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Ectopic hormone secretion from neuroendocrine neoplasms

Clinical and molecular studies

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Abstract

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Ectopic hormone secretion from neuroendocrine neoplasms (NENs) is rare and associated with increased morbidity and mortality. ACTH-secreting NENs causing ectopic Cushing's syndrome (ECS) are well studied, however, existing evidence is limited to small cohorts. The predictive value of hormone secretion in lung-NENs has not been widely studied. The aim of this thesis was to evaluate ACTH secreting NENs and ectopic hormone secretion in lung-NENs.

In the first paper we analysed a cohort of 51 patients with ECS. The secretion of multiple hormones in parallel, high morning cortisol, higher tumour grade, and the presence of diabetes mellitus were associated with shorter overall survival (OS). The development of ECS in non-functioning NENs was associated with tumour progression. Next, we evaluated bilateral adrenalectomy (BA) as a treatment option in ECS. Despite the frequent use of steroidogenesis inhibitor treatment, BA was necessary in a majority of patients. Significantly fewer severe complications were observed in the endoscopic group compared to open surgery (p=0.030). The two-surgeon approach BA should be considered as the method of choice in ECS when compared with other BA approaches, due to fewer complications and shorter operating time (p=0.001). The third paper demonstrated that severe hypokalaemia (< 2.5 mmol/l) at diagnosis was present in 50% of patients with ECS and was associated with shorter OS (12 months vs. 39 months, p=0.009). Hypercortisolaemia in combination with severe hypokalaemia at diagnosis of ECS is an acute life-threatening situation and immediate actions should be considered to reduce cortisol levels, primarily BA. Finally, a separate analysis of hormone secreting lung-NENs, including immunohistochemical staining for hormones was performed. Fifty-two patients out of 127 tested had elevated hormone levels with predominance of serotonin. In this lung-NEN cohort, diffuse calcitonin staining and multiple hormone secretion was associated with shorter OS (p=0.036 resp. 0.002).

In summary, this thesis contributes to a better understanding of a very rare condition and highlights the importance of intensified clinical management in ECS, particularly in cases with multiple hormone secretion. It also demonstrates hormone secretion and high calcitonin expression in tumour tissue in lung-NENs as negative prognostic factors

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To all our patients
with ectopic Cushing's
syndrome who lost their lives

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I **Lase I**, Strele I, Grönberg M, Kozlovacki G, Welin S, Janson ET. Multiple hormone secretion may indicate worse prognosis in patients with ectopic Cushing's syndrome. Hormones (Athens). 2020 Sep; 19(3):351-360. doi: 10.1007/s42000-019-00163-z.

II **Lase I**, Grönberg M, Norlén O, Stålberg P, Welin S, Janson ET. Adrenalectomy in ectopic Cushing's syndrome: A retrospective cohort study from a tertiary care centre. J Neuroendocrinol. 2021 Dec; 33(12):e13030. doi: 10.1111/jne.13030.

III **Lase I**, Grönberg M, Welin S, Janson ET. Hypokalaemia and hypercortisolaemia at diagnosis are negative prognostic factors: a retrospective analysis of the medical management of ectopic Cushing syndrome. ESMO Rare Cancers. 2025;2. doi: 10.1016/j.esmorc.2025.100013.

IV **Lase I**., Löfgren E., Hegedus A., Grönberg M., Welin S., Janson ET. Hormone activity predicts poor prognosis in lung neuroendocrine neoplasms. Manuscript, submitted.

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List of Papers

List of papers by the author not included into the thesis work.

I Ciganoka D, Balcere I, Kapa I, Peculis R, Valtere A, Nikitina-Zake L, **Lase I**, Schiöth HB, Pirags V, Klovins J. Identification of somatostatin receptor type 5 gene polymorphisms associated with acromegaly. Eur J Endocrinol. 2011 Oct;165(4):517-25. doi: 10.1530/EJE-11-0416.

II Falkman L, Sundin A, Skogseid B, Botling J, Bernardo Y, Wallin G, Zhang L, Welin S, **Lase I**, Mollazadegan K, Crona J. Genetics-guided therapy in neuroendocrine carcinoma: response to BRAF- and MEK-inhibitors. Ups J Med Sci. 2024 Apr 10;129. doi: 10.48101/ujms.v129.10660.

III Mollazadegan K, Botling J, Skogseid B, Eriksson B, Falkman L, Zhang L, **Lase I**, Welin S, Sundin A, Crona J. The impact of re-characterizing metastatic pancreatic neuroendocrine tumors: A prospective study. J Neuroendocrinol. 2025 Aug;37(8):e70040. doi: 10.1111/jne.70040.

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Abbreviations

AC	Atypical carcinoid
ACC	Adrenocortical carcinoma
ACTH	Adrenocorticotrophic hormone
ANP	Atrial natriuretic peptide
ATRX	Alpha-thalassemia/mental retardation syndrome X-linked
BA	Bilateral adrenalectomy
CD	Cushing's disease
CDX-2	Caudal type homeobox 2
CRH	Corticotropin releasing hormone
CS	Cushing's syndrome
CT	Computed tomography
DAXX	Death-domain associated protein
DIPNECH	Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia
DOC	11-Deoxycorticosterone
ECS	Ectopic Cushing's syndrome
EPNS	Endocrine paraneoplastic syndrome
ERN	European Reference Networks
FFPE	Formalin-fixed, paraffin-embedded
FSH	Follicle stimulating hormone
G	Grade
GEP-NEN	Gastroenteropancreatic neuroendocrine neoplasm
GH	Growth hormone
GHRH	Growth hormone-releasing hormone
GLP	Glucagon like peptide
HPAC	High proliferative atypical carcinoid
HPF	High power field
IGF	Insulin like growth factor
IHC	Immunohistochemistry
IPSS	Inferior petrosal sinus sampling
i/v	Intravenously
LCNEC	Large cell neuroendocrine carcinoma
LH	Luteinizing hormone
LTA	Laparoscopic transperitoneal adrenalectomy

MC	Mitotic count
MEN	Multiple endocrine neoplasia
MiNEN	Mixed neuroendocrine-non-neuroendocrine neoplasm
MRI	Magnetic resonance imaging
MTC	Medullary thyroid cancer
NEC	Neuroendocrine carcinoma
NEN	Neuroendocrine neoplasm
NET	Neuroendocrine tumour
NICTH	Non-islet cell tumour hypoglycaemia
NOS	Not otherwise specified
NSCLC	Non-small cell lung cancer
OS	Overall survival
Pan-NEN	Pancreatic neuroendocrine neoplasm
PD	Poorly differentiated
PET	Positron emission tomography
POMC	Proopiomelanocortin
PRA	Posterior retroperitoneoscopic adrenalectomy
PRA-1S	PRA performed by 1 surgeon
PRA-2S	PRA performed by 2 surgeons
PTH	Parathyroid hormone
PTHrP	Parathyroid hormone related peptide
p/o	Per os
RB1	Retinoblastoma 1
SCLC	Small cell lung cancer
SCNEC	Small cell neuroendocrine carcinoma
SEER	Surveillance, Epidemiology, and End Results
SI	Steroidogenesis inhibitors
SIADH	Syndrome of inappropriate antidiuretic homone secretion
SSA	Somatostatin analogue
SSTR(s)	Somatostatin receptor(s)
TC	Typical carcinoid
TP53	Tumour protein p53
TTF-1	Thyroid transcription factor-1
UFC	Urine free cortisol
UNL	Multiples of upper normal limit
VIP	Vasoactive intestinal peptide
WD	Well differentiated
WHO	World Health Organization
3β-HSD	3 beta -hydroxysteroid dehydrogenase
5-HIAA	5-Hydroxyindoleacetic acid
^{11}C-HED PET	Carbon-11 hydroxyephedrine PET
^{11}C-5HTP PET	Carbon-11 5-hydroxytryptophan PET
11β-HSD-2	11 beta-dehydrogenase-2

11β-OH	11 beta -hydroxylase
17α-OH	17 alpha -hydroxylase
^{18}F-DOPA PET	Fluorine-18 L-3,4-dihydroxyphenylalanine PET
^{18}F-FDG PET	Fluorine-18 fluorodeoxyglucose PET
18-OH	18-hydroxylase
21-OH	21-hydroxylase
^{68}Ga-DOTA-SSA PET	Gallium-68 DOTA (tetraazacyclododecane tetraacetic acid)-conjugated SSA PET

Introduction

Neuroendocrine neoplasms

Neuroendocrine neoplasms (NENs) constitute a heterogeneous and relatively rare group of tumours with very variable prognosis, arising from cells of neuroendocrine phenotype. These cells have features of both hormone-producing endocrine cells and nerve cells and are located throughout the body. NENs can occur in any organ (however lungs, stomach, pancreas, small intestine, appendix and rectum are the most common localizations) and span from tumours with very low to very high proliferation rate. Most of the well-differentiated NENs express somatostatin receptors (SSTRs).

Even though there are relatively few countries worldwide reporting yearly incidence of NENs (1), a marked increase in incidence over the last decades is observed (Figure 1). By collecting data from the Surveillance, Epidemiology and End Results (SEER) database, the age-adjusted incidence rate has been noted to increase 6.4-fold from 1973 (1.09 per 100 000) to 2012 (6.98 per 100 000) (2). This increase can most likely be explained both by better diagnostic methods and by improved knowledge of this previously less well-known tumour group.

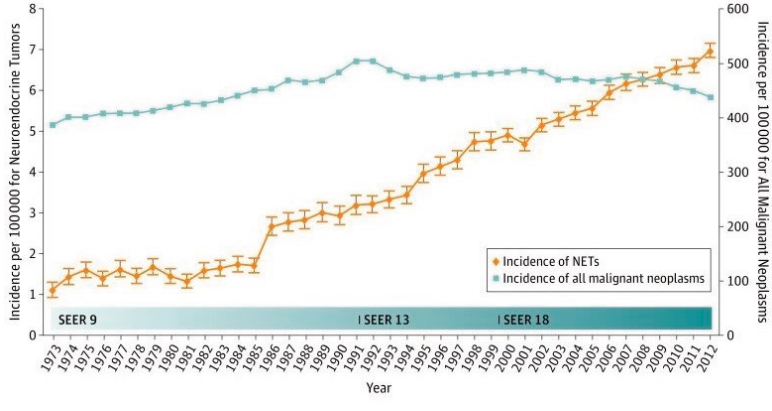


Figure 1. Annual age-adjusted incidence of all neuroendocrine tumours (NETs) and all malignant neoplasms. *Modified from (2).*

Some NENs have the ability to produce excess hormones that give rise to clinical symptoms (functioning NENs) while others release low amounts or inactive forms of hormones to cause symptoms (non-functioning NENs). The hormone secretion in malignancy can be defined as topic or ectopic, depending on whether the hormone is normally secreted from the organ of origin of the tumour or not. Ectopic hormone secretion causing symptoms in malignancy is often termed as endocrine paraneoplastic syndrome (EPNS).

Endocrine paraneoplastic syndromes

Paraneoplastic syndromes are characterized as a group of either neurological or endocrine symptoms in malignancy, caused by production of biologically active substances outside the tissue of their physiological secretion. It is estimated that this phenomenon affects approximately 8-15% of patients with malignant tumours, mostly involving the lung, breast, and the gastrointestinal system (3).

The first case reports of malignancy-associated endocrine disorders were published during the first half of 20th century (4, 5) but definite connection between specific hormones and tumour types were established much later.

EPNS can develop both in NENs and in non-endocrine malignancies, irrespective of their degree of differentiation. In a cohort of 834 patient's, EPNS was found in 2.5% of NENs (6) but the true incidence might be much higher. The summary of EPNS is demonstrated in Table 1. The ectopic hormone secretion can manifest both prior, concomitantly or in an already underlying malignancy in case of progression and/or dedifferentiation (7). Measurable hormones in case of EPNS can both facilitate the diagnostics of tumour disease, monitor response to the treatment and detect early recurrences (7). To come to the correct diagnosis can be challenging, since the tumours responsible for EPNS can be very small. Additionally, the relevant hormone may also be produced by lesions located at sites of their normal physiological secretion, further complicating the differentiation between ectopic and topic sources. Thus, there are four main directions within diagnostics in case of suspected EPNS: (1) assessment of relevant hormones in blood/urine/tumour tissue; (2) assessment of neuroendocrine or organ specific markers in blood/tumour tissue (3) radiological tumour diagnostics; (4) identifying/excluding physiological or other pathological causes of hormonal excess. In parallel with routine radiological methods such as computed tomography (CT) or magnetic resonance imaging (MRI), positron emission tomography (PET) is of great importance - either Gallium-68 DOTA (tetraazacyclododecane tetraacetic acid)-conjugated SSA PET/CT (^{68}Ga-DOTA-SSA PET/CT) for well-differentiated NENs

expressing SSTRs, or Fluorine-18 fluorodeoxyglucose PET/CT (^{18}F-FDG PET/CT) for poorly differentiated tumours or tumours without SSTR expression.

NENs of various origins may secrete ectopic hormones without causing clinical symptoms. This phenomenon is poorly studied and the true incidence is unknown, since testing for ectopic hormones is not included in the standard workup.

Table 1. Summary of endocrine paraneoplastic syndromes (3, 7, 8).

EPNS	**Hormones**
Cushing's syndrome	ACTH; CRH (rarely)
Carcinoid syndrome	Serotonin
Hypercalcaemia	PTHrP; 1,25 dihydroxy-vitamin D3; PTH
Acromegaly	GHRH, GH
SIADH	Vasopressin; ANP
NICTH	IGF-2, IGF-1
Other (rare)	Calcitonin; renin; β-human chorionic gonadotrophin; LH/FSH; prolactin; VIP; GLP-1/2; gastrin; somatostatin;

ACTH, adrenocorticotropic hormone; ANP, atrial natriuretic peptide; CRH, corticotropin releasing hormone; FSH, follicle stimulating hormone; GH, growth hormone; GHRH, growth hormone releasing hormone; GLP, glucagon like peptide; IGF, insulin-like growth factor; LH, luteinizing hormone; NICH, non-islet cell tumour hypoglycaemia; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related protein; SIADH, syndrome of inappropriate antidiuretic hormone secretion; VIP, vasoactive intestinal polypeptide.

Gastroenteropancreatic neuroendocrine neoplasms

Gastroenteropancreatic NENs (GEP-NENs) originate from neuroendocrine cells in digestive organs. Data from the SEER 1973-2007 tumour registry database shows the following distribution of GEP-NENs: small intestine (30.8%), rectum (26.3%), colon (17.6%), pancreas (12.1%), stomach (8.9%), appendix (5.7%) and others (4.3%) (9). The clinical presentation and prognosis depend strongly on the site of the primary tumour. GEP-NENs can be divided into functioning and non-functioning tumours depending on their secretion of hormones. Widespread metastases are common at the diagnosis of non-functioning GEP-NENs as most tumours are well differentiated, grow relatively slowly and do not develop the metabolic dysregulation typical for most low-differentiated tumours. Thus, non-functioning pancreatic NENs (pan-NENs) can develop undetected for many years, presenting with huge tumour burden and tumour mass symptoms. On the other hand, insulin producing pan-NENs, can cause severe hormonal symptoms relatively early during the disease course and the primary tumour may be very small and difficult to detect.

GEP-NENs are categorised based on the tumour proliferation rate (Ki-67) and mitotic count (MC). The World Health Organization (WHO) 2010 classification used only these parameters for NEN grading, while the WHO 2019 classification of GEP-NENs (see Table 2) also includes tumour differentiation (well vs. poorly): GEP-NENs are divided into neuroendocrine tumours (NETs) and neuroendocrine carcinomas (NECs), based on their proliferation rate, degree of differentiation and molecular differences. Mutations in multiple endocrine neoplasia type 1 (MEN-1), death-domain associated protein (DAXX), and alpha-thalassemia/mental retardation X-linked (ATRX) genes are entity-defining for well-differentiated NETs of the pancreas, whereas NECs usually have mutations in tumour protein p53 (TP53) and retinoblastoma 1 (RB1) genes (10).

Table 2. WHO 2019 classification and grading criteria of GEP-NENs (10).

Terminology	**WD/PD**	**MC (mitosis/2mm^2)**	**Ki-67%**
NET, G1	WD	<2	<3
NET, G2		2-20	3-20
NET, G3		>20	>20
NEC, SCNEC	PD	>20	>20
NEC, LCNEC		>20	>20
MiNEN	WD or PD	Variable	

LCNEC, large-cell neuroendocrine carcinoma; MC, mitotic count; MiNEN, mixed neuroendocrine-non-neuroendocrine neoplasm; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumour; PD, poorly differentiated; SCNEC, small-cell neuroendocrine cancer; WD, well differentiated

Pancreatic NENs

Pan-NENs develop from neuroendocrine cells in the pancreatic islets and represent 1-2% of all pancreatic neoplasms. The incidence is approximately 1/100 000 population/year (11) and the majority of pan-NENs are non-functioning (12). The clinicopathological variation is wide: from well differentiated incidentalomas with normal life expectancy to poorly differentiated NECs with a median survival for patients receiving chemotherapy of less than 12 months (13, 14). Pan-NEN usually occur as a single lesion but multiple tumours can be found in patients with MEN-1.

Functioning pan-NENs are associated with a clinical syndrome caused by increased secretion of pancreatic hormones such as insulin, gastrin, glucagon, vasoactive intestinal peptide (VIP), somatostatin or ectopic hormone secretion such as adrenocorticothropic hormone (ACTH), calcitonin, serotonin, growth hormone-releasing hormone (GHRH) and parathyroid hormone related peptide (PTHrP). While secretion of some of these hormones (e.g., somatostatin,

GHRH) give minor clinical symptoms, others, such as ACTH, VIP or insulin, often cause severe morbidity and mortality. Hormonal syndromes can develop in previously non-functioning pan-NENs and hypersecretion of multiple hormones can be observed as well. This development is often associated with radiological progress and impairment of overall survival (OS) (15, 16).

Neuroendocrine neoplasms of the lung

Well-differentiated neuroendocrine neoplasms in the lung (lung-NENs) are rare tumours with increasing incidence, ranging from 0.2-2 per 100 000 population per year (17). This is mainly explained by improved diagnostics and knowledge about this tumour type. Concurrently, the incidence of poorly differentiated tumours is declining in parallel with reduced population smoking rates (2, 18, 19).

Currently, lung-NENs are divided in to typical carcinoid (TC), atypical carcinoid (AC), large cell neuroendocrine carcinoma (LCNEC) and small cell lung cancer (SCLC), depending on differentiation, mitotic count per $2mm^2$ (MC) and presence or absence of necrosis. The first histological recognition of lung-NENs by the World Health Organization (WHO), without dividing them in subgroups, was in 1967 (20). In 1972 *Arrigoni et al.* introduced the grouping into TC and AC, based on mitotic count, necrosis, and cytological features (21), followed by the official recognition of this division by WHO 1981 (22). LCNEC was added to the WHO classification in 1999 and this edition is the basis for the classification that we still use today (23). The histopathological classification of lung-NENs still remains a subject of considerable debate, particularly concerning its possible adjustment to the grading system of GEP-NENs as well as usage of the proliferation marker Ki-67 (24). The latter considerations are important particularly in diagnostics of metastatic disease since there are difficulties to count mitoses and identify necrosis in biopsy material. The current classification of lung-NENs is demonstrated in Table 3.

While TC and AC represent only 1-2% of all lung malignancies, LCNEC and SCLC are represented in higher proportion: up to three and 15% respectively (25). The ratio between TC and AC is described to be about 8-10:1 (17, 25). The prognosis depends strongly on the histopathological subgroup where the 5-year survival in TC is reported to be as high as 87% but only 15% in LCNEC (25). The recently recognized subgroup of highly proliferating AC (HPAC) (Ki-67 >20%) tends to have shorter OS and a higher risk of recurrence, compared to AC with Ki-67 <20%, 53 months vs. 101 months and 66% vs. 9%, respectively. HPAC also seem to respond better to therapies used for

AC than LCNEC (26). The most common sites of metastasis are liver, bone and lung (27).

Table 3. Adjusted summary of WHO 2021 classification of lung-NENs (28-30).

Terminology	**WD/ PD**	**Diagnostic criteria**	**GEP-NEN WHO 2019**
DIPNECH[a]	WD	Pre-invasive condition	*N/A*
TC	WD	No necrosis/MC<2	NET G1
AC	WD	Necrosis and/or MC 2-10	NET G2
HPAC[b]	WD	Necrosis >10 and/or Ki-67 >20%	NET G3
Carcinoid NOS[c]	WD	*N/A* for counting of necrosis and/or MC	NET G1/G2/G3
LCNEC	PD	Large cells and MC >10	NEC
SCLC	PD	Small cells and MC>10	NEC
Mixed tumours	PD	SCLC or LCNEC combined with NSCLC	MiNEN

[a] presence of neuroendocrine cell hyperplasia in the epithelium of at least three separate bronchioles combined with at least three carcinoid tumourlets, constrictive bronchiolitis. Occurring mostly in non-smoking women.
[b] not included officially in recent classification but it is recommended to identify and document these patients;
[c] not included officially in the recent classification but it is recommended to use this nomenclature in case of WD metastastic disease and/or in biopsy material where there are difficulties to assess reliable counting on necrosis and/or MC.
AC, atypical carcinoid; DIPNECH, diffuse idiopathic pulmonary neuroendocrine cell hyperplasia; GEP-NEN, gastroenteropancreatic neuroendocrine neoplasm; HPAC, high proliferative AC; LCNEC, large cell neuroendocrine carcinoma; MC, mitotic count/2mm^2; MiNEN, mixed neuroendocrine-non neuroendocrine neoplasm; *N/A*, not applicable; NEC, neuroendocrine carcinoma; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; PD, poorly differentiated; SCLC, small cell lung cancer; TC, typical carcinoid; WD, well differentiated.

There is a lack of comprehensive studies regarding hormone-secreting lung-NENs: the majority of available publications are case reports or focusing on ectopic Cushing's syndrome (ECS) only. Among hormone-secreting lung-NENs, serotonin is the most commonly secreted hormone (reported in 2-7.6% of cases), followed by ACTH (1-7.6%) and, less frequently, GHRH (17, 27, 31). Hypercalcitonaemia is mainly described in SCLC (32). There are no studies conducted to date, investigating hormone secretion as a prognostic factor in lung-NENs.

Thymic NENs represent 4.3% of all anterior mediastinal malignancies and constitute 0.4% of all NENs according to data from SEER. These tumours are often resistant to chemotherapy and are known for their poor prognosis with a median survival in advanced disease of less than three years (33).

Cushing's syndrome

Cushing's syndrome (CS) is a hormonal disorder with typical signs and symptoms, caused by chronic glucocorticoid excess and disrupted cortisol diurnal rhythm. In healthy individuals this diurnal rhythm is characterized by a peak of cortisol concentrations early in the morning and a nadir around midnight. However, in CS, cortisol levels are on a more constant high level without the normal fluctuations. CS has an estimated incidence of 0.2-5.0 per million people per year and a prevalence of 39-79 per million in various populations (34). The most common cause is iatrogenic, due to long-lasting glucocorticoid therapy. Endogenous CS is caused either by pathology in the pituitary or adrenal glands, or as consequence of ectopic ACTH or corticotropin releasing hormone (CRH) secretion by non-pituitary tumours.

History

Although the presence of the pituitary gland had been known since Antiquity and the adrenal glands were first described by Bartolomeo Eustachius (1520–1574) in 1563 ("glandulae renibus incumbentes"), nothing was known about their function until Thomas Addison (1795-1860) published his monograph "*On the Constitutional and Local Effects of Disease of the Suprarenal Capsules"* in 1855. Adrenal tumours causing different symptoms such as "hypertension, truncal obesity, hirsutism, pyodermia" were described in the early 20th century but mainly with focus on androgen hyperproduction (35-37).

The first complete description of CS was published in 1912 by Harvey Cushing (1869-1939) as "A syndrome of painful obesity, hypertrichosis and amenorrhoea with overdevelopment of secondary sexual characteristics", and in 1932 a basophilic pituitary adenoma was postulated as the cause of the disease (38).

The earliest case report of CS in a patient with SCLC and bilateral adrenal enlargement was published in 1928 as "Case of pluriglandular syndrome: diabetes of bearded women" (4). The definition of the ECS was formulated in the 1960s when a link between malignancies and the clinical picture of CS caused by ectopic ACTH secretion was established (39-42). Already then, authors noted that ECS can cause severe metabolic disturbances and some classical symptoms of CS in ECS can be absent, pointing out that "*The frequent occurrence of hypokalaemia and the frequent absence of some of the physical stigmata of Cushing's syndrome are two points that have been emphasized by previous authors in discussions of tumours associated with hyperadrenocorticism*" (39).

Classification

Endogenous CS is divided in two groups: ACTH-dependent CS (about 80%) and ACTH-independent CS (about 20%) with the following aetiological subgroups, depending on location of the primary pathology, see Table 4.

Table 4. Classification of Cushing's syndrome. *Modified from (34).*

	Aetiology of CS	**Proportion**
ACTH-dependent 70-80%	Cushing' s disease (CD)	60-70%
	Pituitary adenoma	60-70%
	Corticotroph hyperplasia	very rare
	Ectopic ACTH syndrome	5-10%
	Ectopic CRH syndrome	Very rare
ACTH-independent 20-30%	Adrenal adenoma	10-22%
	Adrenal carcinoma	5-7%
	Bilateral macronodular hyperplasia	<2%
	Bilateral micronodular hyperplasia	<2%
	McCune-Albright syndrome	Rare
	Bilateral adenomas or carcinomas	Rare

Clinical presentation

The clinical picture of CS is caused by the peripheral effects of hypercortisolaemia and all tissues and organs of the body can be affected causing a group of heterogeneous signs and symptoms (see Table 5). CS may significantly influence morbidity, mortality and quality of life of the patient. It is a relatively wide variation in the clinical presentation of CS depending to the magnitude and duration of cortisol excess, and patients are often referred to other specialists than endocrinologists or oncologists first, especially in cases with a milder clinical picture (43).

Table 5. Summary of signs and symptoms of Cushing's syndrome.

Organ system	Signs and Symptoms
General	Central obesity, "moon face", "buffalo hump", sleep apnoea, oedema
Metabolic	Hypokalaemia, metabolic alkalosis, glucose intolerance/diabetes
Cardiovascular	Hypertension, dyslipidaemia, thromboembolism, atherosclerosis
Neuropsychiatric	Depression, emotional lability, euphoria, psychosis, insomnia
Skin	Plethora, hirsutism, female balding, striae, bruising, acne, skin atrophy, excess sweating, fungal infections, hyperpigmentation
Musculoskeletal	Osteopenia/osteoporosis, weakness and proximal muscle wasting, growth retardation (in children)
Infection and immune function	Increased frequency and recurrent infections, opportunistic pathogens
Gonadal dysfunction	Oligo-/amenorrhoea, impotence, decreased libido

In rare cases, hypercortisolaemia may occur periodically, combined by periods of normal cortisol secretion, in the literature defined as cyclic CS. In these cases, hypercortisolism can occur regularly or irregularly with periods of normal cortisol levels ranging from days to years. It is probably an under-reported pathology, which provides a greater than usual diagnostic challenge (44). In a systematic review of the literature that was published between 1960 and 2015, *Meinardi et al.* could identify 65 reported cases of cyclic CS, where CD was the underlying cause in 54%, ECS in 26%, and primary adrenal CS in 11%. There were no cases of SCLC causing cyclic CS reported. The range of clinical manifestations reported was wide, from limited or even absent to very severe (44).

Ectopic Cushing's syndrome

ECS, where hypercortisolism is caused by an ACTH or CRH-secreting non-pituitary tumour, is a rare condition, representing only 5-10% of all cases of CS (34, 45). ACTH secretion results in excess adrenal gland cortisol secretion. The normal cortisol feedback mechanism of the hypothalamic-pituitary-adrenal (HPA) axis is disturbed (Figure 2), with loss of circadian rhythm and resulting in severe hypercortisolism (46).

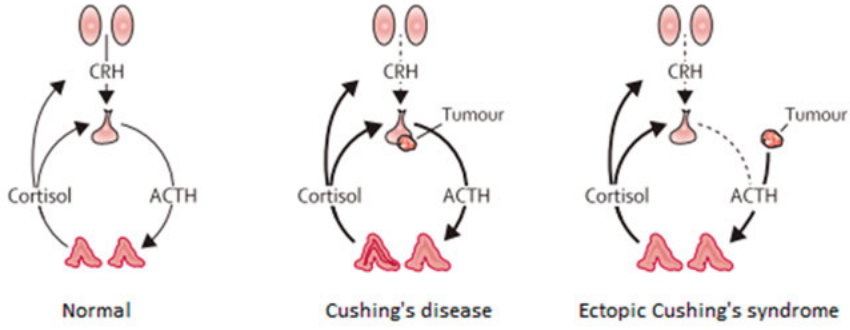


Figure 2. Regulation of hypothalamic-pituitary-adrenal (HPA) axis in healthy individual, compared to one with pituitary adenoma (Cushing's disease) and ectopic Cushing's syndrome. *Modified from (34).*

ECS is the most complex type of the CS in terms of diagnostics and treatment. Previous studies suggest that ECS is more common in women (47-49) and the median age at diagnosis varies from 27-49 years (47, 49-53). ECS can be detected either (1) prior the discovery of the ACTH source or (2) simultaneously with the diagnosis of tumour. Furthermore, ECS can develop in (3) previously known non-functioning NENs, most often in association with tumour progression. Some authors suggest to call (1) + (2) as classic ECS and (3) as paraneoplastic ECS (45).

Sources

Almost all NENs as well as some non-endocrine tumours may be associated with ECS, but the most frequently reported sources are SCLCs, lung and thymic NENs, pan-NENs, medullary thyroid cancer (MTC) and pheochromocytomas (Table 6).

Table 6. Sources reported in the literature of tumours with ectopic ACTH secretion. *Modified from (45)*.

Tumour type	**Percentage reported**
Small cell lung carcinoma	3.3–50
Lung-NEN	5–40
Pancreas-NEN	7.5–25
Thymus-NEN	5–42
Phaeochromocytoma	2.5–25
Medullary thyroid cancer	2–8
Other NENs	≤5
Other miscellaneous tumours	≤5
Occult ECS	12–37.5

SCLC is the most well-known tumour responsible for ectopic ACTH secretion. These tumours are highly malignant, may lack immunohistochemical evidence of neuroendocrine differentiation and patients have an extremely rapid clinical deterioration and short survival despite oncological treatment. The molecular defects behind ectopic ACTH secretion in SCLC are largely unknown (34) but molecular biology studies have suggested different mechanisms for ACTH production in poorly differentiated tumours and NEC. For example, SCLC cells are processing proopiomelanocortin (POMC) in an aberrant way which results in release of ACTH precursors of large molecular weight such as pro-ACTH while NET cells process POMC normally releasing large amounts of intact ACTH to the circulation (54).

Well differentiated lung-NENs are the second most frequently reported cause of ECS with equal occurrence among TC and AC, and with a median size of the tumour being 0.3-6 cm (50, 52). The majority of ACTH producing lung-NENs are relatively well differentiated in contrast to ACTH producing thymic-NENs which usually are aggressive and poorly differentiated (55).

Abdominal NENs are believed to be responsible for 1/3 of ectopic ACTH secreting malignancies and are predominantly located in the pancreas (56). While many ACTH producing lung-NENs can be radically resected at diagnosis, the majority of pan-NENs have lymph node or liver metastases at the onset of ECS (55).

There are published case reports of ECS in connection with other types of malignancies as well, for example paraganglioma (57, 58), prostate cancer (59, 60), peritoneal mesothelioma (61), ovary (62), parotid (63) and neuroendocrine gallbladder cancer (64). Development of ECS has been described in patients with NEN as a part of genetically inherited syndromes such as in MEN-1 and MEN-2 and von Hippel-Lindau syndrome (65-68).

In up to 1/3 of patients with ECS, the source of ACTH secretion is never found (defined as occult ECS) or found after decades of follow-up. It is believed that the major source of occult ECS is lung-NENs (45, 69).

Clinical presentation

The clinical features of ECS are often the same as in CD, but the rapid onset, severity and progress of symptoms may suggest an ectopic source (45). In cases of poorly differentiated and rapidly growing tumours the classical phenotypic features of CD might be lacking (46, 70). The clinical picture is often dominated by metabolic disturbances such as severe hypokalaemia, hyperglycaemia, metabolic alkalosis, severe infections (often by opportunistic agents) as well as cardiovascular and neurocognitive disorders. Many of these patients need intensive care during prolonged periods of time due to the symptoms induced by the cortisol overproduction. Despite the above-mentioned clinical features, the diagnosis of ECS may be missed or delayed. If the patient is initially admitted to a clinic that lack experience of neuroendocrine tumours and the clinical picture is dominated by widespread tumour disease, infections and thromboembolic complications, then it is the presence of severe therapy-resistant hypokalaemia which should guide to the diagnosis of ECS (55).

The background behind the severity of hypokalaemia seen in ECS compared to CD is still not completely understood (53). Historically there have been hypotheses both on a direct stimulatory effect of ACTH on 11-Deoxycorticosterone (DOC) secretion (71) and on a suppression of 11 beta-dehydrogenase-2 (11β-HSD-2) function (72) respectively. However, aldosterone and DOC levels are found to be low in patients with severe ECS (53, 73) and the degree of hypokalaemia is directly correlating to the severity of hypercortisolism regardless of its aetiology (55). Cortisol has the ability to act as a mineralocorticoid *in vivo* by binding to the mineralocorticoid receptor in the kidneys. This capability is downregulated through conversion of cortisol to inactive cortisone by 11β-HSD-2. In conditions of extremely high cortisol levels, this conversion is impaired and cortisol binds to aldosterone receptors. As a result, peripheral effects simulating mineralocorticoid hyperproduction (hypokalaemia, metabolic alkalosis) are often dominating over the symptoms typical for hypercortisolaemia.

Additional symptoms include hyperpigmentation, oedema, severe muscle weakness and arterial hypertension. Hyperpigmentation, which is seldom seen in non-ectopic CS, is reported to be as common as 32-56% in ECS (74, 75) and develops due to melanocyte-stimulating hormone production as a by-product of ACTH synthesis from POMC. The hyperpigmentation can be expressed to varying degrees, usually diffuse, and sometimes only in skin folds.

Extensive hyperpigmentation is mostly seen in ECS due to SCLC, LCNEC or other poorly differentiated and progressing NENs (55). Sometimes hyperpigmentation exists as the only symptom and can be observed best by looking at the mucous membranes. Muscle weakness is much more pronounced in ECS compared to non-ECS and affects not only the proximal muscle groups of the extremities, but also develops elsewhere, e.g., in the myocardium (76).

Diagnostics

The first diagnostic step in a patient with signs and symptoms of CS is to confirm hypercortisolaemia by measuring late-night salivary cortisol and 24-hours urinary free cortisol, followed by measuring of ACTH to distinguish between ACTH dependent and independent CS. The following steps depends strongly on the severity of hypercortisolaemia and the clinical status of the patient. If ACTH and cortisol levels are very high, some suggest direct screening for an ectopic source by whole body thin-slice CT, because (1) MRI of the pituitary may result in both false positive and negative results, (2) functional tests (for example, low- and high-dose dexamethasone test, CRH stimulation or desmopressin test) and sinus petrosus sampling (IPSS) are time consuming, (3) early discovery of an aggressive tumour leading to rapid initiation of surgical and/or oncological treatment is crucial for survival of the patient (55). If the patient's status allows and/or CT does not reveal any source of ectopic ACTH secretion, one should first exclude the pituitary as the source because CD is the most common cause of ACTH-dependant CS.

The choice of radiological method for further diagnostics depends on the site of the suspected primary tumour. While CT is a better method for lung and thymic malignancies, MRI and endoscopic ultrasound should be preferred in suspected pan-NENs. In the case of occult ECS different PET tracers can be helpful. ^{68}Gallium-SSA-PET/CT can reveal SSTR positive tumours, ^{18}FDG-PET/CT - poorly differentiated tumours as well as all subtypes of lung-NENs, independently of proliferation (25). Fluorine-18 L-3,4-dihydroxyphenylalanine PET/CT (^{18}F-DOPA PET/CT) can be useful for diagnosing G1-G2 NETs and MTC and Carbon-11 hydroxyephedrine PET/CT (^{11}C-HED-PET/CT) – for detection of paragangliomas and pheochromocytomas. In case of occult-ECS (particularly due to small lung-NENs) or metastases of MTC Carbon-11 5-hydroxytryptophan PET/CT (^{11}C-5HTP-PET/CT) can be used if available (27, 77). One should take in to consideration the risk of occurrence of opportunistic infections in ECS which can give false positive uptake both on somatostatin receptor imaging and ^{18}FDG-PET.

Immunohistopathological assessment is fundamental for both choice of oncological treatment and prognosis, and should include presence/absence of

general neuroendocrine markers (e.g., Chromogranin A, synaptophysin), proliferation index and differentiation markers (e.g., Ki-67, TP53 or Rb1), specific organ- and neuroendocrine markers (e.g., thyroid transcription factor 1 (TTF-1), pancreatic hormones, serotonin, calcitonin, caudal type homeobox 2 (CDX-2)) and expression of ACTH/CRH in tumour cells.

Since previous studies on pan-NENs have shown worse OS in patients with multiple hormone secretion (15, 16), measurement of other hormones than only ACTH should be considered at diagnosis, progression and in case of new symptoms.

Treatment options of Ectopic Cushing's syndrome

Clinical management of patients with ECS is challenging, complex and demanding multidisciplinary expertise. It involves surgery, oncology, endocrinology and intensive care. Due to the rarity and severity of this condition, patients with ECS should be treated at specialised centres with experience in NENs. The only definitive curative treatment for ECS is the radical resection of the source of ACTH secretion (78).

ECS should be considered as an endocrine emergency requiring an 'emergency response' (55) in terms of diagnostics and treatment. Immediate symptomatic treatment is often needed to control e.g., hypokalaemia, hyperglycaemia, infectious and thromboembolic events by using per oral or intra venous potassium substitution, insulin, anticoagulation treatment and antibiotics. Although spironolactone is very effective to reduce hypokalaemia in ECS, one should be aware that spironolactone may counteract the clinical effects of the steroidogenesis inhibitor (SI) mitotane. Due to an increased risk of infections in ECS it is recommended to consider vaccination for influenza, herpes zoster and pneumococcal disease. Furthermore, due to the frequent occurrence of P. jirovecii pneumonia, systematic prophylaxis with trimethoprim-sulfamethoxazole may be considered (55, 79).

In order to choose the right treatment sequence and strategy one should initially determine whether it is the hormonal or oncological risk that dominates. Endocrine society guidelines recommend urgent treatment (within 24–72 h) of hypercortisolism if life-threatening complications of CS such as infection, pulmonary thromboembolism, cardiovascular complications, and acute psychosis are present (79).

Medical treatment

There are two main treatment directions depending on the severity of hypercortisolaemia and differentiation level of the tumour: treatment of hypercortisolaemia directly by SI or indirectly by oncological treatment, with the hope that tumour shrinkage will be accompanied by a corresponding reduction in ACTH secretion. The latter strategy could be more effective in tumours of lower differentiation. In clinical practice most often both strategies are used in parallel.

Steroidogenesis inhibitors

Recent Endocrine society guidelines recommend SI as the primary treatment for ECS in patients with occult or metastatic ECS followed by bilateral adrenalectomy (BA) (79).

There are several SI available for use as single therapy or in combinations, and they act on different levels of the steroid biosynthesis pathway in the adrenal cortex, see Figure 3.

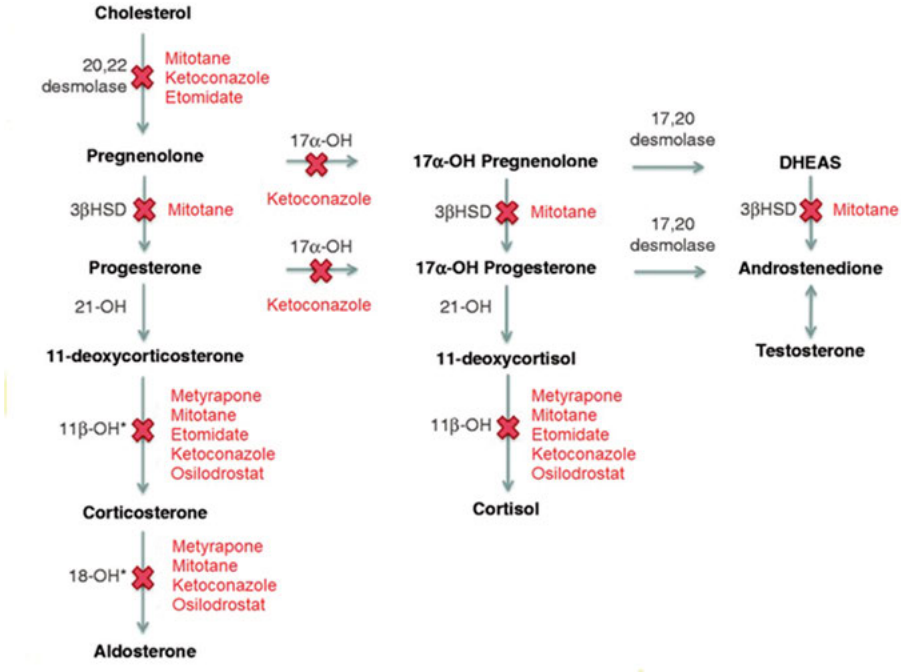


Figure 3. Role of steroidogenesis inhibitors in steroid biosynthesis in the adrenal cortex (17α-OH, 17alpa-hydroxylase; 3β-HSD, 3beta-hydroxysteroid dehydrogenase; 21-OH, 21-hydroxylase; 11β-OH, 11beta-hydroxylase; 18-OH, 18-hydroxylase; *aldosterone synthase). *Modified from (80).*

This treatment should be managed by specialists accustomed to SI dose titration, as well as detection and management of side effects (particularly adrenal insufficiency) and with knowledge about drug-drug interactions. Monitoring of potassium, glucose levels and blood pressure can offer an early indication of the efficiency of these treatments while the results of biochemical analysis of plasma or urinary cortisol may take several days to obtain (55). Two different approaches are used: (1) dose titration to achieve normocortisolaemia or (2) high dosage of SI directly combined with glucocorticoid substitution (block-and-replace). SI are often used preoperatively to improve the general clinical status of the patient and reduce risk for surgical complications (78).

Ketoconazole and metyrapone are the most commonly used SI in ECS while mitotane is mostly used for treatment of adrenocortical cancer (ACC). Osilodrostat is the most recent SI for treatment of CS and was approved for medical use in the European Union in January 2020. No large randomised trials have been performed to investigate the efficacy of the different SI on CS and the choice of therapy is still based on personal experience and the clinical situation.

Ketoconazole is an antifungal medication which is approved for treatment of CS due to its effect on steroid biosynthesis. It has a quick onset of action and mainly gastrointestinal side effects. Due to the risk of severe liver toxicity, close follow-up of liver enzymes is required. Ketoconazole has a high potential for clinically important drug interactions mostly through inhibition of CYP3A4. The recommended dosage is 400-1200 mg/daily, divided in 3-4 doses. Although ketoconazole is effective in the treatment of CS, studies have shown that normocortisolaemia is achieved in only 44% of ECS patients (79).

Metyrapone is the second most used SI in the treatment of CS. It has fast onset of action by rapid absorption and elimination from plasma after oral administration. It attains peak plasma concentrations within one hour after intake. Adverse effects are usually mild and less severe than for ketoconazole. However, metyrapone can cause hypokalaemia, hypertension and oedema by increasing mineralocorticoid precursors and acne and hirsutism by increasing adrenal androgens. Since treatment with metyrapone can lead to very rapid decrease in circulating cortisol levels with a risk for adrenal insufficiency, close monitoring of the patient is needed by a specialist familiar with this treatment. The daily dosage in ECS and adrenocortical carcinoma (ACC) is 500 mg-6 g, divided in 3-4 doses which is higher than in the treatment of CD or benign adrenal disease.

Ketoconazole and metyrapone may be combined in severe cases of ECS.

Mitotane is an adrenolytic agent, approved for treatment of ACC in adjuvant or palliative regime. Sometimes it is used even for treatment of CS but due to the slow onset of action and serious side effects it is less popular.

Mitotane accumulates in the adipose tissue and therefore its onset of action may be delayed for several weeks, during which time the control of hypercortisolism must be achieved with an alternative drug (80). Due to gradual release from adipose tissue, it has a half-life of 18-150 days and can cause adrenal insufficiency for several months after finished treatment (80). It is recommended to monitor mitotane plasma levels on a regular basis to avoid severe toxicity.

Etomidate is the only intravenous SI and is mainly used for patients with very severe hypercortisolism (e.g., accompanied with respiratory failure or psychosis) at intensive care departments, who cannot take per oral medications and are not candidates for BA. It should be administrated through a central venous line and cortisol levels should be monitored several times per day (79).

Osilodrostat is a novel SI, approved for CD by the US Food and Drug Administration and for CS by the European Medicines Agency (EMA). It blocks the final steps in cortisol synthesis and is expected to treat hypercortisolaemia regardless of its cause. It is the only SI that has been assessed in prospective randomized controlled trials (81). Osilodrostat has a long plasma half-life, allowing for a twice daily dosing schedule. Although the initial clinical trials focused on the use of osilodrostat in CD (82, 83), there are promising data on its efficacy for all types of endogenous CS (84, 85). There is only one retrospective study to date on osilodrostat in ECS only, demonstrating good efficacy on severe hypercortisolism (86).

The glucocorticoid receptor antagonist mifepristone is also recommended for the treatment of CS; however, its use is limited due to challenges in dose titration, the absence of reliable biomarker to monitor efficacy (as cortisol levels remain unchanged or may increase), and a high incidence of adverse effects (78, 79).

Oncological treatment

The choice of the oncological treatment strongly depends on localization of the primary tumour and its grade of differentiation, and consists of somatostatin analogues (SSA) for SSTR avid tumours, the m-TOR inhibitor Everolimus, tyrosine kinase inhibitors (e.g., Sunitinib, Cabozantinib) and chemotherapy e.g., Temozolomide/Capecitabine and platinum-based regimes. Peptide receptor radionuclide therapy is suitable for tumours with high SSTR expression (27, 87, 88).

There are a couple of reports published on the benefit of SSA in ECS (89-92). It is believed that SSTR are not downregulated in ACTH secreting NENs despite the chronic hypercortisolism they are exposed to due to aberrant glucocorticoid receptor signalling in the tumour cells (89). Thus, SSA may serve

a dual role in ECS, contributing both to oncologic treatment and concurrently inhibiting ACTH secretion by tumour cells.

Glucocorticoid substitution therapy

Severe glucocorticoid withdrawal syndrome is frequently seen after surgical removal of the tumour producing ACTH or after BA, and recovery may take one year or longer. BA results in a need for lifelong glucocorticoid and mineralocorticoid replacement. In patients in whom surgery of the primary tumour has been performed, glucocorticoid replacement during the recovery phase of the HPA axis is usually needed for 6-12 months. Due to extremely high preoperative cortisol levels in ECS, the substitution therapy may be challenging. Over-replacement of glucocorticoids may lead to higher morbidity (93) and mortality, especially in patients with metastatic NENs, who often have impaired immune function due to oncological treatment. The Endocrine society guidelines recommend glucocorticoid replacement with hydrocortisone, 10-12 mg/m^2/d in divided doses (79).

Surgical treatment

Radical surgery of the primary tumour is the most valuable treatment option although it is not always feasible due to metastatic or occult disease. If radical surgery of the tumour is not possible, normo-cortisolaemia can be achieved either by medical treatment or BA. Control of a hypercortisolaemia with SI must be considered if clinically possible before any invasive therapeutic intervention.

Surgery of the primary tumour

Most of the NENs causing ECS originate from either pancreas or lungs.

The options for radical surgery for pan-NENs strongly depend on the size and location of the tumour, taking into account the anatomical conditions of the region. The best possibilities are for tumours localized in the pancreatic corpus/cauda and for small tumours - when either distal pancreatic resection, radiofrequency ablation or enucleation can be used. Larger NENs located in the pancreatic head often require a pancreaticoduodenectomy (Whipple procedure), and in certain cases, a total pancreatectomy may be needed. If the non-metastatic tumour is considered as non-resectable, neo-adjuvant treatment may also be an option with the following surgery for radical purposes (94).

Surgery represents the treatment of choice in lung-NENs even in the case of regional lymph node metastases. The surgical approach depends on tumour size and location and procedures recommended are: an anatomic pulmonary

resection (e.g., segmentectomy, lobectomy, bilobectomy) or bronchoplastic procedures (e.g., sleeve resections) together with lymph node dissection (27).

Bilateral adrenalectomy

BA can be considered as a life-preserving emergency treatment in patients with very severe ACTH-dependent disease who cannot be promptly controlled by medical therapy (79). BA offers direct reduction of cortisol levels and can be lifesaving.

The first planned open access adrenalectomy was performed 1914 by Perry Sargent (95) and since then different approaches have been developed. During the first decades, only open access surgery was available for BA. Four different approaches (anterior-transperitoneal; lateral-retroperitoneal; posterior-retroperitoneal; thoraco-abdominal) for open adrenalectomy have been used historically and among these only anterior-transperitoneal approach is suitable for BA. Exploration and removing of both adrenals through a single incision is possible with this method. However, transabdominal open surgery results in long recovery time, high mortality and severe operative stress, and the approach is also more difficult in obese patients compared to the retroperitoneal methods (96).

Endoscopic adrenalectomy was reported by *Gagner et al.* for the first time in 1992 (97) and now this approach is considered to be a gold standard for BA (excepted for adrenal carcinomas) (98). Similar to an open access adrenalectomy, different endoscopic approaches have been used historically such as transperitoneal in supine or lateral position and retroperitoneoscopic in prone or lateral position. Laparoscopic lateral transperitoneal adrenalectomy (LTA) is today the most frequently applied surgical method for BA - the patient is placed in a lateral decubitus position to allow gravity-facilitated exposure of the adrenal glands. This approach also allows for exploration of the abdominal cavity and additional surgery, if necessary, e.g., in patients with GEP-NENs. The disadvantage with this method is the long operating time due to the need of patient repositioning during the surgical procedure (96).

Posterior retroperitoneoscopic adrenalectomy (PRA) was introduced in 1995 by Walz (99) and Mercan (100) and is gaining popularity. Using PRA compared to LTA offers a more direct approach to the adrenal glands, shorter operating time (no need for reposition of the patient), less blood loss, faster recovery and it has advantages for patients with obesity or a history of previous abdominal surgery (101). There are centres where PRA is performed by a two-surgeon approach, thus by a simultaneous bilateral approach offering the possibility to decrease the surgical time by up to 50 per cent and to reduce

operative stress (102-104). Different approaches used for BA are demonstrated in Figure 4.

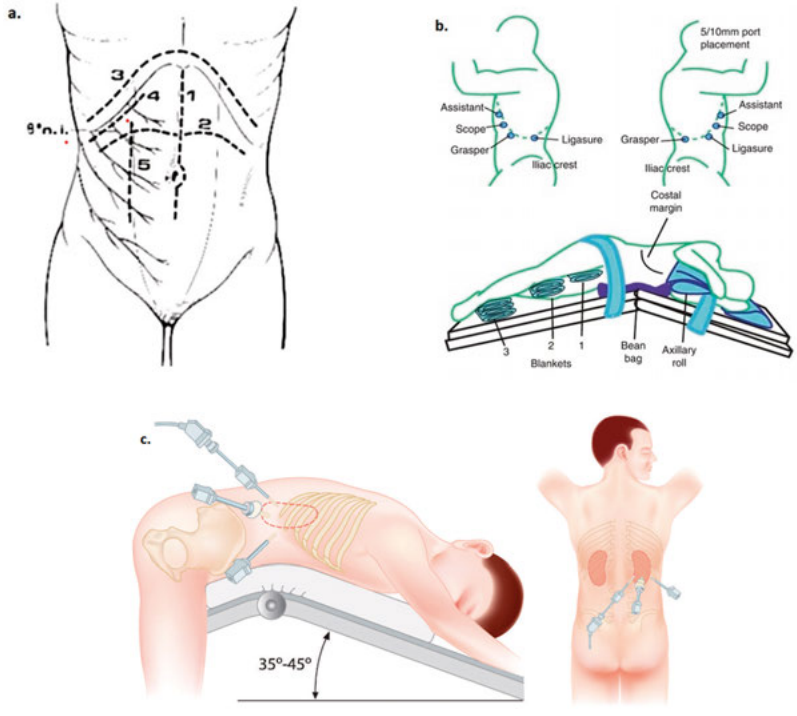


Figure 4. Different approaches for BA: a. open access; b. laparoscopic transabdominal; c. laparoscopic retroperitoneal. *Modified from (105-107).*

CT-guided percutaneous adrenal ablation is mainly used for treating small primary or metastatic adrenal lesions. Data on this approach for patients with hyperplastic adrenals due to ECS are limited but there are several case reports published about minimally invasive ablation techniques as a therapeutic alternative for patients with high operative risk and in whom medical treatment has failed (108-111). Further methods illustrated in case reports are radiofrequency ablation, cryoablation, chemical ablation and microwave ablation. Some disadvantages for those procedures are intraprocedural hypertension due to ablation of normal catecholamine-containing adrenal tissue, requiring pre-procedural administration of adrenergic antagonists and intraprocedural administration of anti-hypertensive agents (111).

Carcinoid syndrome

Carcinoid syndrome, characterised by diarrhoea, flushing, bronchoconstrictive symptoms and carcinoid heart disease, is the most common hormonal syndrome in NENs, usually developing in NENs originating from the small bowel.

The German pathologist Siegfried Oberndorfer introduced the name “carcinoid” when describing these slow-growing tumours in the small bowel 1907 (112). Although the clinical symptoms of carcinoid syndrome were described already in 1930s (113), it was not until the 1950s, when serotonin was identified (114), that the symptoms were definitively linked to serotonin producing tumours (115). The classical fibrotic changes of right-sided heart valves were more specifically described as "carcinoid heart disease" by the Swiss pathologist Walter C. Hedinger in 1961 (116). Historically, severe hormonal symptoms often predominated over those related to tumour burden, until the discovery of somatostatin by *Brazeau et al*. in 1973 (117) and the subsequent introduction of first short-acting in the early 1980s, followed by long-acting, SSA in 1997 for the treatment of carcinoid syndrome (118, 119).

Several substances have been suggested as cause of the carcinoid syndrome e.g., serotonin (as primary marker), histamine, kallikrein, prostaglandins, and tachykinins, among others (120). Measurement of the degradation product of serotonin, 5-hydroxyindoleacetic acid (5-HIAA), either in 24h-urine or serum is the most validated method of diagnosis and follow-up of carcinoid syndrome.

Rarely, even NENs of other origin than the small bowel can produce serotonin. However, the frequency of this remains comparatively poorly characterized in clinical research (121, 122). The true incidence of carcinoid syndrome is difficult to determinate and it highly depends on the selected population (123) and tumour type e.g., it is reported up to 76% in small bowel NENs (124) and 2-7.6% in lung-NENs (31).

Serotonin

Serotonin is a biogenic monoamine, produced from the amino-acid tryptophan in the central nervous system and peripheral neuroendocrine cells, stored in platelets and metabolized in the liver into 5-HIAA. It is involved in many well described neurophysiological processes e.g., sleep, learning, mood as well as vasoconstriction, gut motility and secretion. These effects are mediated through seven different classes of serotonin receptors, spread throughout the body (125).

Interestingly, serotonin has been studied also for having a possible growth factor effect on human tumour cells of different origin as well as for its

possible involvement in cancer cell migration, metastatic dissemination and tumour angiogenesis (126). Besides serotonin producing NENs, the thrombotic environment of the solid tumours with platelet aggregation, nicotine effect on neuroendocrine cells and impaired serotonin metabolism in liver in e.g., cholangiocarcinoma are some of the mechanisms believed to result in elevated serotonin levels in cancer (126). The possible underlying mechanisms for serotonin impact in cancer progression are complex and require further studies. However, dysregulation of serotonin receptor expression in tumour and endothelial cells along with its influence on different intracellular signalling pathways, are likely to represent the most important contributors (125, 126).

Telotristat is an inhibitor of tryptophan hydroxylase which mediates the last step in serotonin synthesis. It is approved for the treatment of serotonin associated diarrhoea in NENs. To date, there are also some promising data on its potential antiproliferative effect both in NENs (127, 128) and other tumours (129).

Hypercalcitonaemia

Calcitonin is a peptide hormone secreted by C-cells of the thyroid, and its serum concentration is typically found elevated in MTC. Other possible reasons for hypercalcitonaemia include chronic kidney failure, hepatic cirrhosis, various medications, smoking, and hypercalcaemia among others.

Rarely, hypercalcitonaemia may have an ectopic source, both produced in NENs and other tumours (32, 130, 131), but there are only few studies published, and these are usually case reports. The largest cohort reported to date includes 32 calcitonin-secreting NENs with similar distribution between origin from pancreas and lung and demonstrates a negative impact of the high calcitonin levels on OS, $p<0.0001$ (130). Meta-analysis of calcitonin secreting lung tumours has demonstrated that a majority of publications are about hypercalcitonaemia in SCLC with only few reports discussing calcitonin secreting TC/AC (32).

Clinical symptoms of high calcitonin levels mimic those of carcinoid syndrome with flushing and diarrhoea. The true incidence of ectopic calcitonin secretion may be underestimated due to various reasons e.g., absence of specific clinical symptoms and limited sampling due to insufficient clinical awareness or synchronous secretion of other hormones. There are no other reports than *Noziéres et al.* (130) to date on the impact of calcitonin on OS in NENs.

Immunohistochemistry

Immunohistochemistry (IHC) is a diagnostic tool that plays an important role in the diagnosis and classification of NENs, in which antibodies are applied to routine histological tissue sections to detect the presence of specific antigens through microscopic evaluation.

The understanding of the concept of antibodies and antigens began with the introduction of "serum therapy" against diphtheria by Emil von Behring in 1890, which later resulted in the first Nobel Prize in Physiology or Medicine in 1901. Later, Albert Hewett Coons, Hugh J Creech, Norman Jones, and Ernst Berliner developed the technique of immunofluorescence in 1941 to localize pneumococcal antigens in infected tissues (132-134). Since then, substantial progress has been made, and the use of IHC allows the determination of multiple clinical factors that influence treatment selection and provide prognostic information.

The main immunohistochemical markers used in diagnostics of NENs are Chromogranin A and Synaptophysin (general markers for NEN), Ki-67 (proliferation marker), hormone markers (e.g., pancreatic hormones, serotonin, ACTH, calcitonin among others), transcription markers for identification of tumour origin (e.g., TTF-1 and CDX-2) as well as other prognostic and therapeutic markers (SSTR2, p53, Rb, ATRX/DAXX).

Historically, IHC was performed on frozen tissue (135), however, today it is most commonly carried out on 4 μm-thick sections of formalin-fixed, paraffin-embedded (FFPE) tissue due to the ease of storage. IHC can be performed either manually or using automated staining platforms (autostainers). After deparaffinization of FFPE tissue with xylene and alcohol, antigen retrieval is performed using high-temperature heating or other methods (e.g., ultrasound or enzyme digestion), to make the epitopes available for immunohistochemical staining (136). Blocking is then carried out to prevent background staining. The tissue sections are subsequently incubated with a primary antibody specific to the antigen of interest, after which the antigen–antibody complex is visualized using an appropriate detection system, often involving an enzyme or fluorophore There are direct and indirect methods in IHC: the direct method is a one-step process in which a labelled primary antibody binds directly to the antigen in the tissue, while the indirect method is a two-step process in which an unlabelled primary antibody first binds to the antigen, followed by a labelled secondary antibody that binds to the primary antibody to increase the signal (137). During antibody validation, appropriate positive and negative control tissues should be selected (136).

The antibodies used can be either monoclonal or polyclonal. Monoclonal antibodies are specific for a single epitope and are usually produced by

immunizing mice with a specific antigen. Antibody-producing B lymphocytes are then harvested and fused with myeloma cells to generate hybridomas that produce identical antibodies. They may show lower sensitivity and reduced reactivity to masked epitopes in FFPE samples but have high specificity, low background staining and give consistent, reproducible results. Polyclonal antibodies are most often obtained from the serum of rabbits after immunization with the antigen of interest. These antibodies recognize multiple epitopes on the same antigen and therefore generally have higher sensitivity. However, they often show lower reproducibility due to batch-to-batch variability and may produce higher background staining due to naturally occurring antibodies (136).

Aims

The aims of the present studies were:

I. To describe clinical symptoms and biomarkers in order to detect predictive and prognostic parameters of clinical significance for ECS patients using a retrospective, comprehensive cohort of NENs treated at a tertiary referral centre.

II. To evaluate different surgical approaches for bilateral BA as a treatment option in ECS.

III. To describe and analyse the medical management of hypokalaemia and hypercortisolaemia in ECS in order to investigate whether both the severity of hypercortisolaemia and hypokalaemia at diagnosis correlates with prognosis.

IV. To examine correlations between immunohistochemical expression and circulating serotonin and calcitonin and their impact on survival in NENs of the lung.

Materials and Methods

All four studies were retrospective cohort studies with additional immunohistochemical analysis for Paper IV. The data were extracted from medical records of patients with NENs, referred to the Department of Endocrine Oncology, Uppsala University Hospital.

All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The studies behind Paper I-IV were approved by the local ethics committee, Swedish Ethical Review Authority (EPM) in Uppsala, Sweden (Dnr 2017/060 Paper I-III, Dnr 2024-08216-01 Paper IV).

Patient characteristics (Paper I-IV)

For Paper I, 886 medical records from year 1986-2014 were screened, to identify patients with ECS. Fifty-one patients (6%) with ECS were identified and further analysed. There were 18 males and 33 females, and the mean age at ECS diagnosis was 52±15 (SD) years. The majority of the tumours were located in the thorax (n=28) followed by pancreas (n=15). There were no patients with ECS due to MTC or pheochromocytoma in our cohort. Five patients had occult ECS despite extensive exploration including octreotide scintigraphy and/or PET. A majority of the thoracic tumours were atypical carcinoids and most of the pan-NENs were G2 tumours. Thirty-four patients had metastatic disease at the time of ECS diagnosis. ECS was diagnosed both prior, simultaneously and after the histopathological diagnosis of a NEN.

For Paper II the already existing database from Paper I was used along with further screening of medical records (2015-2019) where an additional 8 patients with ECS were identified. The cohort for Paper II comprised of 59 patients but six of them were excluded due to lack of follow-up data. Fifty-three patients were available for further analysis including epidemiological data, data on clinical parameters, survival, use of SI, indication and duration of surgery, complications and surgical technique.

The patient cohort for Paper III was based on the existing ECS database, with new patient data extended through the year 2023. Patients with occult ECS (n=5) and with ECS due to prostatic cancer (n=2) were excluded resulting in a final cohort of 55 patients.

For Paper IV, 167 patients with lung-NENs were identified from a database including all patients referred to our centre 2010-2025. There was a clear predominance of women (72%) and AC (50%) in the cohort with median age at diagnosis of 68 years, and 39.5% of the patients had a history of smoking more than 10 pack-years.

Only patients in whom clinical investigations could exclude pituitary/adrenal source of CS by hormonal and functional tests, imaging (CT and MRI), and IPSS or where the clinical picture with very severe hypercortisolaemia together with metastasized NEN indicated strongly in favour for ectopic ACTH secretion rather than pituitary source, were included in the analysis for Paper I-III. Patients with SCLC were not included in either of the papers, since these tumours are not treated at our centre and also may have a different mechanism underlying hormone production than that of NENs.

Clinicopathological data (Paper I-IV)

For Paper I, all the collected clinicopathological parameters (e.g., sex, age at diagnosis of tumour and/or ECS, time from first symptoms of hypercortisolism to diagnosis of ECS, clinical symptoms, complications of ECS, histological data) and biochemical parameters (e.g. potassium and glucose as well as hormone levels including ACTH (ng/l), urine free cortisol (nmol/24h), morning and midnight serum cortisol (nmol/l), and other relevant hormones if the levels were abnormal) were extracted from the medical records at Uppsala University Hospital or local hospitals, at the debut of NENs and/or ECS diagnosis. All data was derived before any symptomatic or tumour-directed treatment was started. Survival data were analysed until March 2018.

For Paper II and III additional data were collected: (1) biochemical parameters after symptomatic or tumour-directed treatment were initiated (Paper II and III); (2) data on the performed treatments: surgery (Paper II) and medical management of hypokalaemia and hypercortisolaemia (Paper III). For Paper III, hormone levels (ACTH, 24h-urine free cortisol (UFC) and morning s-cortisol) were measured at baseline and at the first follow-up after initiation of SI. Survival data were analysed until 31st of December 2019 (Paper II) and 31st of December 2023 (Paper III).

No new immunohistochemical investigations were performed for Paper I-III. In Paper I and II GEP-NENs were classified by the WHO 2010

classification of tumours of the digestive system (138), and thoracic NENs by the WHO 2014 classification of lung cancer (139). For Paper III, grading was updated to the WHO 2019 classification of tumours of the digestive system (30). Lung-NENs were classified according the same classification, depending on differentiation and Ki-67, in order to analyse the whole cohort. In the case of repeated biopsies and histopathological examinations, the highest Ki-67 was used in the analyses.

For Paper IV data on available hormone levels were collected at the diagnosis of the lung-NEN and during follow-up. Sources other than lung-NEN as the cause of hormone secretion were excluded. Tumours were classified according to the WHO classification of thoracic tumours from 2021 (30) if the patient was operated for the primary tumour. If biopsy material was used for diagnostic purposes, well-differentiated tumours were classified as AC in case of necrosis, MC>2 or Ki-67$\geq$10% while the remaining well-differentiated tumours were classified as "carcinoid NOS". Data on immunohistochemical staining for CgA, synaptophysin, and TTF-1 were collected retrospectively and additional stainings were performed for serotonin and calcitonin.

Clinicopathological characteristics for all four studies is demonstrated in Table 7.

Table 7. General characteristics of the cohort of ACTH secreting NENs for Paper I-III and for lung-NENs Paper IV.

Variable		**n (%)**			
		I	II	III	IV
Gender	Male	18 (35)	18 (34)	18 (33)	46 (27)
	Female	33 (65)	35 (66)	37 (67)	121 (72)
Age at diagnosis of tumour (years)	≤ 40	13 (25)	11 (21)	11 (20)	9 (5)
	41-70	28 (55)	31 (59)	35 (64)	92 (55)
	>71	5 (10)	6 (11)	9 (16)	66 (40)
	No tumour found	5 (10)	5 (9)	*N/A*	*N/A*
Primary tumour localization	Pancreas	15 (29)	14 (26)	16 (29)	*N/A*
	Lung- NENs	24 (47)	25 (47)	28 (51)	167 (100)
	Thymus	4 (8)	5 (9)	4 (7)	
	Rectum	0	0	1 (2)	*N/A*
	Unknown	8 (16)	9 (17)	6 (11)[a]	
Metastasis localization at diagnosis of tumour	Liver	23 (45)	22 (41)	35 (64)	46 (27)
	Lymph nodes	12 (23)	13 (24)	25 (45)	77 (46)
	Bone	4 (8)	4 (7)	9 (16)	30 (18)
	Lung	4 (8)	4 (7)	9 (16)	*N/A*
	Other	4 (8)	6 (11)	9 (16)	17 (10) *
	No metastasis	20 (39)	22 (41)	11 (20)	76 (45)
Tumour grade (G):	G1, Ki-67 <=2%	0	0	7	
	G2, Ki-67 3-20%	9	9	24	
	G3, Ki-67 >20%	2	2	6	*N/A*
	NEC, Ki-67>20%	*N/A*	*N/A*	8	
	No data	4	3	10	
Thoracic NENs	Typical	1	1		57
	Atypical	14	15	*N/A*	83
	LCNEC	8	8		7
	Carcinoid NOS	*N/A*	*N/A*		20
	No data	5	6		0

Abbreviations.: NEC, neuroendocrine carcinoma; NEN, neuroendocrine neoplasia; NOS, not otherwise specified; *N/A*., not applicable; LCNEC, large cell neuroendocrine carcinoma.
*4 of 167 patients had brain metastases at diagnosis.

Surgery (Paper II)

Data extracted from medical records and further analysed included surgical approach for BA (open access, LTA, PRA incl. one surgeon approach vs. two surgeons), operating time, post-operative complications and time of hospital stay after surgery. Postoperative complications were graded using the Clavien–Dindo classification (140) and for statistical analyses grades were assessed as mild up to Grade 2 and as severe for Grade 3-5. If BA failed during the first attempt and the patient was re-operated within one week, operating time was counted together from both surgeries. If per-operative conversion

from endoscopic to open access was made, the event was further analysed as open access surgery.

Statistical analysis (Paper I-IV)

The following statistical methods were used in all papers: normally distributed data were described as means ± standard deviation and data with skewed distribution and/or outliers were described as medians, accompanied by the 25th–75th percentile ranges (Q1-Q3) or min-max. The defined event was death from any cause. OS was defined as time from diagnosis of NEN and/or ECS until date of death, or if event was not found, censored at the date of last observation. OS from time of BA until date of death alternatively date of last observation was analysed in Paper II. Kaplan-Meier plots and the log-rank test were used for survival analysis and comparison respectively in all papers. Cox proportional regression was performed for the estimation of hazard ratios (HRs) and confidence intervals (CIs). Spearman's correlation test was used to estimate correlations and, Chi Square or Fisher's exact tests were used for testing relationships between categorical variables. The level of statistical significance was set at 0.05 in all papers. All statistical analyses were performed using IBM SPSS Statistics software (versions 24–28; IBM Corp., Armonk, NY, USA).

Immunohistochemistry (Paper IV)

For Paper IV, additional immunohistochemical staining was performed for serotonin and calcitonin on material previously collected during routine clinical care. FFPE tissue from lung-NENs was used for this study (n=108). The paraffin blocks were stored in room temperature. From these, 4 µm sections were cut and stained with the mouse monoclonal antibody for serotonin (clone 5HT-H209; Dako, Glostrup, Denmark; Cat. No. M0758; dilution 1:300, antibody incubation for 20 min) and a rabbit polyclonal ready-to-use antibody for calcitonin (Dako, Glostrup, Denmark; Cat. No. GA515, dilution: ready to use, antibody incubation for 15 min) according to the manufacturer's instructions using fully automated protocols on a Dako Omnis autostainer (Agilent Technologies/Dako, Glostrup, Denmark). Small intestinal NET and MTC were used as positive controls for serotonin and calcitonin respectively. The author of the thesis and an experienced pathologist evaluated all slides. Stainings were determined as positive if at least one tumour cell showed intense cytoplasmic staining and were categorized into groups according to the percentage

of positive tumour cells. Calcitonin staining was defined as diffuse when more than 50% of tumour cells were positive. Photographs were captured using an Olympus BX43 microscope (10x objective), with an Olympus SC50 camera. Images were acquired using Olympus cellSens Entry (cS) imaging software (version 4.3, Olympus Corporation, Tokyo, Japan).

Results

Not all parameters were available for all patients since data was collected retrospectively. Selection bias may have influenced some findings due to the small cohort of a rare condition from a single tertiary care centre.

Paper I

In more than a half of the patients (n=30), ECS was diagnosed simultaneously with the NEN diagnosis. In 12 cases, ECS developed in previously known non-functioning NEN (n=10) or in NEN secreting other hormones than ACTH (n=2). Ten of those patients had tumour progression at the debut of ECS.

High presence of hyperglycaemia and hypokalaemia at the time of diagnosis of ECS was observed in the cohort with potassium < 3.5 mmol/l in 75% of patients. Potassium levels were negatively correlated to ACTH (ρ=-0.4, p=0.010), UFC (ρ=-0.5, p=0.002), morning cortisol (ρ=-0.6, p<0.001), and evening cortisol (ρ=-0.5, p=0.032) levels. Almost all patients had very high hormone levels as demonstrated in Table 2, Paper 1.

Secretion of other hormones in parallel with ACTH was detected in 41% of cases and was equally occurring between thoracic and pan-NENs. Abnormal levels of 5-HIAA were observed most often followed by calcitonin and, in a majority of patients, hypersecretion of ACTH and other hormones was detected simultaneously.

Average follow-up time from the time of ECS diagnosis to the end of the study period was 33 months (range: 6-83). The most common cause of death in the cohort was progression of NEN, followed by infections and other ECS-related comorbidities. Median OS from the date of ECS diagnosis was 33 months (95% CI 16-50), with a 5-year survival of 30%. The location of the primary tumour did not influence the OS.

Multiple hormone secretion, high morning cortisol, higher tumour grade and diabetes correlated with shorter OS, as demonstrated in Figure 5. Furthermore, hypokalaemia at diagnosis showed a trend towards shorter OS in our cohort: 32 vs. 76 months (p=0.067).

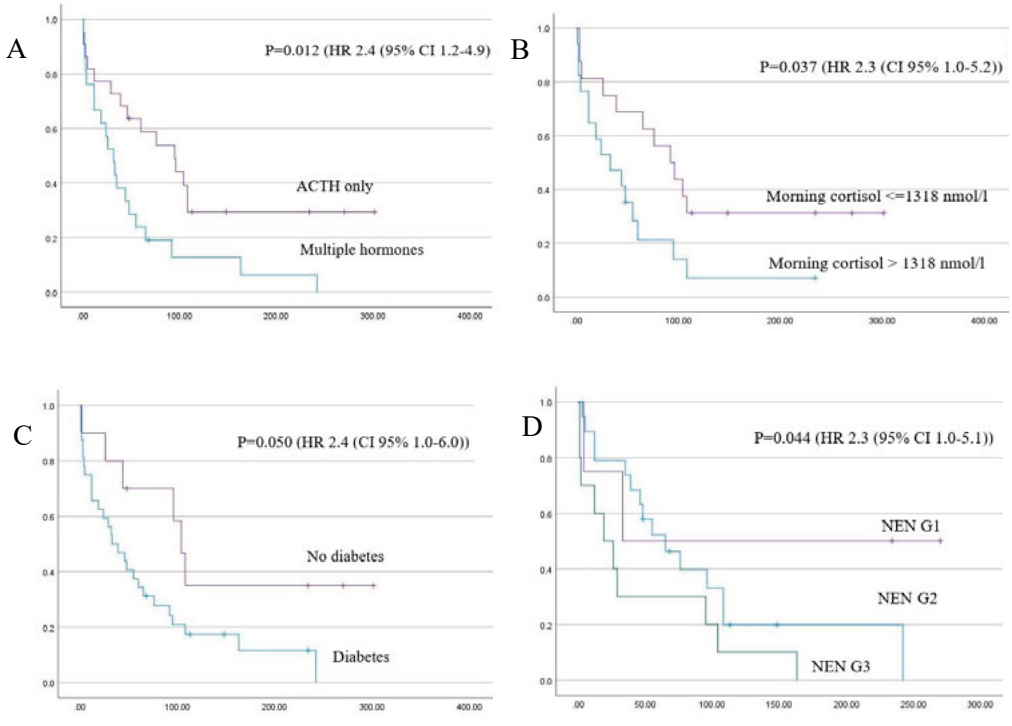


Figure 5. Kaplan-Meier overall survival (OS) curves for patients with ectopic Cushing's syndrome (ECS) according to (A) either tumour secretion of only adrenocorticotropic hormone (ACTH) or ACTH + other hormones (multiple hormone secretion); (B) morning cortisol levels at diagnosis; (C) presence of diabetes at diagnosis of tumour; (D) tumour grade. Statistical analysis performed by using Cox proportional regression.

Paper II

Fifty-three patients with ECS were analysed in this cohort: 37 of those underwent BA while in seven cases ECS resolved after radical surgery of the primary tumour and nine patients were treated with SI and/or antitumoral therapy only. The majority of patients were operated at Uppsala University hospital and the median time from diagnosis of ECS to surgery was two months. Most of the patients (n=32) received SI prior BA and in most cases, ketoconazole was used, as demonstrated in Table 3, Paper II.

Our study covered a period of 30 years and both open and endoscopic approaches for BA were used. We found significantly shorter operating and hospitalization time for the endoscopic approach compared to open access surgery, and the shortest operating time was detected in posterior retroperitoneoscopic

adrenalectomy, performed simultaneously by two surgeons (PRA-2S) as demonstrated in Table 8.

Table 8. Operating and hospitalization time depending on surgical approach in patients undergoing BA due to ECS.

Surgical approach	Operating time in minutes (mean±SD)	p-value	Days at hospital (median)	p-value
Open	246±75	0.004	30	0.005
Endoscopic	162±73		9	
LTA	230	0.001	8	0.957
PRA	121		10	
PRA-1S	184	0.001	12	0.909
PRA-2S	94		10	

BA, bilateral adrenalectomy; ECS, ectopic Cushing's syndrome; LTA, laparoscopic transperitoneal adrenalectomy; PRA, posterior retroperitoneoscopic adrenalectomy; PRA-1S, posterior retroperitoneoscopic adrenalectomy, performed by 1 surgeon; PRA-2S, posterior retroperitoneoscopic adrenalectomy, performed simultaneously by 2 surgeons.

All patients received perioperative prophylaxis against thromboembolic events, antibiotic prophylaxis and perioperative glucocorticoid replacement therapy in different dosage. One year after successful BA, 29 patients received hydrocortisone replacement therapy with more than 30 mg/day.

Median survival after BA was 24 months (min-max: 0-428) with a 5-year survival of 22%. Although there was no operative mortality, four patients died within one month after adrenalectomy due to multiple organ dysfunction syndrome and progression of NEN. Patients treated by radical surgery of the primary tumour or where good biochemical control was achieved by SI had better OS compared to patients who underwent BA, 96 months vs. 29 months respectively. There was no significant difference observed in OS after BA depending on localization of primary tumour or surgical approach, as opposed to multiple hormone secretion which strongly correlated with shorter OS after BA, p=0.009 (HR 2.9 (95% CI 1.3-6.7)). Patients who underwent BA within two months after ECS diagnosis had shorter OS compared to those who were operated at a later stage, probably because of less time for patient stabilization before BA due to more aggressive disease and more acute indications for surgery. We observed a high frequency of postoperative complications as demonstrated in Table 6, which can be explained by severe metabolic disturbances due to hypercortisolaemia preoperatively. However, there were significantly fewer severe complications in the endoscopic surgery group compared to open surgery (p=0.030).

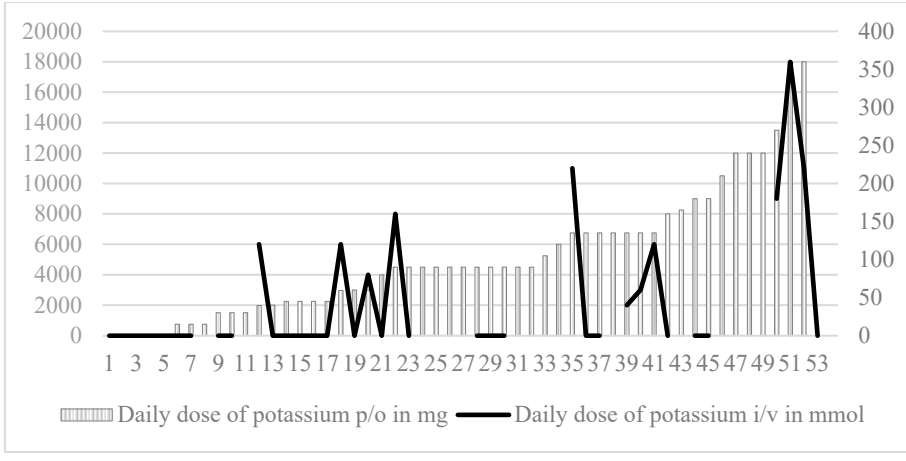
Paper III

Fifty-five patients with ECS were analysed with focus on baseline cortisol and potassium levels and symptomatic medical treatment of hyperkalaemia and hypercortisolaemia in order to identify possible factors influencing prognosis.

Mean baseline potassium levels were low (2.7 ± 0.7 mmol/l) and correlated negatively with baseline hormone levels: ACTH (ρ=-0.3, p=0.013), UFC (ρ=-0.5, p<0.001), and s-cortisol (ρ=-0.6, p<0.001). Severe hypokalaemia at diagnosis (< 2.5 mmol/l) was found in 50% of the patients.

Most of the patients needed potassium substitution (n=48) and spironolactone treatment (n=34) in high doses in order to obtain normal potassium levels as demonstrated in Figure 6. The dose of oral potassium substitution correlated with hormone levels at diagnosis: UFC (ρ=0.6, p<0.001), s-cortisol (ρ=0.5, p<0.001) and ACTH (ρ=0.3, p=0.016).

A



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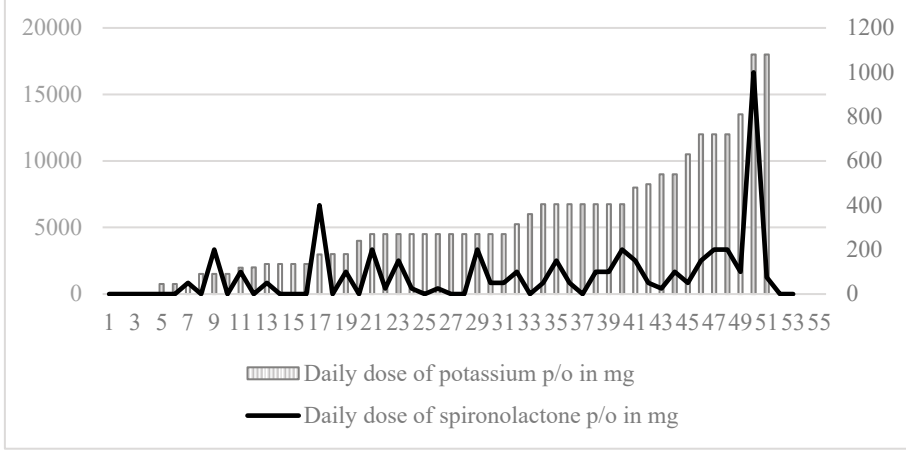

Figure 6. Bar charts for patients with ectopic Cushing's syndrome (ECS) showing (A) dose of potassium substitution therapy in the cohort per patient both per oral (p/o) and intravenously (i/v) and (B) dosage of spironolactone per patient.

To obtain normal cortisol levels, almost all of the patients (n=46) were receiving treatment with SI and despite this, 35 patients proceeded with BA. Although there was a statistically significant reduction of UFC and cortisol levels during SI treatment, the median level of UFC at the first follow-up after SI therapy initiation, was still 2-3 x UNL. Twenty-three patients were receiving SI for only one month or less and these patients had higher median UFC levels at diagnosis than those with longer SI treatment: subacute BA was performed in 18 cases due to severe ECS symptoms while three patients died within 4 weeks, having potassium <2.5 mmol/l and very high UFC at diagnosis (28934-79452 nmol/24h).

At the end of follow-up, 76% of patients had died after a median OS from ECS diagnosis of 26 months (95% CI 13-38). Severe hypokalaemia (<2.5 mmol/l) and hypercorticolaemia at diagnosis, high doses of potassium substitution and tumour differentiation grade were associated with shorter OS (p=0.009, p=0.004, p=0.019 and p=0.024 respectively). Severe hypercortisolaemia at diagnosis remained as an independent negative prognostic factor in multivariate Cox regression analysis, p=0.042 (HR 2.4 (95% CI 1.0-5.8)) regardless of Ki-67 as demonstrated in Table 9.

Table 9. Univariate and multivariate analysis of prognostic factors for survival in ectopic Cushing's syndrome.

Variable		**HR (95% CI)**	**p-value[a]**	**p-value[b]**
Age	>54 vs. ≤54	1.5 (0.8-3.0)	0.191	
Gender	Male vs. female	1.0 (0.6-2.0)	0.840	
Ki-67	>55% vs. 20-55% vs. <20%	2.0 (0.9-4.0)	0.024*	0.343
Serum cortisol nmol/l	>1380 vs. ≤1380	2.8 (1.4-5.8)	0.004*	0.042*
ACTH ng/ml	>151 vs. ≤151	1.2 (0.6-2.3)	0.568	
UFC nmol/24h	>4910 vs. ≤4910	1.1 (0.6-2.0)	0.704	
Potassium mmol/l	≥2.5 vs. <2.5	0.4 (0.2-0.8)	0.009*	0.390

[a] – p-value in univariate analysis
[b]– p-value in multivariate analysis
Hazard ratio (HR) and 95% confidence intervals (CI) obtained from Cox regression models.
ACTH, UFC and cortisol are divided in two groups (low vs. high) by using the median hormone level as cut-off.
ACTH, adrenocortical hormone; UFC, urine free cortisol.

Paper IV

Since the measurement of hormones in patients with lung-NENs without an evident hormone induced syndrome is not currently recommended by guidelines, hormone levels were not available for all 167 patients in the cohort. At least one hormone was measured in 127 patients.

We found elevated hormone levels in 52 patients with lung-NENs and the majority of tumours secreted serotonin, either alone or in combination with other hormones. Forty-two percent of patients with serotonin-secreting tumours did not have any symptoms typical for carcinoid syndrome. The second most commonly elevated hormone was calcitonin (n=20), followed by ACTH (n=13), gastrin (n=1) and VIP (n=1). Statistically significant increase of serotonin and calcitonin levels were observed in case of progressive tumour

disease ($p<0.001$ resp. $p=0.003$) and there was a high prevalence of elevated hormones among patients with recurrence after radical surgery (44%).

In contrast to circulating hormone levels, immunohistochemical staining identified a greater number of calcitonin- than serotonin-positive cases (42% vs. 17%). There was a discrepancy between immunohistochemical findings and circulating hormone levels for serotonin: elevated serotonin levels were observed in 13 of 38 patients with completely negative serotonin staining on IHC. In contrast, calcitonin expression was significantly associated with elevated circulating calcitonin: nine of 16 calcitonin-positive tumours had elevated circulating hormones, compared with one of 18 calcitonin-negative tumours ($p=0.002$).

Median 5-year OS rate was 66% and poorer differentiation, higher age at diagnosis, smoking and presence of lymph node metastases at surgery of the primary tumour were negative prognostic factors. Among patients with metastatic tumour disease at last follow up, median OS was much shorter in case of secretion of one (69 months) or several (33 months) hormones, compared to patients with normal hormone levels (124 months), $p=0.002$. A similar pattern was observed when comparing patients with elevated serotonin and/or calcitonin levels to those with normal levels of these hormones. Calcitonin expression in more than 50% of tumour cells compared to lower or no expression was also associated with reduced survival, 72 months vs. 124 months, $p=0.030$.

Discussion

This thesis focuses on ectopic hormone secretion from NENs, a rare and insufficiently investigated phenomenon that is almost always associated with increased morbidity compared to non-functioning NENs. Several studies have demonstrated higher mortality linked to hormonal activity in NENs (15, 16, 141, 142). The diagnostic approach and clinical management are challenging. Tumours may secrete multiple hormones simultaneously with overlapping symptoms. Furthermore, hormonal syndromes may develop long before the tumour is radiologically localized, and hormone levels may be elevated without an overt hormonal syndrome (121, 142). No guidelines currently recommend measuring ectopic hormones at diagnosis or during disease progression in patients without hormone related symptoms. Large retrospective and prospective multicentre studies specifically evaluating ectopic hormone secretion as a prognostic risk factor are lacking. There is an unmet need to improve understanding of these, often life-threatening, health conditions.

Paper I characterised a single-centre cohort of ACTH secreting NENs, Paper II evaluated surgical treatment, and Paper III assessed biochemical disturbances at diagnosis and medical management. The overall aim was to identify predictive and prognostic factors in ECS. In Paper IV, we extended this focus to ectopic hormone production and secretion in lung-NENs.

Paper I

Paper I aimed to analyse a cohort of NEN patients with ECS, particularly to describe clinical symptoms and biomarkers of predictive and prognostic importance and to identify parameters of clinical significance. In this retrospective cohort from Uppsala ENETS Centre of Excellence and European Reference Networks (ERN) EURACAN G4 domain centre, six percent of all patients with NENs were detected as having ECS which is somewhat higher than in a similar cohort where only about three percent were identified (47). This, together with the fact that the prevalence of ACTH secreting pan-NENs was higher in our cohort (29%) than in previous series (49, 50), could be explained

by selection bias since our study was performed in a specialized centre for endocrine oncology.

More than a half of the patients were diagnosed simultaneously with both a NEN tumour and ECS. In patients in whom ECS developed subsequently, the ECS diagnosis was almost always associated with radiological progression of disease. We could identify multiple hormone secretion, hypokalaemia, high morning cortisol and high blood glucose (diabetes mellitus) as indicators of worse prognosis. Seventy-five percent of patients had potassium levels < 3.5 mmol/l at diagnosis, and the severity of hypokalaemia correlated negatively with hormone levels as well as OS compared to normokalaemic patients (32 vs. 76 months). The same observation was made regarding diabetes being present at diagnosis of ECS and those parameters are confirmed as negative prognostic factors in ECS also by other authors (143).

The most remarkable finding in Paper I was the large proportion of patients with concomitant secretion of other hormones than ACTH and its influence on OS. Multiple hormone secretion has been described in pan-NENs (15, 16, 123, 144-146), suggesting that there is a trend towards shorter survival in patients with multiple hormone secretion (15, 16). To our knowledge there are no studies published regarding the prognostic relevance of multiple hormone production in lung-NENs. We found that multiple hormone secretion was evenly distributed between thoracic and pan-NENs and was leading to significantly worse prognosis compared to that of patients with ECS only, with median OS of 32 months vs. 95 months respectively, p=0.009. Hormones elevated most frequently besides ACTH were 5-HIAA and calcitonin: we suggest that measurement of those hormones at diagnosis of ECS and at the time of radiological progression, should be performed to identify patients who need intensified treatment and follow-up.

Paper II

The main objective in Paper II was to evaluate BA as treatment option in ECS and the effect of different surgical approaches on morbidity and mortality.

Endocrine society guidelines recommend SI as the primary treatment for ECS in patients with (1) occult or (2) metastatic ECS followed by BA (79). However, one should take into account that (1) it can take decades until the source of ACTH is detected in case of occult ECS, (2) prolonged and severe hypercortisolaemia increases the morbidity and risk for severe complications for the patient, and often delays start of oncological treatment. Five patients in our cohort had occult ECS where neither the primary tumour nor metastases were found despite extensive work-up. The longest time period among our

patients from ECS diagnosis and BA until detection of a bronchial NEN with positive ACTH immunohistochemical staining was 34 years and if BA had not been performed, SI treatment would have been required for this patient for several decades. The study period covered almost 30 years and some patients were primarily investigated before the era of PET, which could explain the difficulties to detect small NENs. The major cause of occult ECS is believed to be lung-NENs (45, 69) and detection of small lesions is difficult also with novel PET methods. Even though SI are proven to give good clinical effects on patients with non-ectopic CS, studies have shown that normocortisolaemia induced by e.g., ketoconazole in ECS is achieved in only 44% of patients [63]. Those factors, in combination with the risk for SI side effects during long term use and the high incidence of drug-drug interactions point towards BA as the treatment of choice in occult or metastatic ECS. Of course, there is a definitive place for SI usage in the pre-operative period and in patients who does not classify for surgery. Although severe toxicity of SI in our cohort was low (n=2), 32 patients had persistent hypercortisolaemia despite medical treatment and proceeded to BA.

It is crucial for survival for most of the patients with metastasized ACTH producing NEN to start oncological treatment as soon as possible. However, the trauma caused by surgery can postpone initiation of chemotherapy (147) and fast and minimally invasive surgical procedure, which also result in shorter recovery time after surgery, is highly important. We found that there were fewer severe complications as well as shorter operating and hospitalization time when using the endoscopic approach, compared to open surgery, as well as significantly shorter operating time applying PRA compared to LTA. Similar results have been demonstrated by other authors as well (104, 148). PRA-2S had the shortest operating time and we recommend that this method should be considered as the best choice of surgical approach in ECS.

ECS is characterized by very high cortisol levels making glucocorticoid substitution therapy after BA very challenging. There is a high risk for glucocorticoid withdrawal syndrome and most of the patients' need higher glucocorticoid dosage than normally recommended, especially during the first months after BA. On the other hand - over-replacement of glucocorticoids may lead to higher morbidity and mortality, especially in patients with metastatic NENs, who often have impaired immune function due to oncological treatment. We observed that a majority of our patients received 30 mg of hydrocortisone daily or more one year after BA which is higher than the dosage of 10–12 mg/m^2/d, recommended by the Endocrine society guidelines (79). One third of the patients had residual arterial hypertension and diabetes three months after BA, probably partially depending on too high doses of glucocorticoids.

We observed a relatively high number of surgical complications in our cohort compared to other studies (104, 149, 150). However, it should be noted that most of the papers published include patients with various aetiology of CS (104, 148, 151-153) and are not focusing on ECS only. We suggest that the reason for the higher complication rate in our data depends on specific features of ECS such as more pronounced hypercortisolaemia leading to more severe metabolic disturbances preoperatively than in other types of CS, combined with metastatic tumour disease. Furthermore, presence of e.g., multiple liver metastases, fibrotic processes in the abdomen due to previous surgery or the presence of a large primary tumour in the pancreas can affect the outcome of BA.

Paper III

Paper III aimed to further evaluate the biochemical disturbances at diagnosis, the medical management, and their prognostic implications in ECS.

It is well known that in case of very high cortisol levels, cortisol can bind also to aldosterone receptors, worsening the hypokalaemia. Severe hypokalaemia and hypokalaemic alkalosis are reported to be more frequent and severe in ECS compared to CD (154, 155). Our findings demonstrated very low potassium levels at diagnosis (<2.5 mmol/L) in 50% of patients, which correlated negatively with hormone levels. There may be further contributory factors lowering potassium levels in ECS, including diarrhoea caused by concomitant secretion of other hormones (e.g., serotonin, gastrin, VIP, calcitonin), renal or gastrointestinal potassium losses related to antitumour treatments or side effects of metyrapone. Potassium supplementation doses were high, and many patients required a combination of oral and intravenous administration, often together with high-dose spironolactone. We could confirm that severe hypokalaemia, high doses of oral potassium substitution and spironolactone were correlated to shorter OS. In a cohort of 64 patients with ECS, *Chen et al.* identified hypokalaemia as an independent negative prognostic factor, irrespective of hormone levels (156). In contrast, in our cohort, only severe hypercortisolaemia remained as a statistically significant prognostic factor for shorter survival in multivariate analysis.

SI was used in 46 of 55 patients. However, even if there was a reduction in hormone levels during SI treatment, UFC at the first follow-up after SI therapy initiation remained 2-3 x UNL, and the majority of patients proceeded to BA. There was a subgroup of patients who were treated with SI only 1 month or less: they had significantly higher hormone levels at ECS diagnosis and a

tendency towards shorter OS compared to those receiving SI treatment for a longer period of time.

The main findings in Paper III were that the severity of hypokalaemia and, particularly hypercortisolaemia at diagnosis was associated with shorter OS. Despite SI treatment and high-dose potassium supplementation, many patients required early BA or died. These findings highlight the importance of prompt therapy decisions, especially in patients with severe hypokalaemia, with consideration of early BA if radical surgery of primary tumour is not feasible.

Paper IV

The surprising findings in Paper I, demonstrating a high prevalence of tumours secreting multiple hormones and an associated worse prognosis in this subgroup, encouraged to further investigation of this phenomenon, particularly in lung-NENs, as published data on this topic are very limited.

The true incidence of hormone secreting lung-NENs is unknown since hormones are usually measured only in case of severe hormonal syndrome. The prevalence of carcinoid syndrome and ECS has been reported to be 2-13% (17, 31) and 1-6% (6, 17), respectively. There are no data available on the prevalence of hypercalcitonaemia in well differentiated lung-NENs. Almost nothing is known about the prognostic impact of elevated hormone levels in lung-NENs.

The prevalence of elevated hormone levels among patients with available measurements in our cohort was high: 30% had elevated 5-HIAA levels, and 23% had elevated calcitonin levels, figures which are much higher than previously reported. Thirty-five per-cent of these tumours secreted more than one hormone. Notably, 16 of 38 patients with elevated 5-HIAA and 12 of 20 patients with elevated calcitonin did not show any hormone related symptoms. The hormonal activity of these tumours would likely have gone unrecognised, as current guidelines do not recommend testing all patients.

Positive immunohistochemical staining for serotonin and calcitonin was observed in 17 and 45 of 108 tumours, respectively. Elevated 5-HIAA was found in 34% of serotonin-negative tumours, whereas only 6% of calcitonin-negative tumours secreted calcitonin, suggesting that calcitonin is a more reliable immunohistochemical marker of hormone secretion than serotonin.

Hormone activity in lung-NENs was associated with poorer prognosis: the presence of single or multiple hormone secretion had a significant negative impact on median OS compared with non-secreting tumours despite the absence of hormonal syndrome in more than 40% of cases. This finding highlights the importance of including routine hormone assessment in lung-NENs

at diagnosis and during disease progression, regardless of symptom presence. Calcitonin expression in more than 50% of tumour cells, compared with lower or absent expression, was also associated with poorer survival. Implementing routine calcitonin immunohistochemical staining in all lung-NENs could help identify patients who may require closer disease monitoring and more intensive tumour treatment.

Major Findings

- Multiple hormone secretion, high morning cortisol, higher tumour grade, and diabetes mellitus were correlated with shorter OS in patients with ECS due to ACTH secreting NENs.
- Development of ECS in patients with a non-functioning NEN may indicate tumour progression.
- Multiple hormone secretion should be considered as a bad prognostic sign in ECS patients and should lead to intensified clinical management.
- Despite the frequent use of adrenolytic treatment, BA was necessary in a majority of patients to gain control over ECS.
- The complication rate after BA was high, probably due to the combination of metastatic disease and metabolic disorders caused by high cortisol levels.
- Hypercortisolaemia in combination with severe hypokalaemia (< 2.5 mmol/l) at diagnosis of ECS, indicates an acute life-threatening situation: immediate actions should be considered to reduce cortisol levels, primarily BA.
- The two-surgeon approach BA may be considered as method of choice in ECS when compared to other BA approaches, due to fewer complications and shorter operating time.
- Hormone secretion in lung-NENs is negative prognostic factor.
- Strong calcitonin expression in >50% of tumour cells is associated with poorer outcome in lung-NEN and correlates with elevated calcitonin levels in circulation.

Future directions

ECS is a very rare health condition and studies conducted so far have been limited to just over 100 patients or fewer. To obtain statistical power, most often these cohorts include ACTH secreting tumours of various origins and both low- and high- differentiated tumours. These studies are also often retrospective and cover several decades: the results may be affected by previous documentation in patient records and by treatment strategies that have varied over time. This makes data interpretation difficult. Despite the available recommendations in current guidelines, the effectiveness of treatment is often low and the mortality is high. There is a great need for prospective international studies in ECS with the possibility of analysing significantly larger patient cohorts stratified according to the histological characteristics of the tumours.

Our first and fourth paper demonstrate multiple hormone secretion as a negative prognostic factor in pancreatic and lung-NENs. This phenomenon as a prognostic factor has not been studied in lung-NENs, and there are currently no recommendations for routine hormone sampling at diagnosis and disease progression. We correlated circulating hormone levels with hormone expression in the tumour tissue, but this analysis was limited due to the few patients sampled for circulating hormones at diagnosis. A prospective study should therefore be of value, where IHC for hormone expression is performed and all patients with lung-NENs are hormone sampled at diagnosis and at progression, regardless of symptoms, in order to be able to verify the validity of our findings.

Unlike gastrointestinal NENs, a large proportion of lung-NENs do not show somatostatin receptor expression, and even low-proliferative tumours can have high uptake on ^{18}F-FDG PET. In a future prospective study, it would also be interesting to correlate these findings with hormone activity in lung-NENs at diagnosis and progression.

Sammanfattning på svenska

Neuroendokrina neoplasier (NEN) är en grupp sällsynta tumörer med mycket varierande prognos och med ökande incidens under de senaste decennierna. Ökningen förklaras troligen av förbättrad kunskap om tumörerna samt bättre diagnostiska metoder. NEN kan förekomma i vilket organ som helst, dock är lungor, magsäck, bukspottkörtel, tunntarm, blindtarm och ändtarm de vanligaste lokaliseringarna. Vissa NEN har förmåga att producera olika hormoner som ger upphov till kliniska symtom medan andra inte frisätter hormoner som ger upphov till endokrina symtom eller inte frisätter tillräckligt med hormoner för att orsaka symtom. Hormonutsöndring kan vara topisk (om hormonet utsöndras normalt från tumörens ursprungsorgan) eller ektopisk (om hormonet inte förekommer normalt i tumörens ursprungsorgan). Tidigare studier i området har visat att hormonproduktion i NEN kan vara förknippad med tumörprogress samt sämre prognos. Dessa studier har dock antingen inkluderat NEN av olika ursprung eller fokuserat enbart på pankreas-NEN. Såvitt vi vet finns det inga studier som hittills har analyserat hormonproduktion i lung-NEN som prognostisk faktor.

Fokus för min avhandling är hormonproducerande NEN. De första tre arbeten handlar om ektopisk Cushing's syndrom (ECS) i NEN - ett mycket ovanligt tillstånd med mycket hög sjuklighet och dödlighet. Tumörerna producerar ACTH som i sin tur stimulerar kortisolproduktion i binjurarna. Den normala kortisolåterkopplingsmekanismen i hypotalamus-hypofys-binjureaxeln fungerar inte. Detta resulterar i förlust av dygnsrytmen för utsöndringen av kortisol och medför allvarlig hyperkortisolism. Det fjärde arbetet fokuserar på hormonproduktion i lung-NEN samt hormonuttryck i tumörvävnad.

Vår första studie är en retrospektiv analys av alla patienter med ECS som vårdats på kliniken för onkologisk endokrinologi, Akademiska sjukhuset i Uppsala, mellan 1986 och 2014. Bland 886 patienter med NEN identifierades 6% patienter med ECS vilket är en högre förekomst än tidigare rapporterade siffror för liknande kohorter. Detta kan dock förklaras med att vår kohort kommer från ett högspecialiserat center för NEN. Majoriteten (47%) av ACTH producerade tumörer var lung-NEN följd av pankreas-NEN (29%). Vi kunde visa att utveckling av ECS hos patienter med en tidigare icke-fungerande NEN oftast var förknippad med radiologisk tumörprogress. Högre kortisol nivåer

än medelvärde i kohorten, förekomst av diabetes samt högre tumörgrad var associerad med sämre överlevnad. Det mest intressanta fyndet i det första arbetet var att patienter med multipel hormonsekretion hade mycket kortare överlevnad (32 månader), jämfört med de med endast ACTH-sekretion (95 månader). Vi föreslår att tumörer med multipel hormonsekretion bör följas och behandlas mer intensivt.

Vår andra studie fokuserade på bilateral adrenalektomi och de olika kirurgiska metoder som använts som behandlingsalternativ för patienter med ECS. Hyperkortisolism orsakar svåra metaboliska rubbningar med bl.a. låga kaliumnivåer, höga glukosnivåer samt ökar risken för tromboembolism och svåra infektioner. Ofta behöver dessa patienter intensivvård. Det är viktigt att snabbt kunna reducera kortisolnivåerna för att undvika tidig död samt för att kunna starta onkologisk behandling i tidigt skede. Enligt nuvarande riktlinjerna används läkemedel som påverkar kortisolsyntesen i binjurarna (steroidogeneshämmare) i första hand men dessa är toxiska och effekten blir ofta inte tillräcklig. Ett effektivt sätt att reducera kortisol snabbt är kirurgisk borttagning av båda binjurar. I vår Uppsala kohort genomgick 70% av patienterna med ECS BA med en medeltid från diagnos av ECS till BA på 2 månader. Allvarliga kirurgiska komplikationer upptäcktes hos 12 patienter. Det fanns färre allvarliga komplikationer vid endoskopisk kirurgi jämfört med öppen kirurgi. Den kortaste kirurgitiden var när en endoskopisk metod där 2 kirurger utför adrenalektomi samtidigt på var sin sida användes. Huvudslutaser i den andra arbetet var att: (1) BA var nödvändig för en majoritet av patienterna, (2) komplikationsfrekvensen var hög, troligen som ett resultat av kombinationen av metastatisk sjukdom och metabola störningar orsakade av höga kortisolnivåer och att (3) BA med två kirurger kan betraktas som den metod som föredras vid ECS jämfört med andra BA-metoder på grund av färre komplikationer och en kortare operationstid.

I den tredje studien analyserade vi den prognostiska betydelsen av kortisol- och kaliumnivåer vid diagnos samt vilken symptomatisk behandling som givits i en retrospektiv kohort av 55 patienter med ECS. Hälften av patienterna hade svår hypokalemi vid diagnos och kaliumsubstitution krävdes hos 87% av patienter, oftast i mycket höga doser. Hypokalemi och behov av höga doser kaliumsubstitution var associerade med kortare totalöverlevnad men endast svår hyperkortisolemi var en oberoende negativ faktor för överlevnad i multivariat analys. Steroidogeneshämmare användes initialt hos 46 patienter, men inom 1 månad genomgick 17 patienter BA och 3 avled. Våra slutsatser var att endast svår hyperkortisolemi är en oberoende prognostisk för kortare överlevnad vid ECS. Kombinationen av hyperkortisolemi och svår hypokalemi utgör

en akut livshotande situation, där snabb behandling för att sänka kortisolnivåerna är avgörande, och BA bör övervägas tidigt.

I den fjärde studien analyserade vi en retrospektiv kohort av 167 lung-NEN som har behandlats på Akademiska sjukhuset i Uppsala under de senaste 15 åren. Vi fann något oväntat att nästan 1/3 av alla tumörer (n=52) var hormonproducerande med dominans av serotoninproduktion (n=38). Nya immunohistokemiska färgningar för kalcitonin och serotonin utfördes, och resultaten jämfördes med cirkulerande hormonnivåer. Kalcitoninuttryck i tumörvävnad korrelerade starkt med förhöjda cirkulerande kalcitoninnivåer och diffus kalcitoninfärgning var associerad med kortare överlevnad. Hormonproducerande tumörer hade signifikant kortare överlevnad (62 månader) än icke-producerande tumörer (124 månader). I likhet med den första studien kunde vi även här påvisa att multipel hormonsekretion ytterligare försämrar överlevnaden. Detta är, såvitt vi vet, första studie som analyserat förekomst av serotonin och kalcitonin i tumörvävnad och blod i lung-NEN i relation till överlevnad. Vi föreslår rutinmässig hormontestning vid diagnos och vid progressiv sjukdom samt en mer intensiv behandling vid påvisad hormonproduktion i lung-NEN.

Framtidsperspektiv

ECS är mycket sällsynt hälsotillstånd som kan förekomma vid olika cancerformer, bl.a. vid NEN. Dessa patienter blir mycket sjuka, behöver ofta intensivvård samt kräver multidisciplinärt kliniskt omhändertagande. Man behöver agera snabbt då både svår hyperkortisolism samt cancer i sig utgör ett akut hot för patienternas liv. Pga. sjukdomens sällsynthet är de hittills utförda studierna begränsade till drygt 100 patienter eller färre. Dessutom består dessa kohorter ofta av patienter med mycket lågdifferentierade tumörer så som småcellig lungcancer tillsammans med patienter med högdifferentierade tumörer samt tumörer av olika primärt ursprung. Dessa studier är dessutom oftast retrospektiva och sträcker sig över flera decennier, där resultaten påverkas av tidigare dokumentation i patientjournaler samt av de behandlingsstrategier som har varierat över tid. Detta försvårar tolkning av data. Behandlingsförslagen som finns i nuvarande riktlinjerna har ofta låg effektivitet och mortaliteten är hög. Det finns ett mycket stort behov av prospektiva internationella studier inom ECS, där man har möjlighet att analysera betydligt större patientmaterial stratifierade efter tumörernas histologiska egenskaper och med tydliga behandlingsalternativ.

Vårt första och fjärde arbete visar att ektopisk hormonproduktion är en negativ prognostisk faktor i pankreas- och lung-NENs. Detta fenomen som prognostisk faktor har studerats i mycket begränsad utsträckning vid lung-NEN, och det saknas riktlinjer för rutinmässig hormonprovtagning vid diagnos och

sjukdomsprogress. Vi korrelerade cirkulerande hormonnivåer med hormonuttryck i tumörvävnaden men denna analys var begränsad pga. få patienter provtagits vid diagnos. En prospektiv studie skulle därför vara av värde, där immunhistokemi för hormonuttryck utförs samtidigt som samtliga patienter med lung-NEN hormonprovtas vid diagnos och progress, oavsett symtom, för att med större säkerhet kunna verifiera våra fynd. Till skillnad från gastrointestinala NEN uppvisar en stor andel lung-NEN inget somatostatinreceptoruttryck, och även långsamt växande tumörer kan ha högt upptag på ^{18}F-FDG PET. I en framtida prospektiv studie skulle man även kunna korrelera dessa fynd med hormonell aktivitet vid lung-NEN vid diagnos och progress.

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References

1. Leoncini E, Boffetta P, Shafir M, Aleksovska K, Boccia S, Rindi G. Increased incidence trend of low-grade and high-grade neuroendocrine neoplasms. Endocrine. 2017;58(2):368–79.
2. Dasari A, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, et al. Trends in the Incidence, Prevalence, and Survival Outcomes in Patients With Neuroendocrine Tumors in the United States. JAMA oncology. 2017;3(10):1335–42.
3. Marina Tsoli GKD, Ioannis I. Androulakis, Gregory Kaltsas, and Ashley Grossman. Paraneoplastic Syndromes Related to Neuroendocrine Tumors. In: al. FKASABe, editor. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2020.
4. Brown WH. A CASE OF PLURIGLANDULAR SYNDROME: " DIABETES OF BEARDED WOMEN.". The Lancet. 1928;212(5490):1022–3.
5. Doege KW. FIBRO-SARCOMA OF THE MEDIASTINUM. Annals of Surgery. 1930;92(5):955–60.
6. Daskalakis K, Chatzelis E, Tsoli M, Papadopoulou-Marketou N, Dimitriadis GK, Tsolakis AV, et al. Endocrine paraneoplastic syndromes in patients with neuroendocrine neoplasms. Endocrine. 2019;64(2):384–92.
7. Dimitriadis GK, Angelousi A, Weickert MO, Randeva HS, Kaltsas G, Grossman A. Paraneoplastic endocrine syndromes. Endocr Relat Cancer. 2017;24(6):R173–r90.
8. Kaltsas G, Androulakis, II, de Herder WW, Grossman AB. Paraneoplastic syndromes secondary to neuroendocrine tumours. Endocr Relat Cancer. 2010;17(3):R173–93.
9. Frilling A, Åkerström G, Falconi M, Pavel M, Ramos J, Kidd M, et al. Neuroendocrine tumor disease: an evolving landscape. Endocrine-Related Cancer. 2012;19(5):R163.
10. Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020;76(2):182–8.
11. Öberg K, Eriksson B. Endocrine tumours of the pancreas. Baillière's best practice & research Clinical gastroenterology. 2005;19(5):753–81.
12. Falconi M, Bartsch DK, Eriksson B, Klöppel G, Lopes JM, O'Connor JM, et al. ENETS Consensus Guidelines for the Management of Patients with Digestive Neuroendocrine Neoplasms of the Digestive System: Well-Differentiated Pancreatic Non-Functioning Tumors. Neuroendocrinology. 2012;95(2):120–34.
13. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. Annals of oncology : official journal of the European Society for Medical Oncology. 2013;24(1):152–60.

14. Alese OB, Jiang R, Shaib W, Wu C, Akce M, Behera M, et al. High-Grade Gastrointestinal Neuroendocrine Carcinoma Management and Outcomes: A National Cancer Database Study. The oncologist. 2019;24(7):911–20.
15. Crona J, Norlén O, Antonodimitrakis P, Welin S, Stålberg P, Eriksson B, et al. Multiple and Secondary Hormone Secretion in Patients With Metastatic Pancreatic Neuroendocrine Tumours. The Journal of Clinical Endocrinology & Metabolism. 2016;101(2):445–52.
16. de Mestier L, Hentic O, Cros J, Walter T, Roquin G, Brixi H, et al. Metachronous hormonal syndromes in patients with pancreatic neuroendocrine tumors: a case-series study. Annals of internal medicine. 2015;162(10):682–9.
17. Caplin ME, Baudin E, Ferolla P, Filosso P, Garcia-Yuste M, Lim E, et al. Pulmonary neuroendocrine (carcinoid) tumors: European Neuroendocrine Tumor Society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids. 2015. p. 1604.
18. Hallet J, Law CH, Cukier M, Saskin R, Liu N, Singh S. Exploring the rising incidence of neuroendocrine tumors: a population-based analysis of epidemiology, metastatic presentation, and outcomes. Cancer. 2015;121(4):589–97.
19. Jemal A, Thun MJ, Ries LA, Howe HL, Weir HK, Center MM, et al. Annual report to the nation on the status of cancer, 1975-2005, featuring trends in lung cancer, tobacco use, and tobacco control. Journal of the National Cancer Institute. 2008;100(23):1672–94.
20. L. Kreyberg AAL, and E. A. Uehlinger. . Histological Typing of Lung Tumours. International Histological Classification of Tumours, No. 1. Geneva: World Health Organization; 1967.
21. Arrigoni MG, Woolner LB, Bernatz PE. Atypical carcinoid tumors of the lung. The Journal of thoracic and cardiovascular surgery. 1972;64(3):413–21.
22. Kreyberg L LA, Uehlinger E. Histological Typing of Lung Tumours. 2nd ed. Geneva: World Health Organization.; 1981.
23. Travis WD, Colby, T. V, Corrin, B, Shimosato, Y, Brambilla, Elisabeth et al. . Histological typing of lung and pleural tumours, 3rd ed.: World Health Organization; 1999.
24. Pelosi G, Travis WD. Head-to-head: Should Ki67 proliferation index be included in the formal classification of pulmonary neuroendocrine neoplasms? Histopathology. 2024;85(4):535–48.
25. Grøndahl V, Binderup T, Langer SW, Petersen RH, Nielsen K, Kjaer A, et al. Characteristics of 252 patients with bronchopulmonary neuroendocrine tumours treated at the Copenhagen NET Centre of Excellence. Lung cancer (Amsterdam, Netherlands). 2019;132:141–9.
26. Rubino M, Scoazec JY, Pisa E, Faron M, Spaggiari L, Hadoux J, et al. Lung carcinoids with high proliferative activity: Further support for the identification of a new tumor category in the classification of lung neuroendocrine neoplasms. Lung Cancer. 2020;148:149–58.
27. Baudin E, Caplin M, Garcia-Carbonero R, Fazio N, Ferolla P, Filosso PL, et al. Lung and thymic carcinoids: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up(☆). Annals of oncology: official journal of the European Society for Medical Oncology. 2021.
28. Vocino Trucco G, Righi L, Volante M, Papotti M. Updates on lung neuroendocrine neoplasm classification. Histopathology. 2024;84(1):67–85.
29. Nicholson AG, Tsao MS, Beasley MB, Borczuk AC, Brambilla E, Cooper WA, et al. The 2021 WHO Classification of Lung Tumors: Impact of Advances Since 2015. Journal of Thoracic Oncology. 2022;17(3):362–87.

30. WHO Classification of Tumours Editorial Board. Thoracic tumours: WHO classification of tumours. 5th ed. Lyon, France: IARC, 2021.
31. Halperin DM, Shen C, Dasari A, Xu Y, Chu Y, Zhou S, et al. Frequency of carcinoid syndrome at neuroendocrine tumour diagnosis: a population-based study. The Lancet Oncology. 2017;18(4):525–34.
32. Llewellyn DC, Srirajaskanthan R, Vincent RP, Guy C, Drakou EE, Aylwin SJB, et al. Calcitonin-secreting neuroendocrine neoplasms of the lung: a systematic review and narrative synthesis. Endocrine connections. 2021;10(4):447–61.
33. Gaur P, Leary C, Yao JC. Thymic Neuroendocrine Tumors: A SEER Database Analysis of 160 Patients. Annals of Surgery. 2010;251(6):1117–21.
34. Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. Lancet (London, England). 2015;386(9996):913–27.
35. Lindholm J. Cushing's Syndrome: Historical Aspects. Pituitary. 2000;3(2):97–104.
36. Bullock W, Sequeira JH. On the relation of the suprarenal capsules to the sexual organs. Tr Path Soc London. 1905;56:189.
37. Guthrie L, Emery WdE. Precocious obesity, premature sexual and physical development, and hirsuties in relation to hypernephroma and other morbid conditions. Tr Clin Soc London. 1907;40:175.
38. Cushing H. The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). 1932. Obesity research. 1994;2(5):486–508.
39. Meador CK, Liddle GW, Island DP, Nicholson WE, Lucas CP, Nuckton JG, et al. Cause of Cushing's syndrome in patients with tumors arising from "non-endocrine" tissue. The Journal of clinical endocrinology and metabolism. 1962;22(7):693–703.
40. Liddle GW, Island DP, Ney RL, Nicholson WE, Shimizu N. Nonpituitary Neoplasms and Cushing's Syndrome: Ectopic "Adrenocorticotropin" Produced by Nonpituitary Neoplasms as a Cause of Cushing's Syndrome. Archives of internal medicine (1960). 1963;111(4):471–5.
41. Liddle GW, Nicholson WE, Island DP, Orth DN, Abe K, Lowder SC. Clinical and laboratory studies of ectopic humoral syndromes. Recent progress in hormone research. 1969;25:283–314.
42. Holub D, Katz F. A possible etiologic link between Cushing's syndrome and visceral malignancy. Clin Res. 1961;9:194.
43. Nieman LK. Cushing's syndrome: update on signs, symptoms and biochemical screening. European journal of endocrinology. 2015;173(4):M33.
44. Meinardi JR, Wolffenbuttel BHR, Dullaart RPF. Cyclic Cushing's syndrome: a clinical challenge. European Journal of Endocrinology eur j endocrinol. 2007;157(3):245.
45. Alexandraki KI, Grossman AB. The ectopic ACTH syndrome. Reviews in Endocrine and Metabolic Disorders. 2010;11(2):117–26.
46. Boscaro M, Barzon L, Fallo F, Sonino N. Cushing's syndrome. The Lancet (British edition). 2001;357(9258):783–91.
47. Kamp K, Alwani RA, Korpershoek E, Franssen GJH, de Herder WW, Feelders RA. Prevalence and clinical features of the ectopic ACTH syndrome in patients with gastroenteropancreatic and thoracic neuroendocrine tumors. European journal of endocrinology. 2016;174(3):271–80.
48. Ejaz S, Vassilopoulou-Sellin R, Busaidy NL, Hu MI, Waguespack SG, Jimenez C, et al. Cushing syndrome secondary to ectopic adrenocorticotropic hormone secretion. Cancer. 2011;117(19):4381–9.

49. Aniszewski JP, Young Jr WF, Thompson GB, Grant CS, van Heerden JA. Cushing Syndrome Due to Ectopic Adrenocorticotropic Hormone Secretion. World journal of surgery. 2001;25(7):934–40.
50. Isidori AM, Kaltsas GA, Pozza C, Frajese V, Newell-Price J, Reznek RH, et al. The Ectopic Adrenocorticotropin Syndrome: Clinical Features, Diagnosis, Management, and Long-Term Follow-Up. The Journal of Clinical Endocrinology & Metabolism. 2006;91(2):371–7.
51. Bhansali A, Walia R, Rana SS, Dutta P, Radotra BD, Khandelwal N, et al. Ectopic Cushing's syndrome: Experience from a tertiary care centre. Indian Journal of Medical Research. 2009;129(1):33–41.
52. Ilias I, Torpy DJ, Pacak K, Mullen N, Wesley RA, Nieman LK. Cushing's syndrome due to ectopic corticotropin secretion: Twenty years' experience at the National Institutes of Health. Journal of Clinical Endocrinology and Metabolism. 2005;90(8):4955–62.
53. Torpy DJ, Mullen N, Ilias I, Nieman LK. Association of Hypertension and Hypokalemia with Cushing's Syndrome Caused by Ectopic ACTH Secretion. Annals of the New York Academy of Sciences. 2002;970(1):134–44.
54. Terzolo M, Reimondo G, Ali A, Bovio S, Daffara F, Paccotti P, et al. Ectopic ACTH syndrome: molecular bases and clinical heterogeneity. Annals of oncology : official journal of the European Society for Medical Oncology. 2001;12 Suppl 2:S83–7.
55. Young J, Haissaguerre M, Viera-Pinto O, Chabre O, Baudin E, Tabarin A. MANAGEMENT OF ENDOCRINE DISEASE: Cushing's syndrome due to ectopic ACTH secretion: an expert operational opinion. European journal of endocrinology. 2020;182(4):R29–R58.
56. Isidori AM, Lenzi A. Ectopic ACTH syndrome. Arquivos brasileiros de endocrinologia e metabologia. 2007;51(8):1217–25.
57. Serra F, Duarte S, Abreu S, Marques C, Cassis J, Saraiva M. Cushing's syndrome due to ectopic ACTH production by a nasal paraganglioma. Endocrinology, diabetes & metabolism case reports. 2013;2013:130038.
58. Park HK, Park CM, Ko KH, Rim MS, Kim YI, Hwang JH, et al. A case of Cushing's syndrome in ACTH-secreting mediastinal paraganglioma. The Korean journal of internal medicine. 2000;15(2):142–6.
59. Alwani RA, Neggers SJ, van der Klift M, Baggen MG, van Leenders GJ, van Aken MO, et al. Cushing's syndrome due to ectopic ACTH production by (neuroendocrine) prostate carcinoma. Pituitary. 2009;12(3):280–3.
60. Alshaikh OM, Al-Mahfouz AA, Al-Hindi H, Mahfouz AB, Alzahrani AS. Unusual cause of ectopic secretion of adrenocorticotropic hormone: Cushing syndrome attributable to small cell prostate cancer. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2010;16(2):249–54.
61. Mendoza CF, Ontiveros P, Xibille DX, Rivera MH. Ectopic ACTH secretion (EAS) associated to a well-differentiated peritoneal mesothelioma: case report. BMC ENDOCRINE DISORDERS. 2015;15(1):40.
62. Chentli F, Terki N, Azzoug S. Ectopic adrenocortical carcinoma located in the ovary. European journal of endocrinology. 2016;175(4):K17–23.
63. Dacruz T, Kalhan A, Rashid M, Obuobie K. An Ectopic ACTH Secreting Metastatic Parotid Tumour. Case Reports in Endocrinology. 2016;2016:1–4.
64. Park S, Kim M-H, Kim JH, Kim T-O, Kim TH, So H, et al. Cushing's Syndrome Caused by an ACTH-Producing Neuroendocrine Carcinoma of the Gallbladder. Korean Journal of Medicine. 2014;87(1):67–71.

65. Li X, Su J, Zhao L, Wu J, Ding X, Fang F, et al. Familial Cushing syndrome due to thymic carcinoids in a multiple endocrine neoplasia type 1 kindred. Endocrine. 2014;47(1):183–90.
66. Hasani-Ranjbar S, Rahmanian M, Ebrahim-Habibi A, Soltani A, Soltanzade A, Mahrampour E, et al. Ectopic Cushing syndrome associated with thymic carcinoid tumor as the first presentation of MEN1 syndrome-report of a family with MEN1 gene mutation. Familial Cancer. 2014;13(2):267–72.
67. Singer K, Heiniger N, Thomas I, Worden FP, Menon RK, Chen M. Ectopic Cushing syndrome secondary to metastatic medullary thyroid cancer in a child with multiple endocrine neoplasia syndrome type 2B: clues to early diagnosis of the paraneoplastic syndromes. Journal of Pediatric Endocrinology and Metabolism. 2014;27(9):993–6.
68. Hatipoglu E, Kepicoglu H, Rusen E, Kabasakal L, Gundogdu S, Kadioglu P. Von Hippel Lindau disease with metastatic pancreatic neuroendocrine tumor causing ectopic Cushing's syndrome. Neuro endocrinology letters. 2013;34(1):9–13.
69. Doppman JL, Nieman L, Miller DL, Pass HI, Chang R, Cutler GB, Jr., et al. Ectopic adrenocorticotropic hormone syndrome: localization studies in 28 patients. Radiology. 1989;172(1):115–24.
70. Isidori AM, Kaltsas GA, Pozza C, Frajese V, Newell-Price J, Reznek RH, et al. The ectopic adrenocorticotropin syndrome: clinical features, diagnosis, management, and long-term follow-up. J Clin Endocrinol Metab. 2006;91(2):371–7.
71. Schambelan M, Slaton PE, Jr., Biglieri EG. Mineralocorticoid production in hyperadrenocorticism. Role in pathogenesis of hypokalemic alkalosis. The American journal of medicine. 1971;51(3):299–303.
72. Walker BR, Campbell JC, Fraser R, Stewart PM, Edwards CR. Mineralocorticoid excess and inhibition of 11 beta-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. Clinical endocrinology. 1992;37(6):483–92.
73. Stewart PM, Walker BR, Holder G, O'Halloran D, Shackleton CH. 11 beta-Hydroxysteroid dehydrogenase activity in Cushing's syndrome: explaining the mineralocorticoid excess state of the ectopic adrenocorticotropin syndrome. J Clin Endocrinol Metab. 1995;80(12):3617–20.
74. Shepherd FA, Laskey J, Evans WK, Goss PE, Johansen E, Khamsi F. Cushing's syndrome associated with ectopic corticotropin production and small-cell lung cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1992;10(1):21–7.
75. Howlett TA, Drury PL, Perry L, Doniach I, Rees LH, Besser GM. Diagnosis and management of ACTH-dependent Cushing's syndrome: comparison of the features in ectopic and pituitary ACTH production. Clinical endocrinology. 1986;24(6):699–713.
76. Kamenický P, Redheuil A, Roux C, Salenave S, Kachenoura N, Raissouni Z, et al. Cardiac Structure and Function in Cushing's Syndrome: A Cardiac Magnetic Resonance Imaging Study. The Journal of Clinical Endocrinology & Metabolism. 2014;99(11):E2144–E53.
77. Sundin A, Eriksson B, Bergström M, Långström B, Oberg K, Orlefors H. PET in the diagnosis of neuroendocrine tumors. Ann N Y Acad Sci. 2004;1014:246–57.
78. Ragnarsson O, Juhlin CC, Torpy DJ, Falhammar H. A clinical perspective on ectopic Cushing's syndrome. Trends in endocrinology and metabolism: TEM. 2024;35(4):347–60.

79. Nieman LK, Biller BMK, Findling JW, Murad MH, Newell-Price J, Savage MO, et al. Treatment of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. The Journal of Clinical Endocrinology & Metabolism. 2015;100(8):2807–31.
80. Daniel E, Newell-Price JD. Therapy of endocrine disease: steroidogenesis enzyme inhibitors in Cushing's syndrome. European journal of endocrinology. 2015;172(6):R263–80.
81. Varlamov EV, Han AJ, Fleseriu M. Updates in adrenal steroidogenesis inhibitors for Cushing's syndrome - A practical guide. Best practice & research Clinical endocrinology & metabolism. 2021:101490–.
82. Fleseriu M, Pivonello R, Young J, Hamrahian AH, Molitch ME, Shimizu C, et al. Osilodrostat, a potent oral 11[beta]-hydroxylase inhibitor: 22-week, prospective, Phase II study in Cushing's disease. Pituitary. 2016;19(2):138.
83. Pivonello R, Fleseriu M, Newell-Price J, Bertagna X, Findling J, Shimatsu A, et al. Efficacy and safety of osilodrostat in patients with Cushing's disease (LINC 3): a multicentre phase III study with a double-blind, randomised withdrawal phase. The lancet Diabetes & endocrinology. 2020;8(9):748–61.
84. Tanaka T, Satoh F, Ujihara M, Midorikawa S, Kaneko T, Takeda T, et al. A multicenter, phase 2 study to evaluate the efficacy and safety of osilodrostat, a new 11β-hydroxylase inhibitor, in Japanese patients with endogenous Cushing's syndrome other than Cushing's disease. Endocrine journal. 2020;67(8):841–52.
85. Bessiène L, Bonnet F, Tenenbaum F, Jozwiak M, Corchia A, Bertherat J, et al. Rapid control of severe ectopic Cushing's syndrome by oral osilodrostat monotherapy. European journal of endocrinology. 2021;184(5):L13.
86. Dormoy A, Haissaguerre M, Vitellius G, Do Cao C, Geslot A, Drui D, et al. Efficacy and Safety of Osilodrostat in Paraneoplastic Cushing Syndrome: A Real-World Multicenter Study in France. J Clin Endocrinol Metab. 2023;108(6):1475–87.
87. Panzuto F, Ramage J, Pritchard DM, van Velthuysen MF, Schrader J, Begum N, et al. European Neuroendocrine Tumor Society (ENETS) 2023 guidance paper for gastroduodenal neuroendocrine tumours (NETs) G1-G3. Journal of neuroendocrinology. 2023;35(8):e13306.
88. Garcia-Carbonero R, Sorbye H, Baudin E, Raymond E, Wiedenmann B, Niederle B, et al. ENETS Consensus Guidelines for High-Grade Gastroenteropancreatic Neuroendocrine Tumors and Neuroendocrine Carcinomas. Neuroendocrinology. 2016;103(2):186–94.
89. Feelders RA, Newell-Price J, Pivonello R, Nieman LK, Hofland LJ, Lacroix A. Advances in the medical treatment of Cushing's syndrome. Lancet Diabetes Endocrinol. 2019;7(4):300–12.
90. Phlipponneau M, Nocaudie M, Epelbaum J, De Keyzer Y, Lalau JD, Marchandise X, et al. Somatostatin analogs for the localization and preoperative treatment of an adrenocorticotropin-secreting bronchial carcinoid tumor. The journal of clinical endocrinology and metabolism. 1994;78(1):20–4.
91. Bertagna X, Favrod-Coune C, Escourolle H, Beuzeboc P, Christoforov B, Girard F, et al. Suppression of ectopic adrenocorticotropin secretion by the long-acting somatostatin analog octreotide. The journal of clinical endocrinology and metabolism. 1989;68(5):988–91.
92. Vignati F, Loli P. Additive effect of ketoconazole and octreotide in the treatment of severe adrenocorticotropin-dependent hypercortisolism. The journal of clinical endocrinology and metabolism. 1996;81(8):2885–90.

93. Grossman A, Johannsson G, Quinkler M, Zelissen P. Therapy of endocrine disease: Perspectives on the management of adrenal insufficiency: clinical insights from across Europe. European journal of endocrinology. 2013;169(6):R165–75.
94. Pavel M, Öberg K, Falconi M, Krenning EP, Sundin A, Perren A, et al. Gastroenteropancreatic neuroendocrine neoplasms: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology. 2020;31(7):844–60.
95. Ruffolo LI, Nessen MF, Probst CP, Jackson KM, Ruan DT, Schoeniger LO, et al. Open adrenalectomy through a makuuchi incision: A single institution's experience. Surgery. 2018;164(6):1372–6.
96. Prager G, Heinz-Peer G, Passler C, Kaczirek K, Schindl M, Scheuba C, et al. Surgical strategy in adrenal masses. European journal of radiology. 2002;41(1):70–7.
97. Gagner M, Lacroix A, Bolté E. Laparoscopic Adrenalectomy in Cushing's Syndrome and Pheochromocytoma. The New England journal of medicine. 1992;327(14):1033–.
98. Guerin C, Taieb D, Treglia G, Brue T, Lacroix A, Sebag F, et al. Bilateral adrenalectomy in the 21st century: When to use it for hypercortisolism? Endocrine-Related Cancer. 2016;23(2):R131–R42.
99. Walz MK, Peitgen K, Krause U, Eigler FW. [Dorsal retroperitoneoscopic adrenalectomy--a new surgical technique]. Zentralblatt fur Chirurgie. 1995;120(1):53–8.
100. Mercan S, Seven R, Ozarmagan S, Tezelman S. Endoscopic retroperitoneal adrenalectomy. Surgery. 1995;118(6):1071–6.
101. Vrielink OM, Engelsman AF, Hemmer PHJ, de Vries J, Vorselaars W, Vriens MR, et al. Multicentre study evaluating the surgical learning curve for posterior retroperitoneoscopic adrenalectomy. The British journal of surgery. 2018;105(5):544–51.
102. Lowery AJ, Seeliger B, Alesina PF, Walz MK. Posterior retroperitoneoscopic adrenal surgery for clinical and subclinical Cushing's syndrome in patients with bilateral adrenal disease. Langenbeck's Archives of Surgery. 2017;402(5):775–85.
103. Lombardi CPMD, Raffaelli MMD, de Crea CMD, Bellantone RMD, Fusco AMD, Bianchi AMD, et al. ACTH-dependent Cushing syndrome: The potential benefits of simultaneous bilateral posterior retroperitoneoscopic adrenalectomy. Surgery: Official Journal of the Society of University Surgeons, Central Surgical Association, and the American Association of Endocrine Surgeons. 2011;149(2):299–300.
104. Raffaelli M, Brunaud L, De Crea C, Hoche G, Oragano L, Bresler L, et al. Synchronous bilateral adrenalectomy for Cushing's syndrome: laparoscopic versus posterior retroperitoneoscopic versus robotic approach. World journal of surgery. 2014;38(3):709–15.
105. De Toma G, Polistena A, Cavallaro G. Open Adrenalectomy. In: Valeri A, Bergamini C, Bellantone R, Lombardi CP, editors. Surgery of the Adrenal Gland. Milano: Springer Milan; 2013. p. 145–60.
106. Fingeret AL, Lee JA. Laparoscopic Adrenalectomy: Transperitoneal Approach. In: Chen H, editor. Illustrative Handbook of General Surgery. Cham: Springer International Publishing; 2016. p. 61–72.
107. Hisano M, Vicentini FC, Srougi M. Retroperitoneoscopic adrenalectomy in pheochromocytoma. Clinics (Sao Paulo, Brazil). 2012;67 Suppl 1(Suppl 1):161–7.

108. Chan C, Roberts JM. Ectopic ACTH syndrome complicated by multiple opportunistic infections treated with percutaneous ablation of the adrenal glands. BMJ case reports. 2017;2017.
109. Zener R, Zaleski A, Van Uum SH, Gray DK, Mujoomdar A. Successful percutaneous CT-guided microwave ablation of adrenal gland for ectopic Cushing syndrome. Clinical Imaging. 2016;42:93–5.
110. Maxwell AWPMD, D'Amico KCMD, Dupuy DEMD. Microwave Ablation as Primary Treatment for a Central Adrenocorticotropic Hormone-Secreting Pulmonary Carcinoid Tumor in a Patient with Severe Cushing Syndrome. Journal of Vascular and Interventional Radiology. 2016;27(11):1763–5.
111. Sarma A, Shyn PB, Vivian MA, Ng J-M, Tuncali K, Lorch JH, et al. Single-Session CT-Guided Percutaneous Microwave Ablation of Bilateral Adrenal Gland Hyperplasia Due to Ectopic ACTH Syndrome. CardioVascular and Interventional Radiology. 2015;38(5):1335–8.
112. Oberndorfer S. Karzinoide tumoren des dunndarms. Frankf Zschr Path. 1907;1:426.
113. Scholte A. Ein fall von angioma teleangiectaticum cutis mit chronischer endocarditis und malignem dünnedarmcarcinoid. Beitrage Zur Pathologischen Anatomie. 1931;86:440–3.
114. Page I. Serotonin (5-hydroxytryptamine). Physiological Reviews. 1954;34:563–88.
115. Thorson A, Biorck G, Bjorkman G, Waldenstrom J. Malignant carcinoid of the small intestine with metastases to the liver, valvular disease of the right side of the heart (pulmonary stenosis and tricuspid regurgitation without septal defects), peripheral vasomotor symptoms, bronchoconstriction, and an unusual type of cyanosis; a clinical and pathologic syndrome. Am Heart J. 1954;47(5):795–817.
116. Hedinger CE. Zur Kenntnis der endokardialen Fibrosen bei den Karzinoiden des Dünndarmes. Schweiz Med Wochenschr.1961;91:1061–1064.
117. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, et al. Hypothalamic Polypeptide That Inhibits the Secretion of Immunoreactive Pituitary Growth Hormone. Science. 1973;179(4068):77–9.
118. Bauer W, Briner U, Doepfner W, Haller R, Huguenin R, Marbach P, et al. SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. Life Sci. 1982;31(11):1133–40.
119. Anthony L, Freda PU. From somatostatin to octreotide LAR: evolution of a somatostatin analogue. Curr Med Res Opin. 2009;25(12):2989–99.
120. de Celis Ferrari ACR, Glasberg J, Riechelmann RP. Carcinoid syndrome: update on the pathophysiology and treatment. Clinics. 2018;73:e490s.
121. Milanetto AC, Fassan M, David A, Pasquali C. Serotonin-Secreting Neuroendocrine Tumours of the Pancreas. Journal of clinical medicine. 2020;9(5).
122. Savu C, Melinte A, Lukadi JL, Mirvald C, Savu C, Belu E, et al. Neuroendocrine syndrome in bronchial carcinoid tumors. Exp Ther Med. 2020;20(6):200.
123. Zandee WT, Kamp K, van Adrichem RC, Feelders RA, de Herder WW. Effect of hormone secretory syndromes on neuroendocrine tumor prognosis. ENDOCRINE-RELATED CANCER. 2017;24(7):R261–R74.
124. Janson ET, Holmberg L, Stridsberg M, Eriksson B, Theodorsson E, Wilander E, et al. Carcinoid tumors: analysis of prognostic factors and survival in 301 patients from a referral center. Annals of oncology : official journal of the European Society for Medical Oncology. 1997;8(7):685–90.
125. Balakrishna P, George S, Hatoum H, Mukherjee S. Serotonin Pathway in Cancer. International journal of molecular sciences. 2021;22(3).

126. Sarrouilhe D, Mesnil M. Serotonin and human cancer: A critical view. Biochimie. 2019;161:46–50.
127. Morse MA, Liu E, Joish VN, Huynh L, Cheng M, Duh MS, et al. Antiproliferative Effects of Telotristat Ethyl in Patients with Neuroendocrine Tumors: The TELEACE Real-World Chart Review Study. Cancer Management and Research. 2020;12(null):6607–14.
128. Hodgetts H, Martins MC, Chen L, Hall AR, Luong TV, Mandair D, et al. Telotristat ethyl affects tumour-fibroblast crosstalk in small intestinal neuroendocrine tumours. Journal of neuroendocrinology. 2025:e70094.
129. Awasthi N, Darman L, Schwarz MA, Schwarz RE. Telotristat ethyl, a tryptophan hydroxylase inhibitor, enhances antitumor efficacy of standard chemotherapy in preclinical cholangiocarcinoma models. J Cell Mol Med. 2024;28(17):e18585.
130. Nozières C, Chardon L, Goichot B, Borson-Chazot F, Hervieu V, Chikh K, et al. Neuroendocrine tumors producing calcitonin: characteristics, prognosis and potential interest of calcitonin monitoring during follow-up. European journal of endocrinology. 2016;174(3):335–41.
131. Hillyard CJ, Coombes RC, Greenberg PB, Galante LS, MacIntyre I. Calcitonin in breast and lung cancer. Clinical endocrinology. 1976;5(1):1–8.
132. Ortiz Hidalgo C. Immunohistochemistry in Historical Perspective: Knowing the Past to Understand the Present. Methods Mol Biol. 2022;2422:17–31.
133. Coons AH, Leduc EH, Kaplan MH, Connolly WtTAoJM. Localization of antigen in tissue cells: VI. The fate of injected foreign proteins in the mouse. The Journal of Experimental Medicine. 1951;93(2):173–88.
134. Coons AH, Kaplan MH. Localization of antigen in tissue cells: II. Improvements in a method for the detection of antigen by means of fluorescent antibody. The Journal of experimental medicine. 1950;91(1):1.
135. Coons AH, Creech HJ, Jones RN, Berliner E. The Demonstration of Pneumococcal Antigen in Tissues by the Use of Fluorescent Antibody1. The Journal of Immunology. 1942;45(3):159–70.
136. Magaki S, Hojat SA, Wei B, So A, Yong WH. An Introduction to the Performance of Immunohistochemistry. Methods Mol Biol. 2019;1897:289–98.
137. Ramos-Vara JA. Technical aspects of immunohistochemistry. Vet Pathol. 2005;42(4):405–26.
138. Lloyd RV, Osamura RY, Klöppel G, Rosai J, Bosman FT, Jaffe ES, et al. WHO classification of tumours of endocrine organs. 4. ed. Lyon: International Agency for Research on Cancer; 2017.
139. Travis WD, Brambilla E, Nicholson AG, Yatabe Y, Austin JHM, Beasley MB, et al. The 2015 World Health Organization Classification of Lung Tumors: Impact of Genetic, Clinical and Radiologic Advances Since the 2004 Classification. Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer. 2015;10(9):1243–60.
140. Dindo D, Demartines N, Clavien PA. Classification of Surgical Complications: A New Proposal With Evaluation in a Cohort of 6336 Patients and Results of a Survey. Annals of Surgery. 2004;240(2):205–13.
141. Shrager JB, Wright CD, Wain JC, Torchiana DF, Grillo HC, Mathisen DJ. Bronchopulmonary carcinoid tumors associated with Cushing's syndrome: a more aggressive variant of typical carcinoid. The Journal of thoracic and cardiovascular surgery. 1997;114(3):367–75.
142. Zandee WT, van Adrichem RC, Kamp K, Feelders RA, van Velthuysen MF, de Herder WW. Incidence and prognostic value of serotonin secretion in pancreatic neuroendocrine tumours. Clinical endocrinology. 2017;87(2):165–70.

143. Davi' MV, Cosaro E, Piacentini S, Reimondo G, Albiger N, Arnaldi G, et al. Prognostic factors in ectopic Cushing's syndrome due to neuroendocrine tumors: a multicenter study. European Journal of Endocrinology. 2017;176(4):453–61.
144. Eriksson B, Arnberg H, Lindgren PG, Lorelius LE, Magnusson A, Lundqvist G, et al. Neuroendocrine pancreatic tumours: clinical presentation, biochemical and histopathological findings in 84 patients. Journal of internal medicine. 1990;228(2):103–13.
145. Wynick D, Williams SJ, Bloom SR. Symptomatic secondary hormone syndromes in patients with established malignant pancreatic endocrine tumors. The New England journal of medicine. 1988;319(10):605–7.
146. Chiang HC, O'Dorisio TM, Huang SC, Maton PN, Gardner JD, Jensen RT. Multiple hormone elevations in Zollinger-Ellison syndrome. Prospective study of clinical significance and of the development of a second symptomatic pancreatic endocrine tumor syndrome. Gastroenterology. 1990;99(6):1565–75.
147. Hellman P, Linder F, Hennings J, Hessman O, Eriksson B, Örlefors H, et al. Bilateral adrenalectomy for ectopic Cushing's syndrome - Discussions on technique and indication. World journal of surgery. 2006;30(5):909–16.
148. Porpiglia F, Terzolo M, Fiori C, Bovio S, Destefanis P, Alì A, et al. Bilateral adrenalectomy for Cushing's syndrome: A comparison between laparoscopy and open surgery. Journal of Endocrinological Investigation. 2004;27(7):654–8.
149. Alberda WJ, van Eijck CHJ, Feelders RA, Kazemier G, de Herder WW, Burger JWA. Endoscopic bilateral adrenalectomy in patients with ectopic Cushing's syndrome. Surgical endoscopy. 2012;26(4):1140–5.
150. Szabo Yamashita T, Sada A, Bancos I, Young WF, Dy BM, Farley DR, et al. Bilateral Adrenalectomy: Differences between Cushing Disease and Ectopic ACTH-Producing Tumors. Annals of surgical oncology. 2020.
151. Reincke M, Ritzel K, Oßwald A, Berr C, Stalla G, Hallfeldt K, et al. A critical reappraisal of bilateral adrenalectomy for ACTH-dependent Cushing's syndrome. European journal of endocrinology. 2015;173(4):M23–M32.
152. Vella A, Thompson GB, Grant CS, Van Heerden JA, Farley DR, Young Jr WF. Laparoscopic adrenalectomy for adrenocorticotropin-dependent Cushing's syndrome. Journal of Clinical Endocrinology and Metabolism. 2001;86(4):1596–9.
153. Morris LF, Harris RS, Milton DR, Waguespack SG, Habra MA, Jimenez C, et al. Impact and Timing of Bilateral Adrenalectomy for Refractory ACTH-Dependent Cushing's Syndrome. Surgery. 2013;154(6):1174–84.
154. Stachowska B, Kuliczkowska-Płaksej J, Kałużny M, Grzegrzółka J, Jończyk M, Bolanowski M. Etiology, baseline clinical profile and comorbidities of patients with Cushing's syndrome at a single endocrinological center. Endocrine. 2020;70(3):616–28.
155. Quinkler M, Stewart PM. Hypertension and the Cortisol-Cortisone Shuttle. The Journal of Clinical Endocrinology & Metabolism. 2003;88(6):2384–92.
156. Chen W, Chen L, Pei L, Zeng R, Wu L, Wang C, et al. Diagnostic Strategy and Prognostic Factors for Ectopic Adrenocorticotropic Hormone Syndrome: A Retrospective Study of 64 Cases. Neuroendocrinology. 2023;113(1):92–102.

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