Cushing Syndrome in a child due to Pro-opiomelanocortin (POMC) secretion from a yolk sac tumour

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Abbreviations:

POMC - Pro-opiomelanocortin
ACTH – Adrenocorticotropic hormone
CS – Cushing syndrome
EAS - Ectopic ACTH Syndrome

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Abstract

Context: Pituitary microadenomas and adrenal tumors are most common causes for endogenous Cushing syndrome (CS) in children. Case description: We describe a two-year old girl with Cushing syndrome due to ectopic pro-opiomelanocortin (POMC) production from an abdominal yolk sac tumor. Cortisol concentrations were elevated but adrenocorticotropic hormone (ACTH) concentrations were equivocal. The use of antibodies specifically detecting ACTH precursors revealed that plasma ACTH precursors were elevated. Additionally, an ACTH assay with a low cross-reactivity for precursors showed low concentrations of ACTH. Immunohistochemistry suggested POMC but not ACTH production by the tumor. Conclusion: We describe a yolk sac tumor as a novel source of ectopic POMC production leading to CS in a young girl.

What’s known on this subject:

In adults, Ectopic ACTH Syndrome is most often due to intrathoracic tumors, but cases of carcinoid tumours, neuroblastoma, pheochromocytoma and carcinoma of the pancreas, thymus, thyroid and ovaries have been described.

What this study adds:

To our knowledge, this is the first report of Cushing syndrome in a child due to POMC secretion from a yolk sac tumor.

Introduction
Cushing Syndrome (CS) is due to exposure to excess glucocorticoids. Clinical features include obesity, impaired growth, behavioural changes, facial plethora, hirsutism, muscle weakness and hypertension. Non-iatrogenic CS is rare (two to five per 1,000,000 per year) \(^1\), and paediatric CS is even less common. Pituitary microadenomas producing adrenocorticotropic hormone (ACTH) and adrenal tumors are the most common cause of endogenous paediatric CS. Ectopic ACTH Syndrome (EAS) is extremely rare and accounts for less than one percent of the cases \(^2,3\). In adults, EAS is most often due to intrathoracic tumors, but cases of carcinoid tumours, neuroblastoma, pheochromocytoma and carcinoma of the pancreas, thymus, thyroid and ovaries have been described \(^4\). We describe here, for the first time to our knowledge, a child with CS due to ectopic ACTH precursors from an abdominal yolk sac tumor.

In the human pituitary, POMC undergoes post-translational processing, resulting in the production of pro-ACTH (further cleaved to ACTH, the N-terminal POMC fragment (N-POC) and a small joining peptide) and B-lipotrophin (B-LPH) (which is cleaved to produce G-lipotrophin (G-LPH) and B-endorphin (B-EP)) (Figure 1A). All peptides including POMC are released into the circulation \(^5\). Historically, ACTH precursors were identified as high molecular weight forms of ACTH in EAS tumors \(^6\). More recently, the use of a specific two-site enzyme-linked immunosorbent assay (ELISA) using a pair of monoclonal antibodies, each recognizing a specific epitope in POMC, has enabled the measurement and identification of POMC \(^5,7\). We used this assay to gain insight into the etiology of CS in this patient.

\(\text{Consent}\)
Informed and written consent were obtained from parents.

**Case presentation**

A 2.75 year old girl presented with weight gain (five kilograms in 12 months), increased appetite, body odour, facial acne, pubic hair, lethargy and moodiness. She was the first child of Bulgarian and Maltese parents. There was no relevant past medical history or family history.

Her weight and height were 18.75 kg (+2.69 SDS) and 87.3 cm (-1.38 SDS) (BMI 25 kg/m²). Weight gain in the last 2 months was 2.0 kg whilst height velocity was 0.7 cm/year. Blood pressure was 176/131 mm Hg (>> +2SD). She had a Cushingoid appearance with round facies, facial acne, truncal obesity, mid-scapula fat pad and hypertrichosis. The abdomen was distended without hepatosplenomegaly or palpable masses. She also demonstrated proximal muscle weakness. Tanner stage was B1 P2 A1 M0 (Figure 2).

Initial investigations showed an elevated midnight serum cortisol concentration (1258nmol/L) with loss of circadian variation, increased cortisol excretion in four separate 24-hour urinary samples (>1380nmol/24 hours), incomplete suppression (22%) of cortisol production on a low-dose dexamethasone suppression test (cortisol 1363 nmol/L at 0 min and 1468 and 1054 nmol/l at 24 and 48 hrs [normal < 1.8 µg/dL, <50 nmol/L at 48 hrs]), and 43% suppression on a high dose dexamethasone suppression test (Table 1). An ultrasound of the abdomen was normal. A brain MRI showed a possible microadenoma in the left side of the pituitary.

The child was referred to Great Ormond Street Hospital for Children in London for further investigations. A 24-hour serum cortisol profile showed no circadian rhythm with increased cortisol (35.5µg/dL, 985nmol/L) and ACTH concentration (51.8ng/L,
11.5 pmol/L (normal <5 ng/L) at midnight, and a morning ACTH of 39.9 ng/L (8.8 pmol/L, normal 10-50 ng/L) (data not shown). A corticotropin releasing hormone test (CRH test) (100 mcg CRH) showed a 12% increase in ACTH concentration and no clear increase in cortisol concentration from baseline (Table 2). The baseline production of other pituitary hormones was normal. Dehydroepiandrosterone (DHEAS) and androstenedione were elevated. Potassium was low normal (3.5 mmol/L). The child had an episode of back pain and refused to walk. Orthopaedic investigations including spinal radiographs were normal, and she improved spontaneously.

The CRH test was inconsistent with pituitary-dependent Cushing disease and EAS was considered. A thoracic CT-scan of the chest revealed marked nodular infiltration of the peritoneal surface of the diaphragm. A repeat abdominal ultrasound examination showed a solid pelvic mass (3.5x4.3x3 cm) and a solid heterogeneous infiltrating mass diffusely surrounding the liver and spleen (mimicking prominent adipose tissue) suggesting peritoneal infiltration by the tumor. Lymphadenopathy was present at the level of the porta hepatis. Abdominal MRI (Figure 2) confirmed findings in keeping with malignant infiltrating peritoneal disease. Both adrenals appeared bulky without focal lesions. Alpha-feto protein (AFP) concentration was grossly elevated (>300,000 kU/L) whereas B-HCG (human chorionic gonadotropin) was undetectable. A 99Technetium scan showed increased uptake in multiple ribs and vertebral bodies, femur and humerus compatible with metastatic bone disease. Bone marrow aspiration was normal.

Tumour needle biopsy demonstrated a primitive malignant epithelial tumour with no specific morphological features, expressing AE1/3 (cytokeratin), but CD117, Oct3/4, CD56, desmin, AFP, WT1 and S100 staining were negative. The child received Metyrapone and Ketoconazole, which successfully suppressed cortisol production.
Further treatment consisted of five courses of Cisplatin, Doxorubicin, and Etoposide, which led to reduction of AFP concentrations to 1264kU/L and of cortisol production, so that Metyrapone and Ketoconazole could be stopped. The patient had multiple episodes of sepsis and neutropenia, and on one occasion signs of possible thrombosis, but this improved spontaneously. Adrenal function was suppressed and she required hydrocortisone replacement therapy. However, AFP concentrations increased again. Surgical resection was attempted, but multiple tumor plaques over the peritoneum and intra-abdominal organs prevented complete resection. AFP concentration fell from 17654kU/L prior to surgery to 6590kU/L post-surgery. Histological examination of resected tissue demonstrated sheets of malignant epithelial tumor expressing MNF116, CEA, and AFP but not other markers such as desmin, vimentin, inhibin, WT1, calretinin, CD56, Oct3/4 and CD17; the overall features were strongly suggestive of a malignant yolk sac tumor. The tumor was graded as Grade IV (distant metastases).

### Methods

#### Identification of ectopic ACTH precursor production

**Plasma ACTH and ACTH precursors**

Plasma ACTH was measured initially with a solid phase two-site chemiluminescent immunometric assay (Immulite 2000, Siemens) during diagnostic investigations. This assay has a sensitivity of 5ng/L (1pmol/L) and an inter-assay variability of 6-10%. Cross-reactivity with ACTH precursors in this assay has recently been assessed to be 2.2% (MONAGHAN 2016). After the first and second cycle of chemotherapy, ACTH and ACTH precursors (POMC and pro-ACTH) were measured by ELISA (in house) using the monoclonal antibodies N1C11 and A1A12. Binding of both antibodies to the ACTH precursors is required to generate a signal in the assay; therefore, ACTH or any
of the other peptides derived from POMC and proACTH are not detected. The sensitivity of the precursor ELISA is 8pmol/L and normal adult range of precursors is 7-32pmol/L. Circulating concentrations of ACTH precursors above 100pmol/L are indicative of an ectopic tumour. Measurement of ACTH utilises MAb A1A12 which binds to ACTH (10-18) and MAb A2A3 which binds the cleavage site of ACTH and therefore reduces the cross-reactivity with ACTH-precursors (Figure 1A). This ACTH ELISA has a sensitivity of 1 pmol/L, variability <10% and POMC cross-reacts <3% (unpublished data).

*Immunohistochemistry for ACTH and precursors*

Paraffin sections of tumour biopsy were evaluated following antigen retrieval with mouse monoclonal antibodies A1A12, N1C11, E6B2 and A2A3, which recognise various epitopes of POMC and ACTH as described previously (Figure 1A). Staining was visualized using a Mouse Dako Envision+ System-HRP (DAB) and Gill’s haematoxylin counterstain. Controls were rat pituitary sections (ACTH and POMC positive) and a DMS79 small cell lung cancer xenograft tumour, known to be POMC positive and ACTH negative.

*Results*

*Plasma ACTH and ACTH precursors*

In the first blood sample, taken before the second course of chemotherapy, ACTH precursors were detected at a concentration exceeding the normal adult range (7-32pmol/L) whereas ACTH concentrations (measured with the matching ACTH ELISA) were within the adult normal range. In the second sample, taken just before the 3rd cycle of chemotherapy, the ACTH precursor concentration had decreased considerably (Table 3). A small amount of “ACTH” was detectable in both samples. It
is very likely that this is due to the low cross-reactivity of ACTH precursors in the ACTH assay or endogenous ACTH secreted from the pituitary.

*Immunohistochemistry for ACTH and ACTH precursors in tumor sections*

The tumor showed strong cytoplasmic staining with antibodies A1A12 (recognizes ACTH and ACTH precursors), N1C11 (recognizes N-POC and ACTH precursors) and E6B2 (recognizes beta-endorphin and POMC) but not with the more specific ACTH antibody (A2A3) (Figure 1B). This suggests the presence of POMC, but not mature ACTH, in tumor cells. Immunohistochemistry using A2A3 in a control POMC-producing xenograft tumor did not show any staining (data not shown), confirming specificity of A2A3 for mature ACTH.

*Further treatment*

After establishment of the diagnosis of yolk sac tumour, treatment was changed to Paclitaxel, Ifosfamide and Cisplatin (TIP, 3 courses), after which AFP concentration fell to 2340kU/L and tumor size decreased to 2.7x3.2 cm with only discrete areas of disease intraperitoneally. A further TIP course and high-dose chemotherapy (Paclitaxel, Carboplatin, Etoposide, Cyclophosphamide) with autologous peripheral blood stem cell rescue was given. Despite this, AFP increased again (26444kU/L). Hyperthermic Intraperitoneal Chemotherapy (HIPEC) was administered without long-term success. She further received Gemcitabine, Docetaxel and Metronomic chemotherapy with Cyclophosphamide and Etoposide. Whole abdominal radiotherapy in a dose effective for treatment of germ cell tumours (54Gy) was not possible to deliver. She then continued to receive palliative chemotherapy and unfortunately passed away 1.5 years later.
Discussion

We describe, for the first time to our knowledge, POMC production from a malignant yolk sac tumor as the cause of CS due to ‘ectopic ACTH precursor production’ in a child. Yolk sac tumors (endodermal sinus tumors) are a malignant subtype of germ cell tumor\textsuperscript{13, 14}, characterized typically by AFP and Gli-3 immunoreactivity and may occur in both gonadal and extra-gonadal tissues. Reports of EAS in adults due to ACTH production in teratoma\textsuperscript{15, 16}, ovarian epithelial\textsuperscript{17, 18} and ovarian endometrial carcinoma\textsuperscript{19} exist but EAS has not previously been described in a yolk sac tumor. Two large case series of 90 adults at NIH and 40 adults in London with EAS have recently been published, but yolk sac tumour was not identified as a cause for EAS\textsuperscript{20, 21}.

Another recent case series described ten children with EAS identified during the last 20 years in France\textsuperscript{22}. Seven patients had thoracic neuro-endocrine tumors; one had a liver nested stromal epithelial tumor, one a carcinoma of the thymus and one Ewing’s sarcoma. Of note, positive ACTH staining of the tumour was one of the inclusion criteria. Therefore, POMC/pro-ACTH producing tumours may not have been included, depending on the antibodies used for ACTH detection.

To identify the etiology of the CS in our patient, four different antibodies were used in plasma ELISAs and tumor immunohistochemistry. The ACTH precursor ELISA, which measures both POMC and pro-ACTH, gave the first indication that plasma ACTH precursor concentrations were increased. IHC staining of the tumor with A1A12, N1C11 and, also, E6B2, which binds to POMC but not pro-ACTH, showed
positive staining. This is strong evidence that the tumor produced POMC but did not
cleave it to mature ACTH. Additionally, the tumor did not stain with A2A3, which is
specific for mature ACTH. In addition, the POMC concentration decreased after
chemotherapy, in line with reduction in tumor size and improvement of features of CS.
These data suggest that either POMC is binding to the ACTH receptor (melanocortin-
2 receptor, MC2-R), in the adrenal gland to stimulate cortisol secretion or that POMC
is cleaved in the adrenal gland to allow ACTH to bind and stimulate cortisol secretion.
Indeed, other patients with ectopic “ACTH” syndrome due to ACTH precursor
production have clinical symptoms\textsuperscript{23, 24}, also suggesting that ACTH-precursors bind to
the adrenal MC2-R or are locally cleaved. While it has not been possible to investigate
the bioactivity of POMC because of the high concentrations of purified POMC that is
required, we have shown that POMC can bind to the MC1-R\textsuperscript{25}.

Differentiation between an ACTH-producing pituitary adenoma (Cushing Disease, CD)
and EAS can be difficult. No single biochemical test can differentiate between the two
and responses to dynamic function tests overlap. Bilateral inferior petrosal sinus
sampling (BIPPS)\textsuperscript{22} remains the golden standard to differentiate pituitary Cushing and
EAS (Lacroix, Lancet 2015), but it is difficult to perform, particularly in children. This
case illustrates the potential usefulness of measurement of ACTH-precursors in order
to differentiate between CD and EAS. Peripheral concentrations of ACTH-precursors
are low in CD and high in EAS and a clear relation exists between BIPSS results and
baseline ACTH precursor concentration\textsuperscript{7}. Stewart et al, 1994 ACTH precursor
assessment (by immunoradiometric assay) showed a 100% sensitivity and specificity
in a group of EAS patients and CD patients. Levels of ACTH precursors were between
139 and 18,000pmol/L in EAS patients, between 8 and 73pmol/L in the CD patients
and below 40pmol/L in control subjects. Measuring ACTH precursors can also
distinguish the majority of occult ectopic ‘ACTH’ producing tumours that are not
detected by MRI. Page-Wilson et al, 2014 (reference 9) showed that in their patient
cohort 7 of 11 such patients could be distinguished by ACTH precursor measurement
alone. This gives a sensitivity of 64% and 100% specificity.

Yolk sac tumors are chemosensitive but when complete resection is not feasible, and
there is cisplatin resistance, relapse is frequent and the outcome is poor, as seen in this
case 26. Medical treatment of EAS with Metyrapone and Ketoconazole is effective in
decreasing cortisol production, but is of limited use due to side effects.

Complications of EAS are increased susceptibility for infections and thrombosis.
Therefore, if possible, chemotherapy is started only when cortisol production is
controlled pharmacologically. Antibiotic prophylaxis, PCP prophylaxis and anti-
coagulation could be considered, but currently no guidelines or evidence base exists for
paediatric EAS.

To conclude, we used antibodies to different epitopes on ACTH-precursors in ELISAs
and immunohistochemistry to demonstrate, for the first time, POMC production in a
disseminated malignant yolk sac tumor as the underlying cause of CS in a two-year-old
child. Hence, a diagnosis of aggressive Cushing syndrome in the face of normal ACTH
concentrations should prompt a search for ACTH precursors in EAS.
Acknowledgements

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Authors’ contributions

EG-writing the manuscript, literature search, data collection and interpretation; SM-writing the manuscript, figures and carrying out immunohistochemistry; PS-writing the manuscript, data collection and interpretation; JT-writing the manuscript; CP-writing the manuscript; NS-writing the manuscript and providing histopathology report; OS-writing the manuscript and clinical management of the patient; AW-writing the manuscript, data collection, figures and carrying out immunohistochemistry; MTD-writing the manuscript, literature search, data collection and interpretation, figures.
Tables

Table 1 - Low dose dexamethasone and high dose dexamethasone suppression tests

Table 2 - Corticotropin releasing hormone (CRH) test (100 mcg CRH)

Table 3 - Plasma concentration of ACTH precursors and ACTH after first and second cycle of chemotherapy

Legends to figures

Figure 1 - POMC but not ACTH is produced by the tumor.

IA: Antibodies against epitopes in ACTH and ACTH precursors used in ELISAs and immunohistochemistry.
Immunohistochemistry of tumor sections using mouse antibodies to ACTH-related peptides and horseradish peroxidase conjugated anti-mouse IgG. Staining with A1A12, N1C11 and E6B2 recognizing epitopes on ACTH precursors is positive (brown stain) but staining with A2A3, specific for ACTH, is negative. Primary antibody is omitted in the negative control.

**Figure 2 - Clinical features of the patient described.** A-C Clinical photographs showing Cushingoid features at time of diagnosis. D MRI of the abdomen showing tumor and tumor invasion in peritoneum.

**References**


27.