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Case report

Primary thymic carcinoid with Cushing's syndrome

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Summary. In a 52-year-old Caucasian man osteopoikilosis had been misdiagnosed roentgenologically 2 years before his death. Gradually he developed Cushing's syndrome and ultimately superior vena caval obstruction. At autopsy a primary thymic carcinoid with extensive osteoblastic bone metastasis was found. Immunohistochemically the tumor was shown to be positive for adrenocorticotropic hormone (ACTH), cytokeratin (KL1), neuron-specific enolase, synaptophysin, chromogranin and glucagon. Remarkably the tumour was negative for serotonin despite high urinary hydroxyindolacetic acid levels. Bilateral hyperplasia of the adrenal cortex was found. The adenohypophysis showed a considerable reduction of ACTH-producing cells and numerous Crooke's cells with a characteristic immunohistochemical pattern.

Key words: Thymic carcinoid – Cushing's syndrome – Immunohistochemistry

Introduction

Primary thymic carcinoid was defined as a new entity by Rosai and Higa in 1972. Light microscopically thymic carcinoid is characterized by a *zellballen*-like growth pattern, pseudorosettes and immunohistochemical positivity for neuroendocrine markers, ultrastructurally by typical electron-dense, neurosecretory granules (Wick and Rosai 1988).

About 100 cases of thymic carcinoid have been published in the world literature (Viebahn et al. 1985), about 20 of which showed Cushing's syndrome (Table 1). The association with multiple endocrine adenopathy syndrome (Rosai et al. 1972; Marchevsky and Dikman 1979; Vener et al. 1982), myelolipoma (Harris and El-Katib 1968) and hypertrophic osteoarthropathy (Lowenthal et al. 1974) has been observed in a few cases. Spindle cell variants (Levine and Rosai 1976) and melanocytic differentiation (Ho and Ho 1977) have also been rarely reported.

Here we report on a case of thymic carcinoid with a clinically and pathologically documented Cushing's syndrome and mainly osteoblastic bone metastases in which adrenocorticotropic hormone (ACTH) could be demonstrated immunohistochemically in the tumour. In addition bilateral hyperplasia of the adrenals, a marked reduction of the absolute number of ACTH-positive cells in the adenohypophysis transformed into Crooke's cells with a characteristic immunohistochemical staining pattern was found. Remarkably the tumour showed no immunoreactivity for serotonin while the urinary hydroxyindol-acetic acid values were greatly elevated.

Table 1. Reported cases of thymic carcinoid with Cushing's syndrome in chronological order; the cases listed beneath the line were reported after the definition of the entity by Rosai and Higa in 1972

Year	Author	Number of cases
1945	Frank	1
1961	Camus et al.	1
1963	Micic and Arsenijevic	1
1965	Warter et al.	1
1966	Lemon et al.	1
1967	Miura et al.	1
1968	Harris and El-Katib	1
1968	Albeaux-Fernet et al.	1
1970	Kay and Willson	1
1970	Luton et al.	1
1970	Gilbert-Dreyfus et al.	1
1972	Pimstone et al.	1
1974	Mussini-Montpellier et al.	1
1980	Stewart and Kingston	1
1981	Heitz et al.	1
1982	Wick et al.	5
1984	Huntrakoon et al.	1
1985	Lieske et al.	1
1987	Lagrange et al.	1

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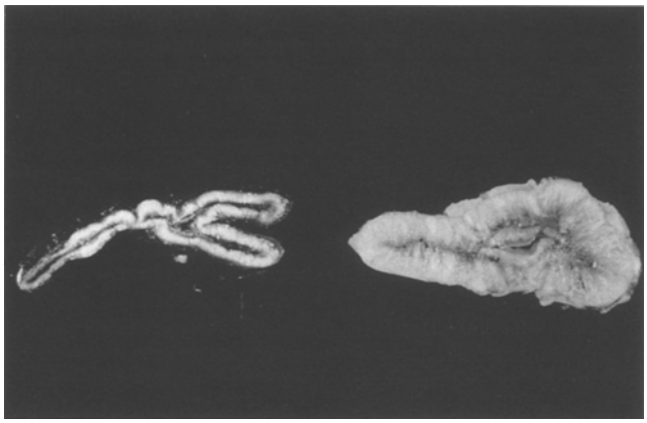

Fig. 1. Comparison of adrenal cortex (*left*) from a normal person with the hyperplastic adrenal cortex (*right*) due to ectopic ACTH stimulation

Case report

Two years before his death osteopoikilosis was misdiagnosed radiologically in a 52-year-old Caucasian male who latter gradually developed hypertension (165/110 mm Hg), superior vena cava syndrome and a Cushing's syndrome with a "moon face". In serum a markedly elevated ACTH (129 pg/ml), cortisol (1299 ng/ml) and dehydroepiandrosterone (4429 ng/ml) level, hypokalemia (2.3 mmol/l) and hyperglycaemia (maximum 220 mg/dl) were found. In urine free cortisol was excessively elevated to 17157 µg/24 h urine and hydroxyindolacetic acid to 13.5 mg/24 h urine. CT of the hypophysis showed no abnormality. Chest radiography showed the upper mediastinum to be enlarged. Biopsies from mediastinoscopy were judged to be from small cell bronchial carcinoma. Eventually the patient died of heart failure probably due to cardiac arrhythmia. At autopsy a metastatic primary thymic carcinoid was found.

For light microscopy the tissue was fixed in 4% formaldehyde. Conventional light microscopical slides were stained with haematoxylin and eosin (H&E), elastic van Gieson (EVG), periodic acid-Schiff (PAS), Congo red and argyrophilic silver impregnation according to Churukian and Schenk 1979. Immunohistochemistry was performed on the primary tumour and the bone metastase by the ABC method (Hsu et al. 1981, modified by Schaefer 1984). For the antibodies used and their working dilutions, see Table 1. For desmin staining the slides were predigested by proteinase K for 3 min. In addition ACTH and cytokeratin (KL1) were examined in the hypophysis separately and in a consecutive double incubation procedure with first the ABC method for detection of ACTH and then the APAAP procedure to detect KL1 (Nakane 1968; True 1990). Vimentin and desmin were also tested in the hypophysis. Ten control adenohypophyses from normal persons were immunostained for comparison.

For electron microscopy the post-mortem tumour tissue was fixed in 3.5% glutaraldehyde, osmicated and routinely processed for final uranyl-acetate and lead citrate staining and was observed in an electron microscope (Phillips CM 10).

Autopsy revealed a large, non-encapsulated, mediastinal mass (10 × 10 cm) with local extension into the pericardium enveloping the thoracic aorta, the common carotids and the brachiocephalic vein. Extensive osteoblastic bone metastases were found in the skull, vertebrae, pelvis and femur. A small metastasis was seen in the cerebral meninges enveloping the facial nerve. Micrometastases were found in the paratracheal and paraaortic lymph nodes. The adrenal cortex was bilaterally diffusely hypertrophied (Fig. 1). The cause of death was heart failure, probably due to cardiac arrhythmia.

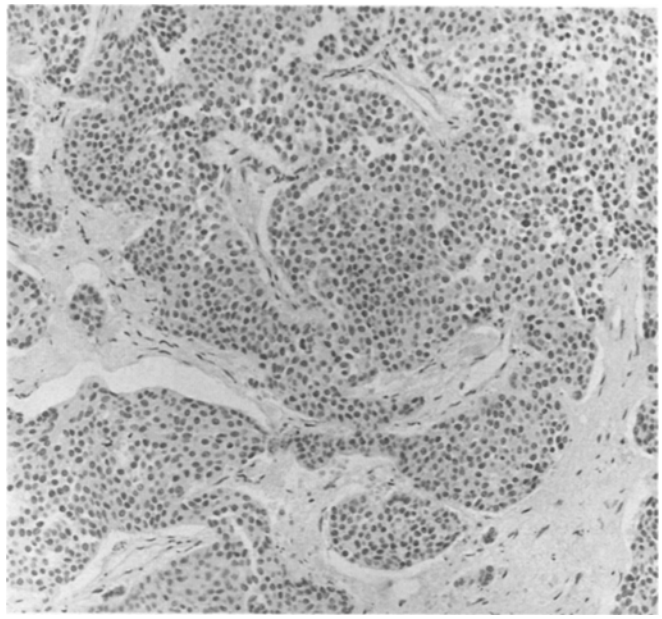

Fig. 2. Microscopical view of the thymic carcinoid. Note the *zellballen* interspersed by fibrovascular septa. H&E, × 170

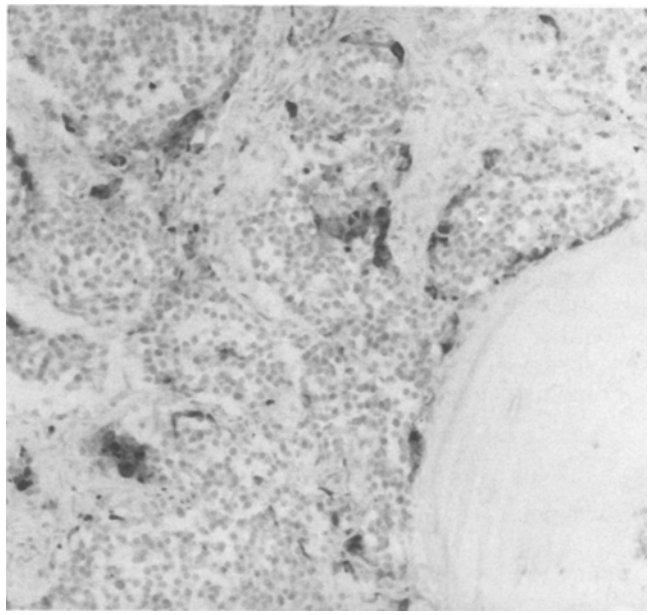

Fig. 3. Pronounced reactivity for ACTH in the femoral bone metastasis in tumour cells residing along osseous trabeculae. ABC, × 170

Histologically the tumour exhibited a lobular, organoid and *zellballen*-like growth pattern (Fig. 2), a few true rosettes, many perivascular pseudorosettes and extensive lymphangiosis blastomatosa. The tumour cells were small, with eosinophilic cytoplasm and round nuclei. Mitotic activity was not conspicuous. There were areas with extended necrosis and haemorrhages. At the outer edges of the primary tumour small residues of thymic tissue with Hasall's corpuscles and thymic cysts were found. The bone metastases were very extensive and found to be strongly osteoblastic. The argyrophilic reaction according to Churukian and Schenk showed numerous intracytoplasmic, positive granules. The Congo stain for amyloid was negative.

Table 2. Panel of primary antibodies used

Antibody	Mono/ polyclonal	Dilution	Source
Synaptophysin	Monoclonal	1:50	Boehringer,
KL 1	Monoclonal	1:50	Immunotech
Chromogranin	Monoclonal	*	Camon
Vimentin	Monoclonal	1:100	Dako
Desmin	Monoclonal	*	Dako
CEA	Monoclonal	1:10	Medac
Serotonin	Monoclonal	1:10	Dako
α-HCG	Monoclonal	1:100	Serolab
Prolactin	Monoclonal	*	Amersham
HMB 45	Monoclonal	1:2000	Enzo
T-200	Monoclonal	1:100	Camon
S-100	Polyclonal	1:100	Dako
NSE	Polyclonal	1:100	Dako
ACTH	Polyclonal	1:300	Dako
Glucagon	Polyclonal	*	Dako
Thyreoglobulin	Polyclonal	*	Dako
Calcitonin	Polyclonal	1:200	Dako
α_1-fetoprotein	Polyclonal	*	Dako
Gastrin	Polyclonal	*	Dako
Somatostatin	Polyclonal	*	Dako
β-HCG	Polyclonal	1:100	Dako

* Prediluted by the supplier

KL1, Cytokeratin; CEA, carcinoembryonic antigen; α-HCG, alpha human chorionic gonadotropin; NSE, neuron specific enolase; ACTH, adrenocorticotropic hormone

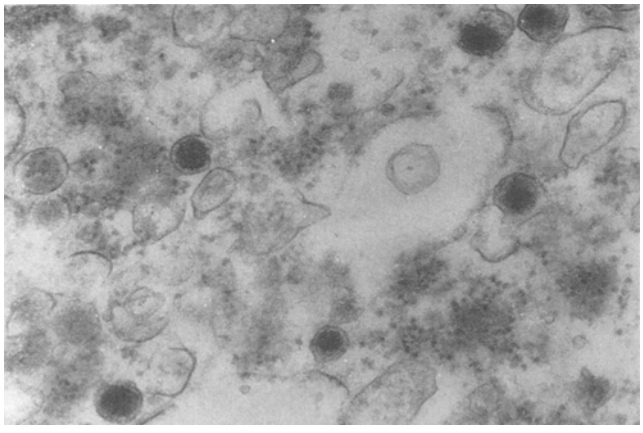

Fig. 4. Electron-dense, neurosecretory granules surrounded by a halo and an outer membrane in the primary tumour cells. ×40875

ACTH and KL1 immunostaining showed a strong, granular, intracytoplasmic reaction most marked in the bone metastasis where the positive cells had a tendency to lie near the bone trabeculae (Fig. 3). In the primary tumour only small groups of tumour cells were positive. Some tumour cells showed co-expression of KL1 and ACTH. The staining pattern for the neuroendocrine markers neuron specific enolase (NSE), chromogranin A and synaptophysin was characterized by a clear intracytoplasmic positivity in the primary tumour and in the metastases, while S-100 was only positive in the bony metastases (Table 2). Glucagon was detectable in small groups of tumour cells in the primary lesion and diffusely in the bone metastasis (Table 2). All other markers proved to be negative.

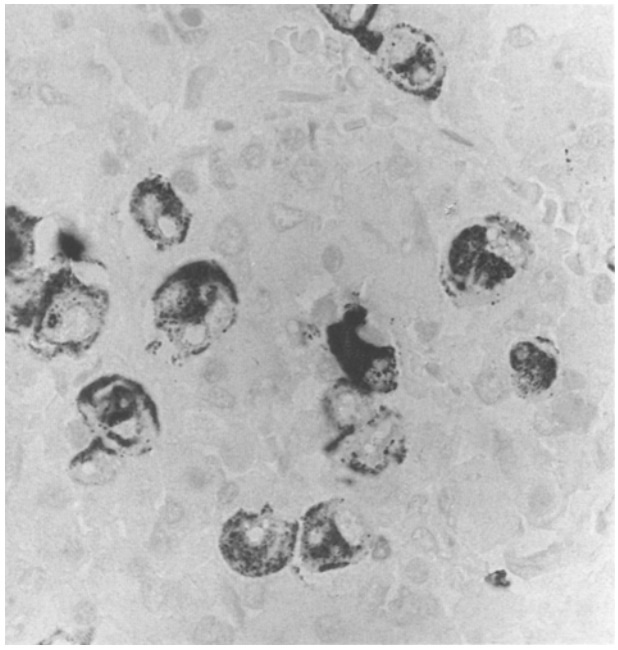

Fig. 5. Double ring positivity for ACTH of Crooke's cells in the adenohypophysis. ABC, ×540. The area between the rings was shown to be positive for KL1 (Fig. 6)

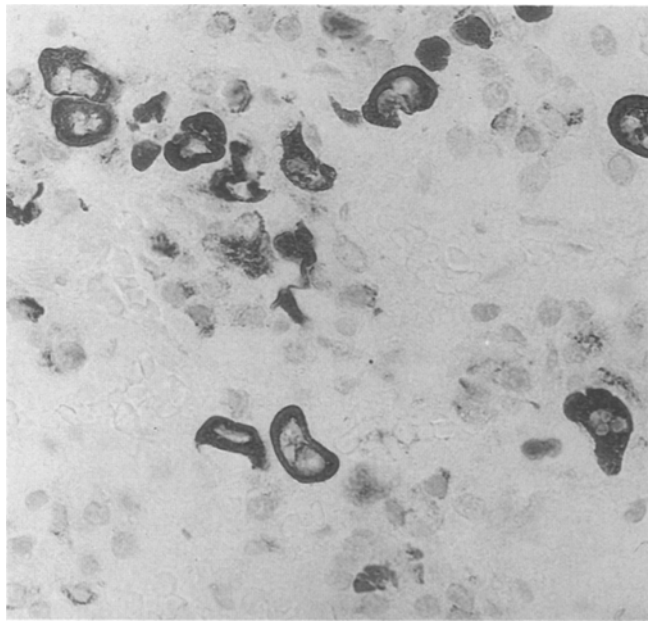

Fig. 6. Single ring positivity for KL1 of Crooke's cells in the adenohypophysis. ABC, ×540

In electron microscopy a moderate amount of electron-dense, membrane bound granules of the halo type with a diameter of 150–500 nm (Fig. 4) and occasionally desmosomes were found.

The hypophysis showed Crooke's cells with double ring, intracytoplasmic positivity for ACTH (Fig. 5). The ring in between was positive for KL1 (Fig. 6). The number of ACTH-positive cells in the adenohypophysis was reduced by approximately 50% compared with ten control adenohypophyses.

Table 3. Immunohistochemical results

	Primary tumour	Bone metastases
KL1	+	+++
ACTH	+	+++
Chromogranin	+++	+++
NSE	+++	+++
Synaptophysin	+++	+++
Glucagon	+	+++
S-100	−	+++

All the other markers examined were negative

Discussion

The differential diagnosis of thymic carcinoid comprises paraganglioma, malignant lymphoma, true thymoma, thymic seminoma and metastasis from other primary neuroendocrine tumours (Wick and Rosai 1988). In this case a carcinoid originating from another site was excluded by autopsy. In addition, residual thymic tissue was found in outer parts of the primary tumour. Other tumours were excluded by the characteristic microscopical and immunohistochemical results.

Statistically males have a three-fold higher incidence of thymic carcinoid and the mean age is approximately 43 years so that this case is typical in these respects (Wick and Rosai 1988).

The lesion showed local complications such as the superior vena cava syndrome due to tumour compression. The pericardium was infiltrated and possibly evoked cardiac arrhythmia. Such local complications have been described by others (Rosai and Higa 1972; Salyer et al. 1976; Wick et al. 1980 and 1982; Wick and Scheithauer 1984; Hartmann et al. 1989).

Ectopic ACTH production in thymic carcinoid has already been observed, but interestingly it is not always associated with Cushing's syndrome (Moriki et al. 1985; Viebahn et al. 1985; Herbst et al. 1987). The amino acid sequence of thymic ACTH has been found to be the 2–38 sequence of pituitary ACTH (Lowry et al. 1976) and ACTH levels can even be used as a tumour marker (Rees et al. 1977). The biological effectiveness of thymic ACTH is well demonstrated in this case since the neoplasm was shown to be positive in immunostaining with ACTH and the serum level of ACTH was greatly elevated. The patient developed Cushing's syndrome with bilateral hyperplasia of the adrenal cortex. Hypophyseal ACTH production was lowered, as can be deduced from the reduced number of ACTH-producing cells in the adenohypophysis and the development of Crooke's cells. These showed a characteristic immunostaining pattern with a double ring positivity for ACTH and the KL1 positivity in between. This pattern has also been described by others in Crooke cells caused by other diseases than ACTH-producing thymic carcinoid (Neumann et al. 1984; Uei 1988). Electron microscopically this pattern has been correlated with the presence of neurosecretory granules in the ACTH-positive area and intermediate filaments in the KL1-positive areas (DeCicco et al. 1972).

KL1 was shown to be strongly positive in the carcinoid, supporting the concept that carcinoids arise from the enterochromaffin cells of endodermal origin (Kultschitzky cells), while true APUDomas of neurogenic origin, like phaeochromocytoma and paraganglioma, are negative for KL1 (Heitz 1987; Hartmann et al. 1989; True 1990). Previously all carcinoids had been thought to arise from the neuroectoderm according to the primary APUD cell concept (Pearse 1969; Judge et al. 1976).

The tumour remarkably showed no reactivity for serotonin, although the hydroxyindolacetic acid was greatly elevated in urine. This possibly is due to the lack of the aromatic L-amino acid decarboxylase, so that only the precursor 5 hydroxytryptophan can be produced by the tumour, as has already been described in a bronchial carcinoid by Sandler et al. (1961). This might also be one of the reasons for the usual absence of the typical carcinoid syndrome in thymic carcinoids, in contrast to bronchial carcinoid and midgut carcinoids (Williams and Sandler 1963). Interestingly a multidirectional thymic carcinoma with neuroendocrine and sarcomatoid components has been recently reported to have been associated with the carcinoid syndrome (Paties et al. 1991).

The carcinoid had a strong positivity of the neuroendocrine markers NSE, chromogranin and synaptophysin, as has been reported by other authors (Moriki et al. 1985; Herbst et al. 1987; Lagrange et al. 1987; Wick and Rosai 1988; Hartmann et al. 1989; Wöckel et al. 1990) and in carcinoids of other sites.

By electron microscopy only a moderate number of neurosecretory granules were detected in the primary tumour. Possibly this is due to the high excretion rate of thymic carcinoids with Cushing's syndrome (Viebahn et al. 1985).

The tumour showed extensive osteoblastic metastases in bones, while other metastases were uncommon and very small. The observed metastatic pattern seems to be characteristic for thymic carcinoid, as has been described by many authors. Other metastatic sites reported in the literature are the skin, liver, brain, optic nerve and lung (Wick and Rosai 1988).

In contrast to classical thymoma and midgut carcinoids, thymic carcinoid is reported to metastasize in up to 73% (Wick and Rosai 1988). This case demonstrates the remarkable malignant potential of thymic carcinoid.

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