Autopsy of a Patient with Cushing's Syndrome Who Was Revealed to Have Pulmonary Tumorlets Producing Ectopic ACTH

ATSUSHI OGO, TOMOAKI ETO, SHINSUKE HIRAMATSU, AKIMASA WATANABE, YOSHIYUKI SAKAI, HIDEYUKI YOSHIKUNI, NORIKO UESUGI* AND OSAMU NAKAJIMA*

Department of Metabolism and Endocrinology, Clinical Research Institute, National Hospital Organization Kyushu Medical Center, 1-8-1 Jigyohama, Chuo-ku, Fukuoka City, 810-8563 Japan

**Department of Pathology, National Hospital Organization Kyushu Medical Center, 1-8-1 Jigyohama, Chuo-ku, Fukuoka City, 810-8563 Japan*

Abstract. The patient, a 78-year-old female with a 10-year history of type 2 diabetes mellitus, was admitted to our department for evaluation of leg edema and general fatigue. Biochemical investigations revealed hypokalemia and elevated serum cortisol and plasma ACTH levels, with a loss of diurnal rhythm and failure of suppression at high doses (8 mg) of dexamethasone. No pituitary tumor or parasellar tumor was detected by contrast-enhanced computed tomography (CT) or magnetic resonance image scan of the pituitary. High resolution CT of the lung and bronchoscopic examination revealed no abnormalities. Abdominal and pelvic CT indicated bilateral, slightly diffuse, adrenal gland hyperplasia only. These findings led to a diagnosis of ACTH-dependent hypercortisolism from an undefined source. Ten days after admission the patient had a fever and was diagnosed with disseminated intravascular coagulation. Despite intensive treatment about 1 month after admission the patient died from progressive multiple organ failure. At autopsy, a histological examination of the periphery of the right middle lobe of the lung revealed the presence of tumorlets. Immunohistochemical staining of the tumorlets revealed scattered cells containing ACTH and many cells containing chromogranin A that were positive for Grimelius staining. In addition, multiple microabscesses were present throughout most tissues of the body. The ectopic hormonal production observed in the present case suggests that pulmonary tumorlets should thus be considered in the differential diagnosis of Cushing's syndrome, and medical treatment to inhibit steroidogenesis should be started immediately to reduce the risk of complications from hypercortisolism.

Key words: Pulmonary tumorlets, Ectopic ACTH syndrome, Autopsy, Diabetes mellitus

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ECTOPIC hormone production is defined as tumor-produced hormone that is not normally produced by the tissue from which the tumor originates, and most of the tumors have proven to be malignant [1]. Wajchenberg *et al.* reported that 10–15% of cases of Cushing's syndrome are caused by ectopic adrenocorticotrophic hormone (ACTH)-producing tumors. Of these tumors,

35–49% were reported to be carcinoid tumors. Of the lung carcinoid tumors, 2% were reported to be ectopic ACTH-producing tumors [2]. In Japan, the ectopic ACTH-producing tumor has been observed in 3.6% of Cushing's syndrome cases and the frequency of the tumors causing ectopic ACTH syndrome was as follows, lung cancer, 49%; carcinoid, 15.7%, malignant thymic tumor, 9.8%; pancreas cancer, 5.9%; thyroid medullary cancer, 5.9%; and pheochromocytoma, 2% [3, 4]. In recent years, an increasing number of pulmonary tumorlets have been reported as a result of the development of more sophisticated radiological imaging techniques. However, ectopic hormone production with clinical significance by pulmonary tumorlets is

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Correspondence to: Atsushi OGO, M.D., Ph.D., Department of Metabolism and Endocrinology, National Hospital Organization, Kyushu Medical Center, 1-8-1 Jigyohama, Chuo-ku, Fukuoka City, 810-8563 Japan

very rare. Although pulmonary tumorlets are still not well understood, the accumulated findings suggest that the concept of the pulmonary tumorlet as a tumor-like lesion or neuroendocrine cell hyperplasia needs to be reconsidered [5, 6]. Histologically, distinguishing pulmonary tumorlets from a carcinoid tumor is difficult, since there are no definite histological criteria [7]. We herein report a case of pulmonary tumorlets producing ectopic ACTH which was revealed at autopsy in a patient with Cushing's syndrome.

Case Report

History and examination (Fig. 1)

A 78-year-old female with a 10-year history of type 2 diabetes mellitus was admitted to our department for

evaluation of leg edema and general fatigue. However, she did not display a cushingoid appearance, such as moon face, hirsutism, acne, buffalo hump or abdominal striae. At this time (Table 1), her glycemic control was poor. Her fasting glucose level was 172 mg/dl and hemoglobin A1c was 8.5%. The patient was treated for diabetes mellitus with 16 units/day of intermediate-acting insulin. A biochemical and endocrinological investigation revealed hypokalemia (1.8 mEq/L) and elevated serum cortisol and plasma ACTH levels (The ACTH-IRMA kit Mitsubishi: Mitsubishi Kagaku Iatron, Inc. Tokyo, Japan) of 55.0 µg/dl and 329 pg/ml respectively, with loss of diurnal rhythm (Table 2). Urinary 17-OHCS, 17-KS and free cortisol excretion were also elevated to 15.2 mg/day, 11.6 mg/day and 1.45 mg/day, respectively. Six months before this admission, she had undergone laminoplasty of the cervical spine at our hospital. At this time, her electrolytes

Fig. 1. Clinical course

Table 1. Laboratory data on admission

Urinalysis	
Protein	(+)
Glucose	(+)
Occult blood	(-)
Ketone	(-)
CBC	
WBC	13500/ μ l
Neu	91.9%
Eos	0.1%
Baso	0.2%
Mono	2.6%
Lympho	5.2%
RBC	$372 \times 10^6/\mu$ l
Hb	11.4 g/dl
Ht	33.6%
Plt	$14.2 \times 10^4/\mu$ l
Blood chemistry	
TP	4.7 g/dl
Alb	2.7 g/dl
T-Bil	2.0 mg/dl
AST	37 IU/l
ALT	46 IU/l
LDH	494 IU/l
ALP	333 IU/l
γ -GTP	48 IU/l
AMY	127 IU/l
CPK	69 IU/l
BUN	12 mg/dl
Cre	0.6 mg/dl
TG	107 mg/dl
T-Chol	156 mg/dl
Na	146 mEq/l
K	1.8 mEq/l
Cl	86 mEq/l
CRP	0.15 mg/dl
Glucose	172 mg/dl
HbA1c	8.5%
Endocrinological data	
ACTH	329 pg/ml
GH	0.20 ng/ml
LH	0.10 mIU/ml
FSH	0.20 mIU/ml
PRL	13.9 ng/ml
TSH	0.032 μ IU/ml
FT4	1.39 ng/ml
Cortisol	55.0 μ g/dl
DHEA-S	3190 μ g/dl
17-OHCS	15.2 mg/day
17-KS	11.6 mg/day
PRA	0.3 ng/ml/hr
Aldosterone	42 pg/ml
CA: AD	79 pg/ml
NA	424 pg/ml
DA	44 pg/ml

Table 2. Diurnal rhythm of ACTH and cortisol, and dexamethasone suppression test

	ACTH (pg/ml)	Cortisol (μ g/dl)
Circadian rhythm		
9 am	329	55.0
5 pm	241	44.9
9 pm	267	45.2
Dexamethasone (Dex) suppression test		
Dex 2 mg	331	63.5
Dex 8 mg	180	46.3

Table 3. Corticotropin-releasing hormone (CRH) test

	0	15	30	60	90	120 min
ACTH (pg/ml)	139	174	160	177	150	150
Cortisol (μ g/dl)	47.1	47.9	67.7	58.4	56.5	61.5

were all at normal levels. Elevated cortisol and ACTH levels were not suppressed by overnight administration of 2 mg or 8 mg of dexamethasone (Table 2). Moreover, following the intravenous administration of 100 μ g of ovine corticotropin-releasing hormone (CRH), serum cortisol and plasma ACTH levels still showed no response (Table 3). Roentgenographic examination of the skull showed a normal sella turcica. No pituitary tumor or parasellar tumor was detected by contrast-enhanced computed tomography (CT) or magnetic resonance image (MRI) scan of the pituitary. High resolution CT of the lung revealed no abnormalities. Bronchoscopic studies were performed; however, these also gave normal results. Abdominal and pelvic CT indicated bilateral and slightly diffuse adrenal gland hyperplasia only. These findings suggested that the diagnosed Cushing's syndrome had been caused by ACTH-dependent hypercortisolism due to either pituitary or ectopic. Ten days after admission, the patient had a body temperature of 38.0°C, blood pressure of 120/60 mmHg, and heart rate of 108/min. Peripheral blood cell count revealed a white blood cell count of $1.93 \times 10^4/\mu$ l, hemoglobin of 11.2 g/dl and a decreased platelet count of $6.2 \times 10^4/\mu$ l. Erythrocyte sedimentation rate was elevated. The prothrombin time was prolonged (14.8 sec), and the activated partial thromboplastin time was also prolonged (54.0 sec). The plasma fibrinogen content was 138 mg/ μ l, and the fibrin/fibrinogen degradative products (FDP) and D-dimer levels were markedly increased to 38.1 μ g/ml and 32.7 μ g/ml respectively, with an anti-thrombin III

Fig. 2. Microscopically, small multiple nodules were identified at the periphery of the right middle lobe of the lung. There were multiple discrete nests of proliferating uniform oval cells. (A and B, hematoxylin and eosin) Magnification: A, $\times 20$; B, $\times 400$. Immunostaining revealing strong positivity for corticotropin (C), chromogranin A (D) and histochemically the cells showed positive staining with the Glimelius (E) stain in the tumorlets. Magnification: $\times 20$.

activity of 30.0%. These data indicated disseminated intravascular coagulation (DIC), and we began treating the patient with antibiotics (Imipenem cilastatin 1 g/day) even though the culture of sputum, urine and blood showed no growth, as well as with 2000 mg/day of gebexate mesilate and 5000 units/day of dalteparin. Despite the intensive therapy, the patient's temperature rose to 40°C and she became hypotensive. She was transferred to the intensive care unit, but she continued to deteriorate, displaying a worsening renal function and severe heart failure, and she also required ventilatory support. Her condition continued to worsen, and

she eventually succumbed to multiple organ failure 14 days after presentation in the intensive care unit.

Autopsy results

Autopsy was performed at 17 hours postmortem. Gross examination of the skull base, sella turcica, cavernous sinus, chest, and abdomen revealed no evidence of an ectopic ACTH-producing tumor. Histological examination of the pituitary gland itself and of the surrounding tissues revealed no neoplasm. The lung was removed and serially sectioned. Microscopically,

Fig. 3. Multiple microabscesses were present in the myocardium (arrows) (hematoxylin and eosin) Magnification: $\times 20$.

small multiple nodules were identified at the periphery of the right middle lobe of the lung. There were multiple discrete nests of proliferating uniform oval cells. The nests varied in size from 1 mm to 3 mm in diameter. Immunohistochemical staining (Automatic immunostaining system: Dako) of the nodules revealed scattered cells containing ACTH (polyclonal rabbit anti-human adrenocorticotropin, 1 : 100 dilution: Dako, Carpinteria, CA, USA) and many cells showed positive staining with the Grimelius and chromogranin A (rabbit anti-human chromogranin A, 1 : 100 dilution: DAKO, Carpinteria, CA, USA) stain (Fig. 2). In addition, multiple microabscesses were present throughout most tissues of the body including the myocardium, lung, kidney, spleen, adrenal glands, and thyroid gland (Fig. 3). The bacterial morphology was similar in all regions and consistent with *S. aureus*.

Discussion

Recently, an increasing number of pulmonary tumorlets have been reported because of the development of radiological imaging techniques. However, clinically significant ectopic hormone production by pulmonary tumorlets is very rare. Only seven such cases of pulmonary tumorlets associated with Cushing's syndrome have ever been reported (Table 4). Sobota & Reed [8] reported the first case of a patient with Cushing's syndrome who had a nodular infiltration of the right lung. A microscopic examination revealed the

presence of multiple bronchial adenomas of different sizes, some of them consistent with a diagnosis of tumorlets. The second patient was reported by Ranchod [9], a 61-year-old woman with Cushing's syndrome, haemoptysis and a left-upper-lobe mass that proved to be a small-cell lung carcinoma with surrounding tumorlets. Immunohistochemical studies revealed positive ACTH staining in both lesions, thus suggesting that the carcinoma originated from one of the tumorlets. Rodgers-Sullivan *et al.* [10] reported the case of a 39-year-old patient who displayed hypokalaemia and an infiltrate in the lower lobe of the right lung. The patient underwent resection of the superior segment of the right lower lobe, and a microscopic examination revealed chronic pneumonitis with abscess formation, and the presence of numerous tumorlets. Endocrinological features were interpreted as being consistent with adrenocortical hyperplasia, and a bilateral adrenalectomy was performed. The patient died 2.5 years after undergoing an adrenalectomy and the postmortem examination revealed metastatic carcinoid tumors in the mediastinum, both lungs, mesentery, liver, spleen and kidneys. Nevertheless, the authors state that the carcinoid tumorlets were a source of excessive production of ACTH. Arioglu *et al.* [11] reported the case of a 9-year-old girl who presented with weight gain, growth arrest and hypertension. The endocrinological findings were consistent with Cushing's syndrome, but the results from MRI of the pituitary were normal. Nine years after a bilateral adrenalectomy, she presented with increased pigmentation, and a CT scan of the chest revealed multiple small nodules confined to the right lower lung lobe. The patient underwent a right lower lobectomy and a microscopic examination revealed the presence of tumorlets. Ozbey *et al.* [12] described a 35-year-old female patient with ectopic ACTH secretion due to multiple peripheral pulmonary carcinoid tumors and tumorlets. The results of a high-dose dexamethasone suppression test led to the diagnosis of Cushing's disease; however, no tumor was identified on sella imaging, and bilateral inferior petrosal sinus sampling was non-diagnostic. CT of the lungs revealed multiple acinar-nodular parenchymal infiltrations confined to the left lung. They decided to explore these nodules with an open lung biopsy. The histopathological diagnosis was multiple peripheral carcinoid tumors and tumorlets of a carcinoid type showing positive immunostaining for ACTH. Povedano *et al.* [13] reported a patient with an aggressive case of

Table 4. Profiles of patients with pulmonary tumorlets associated with Cushing's syndrome

Author	Year	age (yr)/ Gender	Cushingoid appearance	cortisol (µg/dl) ACTH (pg/ml) 17OHCS (mg/day) 17KS (mg/day) u-cortisol (µg/day)	abnormal shadow on chest X-ray	chest CT	broncho- scopy	pituitary MRI	therapy	outcome	microscopic examination	immuno- staining
Sobota	1964	21/F	(+)	ND ND 58.2 42.8 ND	(+)	ND	ND	ND	resection of rt adrenal & part of lt adrenal	Died	multiple bronchial adenomas being consistent with tumorlets	ND
Ranchod	1977	61/F	(+)	ND ND ND ND ND	(+)	ND	normal	ND	lt pneumonectomy	Died	small cell lung carcinoma with surrounding tumorlets	positive ACTH staining
Rodgers- Sullivan	1978	39/M	(-)	36.2 ND ND ND 76.6	(+)	ND	ND	ND	resection of superior segment of RLL & bilateral adrenalectomy	Died	chronic pneumonitis with abscess and numerous tumorlets	ND
Arioglu	1998	9/F	(+)	28 200 ND ND 470	(+)	multiple small nodules in the RLL	ND	normal	medication of ketoconazole & mifepristone and then bilateral adrenalectomy (10 yr) rt lower lobectomy of the lung (19 yr)	cured	tumorlets are seen as a cluster of nodules	ND
Ozboy	2000	35/F	(+)	48 140 ND ND ND	(+)	multiple acinar nodular parenchymal infiltration in LLL	ND	normal	bilateral adrenalectomy & lt pneumonectomy	cured	multiple peripheral cartinoid and tumorlets	positive ACTH staining
Povedano	2001	22/M	(+)	12.3 at 24-00 h 50 209 ND ND	(+)	3 cm nodule in RLL	normal	ND	bilateral adrenalectomy (25 yr) rt lower lobectomy of the lung (45 yr)	cured	peripheral bronchial cartinoid surrounded by tumorlets	positive ACTH staining
Liu	2003	65/M	(-)	ND ND ND ND ND	(+)	cystic lesion containing blood clots	ND	ND	rt lower lobectomy of lung	cured	multiple discrete uniform small nests of tumorlets	positive ACTH staining
Present case		78/F	(-)	55 329 15.2 11.6 1.45	(-)	normal	normal	normal	ND	Died	autopsy revealed small multiple nodule in the RML and multiple discrete nests of uniform oval tumorlet cells	positive ACTH staining

RLL: right lower lobe of lung RML: right middle lobe of lung LLL: left lower lobe of lung ND: not done u-cortisol: urine cortisol

Cushing's syndrome that required a bilateral adrenalectomy, diagnosed 22 years before a 3-cm lung nodule became apparent in a chest X-ray. The biopsy after lung surgery revealed a typical peripheral bronchial carcinoid tumor surrounded by tumorlets. Both the tumorlets and the carcinoid tumor showed strongly positive ACTH immunostaining. Recently, Liu *et al.* [14] reported the case of a 65-year-old male who underwent a right lower lobe lobectomy because of a sudden attack of coughing and hemoptysis. A histological examination revealed multiple, discrete and small uniform nests of tumor cells surrounding the dilated bronchioles. Using immunohistochemistry, the proliferating cells were confirmed to be neuroendocrine in origin with ectopic ACTH production; however, there were no signs or symptoms of Cushing's syndrome. To our knowledge, the patient in our case is the first proven to have pulmonary ectopic ACTH-producing tumorlets, but the ectopic source of the excess corticotropin could not be identified until the autopsy revealed the pulmonary tumorlets that produced the excess corticotropin.

Pulmonary tumorlets, like bronchial carcinoid tumors, arise from Kulchitsky's cells and are immunoreactive to general neuroendocrine markers such as chromogranin A, synaptophysin, and neuron-specific enolase [9]. However, since there are no definite histological criteria, it is difficult to distinguish pulmonary tumorlets from carcinoid tumors. Therefore, the tumor size is arbitrarily used as a diagnostic criterion to distinguish between pulmonary tumorlets and carcinoid tumors. Since the size of most pulmonary tumorlets is less than 3–4 mm even at their largest dimension, a size of less than 5 mm is thus suggested as a guideline to distinguish pulmonary tumorlets from carcinoid tumors [7]. Other morphological features of pulmonary tumorlets include multiple lesions and peripheral location [15]. In this case, small multiple nodules were identified at the periphery of the right middle lobe of the lung and there were multiple discrete nests varying in size from 1 mm to 3 mm in diameter of proliferating uniform oval cells. Immunohistochemical staining of the tumorlets revealed scattered cells containing ACTH and many cells containing chromogranin A and Grimelius, confirming their neuroendocrine origin. These findings were consistent with the characteristics of pulmonary tumorlets and the tumorlets produced ectopic ACTH.

The exact incidence of tumorlets is not known, but in

one study they were found at autopsy in 2 of 1900 patients with no lung disease and 22 of 2400 lung specimens surgically removed for various causes [16]. Therefore, pulmonary tumorlets should be considered in the differential diagnosis of Cushing's syndrome, especially in patients without any apparent nonendocrine tumors. Periodic CT or MRI is necessary to detect small, slow growing lesions such as pulmonary tumorlets.

When the source of endogenous Cushing's syndrome cannot be surgically removed, medical treatment, including glucocorticoid receptor antagonists and other agents is often required, because severe hypercortisolism is associated with life-threatening infection, diabetes and a high surgical mortality and the medical therapy can inhibit steroidogenesis and potentially reduce the risk of complication from hypercortisolism. The administration of the above treatment regimen should thus be considered before performing an adrenalectomy for severe, uncontrollable Cushing's syndrome to improve tissue healing [17, 18]. In this case, the patient who had a 10-year history of type 2 diabetes mellitus displayed a high body temperature 10 days after admission, and the data indicated DIC. We assumed that an acute onset of severe infection and continuous DIC was a possible diagnosis in this patient, even though the culture of sputum, urine and blood showed no growth in spite of the multiple microabscesses which were present throughout most tissues of the body including the myocardium, lung, kidney, spleen, adrenal glands and thyroid gland on the autopsy results. We should have recognized that she was a compromised host and we therefore should have started the medical therapy for hypercortisolism at once, and then investigated the location of the ectopic ACTH producing tumor. Regarding the optimal treatment of this case, based on the above findings, priority should have instead been given to the treatment instead of the diagnosis.

In conclusion, this case demonstrates that pulmonary tumorlets should be considered in the differential diagnosis of Cushing's syndrome or a high serum cortisol level, and in particular in patients without any apparent non-endocrine tumors, who must be treated immediately with medical therapy to inhibit steroidogenesis in order to reduce the risk of complications from hypercortisolism.

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