcino-embryonic antigen; NSE = neuron-specific enolase; ACTH = adrenocorticotropin; SRIF = somatatostatin; BPP = bovine pancreatic polypeptide.

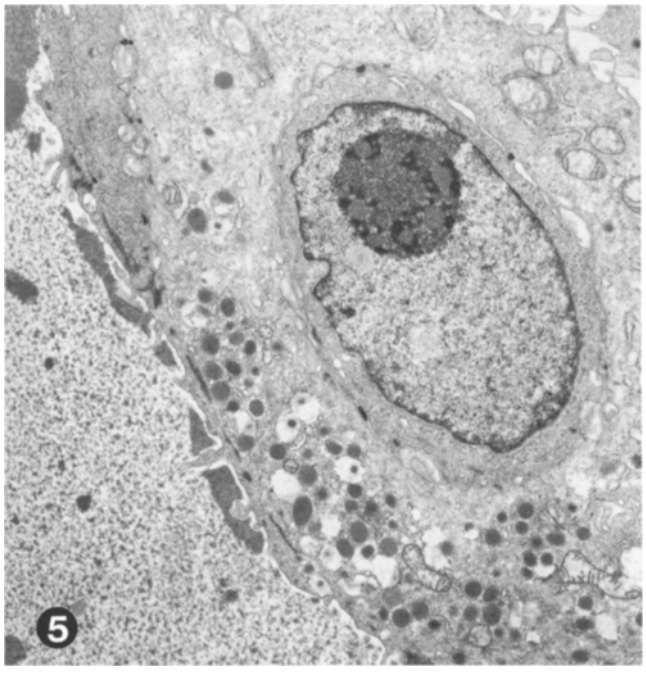

Fig. 5. Cell of the primary larynx tumour displaying neurosecretory granules. ×10000

5.2), chromogranin-A, and the neurofilaments 160 and 200. The detailed immunocytochemical results are listed in Table 2. The cerebral metastasis was also positive for somatostatin and calcitonin, while the skin metastasis was only immunoreactive to somatostatin.

Electron microscopy of the primary tumour after radiation revealed neuroendocrine cells (Fig. 5) that occasionally showed microvilli. The neurosecretory granules were moderately electron dense. Some of them showed a lucent halo between the core and the granule membrane. The average diameter of the granules was 200 nm. Cell junctions were of the zonula adherens type.

Discussion

The neuroendocrine nature of this malignant laryngeal tumour was substantiated by cellular argyrophilia, positive immunoreactivity for chromogranin-A and NSE, and the ultrastructural demonstration of typical neuroendocrine granules. Histologically, the tumour was characterized by a solid pattern with considerable cellular atypia. It can thus be classified as a moderately differentiated neuroendocrine carcinoma – an atypical carcinoid (Wenig et al. 1988). Further immunocytochemical analysis as well as radio-immunological examination of the patient's serum revealed that this laryngeal carcinoid produced and secreted large amounts of calcitonin and, in addition, some somatostatin. The tumour also reacted with a monoclonal monospecific antibody to CEA. Calcitonin and CEA are the markers of medullary thyroid carcinoma, since all of these tumours produce calcitonin and most of them CEA as well (Lloyd et al. 1983; Schröder and Klöppel 1987; Schröder et al. 1988). Amyloid, another marker of medullary thyroid carcinoma, was lacking in our case. However, amyloid is also found inconsistently in medullary thyroid carcinoma, being present in approximately 70% of the cases (Schröder et al. 1988). We consider that this laryngeal tumour closely resembled medullary thyroid carcinoma.

In 1981, Sweeny et al. reported on two extrathyroid tumours with calcitonin and CEA positivity. One tumour was of tonsillar origin; the other arose in the larynx. The latter tumour was also found to secrete calcitonin in the absence of a primary tumour of the thyroid. As we found no other report on a calcitonin secreting tumour of the larynx in the literature, our case obviously represents the second reported laryngeal neoplasm with an elevated calcitonin level. The fact that there are so few laryngeal neuroendocrine carcinomas with elevated calcitonin levels on record is surprising in view of the frequent demonstration of calcitonin at the cellular level. In the series of Woodruff et al. (1985), 23 of the 30 tumours were found to produce calcitonin when examined immunocytochemically. The majority of the recently published case reports on laryngeal carcinoids (Wenig et al. 1988; Grupe Larsen and Krag Jacobsen 1989) have similar findings. Calcitonin may therefore be regarded not only as a marker hormone of medullary thyroid carcinoma but also of well to moderately differen-

tiated neuroendocrine carcinoma of the larynx. The resemblance of the latter tumour to medullary thyroid carcinoma is further emphasized by the finding that many of these neoplasms also express CEA (Woodruff et al. 1985; Wenig et al. 1988). Thus, laryngeal carcinoids have to be included in the differential diagnosis of medullary thyroid carcinoma, particularly when presenting as a calcitonin and CEA positive cervical lymph node metastasis without an obvious primary in the thyroid.

The cells which give rise to the neuroendocrine tumours in the larynx may be of mucosal or submucosal origin (Wenig et al. 1988). The fact that most of the laryngeal neuroendocrine tumours primarily present as submucosal lesions in the supraglottic region (Wenig et al. 1988) and may also produce mucosubstances (Paladugu et al. 1982) supports their origin from cells incorporated in the minor salivary glands of this area (Vrabec and Bartels 1980) rather than from the mucosa cells itself. Whether these neuroendocrine cells represent embryonic rests of C-cells that failed to migrate to the thyroid remains speculative (Sweeny et al. 1981). Undoubtedly, however, the larynx harbours neuroendocrine cells that seem to be capable of expressing calcitonin and CEA, particularly when transformed to neoplastic cells. Tumours that originate from these cells may then be indistinguishable from medullary thyroid carcinoma.

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