Annals of Diagnostic Pathology 38 (2019) 71–79

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Original Contributions

Carcinoid tumors of the thymus and Cushing's syndrome: Clinicopathologic features and current best evidence regarding the cell of origin of these unusual neoplasms

Ann E. Walts*, Joseph Frye, David M. Engman, Alberto M. Marchevsky

Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, Los Angeles, CA, United States of America

ARTICLE INFO

Keywords:
Thymus
Neuroendocrine tumor
Corticotropin-releasing hormone (CRH)
Adrenocorticotropin hormone (ACTH)
Cushing's syndrome
Adrenal

ABSTRACT

It is uncertain whether thymic neuroendocrine tumors (NET) associated with Cushing's syndrome (CS) produce corticotropin-releasing hormone (CRH) and adrenocorticotropin hormone (ACTH) and whether the thymus contains ACTH and/or CRH cells that could originate NET.

The clinicopathologic features of 5 typical (TC) and 6 atypical carcinoids (ATC), 10 additional non-neoplastic thymi, 6 adrenal glands with bilateral nodular hyperplasia and 8 adrenal cortical adenomas were reviewed. Representative slides were immunostained for ACTH and CRH. Four (36.4%) of the 11 patients had CS. The incidence of Masaoka stage IV was higher ($p < 0.0001$) in patients with ATC than TC. Only 2 (18.1%) of the 11 patients were alive at follow-up. Ten NET were CRH immunoreactive and 6 were ACTH immunoreactive. Thymic NET with CS exhibited stronger immunoreactivity for ACTH and CRH than those without CS. Non-neoplastic thymi exhibited scattered ACTH and CRH immunoreactive cells. Normal adrenal cortex and glands with bilateral nodular hyperplasia showed diffuse CRH immunoreactivity while adrenal adenomas showed no or only focal CRH immunoreactivity. Literature review showed no association between thymic NET and adrenal adenomas.

The thymus contains CRH and ACTH immunoreactive cells that are probably the origin of thymic NET. Neoplasms associated with CS exhibit strong immunoreactivity for both hormones, suggesting that CRH probably plays a role in the pathogenesis of CS. As adrenals with bilateral nodular hyperplasia exhibit diffuse CRH immunoreactivity and adrenal cortical adenomas either lack this finding or show few immunoreactive cells, this marker may be useful to distinguish these lesions.

1. Introduction

Neuroendocrine tumors (NETs) comprise 2–5% of primary thymic neoplasms and are currently classified by the World Health Organization (WHO) as typical (TC) and atypical (ATC) carcinoids and high-grade neuroendocrine carcinomas (including large cell neuroendocrine carcinomas and small cell carcinomas) using histopathologic criteria (mitoses and necrosis) similar to those applied to pulmonary NETs [1]. The European Neuroendocrine Tumor Society (ENETS) has implemented a three-tiered grading system for all NETs regardless of site of origin that is based on mitotic and Ki67 indices [2,3]. More recently, the International Agency for Research on Cancer (IARC) and the WHO have proposed a common classification for NETs that classifies lung and thymic carcinoids as grade I and II NETs [4]. However, this classification scheme does not fully take into account that NETs arising in different organs present with different clinicopathologic features and often also follow very different clinical courses. For example, most thymic carcinoid tumors manifest more aggressive clinical behavior than G1 and G2 NETs that arise in the lung and gastrointestinal tract while most carcinoids found as incidental tumors in appendectomy specimens are cured by the procedure. Also, 40%–50% of thymic NETs present as ATCs and 20% as TCs, while 70%–90% of pulmonary NETs are TCs [5]. Thymic NETs are also often associated with endocrinopathies such as Cushing's syndrome or the multiple endocrine neoplasms (MEN) type I syndrome, each present in approximately 25% of patients with thymic NETs [5]. In contrast, Cushing's syndrome is present in fewer than 5% of patients with NETs arising at extrathymic sites [6-10]. There is little information in the literature about the pathogenesis of Cushing's syndrome in patients with thymic NETs and no clear explanation as to why this

* Corresponding author at: Department of Pathology & Laboratory Medicine, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048, United States of America.
E-mail address: Ann.Walts@cshs.org (A.E. Walts).

https://doi.org/10.1016/j.anndiagpath.2018.11.006

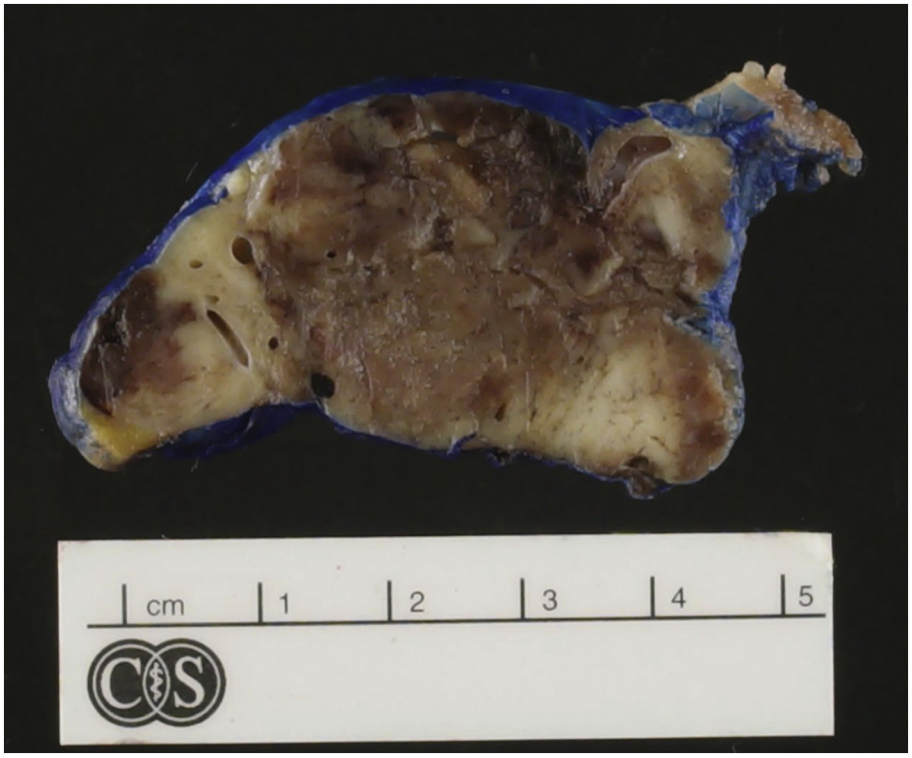

Fig. 1. Gross photo of thymic typical carcinoid in a patient with Cushing's syndrome.

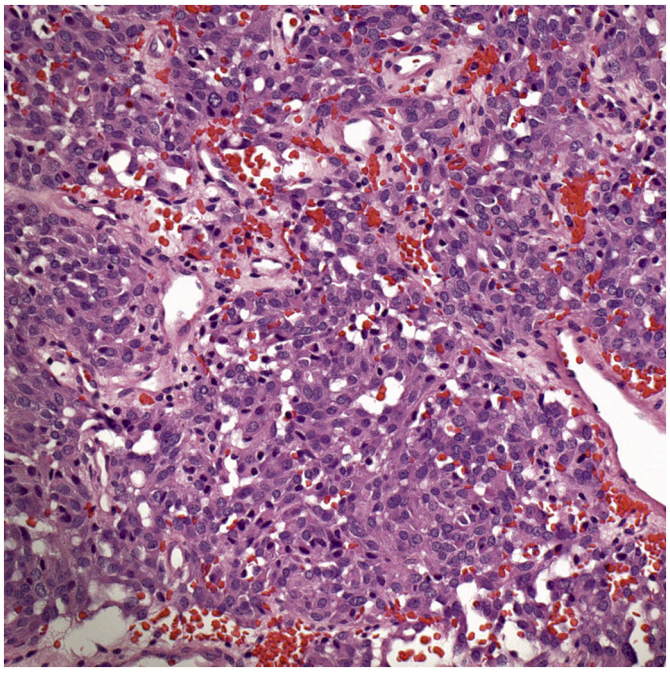

Fig. 2. Thymic typical carcinoid lacks pleomorphism and necrosis and exhibits only rare if any mitoses (same tumor as in Fig. 1); Hematoxylin & eosin x200.

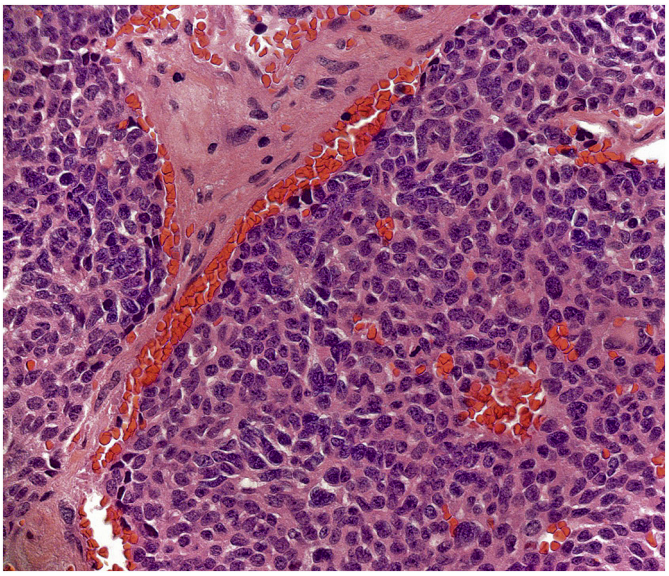

Fig. 3. Thymic atypical carcinoid showing more pleomorphism than seen in Fig. 2 and mitoses; Hematoxylin & eosin ×200.

paraneoplastic syndrome is more frequent in patients with thymic NETs than in patients with NETs arising in other locations. It is also unknown whether the normal human thymus gland contains non-neoplastic neuroendocrine cells that express immunoreactivity for adrenocorticotrophic hormone (ACTH) and/or corticotropin-releasing hormone (CRH) which could then be considered as possible cells of origin for thymic NETs.

A recent case of thymic TC associated with Cushing's syndrome occurring in a 43-year old woman 18 years after resection of an aldosterone-producing adrenal cortical adenoma prompted us to review the literature and our experience with thymic carcinoid tumors, focusing on the clinicopathologic features, prognosis, and immunoexpression of ACTH and CRH in these tumors and in non-neoplastic thymic tissue. Hyperplastic adrenal glands resected from our patients with thymic NETs and associated Cushing's syndrome and a small cohort of adrenal adenomas that served as a comparison group were also reviewed and immunostained for CRH. A systematic literature review was performed to collect best evidence regarding the association between thymic carcinoid tumors and Cushing's syndrome, including information about cell of origin, clinicopathologic features

Table I
Clinico-pathologic features of thymic carcinoid tumors.

Case	Age/gender	TC/ATC	Size (cm)	Masaoka-Koga stage	Cushing's syndrome	Post thymectectomy treatment			Follow-up
						Radiation	Chemo	Somatostatin analogs	
1	43 F	TC	12.5	II a	Yes	Yes	Yes	No	Alive with tumor @ 24 mos.
2	59 F	TC	8	I	Yes	No	No	Yes	Dead with tumor @ 112 mos. bone mets @ 87 mos.
3	22 F	ATC	15.3	IV a	Yes	No	Yes	Yes	Dead with tumor @ 15 mos.
4	48 M	ATC	2.3	IV b	Yes	No	No	No	Dead with tumor @ NA
5	42 M	ATC	15.3	IV b	No	No	Yes	Yes	Dead with tumor @ 21 mos.
6	72 F	TC	5.8	IV b	No	Palliative to bone mets	Yes	No	Alive with tumor @ 103 mos. bone mets @ 97 mos.
7	42 M	ATC	10	IV b	No	Yes	Yes	Yes	Dead with tumor @ 74 mos.
8	55 F	ATC	7.6	IV a	No	No	No	Yes	Dead with no evidence of tumor @ 37 mos.
9	36 F	ATC	NA	IV b	No	Palliative to bone mets	Yes	Yes	Dead with tumor @ 16 mos.
10	63 M	TC	4.2	II	No	No	No	Yes	Dead with no evidence of tumor @ 162 mos.
11	43 M	TC	2	I	No	No	No	No	Dead with no evidence of tumor @ 156 mos.

TC = typical carcinoid; ATC = atypical carcinoid.

and prognosis.

2. Materials and methods

2.1. Patient/case selection

With institutional review board approval that includes a waiver of patient consent, the database of the pathology department at our medical center was searched for: thymic NETs (carcinoids, atypical carcinoids, neuroendocrine carcinomas) between January 1, 1994 and January 1, 2018. Ten randomly selected non-neoplastic thymectomy specimens including 8 normal glands, 1 thymus with simple hyperplasia, and 1 with traumatic hematoma were also retrieved from our files to evaluate whether the non-neoplastic thymus contains neuroendocrine cells that express ACTH and/or CRH immunoreactivity. Clinical information from all cases, including the index case of a 43-year old woman who developed a thymic carcinoid tumor 18 years after resection of an aldosterone-producing adrenal cortical adenoma, was obtained by review of the hospital's electronic medical records. The following data were extracted: age, gender, diagnosis, treatment, and presence of Cushing's syndrome, MEN syndrome and/or other endocrinopathies. Overall survival (OS) data for patients with thymic NETs were obtained from our medical center's tumor registry. Histologic slides and pathology reports from all neoplastic and non-neoplastic cases were retrieved from the surgical pathology files and reviewed by 2 of the authors (AEW and AMM). Thymic NETs were classified according to the 2015 WHO classification and staged according to the Masaoka-Koga staging system [11-13]. Representative formalin fixed, paraffin embedded sections from each tumor and, where present, from adjacent non-neoplastic thymic tissue as well as from all 10 non-neoplastic thymectomy specimens were immunostained for CRH and ACTH.

Three of our patients with thymic NETs and associated Cushing's syndrome had undergone adrenalectomy. Slides from these adrenalectomy specimens, from 8 randomly selected adrenal cortical adenomas (5 aldosterone-producing adenomas from patients with Conn's syndrome and 3 nonfunctioning adenomas) and from 2 normal adrenal glands that had been resected in conjunction with radical nephrectomies were reviewed and a representative formalin fixed paraffin embedded section from each of these specimens was immunostained for CRH.

2.2. Immunohistochemistry

Immunohistochemical detection of CRH and ACTH was performed on 4-μm whole tissue sections using polyclonal CRH antibody (Sigma-Aldrich, St. Louis MO) applied at 1:2500 dilution and prediluted polyclonal ACTH antibody (Cell Marque, Rocklin CA). Staining was performed on the Ventana Discovery Ultra (CRH) or the Ventana Benchmark Ultra (ACTH) automated slide stainer using the on-board retrieval method in citrate pH 6 buffer (CRH) or in high pH buffer (ACTH) (Ventana Medical systems, Tucson AZ). Staining was visualized using the Ventana Chromomap DAB (CRH) or the Ventana ultraview DAB (ACTH) detection system. All slides were counterstained with Mayer's hematoxylin. Placenta and pituitary served as positive controls while placenta and tonsil with omission of primary antibody served as negative controls for CRH and ACTH, respectively.

IHC stained slides were evaluated jointly by AMM and AEW using a double-headed microscope. Diffuse cytoplasmic staining was interpreted as positive for ACTH and CRH; nuclear staining for CRH was interpreted as non-specific. The percentages of tumor cells with positive ACTH or CRH expression were semi-quantitated using the following arbitrary scale: negative; 1%–25% = 1+; 26%–50% = 2+; 51%–75% = 3+; > 75% = 4+. The intensity of staining for each antibody was semi-quantitated using the following arbitrary scale: 0 = absence of staining; 1+ = mild; 2+ = moderate; 3+ = marked.

Table II
Immunoreactivity in thymic carcinoid tumors and adjacent non-neoplastic thymus.

Case	Diagnosis	Cushing's syndrome	Thymic carcinoid tumors				Non-neoplastic thymus			
			CRH		ACTH		CRH		ACTH	
			% of + cells	Intensity	% of + cells	Intensity	% of + cells	Intensity	% of + cells	Intensity
1	TC	Yes	4+	3+	4+	3+	1+[a]	1+[a]	1+[a]	1+[a]
2	TC	Yes	3+	3+	2+	2+	1+[a]	1+[a]	neg	neg
3	ATC	Yes	4+	3+	2+	2+	1+[a]	1+[a]	1+[a]	1+[a]
4	ATC	Yes	4+	4+	4+	3+	NA	NA	NA	NA
5	ATC	No	4+	3+	neg	neg	1+[a]	1+[a]	neg	neg
6	TC	No	neg	neg	2+	2+	neg	neg	1+[a]	1+[a]
7	ATC	No	1+	1+	neg	neg	NA	NA	NA	NA
8	ATC	No	1+	1+	neg	neg	NA	NA	NA	NA
9	ATC	No	2+	2+	neg	neg	NA	NA	NA	NA
10	TC	No	4+	1+	1+	1+	1+[a]	1+[a]	1+[a]	1+[a]
11	TC	No	4+	2+	neg	neg	2+	2+	1+[a]	1+[a]

TC = typical carcinoid; ATC = atypical carcinoid.
% of + cells: 1+ = 1%–25%, 2+ = 26%–50%, 3+ = 51%–75%, 4+ = > 75%.
Intensity = subjective estimate of the intensity of immunoreactivity ranging from 1+ = barely visible to 4+ = strong reactivity.
NA = not available.
[a] Only rare cells were positive.

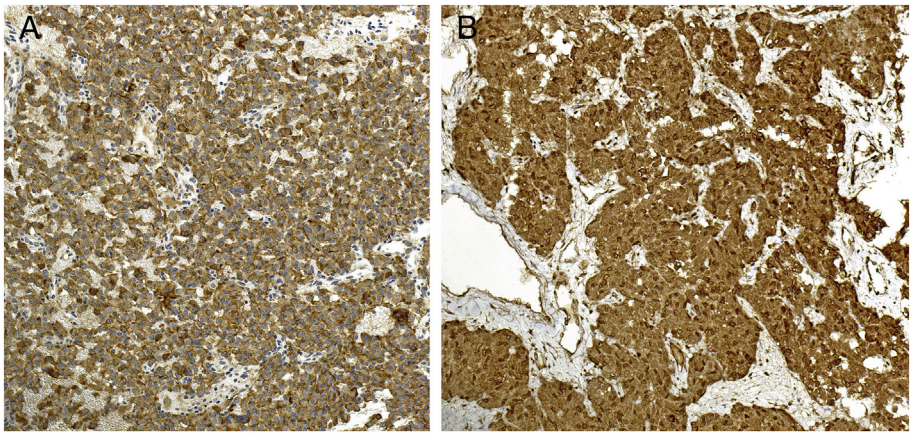

Fig. 4. Thymic typical carcinoid tumor showing CRH (A) and ACTH (B) immunoreactivity in tumor cells; (A, B) ×200.

The same scoring was used to assess immunostaining in non-neoplastic thymic and adrenal tissues.

2.3. Statistical analysis

Results were compared with *t*-test and chi-square tests using Medcalc software (Medcalc, Ostend Belgium).

2.4. Systematic literature review

A systematic review of the English literature between 1988 and 2018 was performed using the following search terms and their combinations: “thymus”, “Cushing's syndrome”, “carcinoid”, “neuroendocrine tumor or neoplasm”, “well differentiated neuroendocrine carcinoma”,“moderately differentiated neuroendocrine carcinoma”, “corticotropin releasing hormone/CRH”, “ACTH”, and “ectopic” using the PubMed database of the National Library of Medicine. The Google search engine was also used to query the Internet for reports of immunohistochemical expression of ACTH and CRH in thymic carcinoids.

3. Results

3.1. Clinicopathologic features of patients with thymic NETs treated at our medical center from 1994 to 2018

A total of 13 patients with NETs who underwent radical thymectomy for the treatment of primary thymic NETs were found in our surgical pathology database. They included 11 carcinoid tumors (Fig. 1) and 2 high-grade neuroendocrine carcinomas (1 large cell neuroendocrine carcinoma and 1 small cell carcinoma). As none of the 2 patients with high-grade neuroendocrine carcinoma presented with Cushing's syndrome, these cases were excluded from the review. The 6 female and 5 male patients with thymic carcinoid tumors ranged in age from 22 to 72 years at diagnosis (median 43 yrs). Their NETs included 5 TCs (Fig. 2) and 6 ATCs (Fig. 3) ranged in size from 2.0 to 15.3 cm (Table I). Thymic TCs appeared to be smaller (6.5 ± 4.0 cm) than ATCs (10.1 ± 5.5 cm) but the difference was not significant. When patients with TC and ATC were compared, there was no significant difference in gender or median age at diagnosis. Seven (63.6%) of the 11 tumors, including all 6 ATC presented as Masaoka-Koga stage IV disease. The incidence of Masaoka-Koga stage IV was significantly higher in the ATCs than in the TCs ($p < 0.001$) at diagnosis. Four (36.4%) of the 11 patients with thymic carcinoid tumors had Cushing's syndrome and none of them had evidence for personal or family history of MEN syndrome in the clinical records. All patients underwent thymectomy;

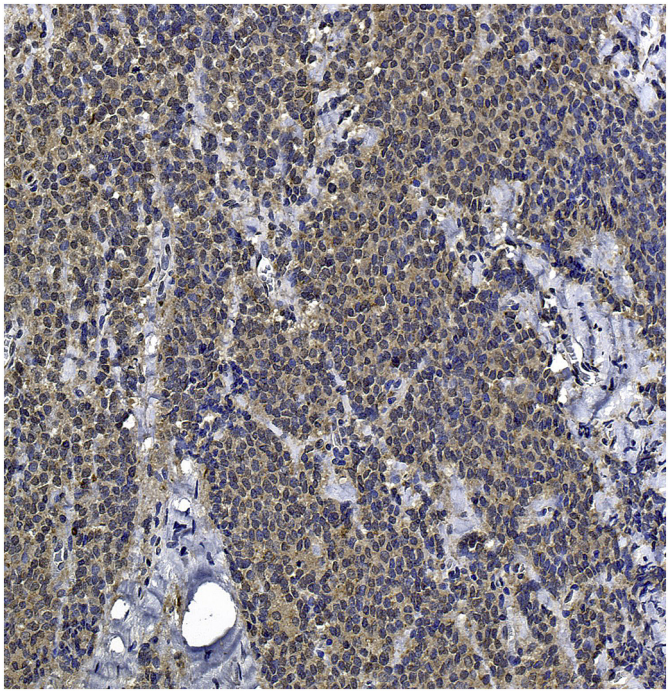

Fig. 5. Thymic typical carcinoid showing cytoplasmic immunoreactivity for CRH in tumor cells; ×200. Immunostain for ACTH was negative in tumor cells.

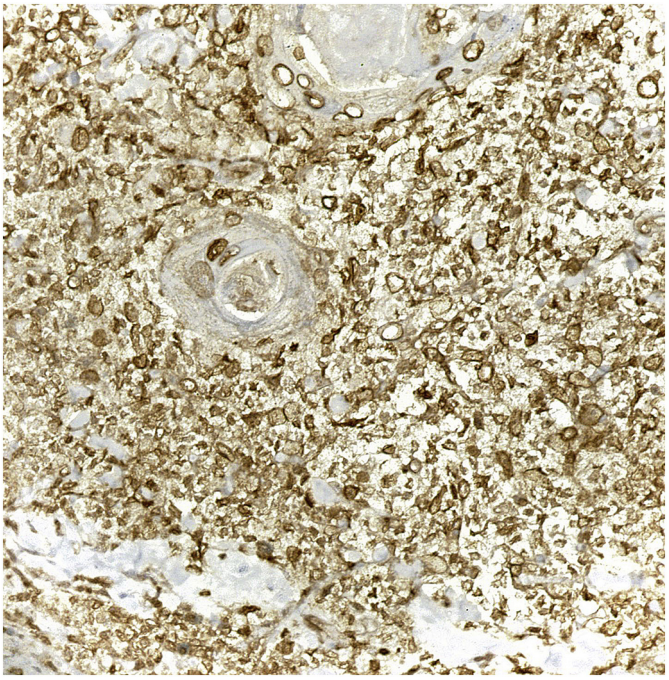

Fig. 6. Neuroendocrine cells in non-neoplastic thymus showing cytoplasmic CRH immunoreactivity; ×400. Nuclear staining was interpreted as nonspecific.

in addition, 7 were treated with somatostatin analogs, 6 were treated with chemotherapy, and 2 received post-operative radiation therapy to the chest (Table I). Three of the 4 patients with thymic NET and Cushing's syndrome had undergone unilateral or bilateral adrenalectomy. They included the index patient who had undergone right adrenalectomy for a 2 cm aldosterone-producing cortical adenoma 18 years prior to the acute onset of Cushing's syndrome and thymectomy and 2 patients who had undergone bilateral adrenalectomy concurrent with their thymic NET/Cushing's syndrome diagnosis.

Only two (18.1%) of the 11 patients with thymic carcinoid were alive at last follow-up, 24 and 103 months post thymectomy (Table I). Both are female with TCs. One of these patients, the index patient, had Cushing's syndrome while the other did not. Both received chemotherapy and radiation. The index patient received adjuvant radiation to the chest for the tumor which was described as inseparable from the left heart border and pericardium. The other patient received radiation to bone metastases. Among the patients who died, the average OS was significantly shorter in those with ATC than in individuals with TC (32.6 ± 21 months vs. 143.33 ± 22.3 mos.) ($p < 0.001$). Only 1 of the 4 patients with Cushing's syndrome was alive at 24 months; the average OS among the others was 32.6 ± 24.76 months. There was no significant difference in average OS between thymic carcinoid patients with and without Cushing's syndrome.

3.2. CRH and ACTH immunostaining in thymic carcinoid tumors at our medical center

Table II shows the results of immunostaining for CRH and ACTH in the thymic carcinoid tumors. Of the 11 thymic carcinoids, 5 were CRH +/ACTH+ (Fig. 4), 5 were CRH+/ACTH-(Fig. 5), and 1 was CRH-/ACTH+. All four (100%) thymic carcinoids in patients with associated Cushing's syndrome stained diffusely and intensely positive for both CRH and ACTH (Fig. 4). Although some staining for CRH was also observed in 6 (66.7%) of the thymic carcinoids without Cushing's, the staining was seen in a lower percentage of the tumor cells and/or was less intense in 5 of these 6 cases. The remaining case was negative for CRH. A similar trend was observed for ACTH. Among patients without Cushing's syndrome, one of the 7 thymic carcinoids was negative for CRH and 5 of the 7 cases were negative for ACTH. Thus, whereas staining for CRH and ACTH was observed in all 4 (100%) of the thymic carcinoids in patients with Cushing's, tumors in patients without the

Table III
Immunoreactivity in non-neoplastic thymus from patients without Cushing's syndrome.

Case	Age/gender	Indication for surgery	Thymus Dx	CRH	ACTH
1	30 M	Papillary thyroid CA	NPD	neg	1+[a]
2	35 F	Papillary thyroid CA	NPD	1+[a]	1+[a]
3	32 F	Completion thyroidect-omy for paillary thyroid CA	NPD	1+[a]	1+[a]
4	49 F	Papillary thyroid CA & parathyroid hyperplasia	NPD	1+[a]	1+[a]
5	24 F	Hyperparathyroidism	NPD	neg	1+[a]
6	57 F	Hyperparathyroidism	NPD, involuted	neg	1+[a]
7	59 F	Hyperparathyroidism	NPD	neg	1+[a]
8	60 M	Cardiomyopathy	NPD, involuted	neg	neg
9	2 M	Trauma	Hematoma	1+[a]	1+[a]
10	44 F	Mediastinal mass	Simple hyperplasia	neg	1+[a]

% of + cells: 1+ = 1%–25%, 2+ = 26%–50%, 3+ = 51%–75%, 4+ = > 75%.
NPD = no pathologic diagnosis.
[a] Only rare cells were positive.

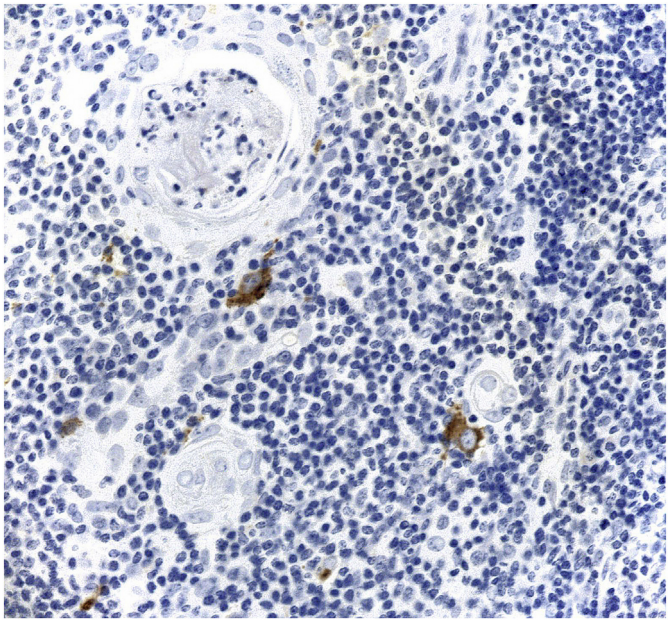

Fig. 7. Neuroendocrine cells in non-neoplastic thymus showing ACTH immunoreactivity; ×400.

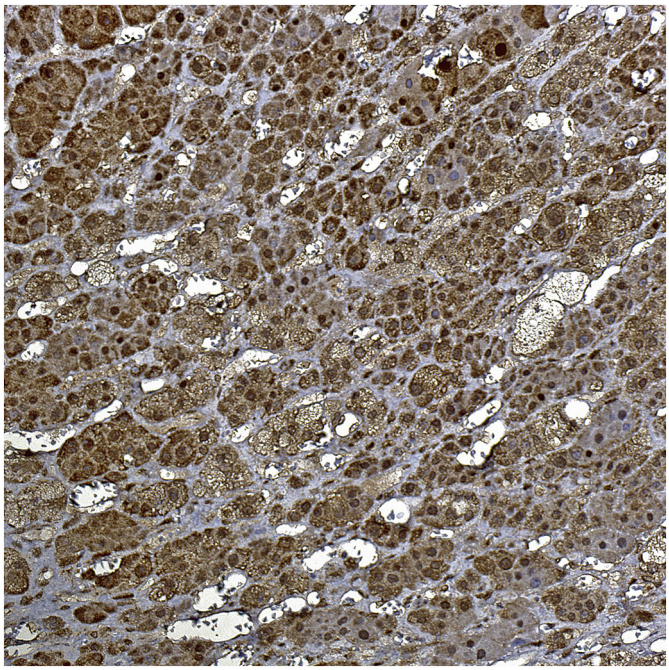

Fig. 8. Adrenal hyperplasia showing diffuse cytoplasmic CRH immunoreactivity; ×200. Nuclear staining was interpreted as nonspecific.

syndrome showed considerably less immunoreactivity for the two epitopes. Immunoreactivity for CRH and ACTH did not appear to correlate with tumor size or histology (TC vs. ATC).

3.3. CRH/ACTH immunostaining in non-neoplastic thymus

Table II also shows the results of immunostaining for CRH and ACTH in the non-neoplastic thymic tissue available in 7 (63.6%) of the 11 thymic carcinoid resection specimens. Three of these patients had associated Cushing's syndrome and the other 4 did not. Table III shows the results of immunostaining for CRH (Fig. 6) and ACTH (Fig. 7) in 10 non-neoplastic thymic tissues without Cushing's syndrome. Tables II and III show that the non-neoplastic thymic tissue adjacent to thymic carcinoid tumors, in simple thymic hyperplasia, and in thymic tissue resected incidentally contain a small number of neuroendocrine cells that exhibited cytoplasmic immunoreactivity for CRH and/or ACTH. In specimens with preserved thymic architecture and absence of involutional changes, these neuroendocrine cells were observed in the thymic medulla. The non-neoplastic thymic tissue in specimens with carcinoid tumors from patients with Cushing's syndrome did not show a significantly greater number of immunoreactive cells than the other specimens.

3.4. CRH immunostaining in adrenal specimens

CRH was expressed in the non-lesional cortex of all adrenal glands in our study. The adrenals from two patients with bilateral adrenal hyperplasia associated with thymic ATC and Cushing's syndrome exhibited strong CRH immunoreactivity (Fig. 8). The adrenal adenoma from the index patient exhibited weak and focal CRH immunoreactivity. This adenoma had been resected 18 years prior to the development of thymic TC and Cushing's syndrome. The patient had no evidence of Cushing's syndrome at the time of adrenalectomy (Table IV). In contrast, CRH was expressed in lesional adrenal tissue in only 2 (25%) of the 8 cortical adenomas from patients without Cushing's syndrome who underwent adrenalectomy for symptoms associated with Conn's syndrome or for diagnosis of an incidental mass seen on imaging and subsequently diagnosed as a nonfunctional adenoma (Fig. 9).

3.5. Literature review of CRH expression and CRH/ACTH co-expression in thymic NETs associated with Cushing's syndrome

Primary NETs of the thymus were first recognized by Rosai and Higa in 1972 [14]. The largest reported series of thymic NETs (160 patients reported to the Surveillance, Epidemiology and End Results (SEER) database over a 33 year period) does not include CRH/ACTH expression nor specify the incidence of Cushing's syndrome among the tumors [15]. The 80 cases reported by Moran & Suster include 5 cases with Cushing's syndrome but no information on CRH or ACTH immunoreactivity is provided [16]. In a more recent report on 12 patients with Cushing's syndrome and thymic NETs, Neary et al. reported immunoreactivity for ACTH in 9 tumors but do not mention CRH [17].

Our literature search revealed only a few reports that include CRH expression or CRH/ACTH co-expression in thymic carcinoid tumors (Table V) [18-23]. The 7 patients (6 males and 1 female) ranged from 13.6 to 63 years of age (median 25 yrs.) at diagnosis. While the information provided is limited, the thymic carcinoid tumors ranged from 6 mm to 13 cm, and at least 4 (50%) were ATCs. Co-expression of CRH and ACTH is reported in 6 (75%) of the tumors although the antibodies and/or the staining protocols utilized differ across the reports and/or are not detailed. In two of the studies, the authors used alternate methodologies (tissue extraction, radioimmunoassay) to document CRH/ACTH production by the tumors [18,19]. Interestingly, 3 reports describe a shift in immunoreactivity for CRH and ACTH when primary and recurrence were compared [18,20,21]. There is no mention of CRH or ACTH expression having been studied in the non-neoplastic thymic tissue in any of these cases, and although at least 3 of these patients underwent bilateral adrenalectomy for persistent Cushing's syndrome, no information regarding CRH or ACTH immunoreactivity in the adrenalectomy specimens is provided [20,22,23].

4. Discussion

Cushing's syndrome results from the presence of excess plasma levels of glucocorticoids, irrespective of the cause, and is distinguished from Cushing's disease in which a pituitary tumor produces excess ACTH that stimulates the adrenal cortex (zona fasciculata) to produce high levels of cortisol. Exogenous administration of glucocorticoids is

Table IV
CRH immunoreactivity in adrenal adenomas, adrenal cortical hyperplasia, and normal adrenal glands.

Case	Age/gender	Indication for adrenaleectomy	Adrenal Dx	Cushing's syndrome	CRH in adrenal lesion % of + cells (intensity)	CRH in normal adrenal cortex % of + cells (intensity)
Thymic carcinoid cases						
1	43 F	Adrenal mass	Cortical adenoma	No in 1998 Yes in 2016	1+ (1+)	
3	22 F	ATC	Bilat hyperplasia	Yes	4+ (3+)	NA
4	48 M	ATC	Bilat hyperplasia	Yes	3+ (3+)	NA
Control adrenal glands						
1	55 M	Renal CA	NPD (incidental adrenalectomy)	No	No lesion	4+ (1+)
2	77 M	Renal CA	NPD (incidental adrenalectomy)	No	No lesion	2+ (2+)
3	66 F	Adrenal mass	Cortical adenoma Nonfunctional	No	neg	2+ (2+)
4	57 M	Adrenal mass	Cortical adenoma Conn's	No	neg	2+ (2+)
5	40 F	Adrenal mass	Cortical adenoma Conn's	No	2+ (2+)	2+ (2+)
6	36 M	Adrenal mass	Cortical adenoma Conn's	No	neg	2+ (1+)
7	40 M	Adrenal mass	Cortical adenoma Conn's	No	neg	3+ (2+)
8	28 M	Adrenal mass	Cortical adenoma Conn's	No	neg	3+ (2+)
9	59 F	Adrenal mass	Cortical adenoma Nonfunctional	Yes	1+ (1+)	2+ (1+)
10	46 M	Adrenal mass	Cortical adenoma Nonfunctional	No	neg	3+ (2+)

ATC = atypical carcinoid.
% of + cells: 1+ = 0–25%, 2+ =25–50%, 3+ = 50–75%, 4+ = > 75%.
Intensity = subjective estimate of the intensity of immunoreactivity ranging from 1+ = barely visible to 4+ = strong reactivity.
NA = not applicable; NPD = no pathologic diagnosis.

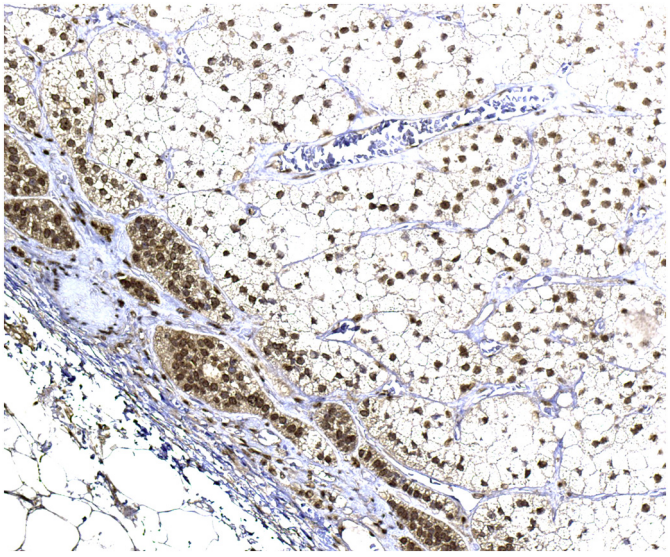

Fig. 9. Adrenal adenoma showing negative immunoreactivity for CRH (upper right) and adjacent compressed rim of normal adrenal showing cytoplasmic immunoreactivity for CRH (lower left); ×200. Nuclear staining was interpreted as nonspecific.

currently the most common cause of Cushing's syndrome. Among the endogenous causes of Cushing's syndrome, 70% of cases are attributable to pituitary adenomas that cause excess corticotropin production (Cushing's disease), about 20% are attributable to an adrenal disorder such as cortical adenoma or nodular/diffuse cortical hyperplasia or adrenocortical carcinoma leading to excess cortisol production and suppression of corticotropin in the pituitary, and about 10% are caused by ectopic secretion of corticotropin by a non-pituitary tumor [24]. Ectopic secretion of corticotropin has been reported in up to 50% of small cell carcinomas of the lung, 15% of thymic carcinoid tumors, 10% of pulmonary carcinoids and 10% of islet cell tumors [25,26]. Smaller percentages of medullary thyroid carcinomas, other carcinoid tumors, pheochromocytomas, and a variety of other tumors have appeared in case reports as sources of ectopic corticotropin and rare cases of Cushing's syndrome secondary to CRH produced by non-pituitary tumors have been described [25,26]. Ectopic CRH production accounts for < 1% of all cases of Cushing's syndrome.

Our findings confirm that thymic NETs are malignant neoplasms that are often large at diagnosis, present at high Masaoka stage and are associated with poor prognosis despite aggressive therapy. It remains controversial whether patients with thymic ATCs have a significantly worse prognosis than those with thymic TCs. Moran and Suster reported that thymic ATC patients have a worse prognosis than those with TC, but other studies and our data showed no significant prognostic differences between the 2 groups [16,27]. The 36.4% incidence of Cushing's syndrome in our patients is higher than the 25% generally quoted in the literature and is considerably higher than in patients with NETs originating at other sites. For example, a recent review by Pedicelli et al. described a 1%–5% incidence of the syndrome in patients with bronchial carcinoids [6]. Bohnnenger et al. [5] noted a tendency for thymic TCs associated with Cushing's syndrome to be smaller than those without Cushing's, but in our small case cohort, there was no such difference.

CRH is the product of a single gene located on the long arm of chromosome 8 and is expressed in a wide variety of tissues including normal hypothalamus, adrenal cortex, heart (myocytes), liver (hepatocytes), lung (bronchial epithelium, alveolar macrophages), pancreas (acini), ovary (stroma, oocyte), immune cells, endothelium, and placenta (synctiotrophoblasts) [28,29]. There is very limited information in the literature regarding the immunohistochemical subcellular localization of CRH. We based our interpretation that nuclear staining for CRH is a nonspecific finding on the studies by Miceli et al. and Rosen et al., although we recognize that this is not optimal as the investigators used different antibodies and staining protocols from each other and from those used in our study [30,31]. Miceli et al. reported diffuse

Table V
Literature review of CRH and ACTH immunoreactivity in thymic carcinoids with Cushing's syndrome.

Reference	Age/gender	Thymus Dx	Size	Masaoka	ACTH	CRH
Ozawa [18]	25 M	Carcinoid, NOS	13 cm	IV	IHC pos	IHC pos Primary: CRH > ACTH Recurrence: ACTH > CRH
Ishikawa [19]	63 M	ATC	5 cm	IV	IHC pos	IHC pos
Markou [20]	25 F	Primary: TC Recurrence: ATC	2 cm	NA	Primary: IHC pos Recurrence: IHC pos	Primary: IHC pos Recurence: IHC neg
Schalin-Jäntti [21]	41 M	Carcinoid, NOS	3.6 cm	NA	Primary: IHC pos Recurrence: IHC pos	Primary: IHC pos focal Recurrence: IHC pos
Karageorgiadis [22]	13.6 M	ATC	NA	NA	IHC pos	IHC pos
	21.3 M	ATC	6 mm	NA	IHC pos	IHC pos
Papadakis [23]	21 M	ATC	NA	NA	IHC pos	IHC pos focal

TC = typical carcinoid; ATC = atypical carcinoid.
NA = not provided.
IHC = immunoreactivity.

cytoplasmic staining for CRH and its two receptor isoforms (CRH-R1 and CRH-R2) using a polyclonal goat anti-CRH antibody from Santa Cruz (C-20; sc-1759) in 100%, 92%, and 60.7% of 51 endometrial cancers, respectively; immunostaining was confined to the para-nuclear/apical regions and nuclei (CRH-R2) in adjacent benign endometrial glands [30]. Rosen et al. reported cytoplasmic staining for CRH and nuclear staining for Re1B-$NF_\kappa B_2$ (a transcription factor) in the placenta of humans and other primates using antibodies from Abnova and Santa Cruz-Cell Signaling Technology, respectively [31]. Their findings raise the possibility that the Sigma CRH antibody used in our study might cross-react with Re1B-$NF_\kappa B_2$ or another transcription factor. The Sigma antibody is not 100% specific and the datasheet for their CRH antibody indicates that there is < 0.01% cross-reactivity of the CRH antibody with human ACTH at 50% binding. More systematic studies of protein and/or mRNA levels employing proteomics or RNA sequencing will be important in elucidating molecular mechanisms of pathogenesis of these tumors.

To our knowledge, the presence of neuroendocrine cells that are immunoreactive for CRH and ACTH in non-neoplastic thymic tissue has not been previously described in human thymus and suggests that these cells could be the cells of origin of thymic NETs. The results of CRH and ACTH immunostains support the suggestion that the association between an adrenal cortical adenoma being followed by the development of a thymic carcinoid tumor and acute onset Cushing's syndrome 18 years later, as observed in our index case, is most likely coincidental. The adenoma exhibited only focal and weak immunoreactivity for CRH whereas the hyperplastic adrenal glands resected from the 2 other patients with thymic carcinoid tumors and Cushing's syndrome showed diffuse strong immunoreactivity for CRH. In addition, out literature review found no previous report of metachronous adrenal adenoma and thymic NET associated with Cushing's syndrome.

An unexpected and somewhat surprising finding in our study was the observation that the cells of adrenal cortical hyperplasia exhibit cytoplasmic CRH immunoreactivity while most adrenal adenomas are negative for this marker. As the histologic distinction between adrenal cortical adenoma and nodular cortical hyperplasia can be difficult in selected cases, this preliminary observation warrants further study. If this difference in CRH immunoreactivity is confirmed with a larger cohort of adrenal lesions, this marker may be helpful in this differential diagnosis.

Declarations of interest

None.

Acknowledgements

Cedars-Sinai Pathology Department Award for Basic, Translational and Clinical Research.

References

[1] Marx A, Chan JKC, Coindre JM, Detterbeck F, Girard N, Harris NL, et al. The 2015 World Health Organization classification of tumors of the thymus: continuity and changes. J Thorac Oncol 2015;10(10):1383–95.
[2] Rindi G, Klöppel G, Couvelard A, Komminoth P, Körner M, Lopes JM, et al. TNM staging of mid gut and hindgut (neuro) endocrine tumors: a consensus proposal including a grading system. Virchows Arch 2007;451(4):757–62.
[3] 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. Ann Oncol 2015;26(8):1604–20.
[4] Rindi G, Klimstra DS, Abedi-Ardekani B, Asa SL, Bosman FT, Brambilla E, et al. A common classification framework for neuroendocrine neoplasms: an International Agency for Research on Cancer (IARC) and World Health Organization (WHO) expert consensus proposal. Mod Pathol 2018; Aug 23. https://doi.org/10.1038/s41379-018-0110-y. [Epub ahead of print].
[5] Bohnenberger H, Dinter H, König A, Ströbel P. Neuroendocrine tumors of the thymus and mediastinum. J Thorac Dis 2017;9(Suppl. 15):S1448–57.
[6] Pedicelli I, Patriciello G, Scala G, Sorrentino A, Gravino G, Patriciello P, et al. Cushing's like syndrome in typical bronchial carcinoid a case report and review of the literature. Int J Surg Case Rep 2016;20S:1–4.
[7] Boddaert G, Grand B, Le Pimpec-Barthes F, Cazes A, Bertagna X, Riquet M. Bronchial carcinoid tumors causing Cushing's syndrome: more aggressive behavior and the need for early diagnosis. Ann Thorac Surg 2012;94(6):1823–9.
[8] Li W-Y, Liu X-D, Li W-N, Dong S-Y, Qu X-H, Gong S-L, et al. Paraneoplastic Cushing's syndrome associated with bronchopulmonary carcinoid tumor in youth: a case report and review of the literature. Oncol Lett 2016;12(1):69–72.
[9] Maragliano R, Vanoli A, Albarello L, Milione M, Basturk O, Klimstra DS, et al. ACTH-secreting pancreatic neoplasms associated with Cushing syndrome. Am J Surg Pathol 2015;39(3):374–82.
[10] Jia R, Sulentic P, Xu JM, Grossman AB. Thymic neuroendocrine neoplasms: biological behaviour and therapy. Neuroendocrinology 2017;105(2):105–14.
[11] Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG. WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart. Lyon: International Agency for Research on Cancer; 2015.
[12] Masaoka A, Monden Y, Nakahara K, Tanioka T. Follow-up study of thymomas with special reference to their clinical stages. Cancer 1981;48(11):2485–92.
[13] Koga K, Matsuno Y, Noguchi M, Mukai K, Asamura H, Goya T, et al. A review of 79 thymomas: modification of staging system and reappraisal of conventional division into invasive and non-invasive thymoma. Pathol Int 1994;44(5):359–67.
[14] Rosai J, Higa E. Mediastinal endocrine neoplasm, of probable thymic origin, related to carcinoid tumor. Clinicopathologic study of 8 cases. Cancer 1972;29(4):1061–74.
[15] Gaur P, Leary C, Yao JC. Thymic neuroendocrine tumors - a SEER database analysis of 160 patients. Ann Surg 2010;251(6):1117–21.
[16] Moran CA, Suster S. Neuroendocrine carcinomas (carcinoid tumor) of the thymus: a clinicopathologic analysis of 80 cases. Am J Clin Pathol 2000;114(1):100–10.
[17] Neary NM, Lopez-Chavez A, Abel BS, Boyce AM, Schaub N, Kwong K, et al. Neuroendocrine ACTH-producing tumor of the thymus - experience with 12 patients over 25 years. J Clin Endocrinol Metab 2012;97(7):2223–30.
[18] Ozawa Y, Tomoyasu H, Takeshita A, Shishiba Y, Yamada S, Kovacs K, et al. Shift from CRH to ACTH production in a thymic carcinoid with Cushing's syndrome. Horm Res 1996;45(6):264–8.
[19] Ishikawa T, Inoue C, Sasaki H, Satou K, Kimura N. Thymic carcinoid associated with ectopic ACTH syndrome. Nihon Kyobu Shikkan Gakkai Zasshi 1996;34(4):471–6.
[20] Markou A, Manning P, Kaya B, Datta SN, Bomanji JB, Conway GS. [^{18}F] fluoro-2-deoxy-D-glucose ([^{18}F]FDG) positron emission tomography imaging of thymic carcinoid tumor presenting with recurrent Cushing's syndrome. Eur J Endocrinol 2005;252:521–5.

[21] Schalin-Jäntti C, Asa SL, Arola J, Sane T. Recurrent acute-onset Cushing's syndrome 6 years after removal of a thymic neuroendocrine carcinoma: from ectopic ACTH to CRH. Endocr Pathol 2013;24:25–9.
[22] Karageorgiadis AS, Papadakis GZ, Biro J, Keil MF, Lyssikatos C, Quezado MM, et al. Ectopic adrenocorticotropic hormone and corticotropin-releasing hormone co-secreting tumors in children and adolescents causing Cushing syndrome: a diagnostic dilemma and how to solve it. J Clin Endocrinol Metab 2015;100(1):141–8.
[23] Papadakis GZ, Bagci U, Sadowski SM, Patronas NJ, Stratakis CA. Ectopic ACTH and CRH co-secreting tumor localized by 68Ga-DOTA-TATE PET/CT. Clin Nucl Med 2015;40(7):576–8.
[24] Pallais JC, Fenves AZ, Lu MT, Glomski K. Case records of the Massachusetts General Hospital: case 18-2018. NEJM 2018;378(24):2322–33.
[25] Wang B-P, Yang L-L, Wang H, He Q, Ma Z-S, Lin Y, et al. An unusual case of ectopic corticotrophin-releasing hormone syndrome caused by an adrenal non-catecholamine secreting pheochromocytoma: a case report. BMC Endocr Disord 2018;18(1):41.
[26] Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. Lancet 2015;386(9996):913–27.
[27] Ströbel P, Zettl A, Shilo K, Chuang W-Y, Nicholson AG, Matsuno Y, et al. Tumor genetics and survival of thymic neuroendocrine neoplasms: a multi-institutional clinicopathologic study. Genes Chromosomes Cancer 2014;53(9):738–49.
[28] Arbiser JL, Morton CC, Bruns GA, Majzoub JA. Human corticotropin releasing hormone gene is located on the long arm of chromosome 8. Cytogenet Cell Genet 1988;47(3):113–6.
[29] Nezi M, Mastorakos G, Mouslech Z. Corticotropin releasing hormone and the immune/inflammatory response. [Updated 2015 Jul 30]. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000.
[30] Miceli F, Ranelletti FO, Martinelli E, Petrillo M, Scambia G, Navarra P, et al. Expression and subcellular localization of CRH and its receptors in human endometrial cancer. Mol Cell Endocrinol 2009;305(1–2):6–11.
[31] Rosen T, Schulkin J, Power M, Tadesse S, Norwitz ER, Wen Z, et al. Comparative immunohistochemistry of placental corticotropin-releasing hormone and the transcription factor Re1B-NF_kB_2 between human and nonhuman primates. Comp Med 2015;65(2):140–3.