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Case report

Calcitonin free oat-cell carcinoma of the thyroid gland

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Summary. Two cases of primary oat-cell carcinoma of thyroid, in a 63-year-old woman and a 73-year-old man, are described. Case 1 was a compound tumour with the oat-cell component merging with a papillary component. Both tumours, in addition to histological features consistent with oat-cell carcinoma, showed immunohistochemical positivity with anti-chromagranin A and anti-synaptophysin antisera. Negative results were obtained when anti-calcitonin and anti-thyroglobulin antisera were employed. Using in situ hybridization, chromogranin A and B messenger RNAs were localized with biotinylated oligonucleotide probes. In contrast, with in situ hybridization, no localization for calcitonin messenger RNA was seen using radioactive and biotinylated probes. It is concluded that these calcitonin-free, small-cell carcinomas should be considered separately from medullary thyroid carcinomas and be regarded as a distinct entity, probably the thyroid equivalent of oat-cell carcinomas of the lung.

Key words: Thyroid – Oat-cell carcinoma – Calcitonin-free small-cell carcinoma

Introduction

The occurrence of extrapulmonary oat-cell carcinomas is well known. They have been described in practically every organ (Ibrahim et al. 1984; Remick et al. 1987) and their occurrence in the kidney constitutes one of the most recently recognized sites (Capella et al. 1984; Tetu et al. 1987). One of the few sites where oat-cell carcinomas have not yet been identified is in the thyroid gland. The purpose of the present paper is to describe two cases of oat-cell carcinoma of the thyroid and to discuss their possible histogenesis.

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Case reports

Both cases were retrieved retrospectively: case 1 from the files of the surgical specimens of the Institute of Pathology of the University of Bologna; case 2 from the post-mortem files of the Department of Pathology of the University of Pavia at Varese.

Case 1. The patient, a 63-year-old lady, presented in August 1974 with dyspnoea due to a large mass of the left thyroid lobe extending to the latero-cervical region. The mass was hard in consistency and appeared adherent to the deep tissues. Right latero-cervical lymph nodes were also enlarged. The patient had undergone total thyroid irradiation (2700 rad total dose) 10 years previously, as "putative" treatment for severe hyperthyroidism, which had been present for at least 30 years. At the time of admission a ^{131}I scintigram revealed a decreased uptake of the right lobe and no uptake of the left lobe. Bronchoscopy revealed reduction of the tracheal calibre at the first rings, although the mucosa appeared unaltered. Thoracic tomography before and after thyroid surgery did not show any lung involvement. A total thyroidectomy was performed along with removal of lymph nodes from both sides of the neck. The patient died shortly afterwards with bilateral pneumonia. No autopsy was performed. The left lobe of the thyroid together with the isthmus measured $7 \times 6.5 \times 3$ cm; it was nodular and hard, with-ish with yellowish patches on cut surface. The right lobe measured $5 \times 3 \times 1$ cm. The latero-cervical lymph nodes (three from the left side and two from the right side) were large and showed macroscopic evidence of metastatic deposits.

Case 2. The patient, a 73-year-old man, was admitted in March 1974 to Varese University Hospital as a consequence of severe anaemia. The patient had a history of pernicious anaemia.

On physical examination bilateral latero-cervical lymph node enlargement was found together with a palpable nodule on the left thyroid lobe. Routine chest radiographs showed both lungs free from any neoplastic involvement. His health deteriorated and he died in April 1974.

Autopsy revealed a nodule in the left lobe of thyroid which measured 5 cm across. The nodule had invasive margins and was very close to the capsule. It was firm and white with occasional yellow necrotic patches.

The latero-cervical lymph nodes from both sites were enlarged; they were packed together and showed macroscopic evidence of metastatic deposits. The right supraclavicular lymph nodes showed similar features. Both lungs displayed signs of panlobular emphysema, but no clear neoplastic nodules were present within the bronchi and the lung parenchyma. Several small subpleural whitish nodules

Table 1. Antisera employed for immunohistochemistry

Antisera	M/P	Source	Dilution
Thyroglobulin	P	Dakopatts (Denmark)	1:30000
Calcitonin	P	Ortho Diagnostic Systems (Milan, Italy)	1:1
ACTH	P	Ortho Diagnostic Systems	1:1
ADH	P	U.C.B. (Bruxelles)	1:1000
5HT	P	I.N.C. (Stillwater, California)	1:500
Bombesin	P	Milab (Denmark)	1:1000
NSE	P	Dakopatts	1:200
Synaptophysin	M	Biogenex (San Ramon, California)	1:7
Chromogranin A	M	R. Lloyd Michigan, USA	1:120
Keratin-EAB 902	M	Ortho Diagnostic Systems	1:1500
Keratin-EAB 903	M	Ortho Diagnostic Systems	1:1500
CLA	M	Ortho Diagnostic Systems	1:1
EMA	M	Dakopatts	1:200

M, Monoclonal; P, polyclonal

measuring a few millimetres across were scattered in both organs. Several large metastatic nodules were also visible in the liver.

Materials and methods

Tissues from both cases were fixed in 10% formalin and embedded in paraffin.

The following stains were performed: haematoxylin and eosin (H & E); Grimelius' method; Congo red for amyloid; alcian blue pH 2.5 followed by periodic acid-Schiff (PAS) before and after diastase digestion. For immunohistochemistry the strepto-avidin-biotin peroxidase complex was used (Hsu et al. 1981). The list of the primary antisera employed together with the respective dilutions is reported in Table 1.

In situ hybridization for calcitonin messenger RNA was per formed with radioactive (^{35}S) and biotinylated probes as previously described (Hankin and Lloyd 1990). Chromogranin A and B messenger RNAs were localized by in situ hybridization with biotinylated oligonucleotide probes as previously described (Lloyd et al. 1989).

Results

Case 1

The neoplastic nodule is composed of two neoplastic components which are closely adjacent, but separated (Fig. 1). One component, defined as type A, constitutes about 80% of the cellular population of the tumour, and is formed by sheets, nests and trabeculae of round to spindle-shaped neoplastic elements. These have scanty eosinophilic granular cytoplasm. Their nuclei, round to ovoid, are hyperchromatic and only very small nucleoli are visible (Fig. 2). Mitoses are frequent and small groups of necrotic elements with pyknotic nuclei are also seen. The stroma is scanty and the vascular supply to the tumour is formed by small capillaries. The neoplastic

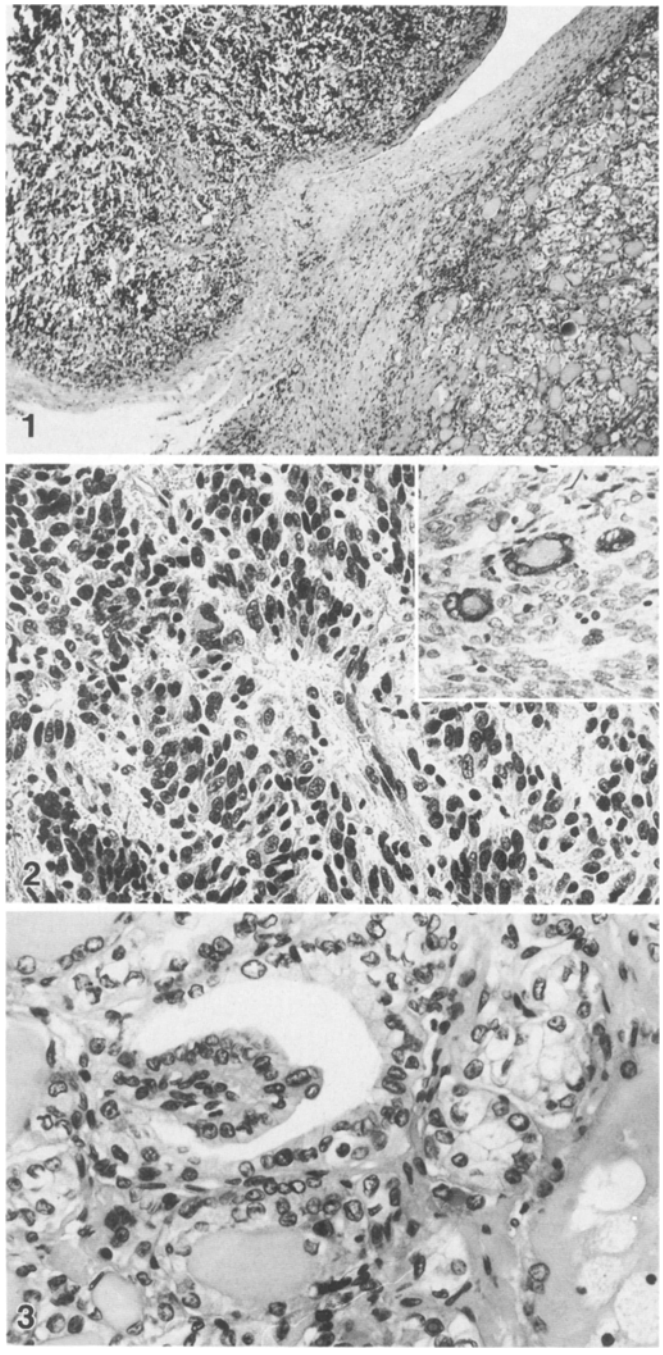
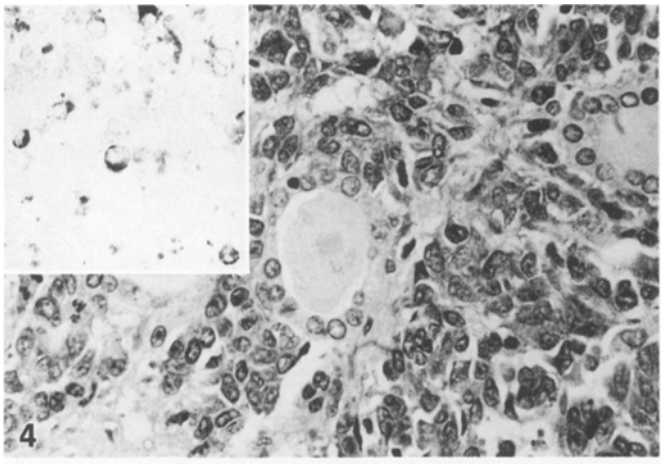


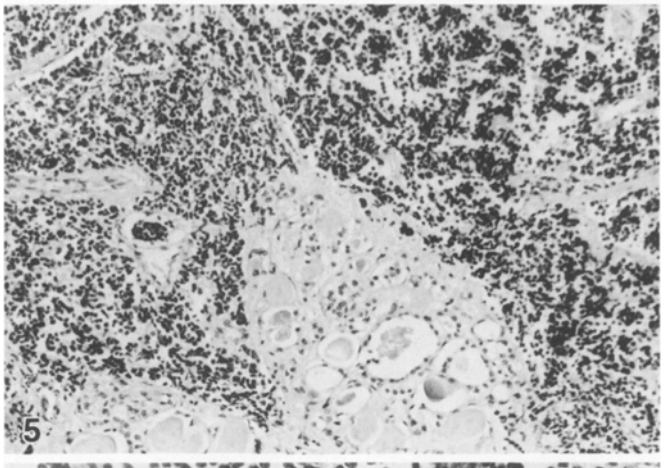
Fig. 1. Case 1: the two neoplastic components are separated by a large fibrous band. H&E, × 51

Fig. 2. Case 1: nests and trabeculae of round to spindle-shaped neoplastic elements. In the *inset*, three residual follicular structures are stained with anti-thyroglobulin antiserum. H&E, × 128; *inset*: strepto-avidin-biotin-peroxidase, × 128

Fig. 3. Case 1: a small papillary structure abuts within a follicular space. The nuclei are vesicular and faintly stained. H&E, × 320

cells extend to the perithyroidal fat, and within the mass occasional residual thyroid follicles are visible (Fig. 2). The other component of the tumour, defined as type B, is formed by small follicles and nests of cuboidal cells with poorly stainable cytoplasm. Occasional small





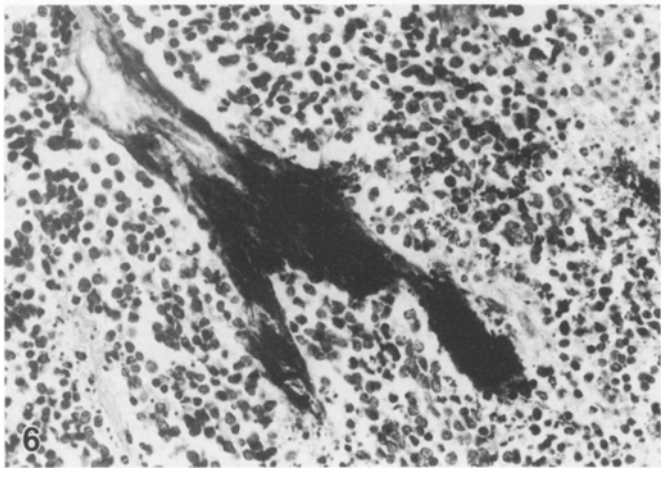


Fig. 4. Case 1: most of the neoplastic cells stain with anti-synaptophysin antiserum, while the epithelium of the entrapped follicles appears unstained. In the inset are shown the rare chromogranin immunoreactive elements. No nuclear counterstain has been used here. Strepto-avidin-biotin-peroxidase, ×320; *inset*, ×320

Fig. 5. Case 2: large sheets of neoplastic elements are intermingled with residual follicular structures. H&E, ×128

Fig. 6. Case 2: necrotic tissue is abundant and perivascular DNA incrustations (Azzopardi's phenomenon) are numerous. H&E, ×175

papillary structures abut on the follicular lumina, where a homogeneous, strongly eosinophilic colloid is present. The nuclei of follicular cells are vesicular, frequently with faint staining. Occasional intranuclear cytoplasmic invaginations are seen. In addition chromatin bars, reminiscent of nuclear groovings (Chan and Saw 1986), are also seen (Fig. 3). All these features are consistent with the diagnosis of papillary carcinoma. Grimelius'stain, alcian blue and Congo red methods are consistently negative throughout the tumour. Staining with anti-chromogranin A and anti-synaptophysin antisera are positive only within the cytoplasm of the neoplastic type A elements.

Chromogranin A immunoreactive elements are strongly positive and constitute about 5% of the total neoplastic population. In contrast most of the neoplastic elements react with anti-synaptophysin antiserum (Fig. 4). Anti-thyroglobulin and keratin antisera stain only the type B component. In addition, occasional entrapped follicles are stained by these same antisera in the type A component. Anti-epithelial membrane antigen (EMA) and anti- neuron specific enolase (NSE) antisera stain numerous elements in both components. All other antisera employed give negative results. The five latero-cervical lymph nodes contain type A tumour.

Case 2

The nodule is formed by large sheets of neoplastic elements occasionally intermingled with residual follicular structures (Fig. 5). Large areas of necrosis are present in areas in which perivascular DNA incrustations (Azzopardi 1959) are abundant (Fig. 6). The neoplastic elements display scanty cytoplasm and are mostly round in shape. Their nuclei are round and hyperchromatic with no nucleoli visible. Mitoses are numerous. The stroma is scanty. Grimelius' method and alcian blue and Congo red stains are consistently negative. Numerous neoplastic cells show NSE immunoreactivity, while rare elements are stained with anti-chromogranin and anti-EMA antisera. All the other antisera, in the tumour, give consistently negative results.

The metastatic deposits in the latero-cervical lymph nodes, pleura and liver show features identical to those of the thyroid tumour.

Chromogranin A and B messenger RNAs are localized in both tumours (Figs. 7–9). Calcitonin messenger RNA is not detectable in the tumours while control tissue sections of a medullary thyroid carcinoma has a positive hybridization signal.

Discussion

Both thyroid tumours (type A pattern in case 1, and case 2) show structural (trabeculae), cytological (spindle to lymphocyte-like elements) and nuclear (hyperchromatic without nucleoli) features consistent with the criteria described by Azzopardi (1959) for oat-cell carcinomas. Large areas of necrosis together with Azzopardi's phenomenon (perivascular DNA incrustations) as seen in case 2 are also features of oat-cell carcinomas. Im-

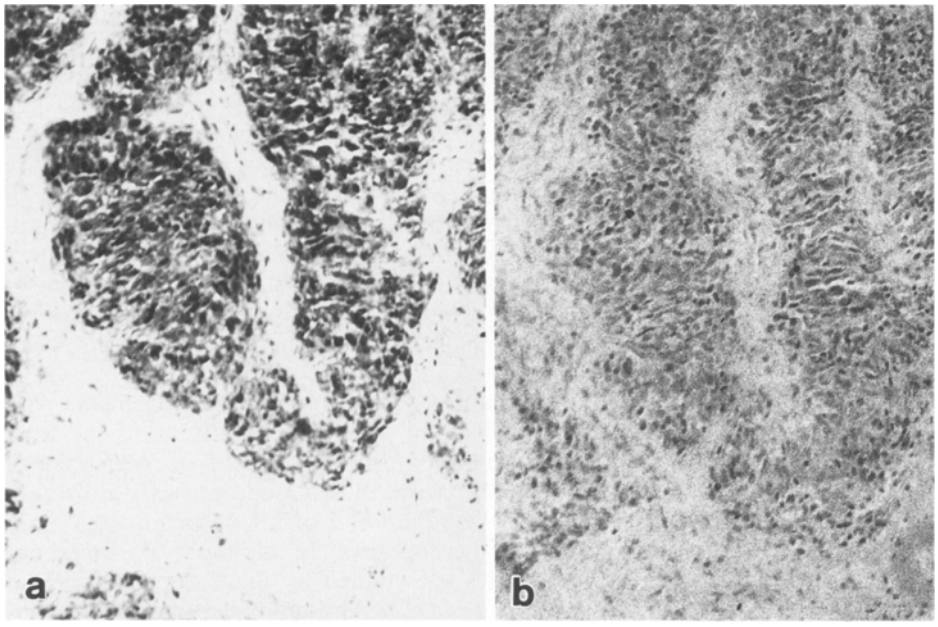


Fig. 7a, b. Case 1: in situ hybridization with a biotinylated chromogranin A probe. The tumour cells show a positive hybridization signal (**a**), as opposite to a negative control slide (**b**) in which no hybridization signal is visible as the biotinylated probe was omitted. **a, b** ×51

munologically, the positivity with anti-chromogranin A and anti-NSE antisera in both cases, and with anti-synaptophysin in case 1, are additional elements consistent with endocrine differentiation in both tumours. Chromogranin A has been described to be present in oat-cell carcinomas by Weiler et al. (1988), and synaptophysin by Kayser et al. (1988). In addition to oat-cell neuroendocrine features, case 1 displays follicular and micropapillary structures together with "clear" nuclei which also show intracytoplasmic invaginations and the phenomenon of "grooving" consistent with the diagnosis of papillary carcinoma.

Both tumours had a very aggressive behaviour and the two patients died shortly after admission. In both cases extensive radiological investigations were performed which helped to exclude other sites of origin of the tumours. This was further proven by the autopsy in case 2, where the involvement of other organs, including the lungs, was consistent with metastatic deposits from the thyroid tumour.

There are conflicting data in the literature concerning anaplastic, small-cell thyroid carcinomas as a distinct entity. The possibility is accepted in early standard textbooks where small-cell carcinomas were classified into compact and diffuse subtypes (Meissner and Warren 1969). Nevertheless, Heimann et al. (1978) denied this possibility and considered all small-cell thyroid tumours as malignant lymphomas. Rayfield et al. (1971) and Tobler et al. (1984) have shown that the majority of small-cell anaplastic thyroid tumours are lymphomas and have suggested that true anaplastic small-cell carcinomas of the thyroid are extremely rare. Carcangiu et al. (1985) stated that small-cell thyroid carcinomas are vanishingly rare and can be regarded as subtypes of medullary or poorly differentiated (insular) carcinomas. Nieuwenhuijzen Kruseman et al. (1982) described five cases of small-cell, anaplastic thyroid carcinoma of which two were argyrophylic and calcitonin-positive.

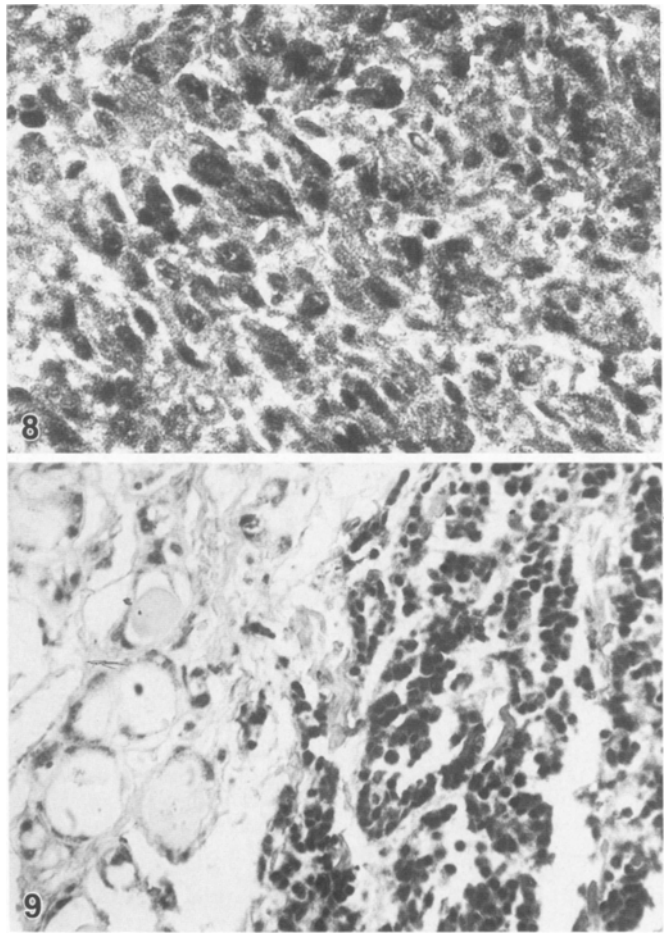


Fig. 8. Case 1: in situ hybridization with a biotinylated chromogranin A probe. Numerous tumour cells show a positive signal as indicated by the blue colour in the cytoplasm. ×320

Fig. 9. Case 2: in situ hybridization with biotinylated chromogranin B probe. A positive hybridization signal is shown by numerous neoplastic cells. Some nuclei of the thyroid follicular cells display a non-specific signal. ×128

The authors concluded that there was medullary thyroid carcinoma differentiation in these tumours. The illustration of one of these cases depicted features very similar to those observed in the present cases. The extraordinary high percentage of calcitonin-positive, anaplastic tumours in this latter series, most likely can be explained by the use of polyspecific antisera, since these results could not be reduplicated in the extensive investigation of Carcangiu et al. (1985). Two additional cases similar to the present ones were reported by Mendelsohn et al. (1980) as anaplastic variants of medullary thyroid carcinomas (MTC). Both these cases were regarded as the thyroid equivalent of oat-cell carcinoma and probably as the small-cell counterpart of MTC.

In contrast to what is classically seen in MTC and in the cases reported by Nieuwenhuijzen Kruseman et al. (1982) and by Mendelsohn et al. (1980), neither of the cases described here has calcitonin localized either with immunohistochemistry or by in situ hybridization. It is possible that the lack of calcitonin in the neoplastic cells results from a structural dedifferentiation of these elements with loss of some functional properties. As a consequence, in view of better evidence in favour of a C-cell derivation, it appears that the present cases ought to be kept separate from the anaplastic variants of MTC and the more generic term of oat-cell or small-cell carcinoma seems more appropiate.

Case 1 is also associated with a papillary carcinoma. As pointed out by Remick et al. (1981) the association of extrapulmonary oat-cell carcinomas with other types of carcinomas in the same sites is a common occurrence. This association has been considered to be the result of a divergent differentiation of a common precursor (Cook et al. 1976; Eusebi et al. 1978) and the neural crest origin has therefore been denied for most of these cases. Nieuwenhuijzen Kruseman et al. (1982) thought that the origin of their cases was from C-cells especially after they showed calcitonin production by two tumours. This statement has been challenged by Golouh et al. (1985), who described a case of mucus- and calcitonin-producing signet-ring cell carcinoma of the thyroid and concluded that the neoplastic elements might have had an origin other than the neural crest derived C-cells. The final resolution of this problem must await further experimental evidence.

In conclusion, our findings indicate that the small-cell carcinomas described in this report should be separate from MTC and should be regarded as a distinct entity, probably the thyroid equivalent of oat-cell carcinomas of the lung.

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