ESA CLINICAL WEEKEND 2023

www.esaclinicalweekend.org.au

24 - 26 November 2023

voco, Brisbane, Australia

FULL CASE STUDIES

esa
endocrine society of australia

Don’t hold your breath – from looking blue to feeling blue

Arunan Sriravindrarajah[2, 1], Christopher Muir[2, 1]

1. St Vincent’s Hospital, Sydney, NSW, Australia

2. Faculty of Medicine, University of New South Wales, Sydney, NSW, Australia

Case presentation

A 60-year-old male presented with worsening exertional shortness of breath, fatigue, and 4kg weight loss. This occurred on a background of stable Eisenmenger’s syndrome secondary to congenital peri-membranous ventricular septal defect, severe pulmonary hypertension, and chronic hypoxic respiratory failure (baseline oxygen saturation 75-80%). Additional background history included secondary polycythaemia requiring regular venesection, atrial flutter, embolic cerebrovascular accident, osteoporosis, epilepsy, gout, migraine, and asthma. Regular medications included bosentan, sildenafil, warfarin, levetiracetam, allopurinol and inhaled budesonide. On examination, he was normotensive with systolic blood pressure 120-130mmHg and resting heart rate 80-90 beats per minute.

Investigations

Initial investigation with chest X-ray reported upper lobe changes suggestive of fibrosis or infection. Subsequent high-resolution CT-imaging identified non-specific features favouring infective/inflammatory changes, but also incidentally identified a 47x37mm soft tissue density in the left para-aortic region. Dedicated CT-imaging confirmed a 47x38mm heterogeneously enhancing para-aortic lesion anteroinferior to the left renal vein with a small focus of hypodensity possibly reflecting an area of cystic change. No adrenal lesion, other sympathetic/parasympathetic lesions or intrabdominal lymphadenopathy were identified.

Biochemical investigations demonstrated elevation of plasma normetanephrines (7,960pmol/L; reference range [RR]<800) and chromogranin A (425ug/L; RR<93). Plasma metanephrines were not elevated (291pmol/L; RR<450). Repeat investigations confirmed significantly elevated plasma normetanephrines (5,770pmol/L).

Based on the CT findings and biochemical profile, the mass lesion was thought to represent a paraganglioma. Additional imaging with Ga-68 DOTATATE PET-CT again confirmed the presence of a 34x42mm left para-aortic soft tissue mass with heterogeneous DOTATATE avidity (SUVmax 14.5) consistent with paraganglioma and high somatostatin receptor density. There were no additional sites of DOTATATE avid disease (Figure 1).

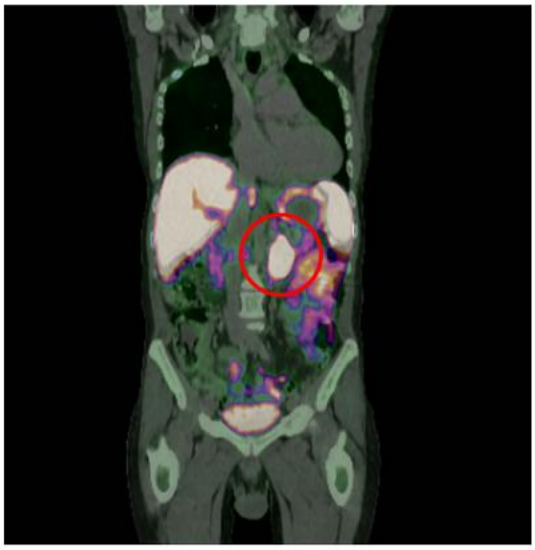


Figure 1. Ga-68 DOTATATE PET-CT demonstrating DOTATATE avid para-aortic mass

Management

Alpha-adrenoreceptor blockade using prazosin 1mg twice daily was commenced in preparation for surgical resection. Following 12 weeks of blockade, he was admitted for laparoscopic resection of the suspected para-aortic paraganglioma. The perioperative course was uncomplicated and resection of the lesion was successful without intra-operative or post-operative hypotension or hypertension.

Outcome and follow-up

Histopathology confirmed a 5.5cm tumour with positive immunohistochemical staining for chromogranin A, synaptophysin, CD56, and succinate dehydrogenase B (SDHB) suggestive of paraganglioma. Lymphatic invasion was present without concomitant vascular invasion, capsular invasion, or involvement of the surgical margins. Ki-67 measured 2% with <1 mitoses per high powered field.

Four weeks post-operatively, plasma normetanephrines declined to a nadir level of 1,420umol/L (RR<800). At the time, ongoing mild elevation in plasma normetanephrines was attributed to his underlying cyanotic congenital heart disease (CCHD).

He remained asymptomatic with a mean blood pressure of 115/65mmHg on home monitoring. However, plasma normetanephrine levels were monitored and progressively increased over the next 9 months to 3,830umol/L.

Repeat CT-imaging did not identify recurrence at the site of prior resection. However, Ga-68 DOTATATE PET-CT identified new DOTATATE avid lesions within the T8 vertebral body (SUVmax 13.2) and left anterior 5th rib (SUVmax 4.0) suggestive of metastatic paraganglioma (Figure 2). Following discussion in a neuroendocrine tumour multidisciplinary team meeting, he commenced peptide receptor radionuclide therapy (PRRT) with lutetium-177, with a plan for 8 cycles in total. Repeat DOTATATE imaging performed after the first dose (7,927 MBq) demonstrated accumulation of lutetium within the T8 vertebral body lesion, although uptake in the rib lesion was not readily identified.

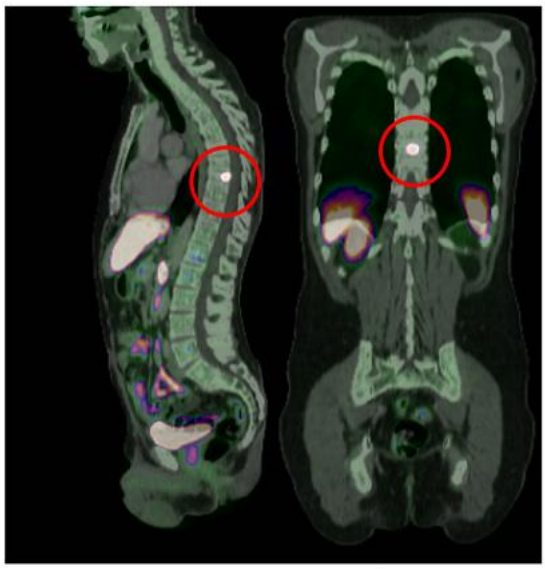


Figure 2. Repeat Ga-68 DOTATATE PET-CT demonstrating DOTATATE avid metastatic T8 vertebral lesion

Discussion

Phaeochromocytoma and paraganglioma (PPGL) are rare neuroendocrine tumours arising from neural crest cells with an estimated incidence of 2-8 per million persons.(1) Metastatic PPGL is rarer accounting for less than 25% of all PPGL.(1) Risk factors for metastatic PPGL include larger tumour size (>4cm for paraganglioma), large vessel invasion, extra-adrenal location, lymph node spread, SDHB pathogenic variant, and elevated Ki-67 or mitotic index.(1) Metastatic PPGL has a 5-year survival rate between 50-70%.(1)

Diagnosis of PPGL involves biochemical testing for plasma free metanephrines or urinary fractionated metanephrines.(2) Whilst sensitivity of testing is high, both pre-test and post-test probability remains low given the rarity of PPGL, and false positive results are common. Medications such as tricyclic antidepressants and alpha-2 adrenoreceptor blockers are well-known causes of false positive results.(2) Additionally, conditions that stimulate sympathetic activity, such as congestive cardiac failure and pulmonary hypertension have also been associated with elevated noradrenaline levels, and thus affect the interpretation of testing for PPGL.(2-3)

Despite challenges with biochemical confirmation of PPGL in patients with CCHD, a high index of suspicion is required as these patients possess higher risk of PPGL. Epidemiological studies have shown an increased likelihood of PPGL and carotid body hyperplasia in people living at high altitudes or with diseases associated with chronic hypoxaemia.(4-5) These patients tend to present with PPGL at a younger age, be less symptomatic, exhibit more aggressive disease such as multiple or recurrent PPGL, and secrete predominately noradrenaline.(5-7) This phenotype is similar to PPGL tumours derived from the pseudohypoxic cluster 1 profile arising from mutations in endothelial PAS domain-containing protein 1 (EPAS1), Von Hippel-Lindau (VHL) or succinate dehydrogenase (SDHx) genes. (5-6). These mutations constitutively activate hypoxia signaling pathways via altered regulation of hypoxia inducible factor alpha (HIFα). HIFα normally undergoes oxygen-dependent hydroxylation via prolyl hydroxylase, ubiquitination via VHL proteins and proteasomal degradation. However, under hypoxic conditions or when cluster 1 mutations simulate ‘pseudohypoxia’, HIFα avoids degradation and co-activates with HIFβ to trigger a downstream cascade that contributes to tumorigenesis and increased risk of developing PPGL.(6)

In 2018, a landmark case series of five patients with CCHD and PPGL demonstrated 80% of cases harboured a pathogenic somatic EPAS1 mutation.(7) In contrast, EPAS1 is the causative mutation in only 5% of unselected PPGL.(8) The disproportionate presence of EPAS1 mutations suggests it may be the key driver for increased risk of PPGL in CCHD patients. Since this initial report, additional cases of EPAS1 mutated tumours in patients with CCHD have been published, as well as in other conditions associated with chronic hypoxaemia such as sickle cell anaemia.(9) EPAS1 mutant tumours may be more aggressive due to downregulation of genes involved in chromaffin cell differentiation and increased expression of the oncogene myc and cyclin D1.(8)

Treatment options for metastatic PPGL are limited but include PRRT with lutetium-177, iobenguane I-131 (MIBG), chemotherapy, and targeted molecular therapies.(1) Available data for PRRT in PPGL report a disease control rate of 84%.(10) PRRT is generally well tolerated, although grade 3 and above neutropenia, thrombocytopenia, lymphopenia, and nephrotoxicity are observed in 3%, 9%, 11%, and 4% of patients respectively.(10) However, given the emerging nature of PRRT, and the rarity of PPGL in CCHD patients, there is limited experience with its use in EPAS1 mutated PPGL and patients with CCHD.

Take home messages

- The incidence of PPGL is increased in patients with CCHD
- Sympathetic neurohormonal activation associated with CCHD can result in elevated plasma free metanephrines and urinary fractionated metanephrines, which makes biochemical confirmation of PPGL challenging in patients with CCHD
- Chronic hypoxaemia in patients with CCHD is associated with somatic mutations in the EPAS1 gene
- Activating EPAS1 mutations stabilises the HIF2α protein, which promotes signalling pathways associated with tumorigenesis, such as angiogenesis, metabolism, and cell growth
- PPGL in patients with CCHD may be more aggressive compared to spontaneous PPGL or PPGL arising from other genetic syndromes
- Treatment options for metastatic PPGL are limited, but PRRT with lutetium-177 is an emerging therapy and appears promising in terms of disease control and side-effect profile

References

1. Fishbein, L. et. al. The North American Neuroendocrine Tumor Society Consensus Guidelines for Surveillance and Management of Metastatic and/or Unresectable Pheochromocytoma and Paraganglioma. Pancreas. 2021;50(4):469-493
2. Eisenhofer, G., Pamporaki, C. and Lenders, J.W.M. Biochemical assessment of pheochromocytoma and paraganglioma. Endocr Rev. 2023;bnad011
3. Viquerat, C.E. et. al. Endogenous catecholamine levels in chronic heart failure. Relation to the severity of hemodynamic abnormalities. Am J Med. 1985;78(3):455-460
4. Saldana M.J., Salem L.E. and Travezan R. High altitude hypoxia and chemodactomas. Hum Pathol. 1973;4(2):251-263
5. Opotowsky A.R. et.al. Pheochromocytoma and paraganglioma in cyanotic congenital heart disease. J Clin Endocrinol Metab. 2015;100(4):1325-1334
6. Nolting, S. et. al. Personalized management of pheochromocytoma and paraganglioma. Endocr Rev. 2022;43(2):199-239
7. Vaidya A. et. al. EPAS1 mutations and paragangliomas in cyanotic congenital heart disease. NEJM. 2018;378(13):1259-1261
8. Dahia P.L.M. Pheochromocytoma and paraganglioma pathogenesis: learning from genetic heterogeneity. Nat Rev Cancer. 2014;14:108-119
9. White, G. et. al. Somatic EPAS1 variants in pheochromocytoma and paraganglioma in patients with sickle cell disease. JCEM. 2023;dgad2311
10. Satapathy, S., Mittal, B.R., and Bhansali, A. Peptide receptor radionuclide therapy in the management of advanced pheochromocytoma and paraganglioma: A systematic review and meta-analysis. Clin Endocrinol. 2019;91(6):718-727

The Clinical Conundrum of Inconclusive AVS for Primary Aldosteronism

Bella Halim[1], Nirupa Sachithanandan[1]

1. Endocrinology Department, St Vincent's Hospital Melbourne, Melbourne, VIC, Australia

Primary aldosteronism (PA) requires adrenal venous sampling (AVS) to subtype and identify patients who might benefit from surgery. However, AVS can be technically challenging. Some PA patients are denied adrenalectomy due to lack of lateralization.

We present a case of a 37 year old gentleman, who was referred to our endocrinology clinic for investigation of possible primary aldosteronism (PA). He had hypertension and hypokalaemia at 2.6mmol/L – this is despite being on ACE inhibitor and K replacements. His antihypertensive was changed to verapamil 80mg TDS and 2 weeks after the swap, his renin was 4 mIU/L and his aldosterone was 607 pmol/L.

His initial investigation result are listed on **Table 1** – apart from elevated aldosterone, other screening tests for secondary causes of hypertension were unremarkable. His physical examination was also unremarkable. His initial CT showed indeterminate 8mm adrenal nodule (29 Houndsfield Unit) on the medial aspect of the right adrenal body **(Image 1).** His other medical history included obesity, obstructive sleep apnea and diabetes, which was diagnosed during this presentation. His family history included hypertension on his paternal side.

Test	22/5/20	25/5/20
Blood pressure (mmHg)	137/83	
Medications	verapamil IR 80mg TDS	
K supplement	Span K 5 tablets TDS	
Sodium (mmol/L)	144	143
Potassium (mmol/L)	2.7	4
Urea (mmol/L)	4.2	5
Creatinine (umol/L)	68	84
eGFR (mL/min/1.73m2)	>90	>90
TSH (mIU/L)	1.67	
Free T4 (pmol/L)	19.1	
Free T3 (pmol/L)	5.4	
Cortisol (nmol/L)	341	
ACTH (pmol/L)	6.7	
Normetanephrines (pmol/L)	389	
Metanephrines (pmol/L)	114	
Renin (mIU/L)	0	4
Aldosterone (pmol/L)		607
Aldosterone/Renin Ratio		152

Table 1. Initial Investigations

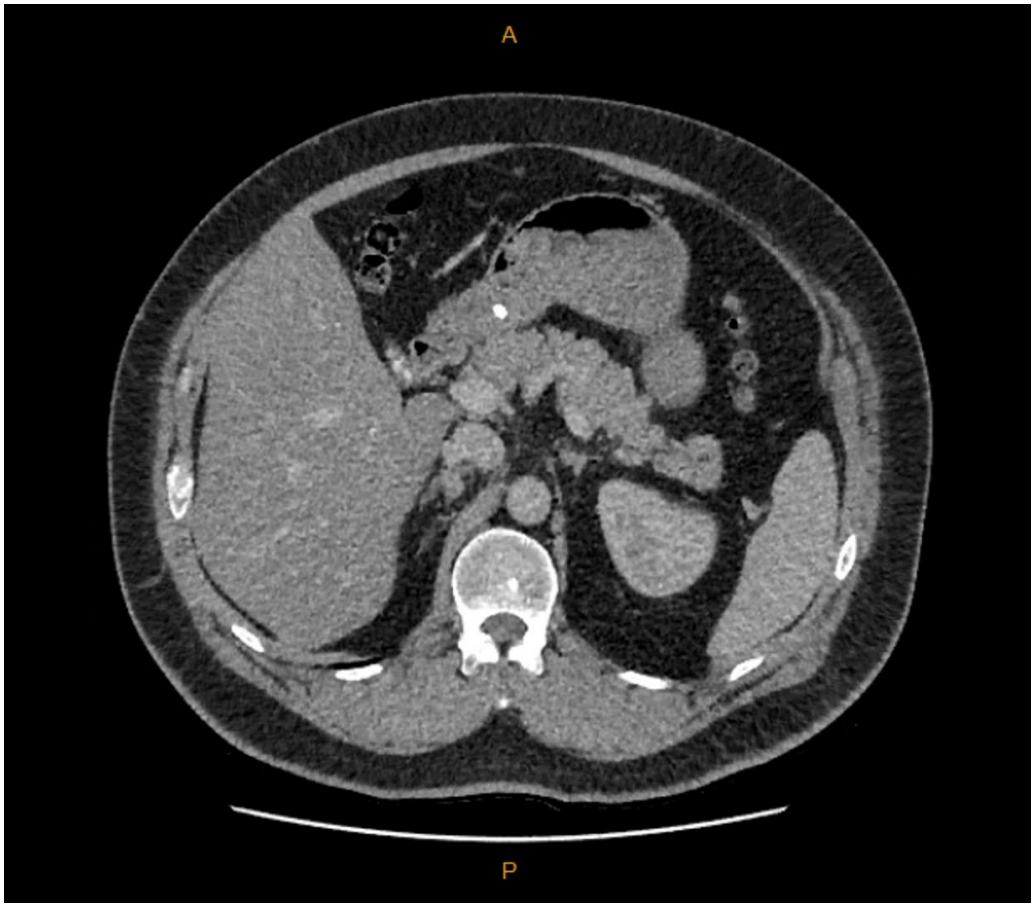


Image 1. CT of the adrenals

Following referral to endocrinology department and hypokalaemia correction, he underwent saline suppression test (SST) **(Table 2.**). His renin and aldosterone were 1100 pmol/L pre-SST and 777pmol/L post-SST confirming primary aldosteronism. He underwent AVS that showed non-lateralisation **(Table 3) with both adrenal vein aldosterone / cortisol ratios lower than simultaneous peripheral vein aldosterone/cortisol ratios indicating 'double down' AVS.**

	08:20am Before Saline	12:45pm After Saline
Renin (mIU/L)	2.6	
Aldosterone (pmol/L)	1100	777
Aldosterone/Renin Ratio (pmol/L)	423.1	
Cortisol (nmol/L)	253	191

Table 2. Saline Suppression Test

1st Adrenal Vein Sampling								
Time	Right adrenal aldosterone	Right adrenal cortisol	Right peripheral aldosterone	Right peripheral cortisol	Right peripheral aldosterone/cortisol	Right adrenal aldosterone/cortisol	Selectivity index	Lateralization index (L/R)
10:43	4120	7560	1150	486	2.37	0.54	15.56	
10:56	11280	14800	1180	540	2.19	0.76	27.41	1.06
Time	Left adrenal aldosterone	Left adrenal cortisol	Left peripheral aldosterone	Left peripheral cortisol	Left peripheral aldosterone/cortisol	Left adrenal aldosterone/cortisol ratio	Selectivity index	
9:39	8360	11680	1260	494	2.55	0.72	23.64	
9:43	10440	10920	1250	492	2.54	0.96	22.20	
9:28	3212	9480	556	472	1.18	0.34	20.08	

Table 3. 1st Adrenal Vein Sampling

Given the initial AVS result, he underwent dexamethasone suppression test to rule out glucorticoid remediable aldosteronism (GRA). His cortisol and aldosterone before dexamethasone was 659 nmol/L and 1320 pmol/L respectively and after dexamethasone they were 21 nmol/L and 306 pmol/L. Given this result, he underwent genetic testing for GRA, which subsequently came back negative for FH-1. In the meantime, his BP was controlled with eplerenone 25mg BD, perindopril 10mg OD, amlodipine 10mg OD and atenolol 25mg OD. He also underwent testicular US that ruled out ectopic aldosterone source.

His case was discussed in endocrinology radiology meeting and the consensus was to undergo repeat adrenal vein sampling given results of the previous AVS which showed that the adrenal aldosterone/cortisol ratios were both lower than the peripheral aldosterone/cortisol ratios, a phenomenon known as apparent bilateral adrenal suppression or double down AVS.

He underwent a repeat AVS which showed adrenal A/C ratios higher than peripheral bilaterally and lateralization **(Table 4)**. Following this, he had surgical removal of the right adrenal adenoma. On follow up, he was normotensive and normokalaemic with ramipril 2.5mg daily and subsequently discharged from endocrinology clinic. His repeat renin and aldosterone were 9.6 mIU/L and 101 pmol/L respectively with ARR of 10. A summary of his electrolytes, medication and blood pressure can be seen in **Table 5**.

2nd Adrenal Vein Sampling (before ACTH)								
Time	Right adrenal aldosterone	Right adrenal cortisol	Right peripheral aldosterone	Right peripheral cortisol	Right peripheral aldosterone/cortisol	Right adrenal aldosterone/cortisol	Selectivity index	Lateralization index (R/L)
11:37	514	169	755	222	3.4	3.04	0.76	
11:48	475	157	694	192	3.61	3.03	0.82	
11:48	458	157	759	156	4.87	2.92	1.01	
11:52	10140	6322	686	194	3.54	1.6	32.59	6.98
11:53	13520	8482	728	196	3.71	1.59	43.28	6.89
Time	Left adrenal aldosterone	Left adrenal cortisol	Left peripheral aldosterone	Left peripheral cortisol	Left peripheral aldosterone/cortisol	Left adrenal aldosterone/cortisol ratio	Selectivity index	Contralateral suppression index
10:53	1080	4702	1070	299	0.23		15.73	0.06
10:55	1180	5100	1000	306	0.23		16.67	0.07
11:04	2330	12632	987	324	0.18		38.99	0.06
11:06	2170	10330	1030	326	0.21		31.69	0.07
11:16	811	285	1090	321	2.85		0.89	
11:18	868	294	976	288	2.95		1.02	
11:22	783	888	959	280	0.88		3.17	
2nd Adrenal Vein Sampling (after ACTH)								
Time	Right adrenal aldosterone	Right adrenal cortisol	Right peripheral aldosterone	Right peripheral cortisol	Right peripheral aldosterone/cortisol	Right adrenal aldosterone/cortisol	Selectivity index	Lateralization index (R/L)
13:16	17120	3461	1430	476	3	4.95	7.27	24.53
13:16	17120	3614	1610	474	3.4	4.74	7.62	18.82
13:24	524000	16112	1530	476	3.21	35.52	33.85	72.25
13:24	50800	16176	1530	469	3.26	3.14	34.49	4.21
13:25	252800	18092	1520	468	3.25	13.97	38.66	20.86
13:25	1890	1034	1400	482	2.9	1.83	2.15	
13:26	3640	464	1680	488	3.44	7.84	0.95	
Time	Left adrenal aldosterone	Left adrenal cortisol	Left peripheral aldosterone	Left peripheral cortisol	Left peripheral aldosterone/cortisol	Left adrenal aldosterone/cortisol ratio	Selectivity index	Contralateral suppression index
12:59	4720	23404	1500	463	3.24	0.2	50.55	0.06
13:00	5610	22284	1470	473	3.11	0.25	47.11	0.08
13:03	24240	5376	1560	468	3.33	0.45	11.49	0.14
13:08	1770	2370	1630	476	3.42	0.75	4.98	0.22
13:10	8240	12304	1520	472	3.22	0.67	26.07	0.21
13:11	5140	10068	1590	454	3.5	0.51	22.18	0.15
13:12	4960	10824	1510	474	3.19	0.46	22.84	0.14

Table 4. 2nd Adrenal Vein Sampling

Test	22/5/20	25/5/20	15/7/20	14/1/21	21/9/21	2/7/22	14/10/22
Blood pressure (mmHg)	137/83		140/100	125/85	130/80		
Medications	verapamil IR 80mg TDS		verapamil SR 180mg mane and verapamil IR 80mg BD	spironolactone 50mg BD, perindopril 10mg OD and amlodipine 10mg OD	eplerenone 50mg daily, atenolol 25mg BD, perinodpril 10mg OD and amlodipine 10mg OD	eplerenone 50mg daily, atenolol 25mg BD, perinodpril 10mg OD and amlodipine 10mg OD	ramipril 2.5mg daily
K supplement	Span K 5 tablets TDS		Slow K 6 tablets mane, 6 tablets midi and 7 tablets nocte			Slow K 4 tablets daily	nil
Sodium (mmol/L)	144	143	143	142	146	147	145
Potassium (mmol/L)	2.7	4	4	4.7	3.7	3.9	4.4
Urea (mmol/L)	4.2	5	7.7	9.3	6	5.1	6.1
Creatinine (umol/L)	68	84	109	108	97	87	90
eGFR (mL/min/1.73 m2)	>90	>90	74	75	85	>90	>90
TSH (mIU/L)	1.67						
Free T4 (pmol/L)	19.1						
Free T3 (pmol/L)	5.4						
Cortisol (nmol/L)	341						
ACTH (pmol/L)	6.7						
Normetanephrines (pmol/L)	389						
Metanephrines (pmol/L)	114						
Renin (mIU/L)	0	4			8		9.6
Aldosterone (pmol/L)		607			1010		101
Aldosterone/ Renin Ratio		152			126		10

Table 5. Summary of electrolytes, medications and blood pressure.

Discussion

PA is associated with increased cardiovascular morbidity and mortality, even when treated with mineral corticoid receptor (MR) antagonist (1). Aldosterone production can be unilateral, caused by aldosterone producing adenoma (APA) and bilateral, caused by bilateral adrenal hyperplasia (BAH). Other rarer causes include adrenal carcinoma, familial hyperaldosteronism, glucorticoid-remediable aldosteronism and ectopic aldosterone-producing adenoma. Diagnostic process in PA can be simplified into 3 distinct steps: case finding test, confirmatory test and subtype evaluation test. Subtyping is important as unilateral adrenalectomy for APA is associated with biochemical cure and clinical cure (2).

AVS using digital subtraction or fluoroscopic guidance is the gold standard for subtyping PA. Using CT alone in subtyping PA might miss APA or identify a non-functioning adrenal incidentaloma, which are common in patients older than 40 years of age(3).

However, AVS can be technically challenging with variable success rates, depending on the case volume of centers. Continuous ACTH infusion can be used during AVS to minimize stress induced aldosterone fluctuations, maximize aldosterone secretion from APA and maximize the cortisol gradient from adrenal vein to inferior vena cava, which helps in confirmation successful sampling.

One of the ways to ensure that the catheter has been placed in the correct location is by using selectivity index (SI), which is defined as adrenal vein/peripheral vein cortisol ratio ³ 3.0 without ACTH and 5.0 with ACTH (4) . Subsequently, aldosterone/cortisol (A/C) ratios are compared between adrenal and peripheral samples to differentiate PA – lateralization index (LI) ³ 4.0 is in keeping with unilateral PA .

Rarely, adrenal aldosterone/cortisol ratios are lower than the peripheral ratios on both sides despite successful adrenal vein cannulation – this phenomenon is referred as apparent bilateral aldosterone suppression (ABAS), and is not diagnostic of unilateral disease or bilateral adrenal hyperplasia by any criteria. This prevalence of this finding has been reported to range between 2.5 – 9.5% (5; 6). Causes for this include inadvertent superselective cannulation, venous anomalies, ectopic aldosterone secretion or co-secretion of cortisol (4; 7). Management of ABAS result is challenging and there is no guidelines or literature review performed to guide further management when such result is encountered (4).

A retrospective review of 22 out of 37 patients with ABAS despite adequate sampling who then underwent repeat AVS shows that 10 had unilateral aldosterone production, 8 had bilateral aldosterone production and 4 were inconclusive – there was a small preponderance of males in those with ABAS (5). Another retrospective study in a high volume centre for AVS showed that 4 out of 61 cases had ABAS and repeat AVS in 3 patients showed right lateralization and biochemical cure following surgery (8). It is unclear if ACTH administration during AVS can rectify ABAS given conflicting data (9; 10).

This case highlights that AVS requires careful interpretation and more attention should be paid to the A/C ratios as it took 2 years for our patient to undergo repeat AVS, which led to curative surgery for his condition.

Take away message:

AVS can be challenging, even in the hands of qualified radiologists.

ABAS in AVS is underrecognized and when ABAS is encountered, lateralization cannot be determined.

Bilateral low aldosterone-cortisol ratio could be due to inadvertent superselective cannulation, venous anomalies, ectopic aldosterone production or co-secretion of cortisol.

In cases of inconclusive AVS, the result should be reviewed in a multidisciplinary meeting and patients should be offered repeat AVS if suspicion of unilateral PA is high.

References

1. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. The lancet Diabetes & endocrinology 2018;6:51-59
2. Young WF. Primary aldosteronism: renaissance of a syndrome. Clinical endocrinology 2007;66:607-618
3. Magill SB, Raff H, Shaker JL, Brickner RC, Knechtges TE, Kehoe ME, Findling JW. Comparison of adrenal vein sampling and computed tomography in the differentiation of primary aldosteronism. The Journal of Clinical Endocrinology & Metabolism 2001;86:1066-1071
4. Wolley M, Thuzar M, Stowasser M. Controversies and advances in adrenal venous sampling in the diagnostic workup of primary aldosteronism. Best Practice & Research Clinical Endocrinology & Metabolism 2020;34:101400
5. Wolley M, Gordon RD, Pimenta E, Daunt N, Slater GJ, Ahmed AH, Stowasser M. Repeating adrenal vein sampling when neither aldosterone/cortisol ratio exceeds peripheral yields a high incidence of aldosterone-producing adenoma. Journal of Hypertension 2013;31:2005-2009
6. Shibayama Y, Wada N, Umakoshi H, Ichijo T, Fujii Y, Kamemura K, Kai T, Sakamoto R, Ogo A, Matsuda Y. Bilateral aldosterone suppression and its resolution in adrenal vein sampling of patients with primary aldosteronism: analysis of data from the WAVES-J study. Clinical endocrinology 2016;85:696-702
7. DePietro DM, Fraker DL, Wachtel H, Cohen DL, Trerotola SO. “Double-down” adrenal vein sampling results in patients with apparent bilateral aldosterone suppression: utility of repeat sampling including super-selective sampling. Journal of Vascular and Interventional Radiology 2021;32:656-665
8. Tan SYT, Ng KS, Tan C, Chuah M, Zhang M, Puar TH. Bilateral aldosterone suppression in patients with right unilateral primary aldosteronism and review of the literature. Journal of the Endocrine Society 2020;4:bvaa033
9. Shibayama Y, Wada N, Naruse M, Kurihara I, Ito H, Yoneda T, Takeda Y, Umakoshi H, Tsuiki M, Ichijo T. The occurrence of apparent bilateral aldosterone suppression in adrenal vein sampling for primary aldosteronism. Journal of the Endocrine Society 2018;2:398-407
10. Wolley MJ, Ahmed AH, Gordon RD, Stowasser M. Does ACTH improve the diagnostic performance of adrenal vein sampling for subtyping primary aldosteronism? Clinical endocrinology 2016;85:703-709

A hairy problem in MEN-1

Monica Majumder[1], Roderick J Clifton-Bligh[1, 2, 3]

1. Royal North Shore Hospital, St Leonards, NSW, Australia

2. University of Sydney, Sydney , NSW, Australia

3. Kolling Institute, St Leonards, NSW, Australia

Case Study

A 36 year old female presented to her general practitioner with fatigue and low mood in 2015. Her initial work up demonstrated primary hyperparathyroidism with hypercalcaemia. She denied history of renal calculi or osteoporosis. Her other comorbidities include impaired glucose tolerance on metformin and hypercholesterolaemia on atorvastatin. Her mother and sister had osteoporosis otherwise she had no other significant family history.

Her parathyroid sestamibi (Figure 1) demonstrated uptake in the right lower lobe consistent with a parathyroid adenoma. She subsequently underwent a parathyroidectomy. The histopathology demonstrated a large, fat deplete right superior parathyroid adenoma (weight 1949g). Post operatively, she had a persistently elevated corrected calcium of 2.72mmol/L [2.10-2.60] with an elevated PTH 123 ng/L [15-68] and vitamin D of 67nmol/L [50-140]. She was referred to the endocrinology clinic for review. On examination she weighed 71 kg. Her height was 163 cm. Her blood pressure was 110/70 mmHg. She had no palpable neck lump. Her systems review was unremarkable.

At this time, a 4D CT parathyroid scan demonstrated two nodules suspicious for left sided parathyroid adenomas, posterior to the upper left lobe (11 mm) and posteroinferior to the lower left lobe (5 mm). In February 2016, subtotal parathyroidectomy and thymectomy, leaving part of the left inferior parathyroid *in situ*. Post operatively, serum PTH remained mildly elevated with normocalcaemia. Biochemical measurements of pancreatic and pituitary hormones were normal (insulin 9.8 mIU/L [<10], prolactin 454 mIU/L [110-560], IGF-1 212 ug/L [108-247], TSH 0.562 mIU/L [0.4-3.5], gastrin 22 pmol/L [<50], chromogranin A [≤ 3], glucagon 106 pg/mL [50-150], pancreatic polypeptide 36.3 pmol/L [0-300 pmol/L]). Given her age and multi-gland parathyroid disease, genetic testing was performed which found a variant of unknown significance in *MEN1* (c.487G>C). Abdominal imaging was recommended but not performed and the patient was temporarily lost to follow-up.

In 2019, she reported a 6 month history of amenorrhoea, weight gain and hirsutism. She also noted labile mood and felt she was quick to anger. Her repeat hormonal screening saliently demonstrated an elevated testosterone level 8.1nmol/L [0.4-1.4], and DHEAS 23 umol/L [1.9- 7.3]. She also had a low FSH 1.0 IU/L, LH 0.5 IU/L and her oestradiol was 373pmol/L. She completed a CT chest, abdomen and pelvis (Figure 3) which demonstrated an 8cm right adrenal lesion. Given the significant concern for malignancy in the setting of a large virilising tumour, she was referred for right adrenalectomy. The histopathology, as seen in figure 4, demonstrated a 6.5cm oncocytic adrenal cortex carcinoma which was contained with a 2mm margin, confirming a R0 resection. Her Weiss score was 5, and Ki-67 was 15% in hotspots. There was nil lymph node involvement. She underwent adjuvant radiotherapy following surgery. Her virilising symptoms resolved post surgery.

As part of her screening, in 2020 her MRI brain demonstrated a 4mm rounded hypointense focus in the left adenohypophysis on T2 weighted imaging which was consistent with a microadenoma. Her repeat pituitary profile was unremarkable.

Three years following her diagnosis of ACC, she completed an FDG-PET scan which demonstrated two areas suspicious for recurrence at the abdominal wall anterior to segment III and posterior abdominal wall near the quadratus laborum. A CT abdomen and pelvis confirmed two foci disease at the falciform ligament of the liver and posterior to the kidney. Her MRI liver showed no evidence of intrahepatic metastasis. She underwent a laparotomy for resection of these lesions. Subsequent CT imaging demonstrated a 7mm pararenal deposit concerning for remnant disease. She was commenced on mitotane therapy with cortisone replacement as well as chemotherapy (EDP- etoposide, doxorubicin and cisplatin). She was eventually transitioned to mitotane 200mg daily for ongoing maintenance therapy and cortisone replacement.

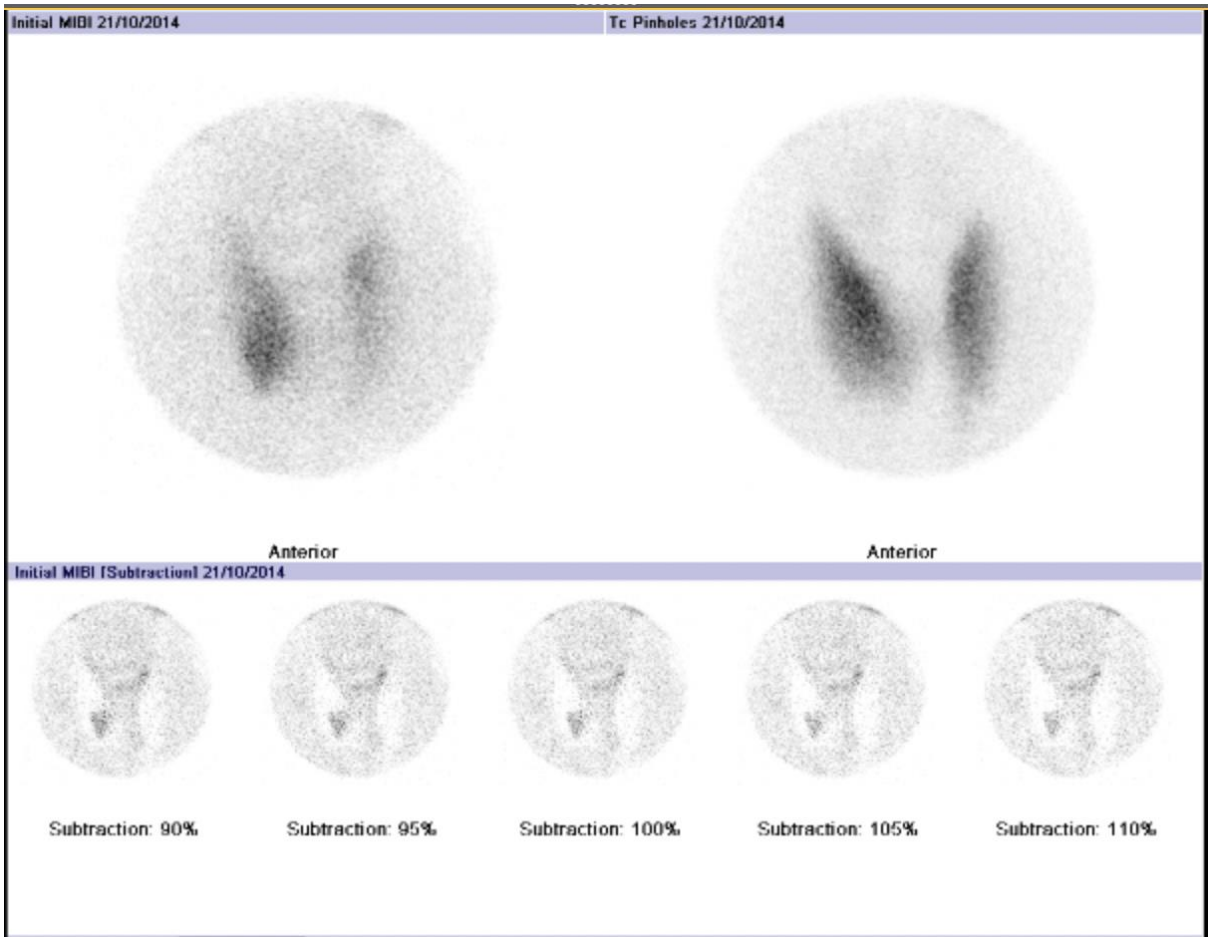


Figure 1: 2014 parathyroid sestamibi demonstrating right parathyroid adenoma

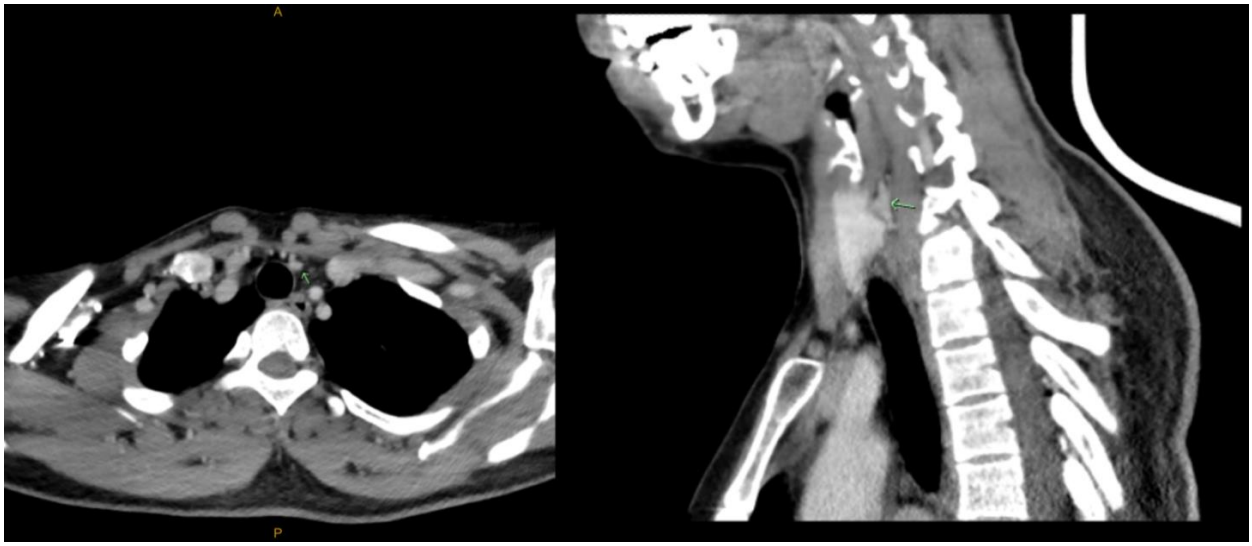


Figure 2: 2015 4D computed tomography neck demonstrating 2x parathyroid adenomas

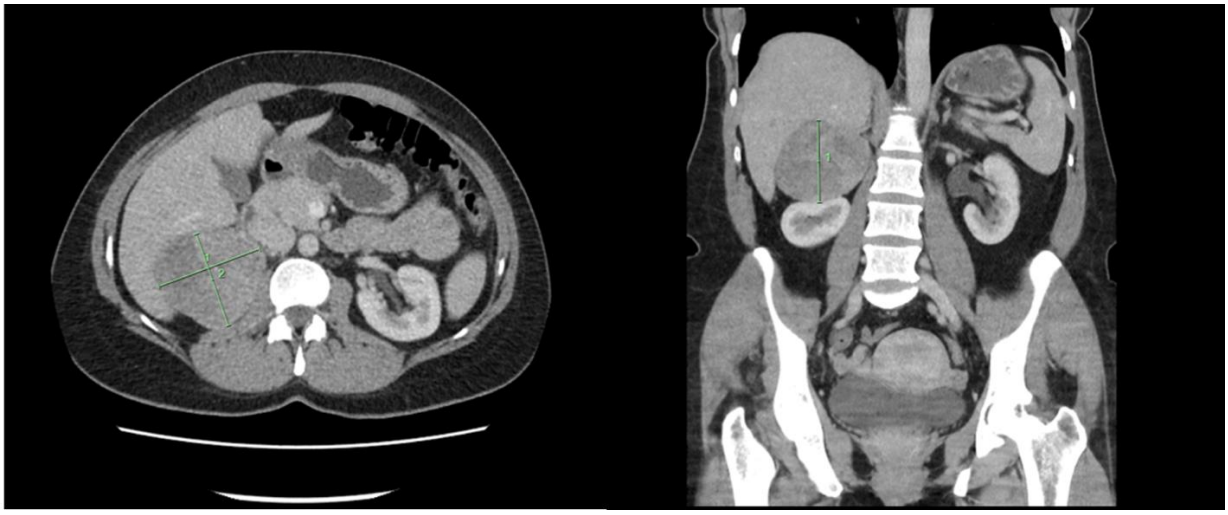


Figure 3: 2019 Computed Tomography Abdomen demonstrating right adrenal lesion

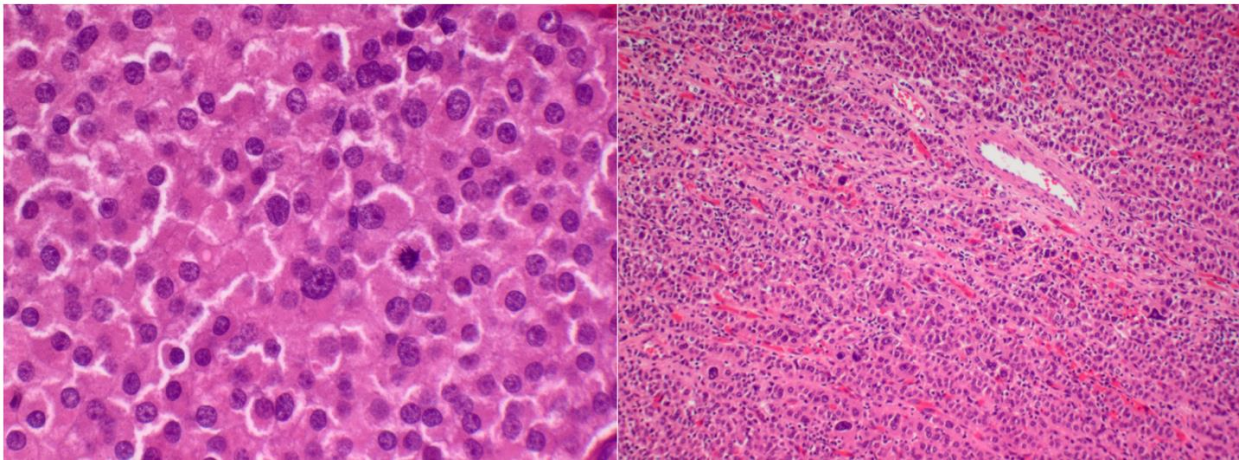


Figure 4: H&E stain of adrenal sample demonstrating adrenal cell carcinoma

Discussion

MEN-1 is a condition characterised by germline mutations of the tumour suppressor gene menin. It follows an autosomal dominant pattern of inheritance. It is characteristically associated with pancreatic, pituitary and parathyroid lesions but involvement of other glands is commonly seen.[1]

Adrenal lesions occur in 20-55% of MEN-1 patients, ranging from benign adenomas, hyperplasia to malignancy. [2] Adrenal cell carcinoma (ACC) is a rare occurrence in MEN-1. In a cohort of 715 MEN-1 patients across France and Belgium, 1.4% were found to have ACC.[3] ACC has been found to be tenfold more common in patients with MEN-1 with adrenal lesion when compared to sporadic adrenal lesions.[3] ACC confers a poor prognosis with 5 year mortality rates of 75-90% and median overall survival of 14-17 months.[4]

The clinical presentation of ACC is heterogenous as they can be functional or non-functional lesions. In the setting of functional lesions, individuals can develop signs of virilisation, hypercortisolism or both. [3] There have been reported cases of Cushing's syndrome with benign and malignant adrenal lesions in the setting of MEN-1. [3, 5] A retrospective analysis examining this association in MEN-1 patients found that three out of 19 patients with Cushing's syndrome was of adrenal origin. Two of these cases were related to ACC. [6]

The contribution of genetics in the development of ACC in MEN-1 is poorly understood. It has been postulated that loss of function mutations of menin together with modified β-catenin pathway and p53/Rb signalling plays a role in tumorigenesis. [2, 3] In a genomic analysis of metastatic ACC, a consistent mutation profile was not found however a higher mutation rate was identified in these patients.[4]

Menin is a nuclear scaffold protein involved in regulation of gene transcription through influencing chromatin remodelling and various interactions with transcription factors (JUND, NFKB, SMAD3). MEN-1, which encodes menin, acts as a tumour

suppressor gene. [2, 3] In a single centre, retrospective study of 121 MEN-1 patients 33.9% had an adrenal lesion of which 4.7% were ACC. Analysis of the ACC samples demonstrated mutations in MEN1 including homogenous frameshift mutation and loss of heterozygosity of the MEN-1 gene on chromosome 11q13 with negative menin staining. These changes were biallelic. [2] This is supportive evidence that menin may play a key role in oncogenesis in the setting of MEN-1.

Genomic analyses of The Cancer Genome Atlas (TCGA) samples identified CTNNB1, TP53, CDKN2A, RB1 and MEN1 as significant mutations in ACC. [7, 8] Alterations in some of these pathways, including MEN1 suggests the Wnt/beta-catenin pathway may play a central role in carcinogenesis.[2, 4] β-catenin is integral to embryonic adrenal development as well as maintenance of the adrenal cortex in adults. Histopathological analysis of MEN-1 associated ACC tissue has often demonstrated nuclear β-catenin staining, with heterozygosis CTNNB1 mutation and positive P53 staining. [2] Coincident β-catenin activation and TP53 inactivation has been shown to predict poor outcomes in ACC and are notably some of the most commonly reported mutations. [9]

These mutations are not yet actionable. Treatment of ACC generally relies on surgical resection, radiotherapy and systemic therapy with mitotane or chemotherapy.

Take home messages

- While adrenal lesions can be commonly seen in MEN-1, ACC is a rare manifestation.
- These can present as functional or non-functional lesions.
- The genetics are poorly understood, but loss of menin and dysregulation of the Wnt/beta-catenin pathway are postulated to play a key role.

References

1. Ohara N, Kaneko M, Ikeda M, et al. Lung adenocarcinoma and adrenocortical carcinoma in a patient with multiple endocrine neoplasia type 1. *Respiratory Medicine Case Reports* 2017; 20: 77-81. DOI: https://doi.org/10.1016/j.rmcr.2016.12.002.
2. Wang W, Han R, Ye L, et al. Adrenocortical carcinoma in patients with MEN1: a kindred report and review of the literature. *Endocr Connect* 2019; 8: 230-238. 2019/02/06. DOI: 10.1530/ec-18-0526.
3. Gatta-Cherifi B, Chabre O, Murat A, et al. Adrenal involvement in MEN1. Analysis of 715 cases from the Groupe d'étude des Tumeurs Endocrines database. *European Journal of Endocrinology* 2012; 166: 269-279. DOI: 10.1530/eje-11-0679.
4. Fojo T, Huff L, Litman T, et al. Metastatic and recurrent adrenocortical cancer is not defined by its genomic landscape. *BMC Medical Genomics* 2020; 13: 165. DOI: 10.1186/s12920-020-00809-7.
5. Simonds WF. Expressions of Cushing's syndrome in multiple endocrine neoplasia type 1. *Frontiers in Endocrinology* 2023; 14. Mini Review. DOI: 10.3389/fendo.2023.1183297.
6. Waldmann J, Bartsch DK, Kann PH, et al. Adrenal involvement in multiple endocrine neoplasia type 1: results of 7 years prospective screening. *Langenbecks Arch Surg* 2007; 392: 437-443. 2007/01/20. DOI: 10.1007/s00423-006-0124-7.
7. Zheng S, Cherniack AD, Dewal N, et al. Comprehensive Pan-Genomic Characterization of Adrenocortical Carcinoma. *Cancer Cell* 2016; 29: 723-736. 2016/05/12. DOI: 10.1016/j.ccell.2016.04.002.
8. Assié G, Letouzé E, Fassnacht M, et al. Integrated genomic characterization of adrenocortical carcinoma. *Nature Genetics* 2014; 46: 607-612. DOI: 10.1038/ng.2953.
9. Ragazzon B, Assié G and Bertherat J. Transcriptome analysis of adrenocortical cancers: from molecular classification to the identification of new treatments. *Endocr Relat Cancer* 2011; 18: R15-27. 2011/01/07. DOI: 10.1530/erc-10-0220.

When nothing else works, try steroids: an unconventional treatment for hyperglycaemia in a patient with type 1 diabetes.

James Nolan[1], Ee Mun Lim[2, 3]

1. Department of Endocrinology and Diabetes, Fiona Stanley Hospital, Perth, WA, Australia

2. Department of Biochemistry (QEII), PathWest Laboratory Medicine Western Australia, Perth, WA, Australia

3. Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, WA, Australia

Case

MO is a 58-year-old woman of Malaysian Chinese descent with a background of type 1 diabetes mellitus (T1DM), who has experienced significant deterioration in glycaemic control recently in association with generalised lipoatrophy, most marked at her insulin pump cannula sites.

She was diagnosed with T1DM in 2012. GAD-65 and IA2 autoantibodies were positive, measuring 675 U/mL (<5) and 3.4 (<1.1 U/mL), respectively. She transitioned to continuous subcutaneous insulin infusion (CSII) and continuous glucose monitoring (CGM) in 2015. Microalbuminuria has been present since 2012 with preserved renal function (eGFR >90). She has no other microvascular complications. Her other past medical history includes idiopathic angioedema, autoimmune urticaria and vitiligo. She takes ramipril 5mg daily and NovoRapid via Continuous Subcutaneous Insulin Infusion (CSII). She has a strong family history of type 2 diabetes mellitus, affecting her father and three brothers; and systemic lupus erythematosus (SLE) nephritis in her younger sister and end-stage renal failure of unknown cause in her mother. She migrated from Malaysia in 2006 and is recently retired from administrative work. She is a non-smoker, non-drinker and lives with her husband and two healthy daughters.

Lipoatrophy first developed at the patient's insulin injection sites in February 2015. Despite rotating the injection sites, lipoatrophy persisted, and in September 2019, facial fat wasting became evident. Serial body composition scans from October 2020 to June 2022 showed a significant decline in total body fat from the 13th to the 2nd centile. Omalizumab injections for autoimmune urticaria were ceased due to atrophy at injection sites.

Glycaemic control worsened from 2021 to 2022 (HbA1c 8.8% to 9.8%), marked by significant postprandial hyperglycaemia and nocturnal hypoglycaemia, which MO managed by disconnecting her insulin pump overnight. Strategies including dietary carbohydrate restriction, exercise and trial of oral agents (including empagliflozin, linagliptin and pioglitazone) had limited effect and concerns arose due to 13kg of weight loss over 6 months. MO presented with DKA in April 2022. Total intravenous insulin requirement over 23 hours was 275 units. Upon transition to pump therapy, overnight hypoglycaemia was observed. Insulin therapy was intensified prior to discharge and causes of severe insulin resistance were investigated.

Serum insulin was 15,000 mU/L (<12), proinsulin >100 pmol/L (<13.3) and C peptide <0.05 nmol/L. The absurdly increased insulin level prompted polyethylene glycol (PEG) precipitation and insulin post PEG was markedly lower at 200mU/L. Her insulin antibodies were also markedly increased at 10,350 U/mL (0-0.5), confirming a diagnosis of insulin autoimmune syndrome (IAS). Nephrotic range proteinuria was identified (345 mg/mmol). Elective admission was arranged for renal biopsy and treatment induction for IAS. Treatment consisted of IV hydrocortisone 100mg BD for 3 days, stepping down to oral prednisolone 50mg daily and rituximab 1000mg infusion (with repeat infusion 2 weeks later). Mycophenolate 360mg BD was started day 6. Total daily insulin dose was 162 units on admission, reducing to 60 units by day 6 with marked glycaemic improvement. Serum insulin was monitored to assess treatment response, reducing from a peak of 27,000 mU/L to 1100 mU/L. Renal biopsy subsequently confirmed membranous nephropathy secondary to lupus nephritis.

MO has experienced sustained improvement in glycaemic control over one year of follow up: HbA1c is 7.3%, GMI 7.1% and time in range 75% on CGM with 0% time below range. Total daily insulin dose ranges from 42 to 67 units. Insulin levels are stable at 240 mU/L. She has returned to her baseline weight and lipoatrophy has resolved. SLE nephropathy is quiescent and urine ACR normal.

Discussion

Insulin autoimmune syndrome (IAS) is characterized by hyperinsulinaemic hypoglycaemia due to the presence of insulin autoantibodies (IAA) and was first described by Hirata in 1970 [1]. While the original definition of IAS is restricted to patients without prior exogenous insulin exposure, IAS occurs in patients receiving exogenous insulin, including those with T1DM and has been termed exogenous IAS (EIAS) [2,3].

Certain HLA haplotypes confer a higher risk, such as HLADR4 in Asian patients [2, 4]. Other risk factors include autoimmune conditions (Graves' disease, SLE, rheumatoid arthritis, ankylosing spondylitis) and medications (methimazole, carbimazole, alpha-lipoic acid and other medications containing sulfhydryl groups) [2].

In patients with IAS, the postprandial glucose rise triggers insulin release, which is bound and sequestered in IAAs, rendering it ineffective and triggering further insulin release [5]. In the setting of endogenous insulin deficiency of T1DM, exogenous insulin is sequestered by IAAs in a similar fashion [2]. Complexed insulin later dissociates, producing postprandial hypoglycaemia and, less commonly, fasting hypoglycaemia. [5]. Persistent hyperglycaemia without hypoglycaemia has been described in cases with IAAs with higher insulin affinity, leading to low levels of free insulin and development of severe insulin resistance. The glycaemic disturbance caused by IAS is determined by the patient's own counter-regulatory responses and the characteristics of IAAs including antibody affinity, avidity and quantity [2].

Once the diagnosis is suspected, IAS is confirmed by identifying IAAs and demonstration of insulin-IAA complexes through methods such as PEG precipitation which allows for quantification of total and unbound insulin [2]. In patients with diabetes, EIAS poses a diagnostic challenge given the common occurrence of hypoglycaemia and considerations of erratic or surreptitious insulin administration, which may lead to under-diagnosis of a potentially life-threatening condition [3]. Similar to MO's case, patients with EIAS have a higher frequency of autoimmune conditions and lipodystrophy [3].

IAS is often self-limiting [2], particularly where a causative medication is ceased [5]. Conservative management strategies include dietary modifications with frequent small carbohydrate meals and acarbose, which attenuates prandial insulin release. In more severe cases, glucocorticoids, immunosuppression and plasmapheresis have been used [2,4]. Rituximab, an anti-CD20 monoclonal antibody, has demonstrated efficacy in case reports of IAS in patients with T1DM [7]. Despite concerns that glucocorticoids may exacerbate hyperglycaemia and lead to diabetic ketoacidosis, MO's hyperglycaemia and insulin

requirements rapidly improved in response to glucocorticoids and immunosuppression, in keeping with similar reports in the literature [2]. The ratio of free to total insulin has been proposed to assess treatment response [4]. In MO, serum insulin levels declined in parallel with improvement in insulin resistance, suggesting that total insulin is an effective method to assess disease response.

In SLE, several autoantibodies, including IAAs, can be spontaneously produced or inducible on exposure to antigen [8]. While IAAs are often present in low titre in the general population and in patients with SLE, the simultaneous development of severe insulin resistance from IAS and nephrotic-range proteinuria from undiagnosed SLE, suggests SLE as a possible driver of IAS in this patient.

Take home points

- IAS occurs due to development of IAAs, leading to hyperinsulinaemic hypoglycaemia and, less commonly, refractory hyperglycaemia and insulin resistance.
- Variability in presentation of IAS occurs due to differences in IAA affinity, avidity and titre, as well as the individuals' capacity to mount a counterregulatory response to glycaemic changes.
- Diagnosis of EIAS is particularly challenging in patients with T1DM, in whom prevalence is felt to be underestimated and hypoglycaemia common, underscoring the importance of identifying clues for IAS on history and physical examination. The result of measured serum insulin may be absurdly high and much lower insulin levels seen post PEG.
- Glucocorticoid therapy and immunosuppression are a safe and rapidly effective treatments for IAS in patients with diabetes.

References

1. 1. Hirata Y, Ishizu H, Ouchi N. Insulin autoimmunity in a case of spontaneous hypoglycemia. J Jpn Diabetes Soc. 1970;13:312–320.
2. 2. Cappellani D, Macchia E, Falorni A, Marchetti P. Insulin Autoimmune Syndrome (Hirata Disease): A Comprehensive Review Fifty Years After Its First Description. Diabetes Metab Syndr Obes. 2020 Apr 1;13:963-978. doi: 10.2147/DMSO.S219438. PMID: 32308449.
3. 3. Liu Y, Ping F, Yu J, Lv L, Zhao Y, Qi M, Li W, Xu L, Yu M, Li M, Zhang H, Li Y. Hypoglycemia Caused by Exogenous Insulin Antibody Syndrome: A Large Single-Center Case Series From China. J Clin Endocrinol Metab. 2023 Feb 15;108(3):713-717. doi: 10.1210/clinem/dgac578. PMID: 36219196.
4. 4. Liu H, Liang S, Li Y, Fu J, Chen S, Li M, Zhu H, Pan H, Wang O, Yuan T, Mao J, Qin Y, Li Y. A Novel Type of Extreme Insulin Resistance: Nonhypoglycemic Insulin Autoimmune Syndrome. J Clin Endocrinol Metab. 2021 Mar 25;106(4):1051-1061. doi: 10.1210/clinem/dgaa911. PMID: 33382420.
5. 5. Boro H, Gupta U, Singh C, Malhotra R, Khadgawat R. Insulin Autoimmune Syndrome - A Case Series. Eur Endocrinol. 2020 Oct;16(2):168-171. doi: 10.17925/EE.2020.16.2.168. Epub 2020 Oct 6. PMID: 33117451; PMCID: PMC7572167.
6. 6. Boro H, Gupta U, Singh C, Malhotra R, Khadgawat R. Continuous glucose monitoring and Rituximab treatment in insulin autoimmune syndrome. Diabetes Metab Syndr. 2021 Nov-Dec;15(6):102294. doi: 10.1016/j.dsx.2021.102294. Epub 2021 Sep 20. PMID: 34562871.
7. 7. Censi S, Mian C, Betterle C. Insulin autoimmune syndrome: from diagnosis to clinical management. Ann Transl Med. 2018 Sep;6(17):335. doi: 10.21037/atm.2018.07.32. PMID: 30306074; PMCID: PMC6174196.
8. 8. Lidar M, Braf A, Givol N, Langevitz P, Pauzner R, Many A, Livneh A. Anti-insulin antibodies and the natural autoimmune response in systemic lupus erythematosus. Lupus. 2001;10(2):81-6. doi: 10.1191/096120301669081314. PMID: 11237130.

Ectopic Cushing's Syndrome: seaCRHing for the cause

Raquel Maggacis[1], Anish Menon[1], Warrick Inder[1], Emily Mackenzie[1]

1. Princess Alexandra Hospital, Woolloongabba, QLD, Australia

A 79 year-old gentleman was admitted from medical oncology outpatients with severe hypokalaemia to 2.3 mmol/L (3.5-5.0) with an associated metabolic alkalosis identified on routine outpatient pathology. Also identified was new hyperglycaemia, glucose 16.5 mmol/L (3.0-7.7), and an elevated early morning cortisol of 1380 nmol/L (190-610).

His background was significant for metastatic squamous cell carcinoma of the right leg with right groin nodes and pulmonary nodules. He had been commenced on neoadjuvant pembrolizumab earlier that year as part of a trial. His other medical history included chronic obstructive pulmonary disease, hypertension, infective endocarditis, peripheral vascular disease, venous insufficiency and an ex-smoker with a 160 pack year history. His regular medications included atorvastatin 40mg nocte, folic acid 5mg mane, magnesium 1g nocte, Olmesartan/hydrochlorothiazide 40/12.5mg mane, potassium chloride modified release 1200mg mane (started the month prior), pregabalin 150mg BD, thiamine 100mg mane and tiotropium/olodaterol 2.5mcg/2.5mcg. He was retired and lived alone.

In the setting of new-onset hypokalaemia, hypercortisolism and hyperglycaemia, an Endocrine consult was sought. Assessment was significant for easy bruising; there was no weight gain, recurrent infection or mood change. He reported developing bilateral lower limb oedema from approximately 2 weeks' prior to admission, on the left lower limb to the knee and the right lower limb to the groin. Clinically there were no moon facies, there were mild dorsocervical and supraclavicular fat pads, central adiposity without abdominal striae and bruising to arms and legs. He had proximal weakness with hip flexion 3/5 bilaterally. He was commenced on spironolactone 50mg daily, quickly increased to 50mg twice daily, telmisartan 80mg daily and planned for further testing to screen for Cushing's syndrome. Biochemistry indicated marked cortisol excess with 24 hour urinary free cortisol 13600 nmol/24 h (10-165) and late night salivary cortisol 76 nmol/L (<5). A high dose 4mg intravenous dexamethasone suppression test showed clear evidence of Cushing's syndrome, with cortisol increasing from 1930 nmol/L to 3620 nmol/L on day 2, and no suppression seen on day 1. ACTH (adrenocorticotropic hormone) was inappropriately normal at 46 ng/L (10-50), consistent with ACTH-dependent Cushing's syndrome. Of note, urine biogenic amines were negative.

An MRI (magnetic resonance imaging) pituitary was performed and, in keeping with the hypothesis of ectopic ACTH-dependent Cushing's syndrome, did not indicate any evidence of pituitary adenoma or intracranial metastasis. A whole body fluorodeoxyglucose (FDG)-positron emission tomography (PET) was consistent with disease progression, showing increased size of the known pulmonary, iliac and inguinal nodal metastases (Image 1).

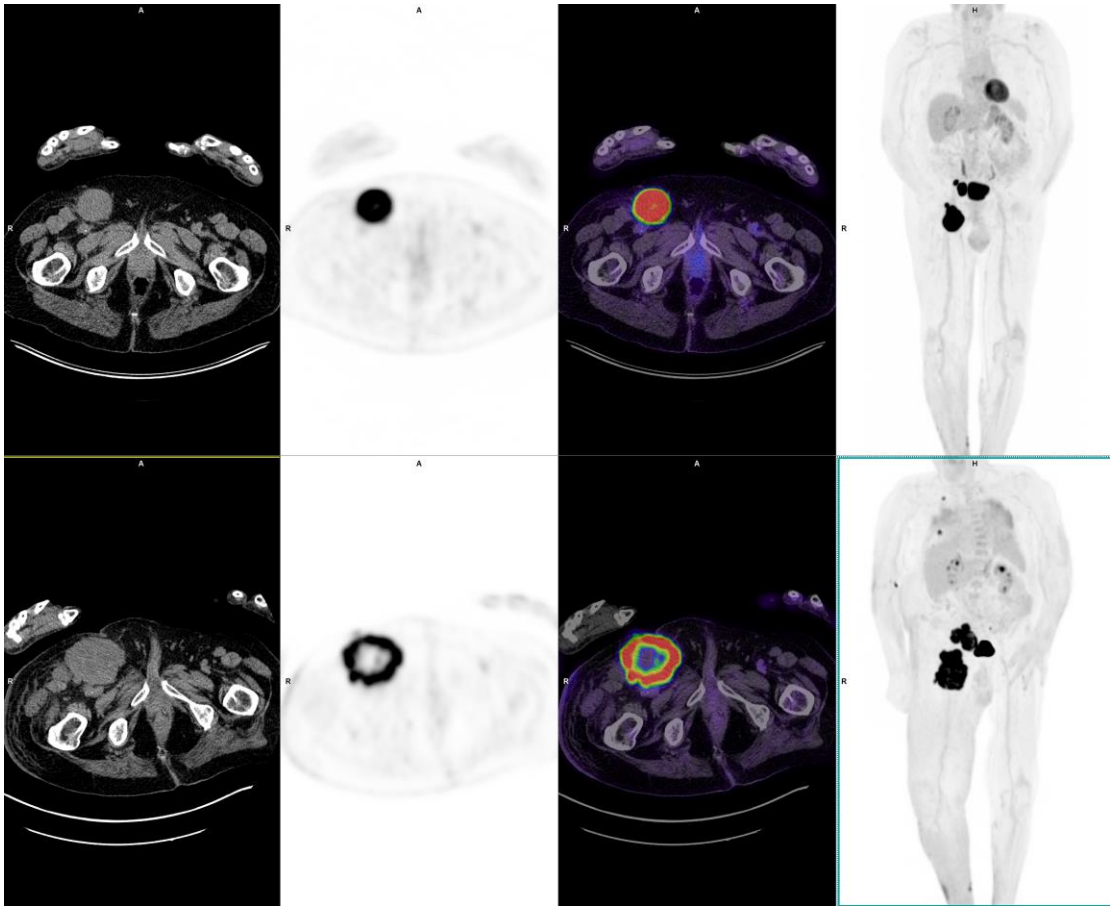
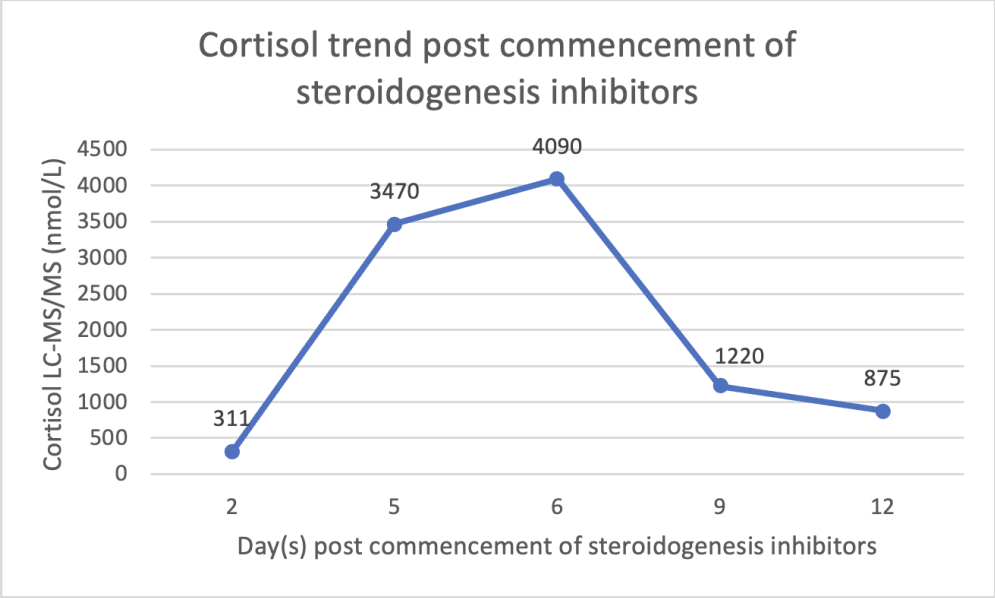


Image 1: FDG-PET/CT showing increased size and central photopenia suggestive of necrosis comparing July 2022 (top row; 66x55mm) and September 2022 (bottom row; 94x86mm), showing increased. There is increased compression of the right proximal external iliac vein by the iliac metastases.

Medical management of Cushing's syndrome was implemented, including ketoconazole 200mg TDS, metyrapone 500mg TDS and mitotane 500mg TDS. This allowed for reduction in spironolactone to 50mg once daily and saw near-normalisation of his 24 hour urinary free cortisol. Two weeks after commencement, metyrapone was increased to 1g TDS; mitotane and ketoconazole were continued at the same dose. Ultimately there was never adequate suppression of hypercortisolism. The trend in LC-MS/MS cortisol can be seen in table 1.



Radiation therapy to the right groin and pelvic lymph nodes was commenced in an effort to control the hypercortisolism. To confirm the diagnosis, ACTH immunohistochemistry was arranged on prior core biopsies obtained of the right inguinal squamous cell carcinoma metastatic deposits; ACTH immunohistochemistry was negative. He underwent a ^{68}Gallium DOTATATE PET, which showed hypertrophic adrenal glands with moderately increased uptake; there was no intense avidity identified elsewhere on the DOTATATE PET as a potential cause for hypercortisolism.

Unfortunately, the patient clinically deteriorated rapidly and his family opted for palliation. He passed away peacefully. ACTH immunohistochemistry was then arranged on a previous biopsy of a right upper lobe metastatic deposit,; again, ACTH immunohistochemistry was negative.

Given this, in consultation with Professor Roger Smith's laboratory at the Hunter Medical Research Institute, University of Newcastle, analysis of plasma corticotrophin releasing hormone (CRH) was undertaken using radioimmunoassay(1). Due to logistical reasons, tissue immunohistochemistry for CRH was not able to be performed. The plasma CRH concentration was grossly elevated at 66.3 pg/mL, checked against three different dilutions; CRH levels in healthy males using a similar assay were typically 2-3 pmol/L (9-14 ng/ml)(2). Thus, the final diagnosis was CRH-mediated ectopic Cushing's syndrome secondary to metastatic squamous cell carcinoma.

Discussion

Corticotrophin-releasing hormone-like activity as a potential mechanism for the development of ectopic ACTH-dependent Cushing's syndrome was in 1971 after studying peptides extracted from two patients, one with a pancreatic neuroendocrine tumour and the other with lung oat (small) cell carcinoma(3). The first case of ectopic CRH secretion was in 1984, identified in a patient with ACTH-dependent Cushing's syndrome in the setting of metastatic prostate cancer, including to the hypothalamus; post-mortem tumour immunohistochemistry was negative for ACTH but positive for CRH(4). This was subsequently described post-mortem in case studies of patients with an intrasellar gangliocytoma and metastatic medullary thyroid carcinoma(5). Phaeochromocytomas may also secrete corticotrophin-releasing hormone, as described in a 2020 meta-analysis, usually presenting with clinical Cushing's syndrome(6).

A literature review conducted in 2019 identified 75 cases of Cushing's syndrome secondary to ectopic CRH production from 1971-2018, ACTH and CRH co-secreting tumours most commonly originated from bronchial carcinoid tumour, thymic carcinoid tumour, pancreatic tumour, phaeochromocytoma and medullary thyroid cancer(3). Considering tumours that secreted CRH only, medullary thyroid carcinoma and phaeochromocytoma were most common(3, 5). A 2010 literature review identified Cushing's syndrome secondary to CRH-only secretion in 20 cases total, with other primary malignancies including prostate cancer, small cell lung carcinoma, small cell carcinoma (occult primary) and carcinoid tumour(5).

A case study from the Bethesda Institute of a 21 year-old patient with ectopic ACTH and CRH co-secretion from a thymic neuroendocrine tumour showed that ^{68}Ga-DOTATATE PET/CT was assistive for diagnosis, however FDG PET/CT was non-diagnostic, similar to this case(7).

To our knowledge, this is the first case of ectopic CRH-mediated Cushing's syndrome secondary to metastatic squamous cell carcinoma with documented elevated CRH levels, though it has previously been postulated(8). Ectopic CRH production presents a particular diagnostic challenge in dynamic endocrine testing. As CRH stimulates pituitary ACTH production, cases may respond

similarly to Cushing's disease to high dose dexamethasone tests, CRH stimulation tests and bilateral inferior petrosal sinus sampling, thus potentially leading to false results, when the diagnosis might not be straightforward at the outset(9). CRH levels and or tissue immunohistochemistry might be useful in such circumstances. Control of hypercortisolaemia was not achieved prior to the patient's passing; there is burgeoning evidence for the efficacy of the 11-beta hydroxylase inhibitor osilodrostat in ectopic Cushing's syndrome for rapid control of hypercortisolism(10).

Take Home Messages

1. Ectopic Cushing's syndrome is clinically aggressive and requires prompt detection and treatment
2. CRH-mediated Cushing's syndrome is a rare but important entity of which to be cognisant, particularly in the setting of diagnostic pitfalls and risk of false positives when undertaking endocrine dynamic testing to differentiate between Cushing's disease and ectopic Cushing's syndrome.
3. Implementing a "triple therapy" steroidogenesis blocking regimen with replacement glucocorticoids can achieve control of hypercortisolaemia. There is growing evidence for the consideration of osilodrostat for targeted, rapid control of hypercortisolism.

References

1. 1. Smith R, Smith JI, Shen X, Engel PJ, Bowman ME, McGrath SA, et al. Patterns of Plasma Corticotropin-Releasing Hormone, Progesterone, Estradiol, and Estriol Change and the Onset of Human Labor. The Journal of Clinical Endocrinology & Metabolism. 2009;94(6):2066-74.
2. 2. Inder WJ, Hellemans J, Ellis MJ, Evans MJ, Livesey JH, Donald RA. Elevated basal adrenocorticotropin and evidence for increased central opioid tone in highly trained male athletes. The Journal of Clinical Endocrinology & Metabolism. 1995;80(1):244-8.
3. 3. Nakhjavani M, Amirbaigloo A, Rabizadeh S, Rotondo F, Kovacs K, Ghazi AA. Ectopic cushing's syndrome due to corticotropin releasing hormone. Pituitary. 2019;22(5):561-8.
4. 4. Carey RM, Varma SK, Drake CR, Thorner MO, Kovacs K, Rivier J, et al. Ectopic Secretion of Corticotropin-Releasing Factor as a Cause of Cushing's Syndrome: A Clinical, Morphologic, and Biochemical Study. N Engl J Med. 1984;311(1):13-20.
5. 5. Shahani S, Nudelman RJ, Nalini R, Kim HS, Samson SL. Ectopic corticotropin-releasing hormone (CRH) syndrome from metastatic small cell carcinoma: a case report and review of the literature. Diagn Pathol. 2010;5:56.
6. 6. Elliott PF, Berhane T, Ragnarsson O, Falhammar H. Ectopic ACTH- and/or CRH-Producing Pheochromocytomas. J Clin Endocrinol Metab. 2021;106(2):598-608.
7. 7. 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.
8. 8. Boon ES, Leers MP, Tjwa MK. Ectopic Cushing's syndrome in a patient with squamous cell carcinoma of the lung due to CRF-like production. Monaldi Arch Chest Dis. 1994;49(1):19-21.
9. 9. Young J, Deneux C, Grino M, Oliver C, Chanson P, Schaison G. Pitfall of Petrosal Sinus Sampling in a Cushing's Syndrome Secondary to Ectopic Adrenocorticotropin-Corticotropin Releasing Hormone (ACTH-CRH) Secretion. The Journal of Clinical Endocrinology & Metabolism. 1998;83(2):305-8.
10. 10. 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-L5.

Broken bones, miserable moans, and unfortunate unknowns

Tomasz J Block[1, 2], Michael JW Thompson[1]

1. Department of Diabetes and Endocrinology, Royal Hobart Hospital, Hobart, TAS, Australia

2. Department of Diabetes, Monash University, Melbourne, VIC, Australia

Case Summary

A 62-year-old female presented to a new specialist for review of profound skeletal fragility with numerous fractures despite 5 years of potent antiresorptive and osteoanabolic treatment. She was initially diagnosed with severe osteoporosis at age 58, when she presented with 12-months of persistent lumbar, hip, and bilateral foot pains associated with prominent myalgias. Whole body bone scan with SPECT CT demonstrated several minimal trauma vertebral, rib, pelvic and metatarsal fractures. Initial BMD demonstrated a T-score of -3.3 at the lumbar spine (0.779 g/cm^2) and -2.9 at the femoral neck (0.655 g/cm^2). She was an ex-smoker with a 40 pack-year history and had a BMI of 14.6 kg/m^2. There were no syndromic features, nor any history of early onset skeletal fragility or family history of minimal trauma fractures. There were no other significant risk factors for osteoporosis.

Investigation for secondary causes of skeletal fragility included a normal FBC, tryptase, TFT, PTH, HbA1c, corrected calcium and renal function. ALP was 133 U/L (N 30-115). Other LFTs were normal and 25-hydroxyvitamin D was 68 nmol/L. Myeloma assessment, inflammatory & autoimmune markers and 24-hour urinary free cortisol were normal.

Initial treatment comprised optimising dietary calcium, vitamin D supplementation and denosumab 60 mg 6-monthly. However, over the next 12-months her myalgias continued and resulted in progressive disability. Repeat bone scan demonstrated new focal lesions in the left clavicle, left rib cage, bilateral ulnar and right radius, with progressive uptake in bilateral femoral heads. Oncologist review and staging CT of the chest, abdomen, and pelvis, ^{18}FDG-PET, bilateral mammography, pap smear, skin check and colonoscopy did not reveal any signs of malignancy. Bilateral hip MRI demonstrated a 12-mm lesion in the right acetabulum of indeterminate appearance, lacking FDG or scintigraphic uptake. She was subsequently treated with 18-months of teriparatide and then continued regular denosumab. Despite an improvement in BMD with a lumbar spine T-score of -1.7 (+0.22 g/cm^2, +28%) and -2.4 at the hip (+0.065 g/cm^2, +10%) within 4 years, she continued to experience numerous minimal/no trauma fractures, one of which required a right dynamic hip screw.

Upon referral to an endocrinologist, it was noted she had an acquired and persistent moderate to severe hypophosphatemia (~0.40 mmol/L) over the last 5 years, with a normal serum phosphate 10 years prior. Renal phosphate wasting was demonstrated by a markedly reduced tubular maximum reabsorption of phosphate to glomerular filtration rate (TmP/GFR) of 0.29 (N >0.84) without evidence of a proximal tubulopathy with normal urine glucose and plasma bicarbonate. 1,25 dihydroxyvitamin D was 74 (N 50-190), and IGF-1 and iron studies were normal. FGF23 was elevated at 310 ng/L (N 23.2 - 95.4). A subsequent ^{68}Ga-DOTA PET/CT demonstrated an avid focus in the right acetabulum, corresponding to a 13mm well-circumscribed lucent and stable lesion on CT, present on previous MRI studies. A diagnosis of tumour-induced osteomalacia (TIO) due to a presumed benign FGF-23 producing phosphaturic mesenchymal tumour was made.

She was commenced on phosphate and calcitriol with rapid improvements in muscle strength and reduced myalgias. Serum phosphate increased from 0.37 to 0.78 mmol/L (N 0.8-1.5) within 4 weeks. Currently, she is awaiting an orthopaedic opinion regarding potential resection of the lesion, which may be complicated due to existing metalware in the affected joint. Burosumab via compassionate access is being arranged if definitive treatment is not viable.

Tumour Induced Osteomalacia

TIO is a rare acquired paraneoplastic syndrome characterised by overproduction of FGF23 by ectopic phosphaturic mesenchymal tumours of the mixed connective tissue type. (1) FGF23 is produced by osteocytes and is the principal regulator of phosphate homeostasis. It acts at the proximal renal tubule to reduce expression of the sodium phosphate cotransporters NaPi-2a/2c causing decreased tubular phosphate reabsorption. FGF23 also inhibits expression of 1-alpha-hydroxylase in the proximal tubules, leading to reduced concentration of calcitriol and subsequent decreased intestinal phosphate and calcium absorption. (2) The resulting hypophosphatemia, phosphaturia, and low or inappropriately normal concentration of calcitriol, lead to muscle weakness, bone pain, osteomalacia, and ultimately fragility fractures. (3) The occult nature, small size, slow growth, and often obscure anatomical location of an underlying tumour leads to an average delay from symptom onset to diagnosis of 2.9 ± 2.3 years, resulting in skeletal deformities and severe disability. (4)

Once TIO is suspected on biochemical and clinical abnormalities, successful tumour localisation is best performed utilising whole-body ^{68}Ga-DOTA PET/CT imaging, which demonstrates the highest sensitivity and specificity. (5) After successful detection of neoplastic lesions with functional imaging, precise localisation is performed using anatomical imaging typically involving CT or MRI. Complete tumour resection is the only curative treatment for TIO. This results in prompt reversal of the biochemical abnormalities over days and remineralisation of affected bone over 12 months. (6) In cases of incompletely resected tumours, adjuvant radiotherapy has been successfully used in a few patients (7). If tumour localisation or resection is not possible, conventional medical treatment is with oral phosphate and calcitriol supplementation. The aim is to increase serum phosphate to the lower limit of the age-appropriate normal range, normalise ALP, and maintain PTH within the normal range. However, sustained adherence to this therapy is often poor due to treatment burden and gastrointestinal side effects. Major long-term complications include nephrocalcinosis, nephrolithiasis, and secondary/tertiary hyperparathyroidism. (8)

Burosumab is a novel humanised monoclonal antibody against FGF23. It has demonstrated efficacy in two small trials and corrects the biochemical and radiological abnormalities, and physical symptoms associated with osteomalacia in TIO. (9, 10) It is currently PBS listed for X-linked hypophosphataemia, but not for TIO, making access difficult.

Take Home Points

- In a patient with multiple/unusual minimal trauma fractures despite potent osteoporosis therapy, it is imperative to consider rarer causes.
- Persistently low serum phosphate concentration in a patient with fragility fractures should prompt investigation for osteomalacia, FGF-23 excess, and renal phosphate wasting.
- The diagnosis of tumour-induced osteomalacia is often delayed by several years resulting in significant skeletal deformities and severe disability.
- ^{68}Ga-DOTA PET/CT is the most sensitive and specific imaging modality to detect mesenchymal tumours hypersecreting FGF23.
- Tumour resection affords curative treatment, however burosumab has demonstrated effectiveness in small trials and avoids the problems with phosphate and calcitriol supplementation.

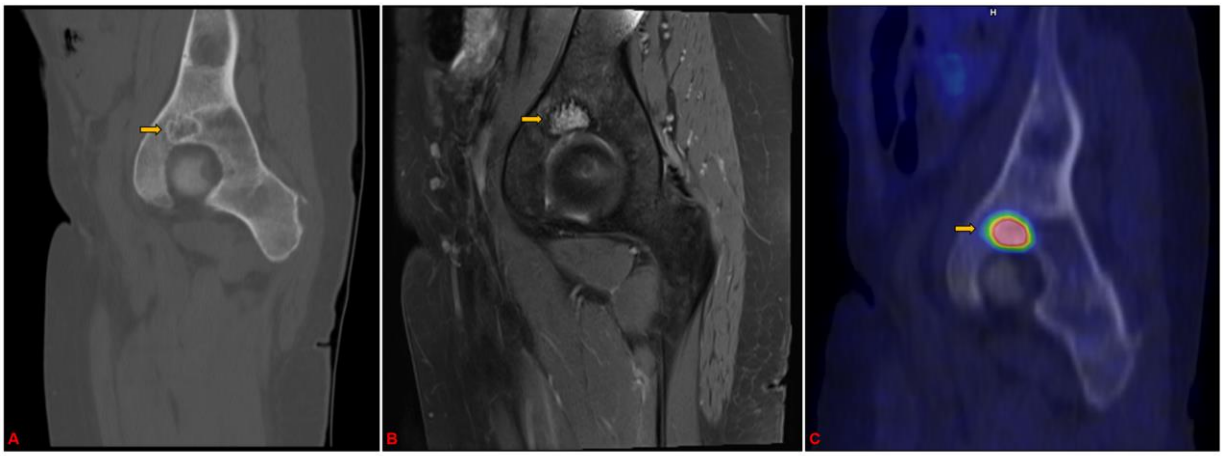


Figure 1. Presumed 13 mm phosphaturic mesenchymal tumour in the right acetabulum with sagittal views. **A:** Computed tomography. **B:** Fat suppressed proton density weighted magnetic resonance imaging. **C:** Gallium-68 DOTATATE positron emission tomography.

References

1. Folpe AL, Fanburg-Smith JC, Billings SD, Bisceglia M, Bertoni F, Cho JY, et al. Most osteomalacia-associated mesenchymal tumors are a single histopathologic entity: an analysis of 32 cases and a comprehensive review of the literature. Am J Surg Pathol. 2004;28(1):1-30.
2. Shimada T, Hasegawa H, Yamazaki Y, Muto T, Hino R, Takeuchi Y, et al. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. J Bone Miner Res. 2004;19(3):429-35.
3. Jonsson KB, Zahradnik R, Larsson T, White KE, Sugimoto T, Imanishi Y, et al. Fibroblast growth factor 23 in oncogenic osteomalacia and X-linked hypophosphatemia. N Engl J Med. 2003;348(17):1656-63.
4. Florenzano P, Hartley IR, Jimenez M, Roszko K, Gafni RI, Collins MT. Tumor-Induced Osteomalacia. Calcif Tissue Int. 2021;108(1):128-42.
5. Zhang J, Zhu Z, Zhong D, Dang Y, Xing H, Du Y, et al. 68Ga DOTATATE PET/CT is an Accurate Imaging Modality in the Detection of Culprit Tumors Causing Osteomalacia. Clin Nucl Med. 2015;40(8):642-6.
6. Chong WH, Andreopoulou P, Chen CC, Reynolds J, Guthrie L, Kelly M, et al. Tumor localization and biochemical response to cure in tumor-induced osteomalacia. J Bone Miner Res. 2013;28(6):1386-98.
7. Mishra SK, Kuchay MS, Sen IB, Garg A, Baijal SS, Mithal A. Successful Management Of Tumor-Induced Osteomalacia with Radiofrequency Ablation: A Case Series. JBMR Plus. 2019;3(7):e10178.
8. Huang QL, Feig DS, Blackstein ME. Development of tertiary hyperparathyroidism after phosphate supplementation in oncogenic osteomalacia. J Endocrinol Invest. 2000;23(4):263-7.
9. Jan de Beur SM, Miller PD, Weber TJ, Peacock M, Insogna K, Kumar R, et al. Burosumab for the Treatment of Tumor-Induced Osteomalacia. J Bone Miner Res. 2021;36(4):627-35.
10. Imanishi Y, Ito N, Rhee Y, Takeuchi Y, Shin CS, Takahashi Y, et al. Interim Analysis of a Phase 2 Open-Label Trial Assessing Burosumab Efficacy and Safety in Patients With Tumor-Induced Osteomalacia. J Bone Miner Res. 2021;36(2):262-70.

An unexpected turn of events in the complex obesity clinic

Alexandra Matthews[1], Benjamin Ong[2], Matthew Lumchee[1], Jack Lockett[1, 3]

1. Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

2. Department of Medical Imaging, Princess Alexandra Hospital, Brisbane, Queensland, Australia

3. PA-Southside Clinical Unit and Mater Research Institute-UQ, Faculty of Medicine, University of Queensland, Brisbane, Queensland, Australia

Case

A 55-year-old female was referred to the complex obesity clinic for weight management: weight 170kg, body mass index (BMI) 61kg/m2. Background medical history included type 2 diabetes mellitus treated with metformin and insulin glargine (HbA1c 6.6%), obstructive sleep apnoea (on CPAP), dyslipidaemia, hypertension, asthma, gastro-oesophageal reflux disease and primary hypothyroidism. Semaglutide was commenced and insulin substituted with empagliflozin, to assist with weight loss. The patient achieved weight loss of 70kg in ten months, however HbA1c worsened to 8.5% and hypokalaemia developed. There was a history of peripheral oedema and of recurrent vaginal candidiasis. There was no history of exogenous glucocorticoid exposure or alcohol intake. The patient was post-menopausal and there was no history of fracture, with no prior bone mineral density scan performed. She did not have mood disturbance.

Physical examination yielded BMI of 37kg/m2 with truncal obesity and mild facial hirsutism. Blood pressure was elevated at 150/70mmHg. There was no appreciable facial rounding or plethora, striae, bruising, proximal myopathy, peripheral oedema nor skin changes.

Investigation for Cushing's syndrome (CS) was undertaken given the clinical history. A 1mg dexamethasone suppression test (DST) failed to suppress with cortisol decrementing from 500nmol/L to 230nmol/L (<50nmol/L), baseline adrenocorticotropic hormone (ACTH) 3.6pmol/L (1.1-11.1pmol/L), and 24hr urinary free cortisol (UFC) was elevated twice the upper limit of normal at 214nmol/24h (10-120nmol/24h). A 4mg intravenous DST and corticotrophin-releasing hormone (CRH) stimulation test were both consistent with a pituitary source of ACTH (Table 1).

Initial magnetic resonance imaging (MRI) pituitary demonstrated partial empty sella with no adenoma identified, however motion artifact impacted interpretation. Inferior petrosal sinus sampling (IPSS) was consistent with Cushing's disease (CD), with exceptionally high central-to-peripheral ACTH gradient post-CRH administration (Table 2). A DOTATATE-PET/MRI was completed to exclude an ectopic source of CRH production, with no neuroendocrine tumour identified, and computed tomography yielded only slightly bulky adrenal glands. Repeat MRI pituitary, using recently described sequences more sensitive for corticotroph lesions, demonstrated a 2.5mm hypointense focus in the right lateral pituitary on T1 VIBE sequence, suggestive of an adenoma.

While awaiting localisation and further management, medical therapy with metyrapone was commenced. This was limited by hirsutism and inadequate hypercortisolism control and therefore the patient was switched to osilodrostat, commencing at 2mg twice daily. Osilodrostat dosage was titrated every 3 weeks based on UFC and serum cortisol results. After 16 weeks, the UFC normalised to 106nmol/24h, with a morning cortisol of 327nmol/L on 4mg mane, 5mg nocte. The osilodrostat has been well tolerated, with resolution of hirsutism, and no reported side-effects.

The most recent MRI pituitary with supplementary sequences, performed six months after commencement of osilodrostat, has demonstrated a 4mm lesion in the right inferior pituitary tissue suggestive of a small pituitary adenoma. Given she was thought to be high risk from a neurosurgical perspective, after discussion at the pituitary multi-disciplinary team (MDT) meeting, Gamma Knife stereotactic radiosurgery to the right-half of the sella was recommended. This was recently administered with a plan for repeat MRI pituitary in six months.

Discussion

CS remains one of the most challenging endocrine conditions to recognise and diagnose. Many of the clinical features associated with hypercortisolism overlap with common medical conditions in the general population (e.g. obesity, hypertension and diabetes mellitus). It is important to note that there is no single "typical" presentation for CS and some patients have atypical presentations with only isolated signs or symptoms. The clinical features vary and depend on the duration and severity of hypercortisolism, along with the individual patient's sensitivity to excessive cortisol (1). Recent onset of hypertension, glucose intolerance or diabetes mellitus are typical presenting symptoms. Furthermore, suspicion should be raised if there is worsening control of pre-existing diabetes mellitus or hypertension, as was seen in this patient's case (1). This patient did not possess any of the clinical features with the highest discriminatory index for CS, including proximal myopathy, plethora, violaceous striae, easy bruising or thin skin (2).

After confirmation of ACTH-dependent CS, as was demonstrated in this case, localisation of the source can be challenging due to the often occult nature of corticotroph and neuroendocrine tumours. MRI is the imaging modality of choice for detecting ACTH-producing pituitary microadenomas, however no pituitary lesion is seen on MRI in up to 50% of cases (3). The development and widespread utilisation of 3 Tesla MRI (3T MRI) has resulted in much higher tumour detection rates (4). Alternative MR protocols may allow detection of tumours that have not been previously identified. One of these is T1-weighted gadolinium enhanced 3D-spoiled gradient echo (3D-SGE) MRI which allows for much better soft tissue contrast definition, with a reported detection rate for corticotroph tumours of 80-90% (3). Additional sequences that may be helpful are FLAIR with gadolinium enhancement (to

detect delayed contrast washout in an adenoma), along with isotropic 3D-fast spin echo (e.g. SPACE, VIBE) that produce high resolution 3D images.

Functional imaging may be an option if imaging remains indeterminate, with several radioligands having been used with some success, such as 68Ga (-DOTA)-CRH PET. Consultation with an expert neuroradiologist, with support from a pituitary MDT, is recommended (3). In this case, supplementary MR sequences utilised to assist with localisation of the adenoma included 3D-T2 FLAIR, T1 VIBE and T2 SPACE.

The decision to pursue surgery for MRI-negative or MRI-inconclusive CD remains highly controversial. Endoscopic endonasal transsphenoidal surgery (ETSS) is the surgical management of choice (5). Overall, ETSS performed by an expert neurosurgeon does appear to have a good prognosis in patients with MRI-negative CD (5). There is a reported short and long-term remission rate of 93% and 80%, respectively, in patients with MRI-negative or inconclusive disease who undergo ETSS (6). Other second-line treatment options include bilateral adrenalectomy, radiotherapy and medical treatments (1).

CS is associated with major morbidity and mortality and medical therapy to treat the hypercortisolism may be required in select cases. Patients with CD potentially warranting medical therapy include those who are not surgical candidates, patients with persistent/recurrent disease post-operatively and those who require pre-operative optimisation prior to resection of the causative lesion (7). Osilodrostat is a new medical therapy available to treat hypercortisolemia. It is an 11β-hydroxylase and aldosterone synthase inhibitor that is effective at controlling hypercortisolism and carries an acceptable safety profile (8). The most common side-effects are features of hyperandrogenism in females (including acne and hirsutism) and features of mineralocorticoid excess owing to an increase in aldosterone and cortisol precursors (7), however these may be lower than in metyrapone due to apparent inhibition of 17α-hydroxylase and 21-hydroxylase (9). The control of hypercortisolism is more rapid than metyrapone (10), and careful monitoring is required to avoid hypoadrenalism. The recommended starting dose is 2mg twice daily and should be slowly uptitrated in 1-2mg increments(7). Osilodrostat has been approved by the Therapeutic Goods Administration in 2023 for treatment of endogenous CS in adults.

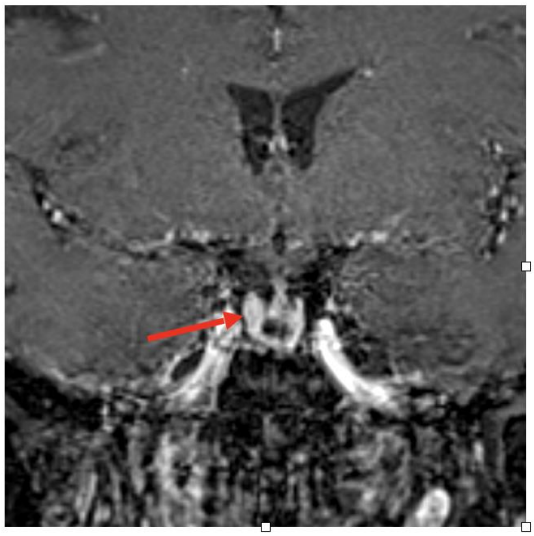
Take-home messages:

1. CS is a challenging diagnosis with a broad variation in clinical presentation.

1. In ACTH-dependent CS, localisation of the source of ACTH can be exceedingly difficult.

1. New MRI sequences available for inconclusive or MRI-negative CD can improve the detection rate of corticotroph adenomas and expert neuroradiologist advice should be sought.

1. Osilodrostat is a new medical therapy for endogenous CS that is effective at controlling hypercortisolism and carries an acceptable safety profile.



The most recent MRI demonstrating a 4mm lesion in the right inferior pituitary suggestive of a small adenoma on the coronal thin slice T1 VIBE scan

Table 1

Intravenous 4mg Dexamethasone Suppression Test (DST)		
Time	**Cortisol (nmol/L)**	
Day 1		
- 60 minutes (basal, 08:30)	436	
- 5 minutes	341	
0 minutes	*Intravenous 4mg dexamethasone infusion commenced*	
+ 3 hours	206	
+ 4 hours	106	
+ 5 hours	130	
Day 2		
+ 23.5 hours (09:00)	327	
+ 24 hours (09:30)	373	
Peripheral Corticotrophin-Releasing Hormone (CRH) Test		
Time	**ACTH (ng/L)**	**Cortisol (nmol/L)**
- 5 minutes	20	439
- 1 minute	18	371
0 minutes	*Intravenous CRH administered*	
15 minutes	240	859
30 minutes	230	1090
45 minutes	180	1490
60 minutes	130	1470
90 minutes	67	1150

For intravenous 4mg DST, Day 2 serum cortisol level (mean of +23.5h and +24h cortisol values) >130 nmol/L or >20% of baseline cortisol (Day 1 at -60 minutes) is consistent with Cushing's Syndrome. Cushing's disease tends to partially suppress on Day 1 with rebound increase on Day 2. For CRH test, peak ACTH increment of > 50% from mean basal values or increase in peak cortisol concentration > 30% from mean basal values are consistent with Cushing's disease.

Table 2

Inferior Petrosal Sinus Sampling (IPSS)								
Time	**ACTH (ng/L)**					**Prolactin (mU/L)**		
	Peripheral	*Left*	*Left: Peripheral ratio*	*Right*	*Right: Peripheral ratio*	*Peripheral*	*Left*	*Right*
-5 minutes	23	810	35	790	34	201	7205	3392
-2 minutes	21	1400	67	990	47	195	6020	3094
0 minutes	*IV CRH administered*							
+2 minutes	22	25035	1138	27900	1268	196	4419	3590
+5 minutes	63	27599	438	21653	344	189	7442	6392
+10 minutes	140	14383	103	220	2	233	6838	260
+15 minutes	200	10000	50	240	1	356	5019	275

A central to peripheral ACTH ratio of ≥ 2 pre-CRH and/or ratio of ≥ 3 post-CRH is consistent with Cushing's disease.

References

1. Fleseriu, M., Auchus, R., Bancos, I., Ben-Shlomo, A., Bertherat, J., Biermasz, N. R., Boguszewski, C. L., Bronstein, M. D., Buchfelder, M., Carmichael, J. D., Casanueva, F. F., Castinetti, F., Chanson, P., Findling, J., Gadelha, M., Geer, E. B., Giustina, A., Grossman, A., Gurnell, M., … Karavitaki, N. (2021). Consensus on diagnosis and management of Cushing's disease: a guideline update. The Lancet. Diabetes & Endocrinology, 9(12), 847–875. https://doi.org/10.1016/S2213-8587(21)00235-7
2. Nieman, L. K., Biller, B. M. K., Findling, J. W., Newell-Price, J., Savage, M. O., Stewart, P. M., & Montori, V. M. (2008). The Diagnosis of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. The Journal of Clinical Endocrinology and Metabolism, 93(5), 1526–1540. https://doi.org/10.1210/jc.2008-0125
3. Bashari, W. A., Gillett, D., MacFarlane, J., Powlson, A. S., Kolias, A. G., Mannion, R., Scoffings, D. J., Mendichovszky, I. A., Jones, J., Cheow, H. K., Koulouri, O., & Gurnell, M. (2022). Modern imaging in Cushing's disease. Pituitary, 25(5), 709–712. https://doi.org/10.1007/s11102-022-01236-w

4. Erickson, D., Erickson, B., Watson, R., Patton, A., Atkinson, J., Meyer, F., Nippoldt, T., Carpenter, P., Natt, N., Vella, A., & Thapa, P. (2010). 3 Tesla magnetic resonance imaging with and without corticotropin releasing hormone stimulation for the detection of microadenomas in Cushings syndrome. Clinical Endocrinology (Oxford), 72(6), 793–799. https://doi.org/10.1111/j.1365-2265.2009.03723.x
5. Cristante, J., Lefournier, V., Sturm, N., Passagia, J. G., Gauchez, A. S., Tahon, F., Cantin, S., Chabre, O., & Gay, E. (2019). Why We Should Still Treat by Neurosurgery Patients with Cushing Disease and a Normal or Inconclusive Pituitary MRI. The Journal of Clinical Endocrinology and Metabolism, 104(9), 4101–4113. https://doi.org/10.1210/jc.2019-00333
6. Sharifi, G., Amin, A. A., Sabahi, M., Echeverry, N. B., Dilmaghani, N. A., Mousavinejad, S. A., Valizadeh, M., Davoudi, Z., Adada, B., & Borghei-Razavi, H. (2022). MRI-negative Cushing's Disease: Management Strategy and Outcomes in 15 Cases Utilizing a Pure Endoscopic Endonasal Approach. BMC Endocrine Disorders, 22(1), 154–154. https://doi.org/10.1186/s12902-022-01069-5
7. Perosevic, M., & Tritos, N. A. (2023). Clinical Utility of Osilodrostat in Cushing's Disease: Review of Currently Available Literature. Drug Design, Development and Therapy, 17, 1303–1312. https://doi.org/10.2147/DDDT.S315359
8. Gadelha, M., Bex, M., Feelders, R. A., Heaney, A. P., Auchus, R. J., Gilis-Januszewska, A., Witek, P., Belaya, Z., Yu, Y., Liao, Z., Ku, C. H. C., Carvalho, D., Roughton, M., Wojna, J., Peoncelli, A. M., & Snyder, P. J. (2022). Randomized Trial of Osilodrostat for the Treatment of Cushing Disease. The Journal of Clinical Endocrinology and Metabolism, 107(7), E2882–E2895. https://doi.org/10.1210/clinem/dgac178
9. Bonnet-Serrano, F., Poirier, J., Vaczlavik, A., Laguillier-Morizot, C., Blanchet, B., Baron, S., Guignat, L., Bessiene, L., Bricaire, L., Groussin, L., Assié, G., Guibourdenche, J., & Bertherat, J. (2022). Differences in the spectrum of steroidogenic enzyme inhibition between Osilodrostat and Metyrapone in ACTH-dependent Cushing syndrome patients. European Journal of Endocrinology, 187(2), 315–322. https://doi.org/10.1530/EJE-22-0208
10. Detomas, M., Altieri, B., Deutschbein, T., Fassnacht, M., & Dischinger, U. (2022). Metyrapone Versus Osilodrostat in the Short-Term Therapy of Endogenous Cushing's Syndrome: Results From a Single Center Cohort Study. Frontiers in Endocrinology (Lausanne), 13, 903545–903545. https://doi.org/10.3389/fendo.2022.903545

A twisted tale of two thyroid pathologies

Amanda Ji[1], Ashim Sinha[1], Santosh Chaubey[1]

1. Cairns Hospital, Cairns North, QUEENSLAND, Australia

Case summary:

A 47-year-old female was referred to endocrine clinic with two-year history of abnormal thyroid function test. This was identified on her routine blood test with prior tests showing euthyroid. Her symptoms were long-standing fatigue and intermittent palpitations. She has complex medical histories which includes multifocal right breast cancer diagnosed at the age of 34 years with negative BRAF mutation. She was treated with bilateral mastectomy with adjuvant chemoradiotherapy followed by 4 years of tamoxifen therapy. This was complicated by chemotherapy related non-ischaemic dilated cardiomyopathy requiring required ICD defibrillator and pacemaker insertion. She also has fibromyalgia, seronegative arthropathy and anxiety linked to multimorbidity.

On examination, she was normotensive with normal heart rate. Diffuse goitre was noted without any opthalmopathy.

Thyroid function tests demonstrated elevated TSH of 5-7mIU/L (0.3-3.5mIU/L) and free thyroxine (FT4) of 18-23pmol/L since 2021 (Table 1 and Figure1). Free triiodothyronine (FT3) was also elevated at 6-7pmol/L respectively. This was seen with different assays done in another laboratory with TSH of 6.08mU/L (0.55-4.78mU/L) with inappropriately raised FT4 of 26.1 (11.5-22.7pmol/L) and FT3 of 8.1 (3.5-6.5pmol/L). Antibodies to thyroglobulin, thyroid peroxidase, thyroid stimulating immunoglobulin, thyroid receptor antibodies were absent (Table 2). Immunoassay interferences were excluded.

Therefore, initial impression for this presentation was that of central hyperthyroidism due to TSH-secreting adenomas (TSHoma) or resistance to thyroid hormone (RTH). RTH was excluded based on negative family history, previously euthyroid state and negative THRB gene test. On further investigation, SHBG was 58nmol/L (20-110nmol/L) and free alpha glycoprotein subunit was elevated at 5.35IU/L (Premenopausal: 0.00-0.60IU/L, Postmenopausal 0.00-1.30IU/L). Thyrotropin-releasing hormone test was done and was consistent with the diagnosis of TSHoma (Table 3). The remaining anterior pituitary panel were normal apart from raised prolactin level of less than two times upper limit of normal on two occasions (Table 4). Due to patient's claustrophobia and her preference, MRI pituitary could not be performed. CT pituitary with contrast was performed as an alternative which did not identify any pituitary adenoma (Figure 2).

An ultrasound of neck was performed which demonstrated mildly heterogenous thyroid parenchyma with generalised increased vascularity. Thyroid nodules were also seen with the largest nodule of 1.5cm characterised as TIRAD 4 which underwent FNA. The subsequent biopsy of this nodule demonstrated Bethesda category 4, suggestive of a Hurthle cell neoplasm. She underwent hemithyroidectomy in March 2023 with no postoperative complication. The histopathology showed multifocal papillary thyroid carcinoma with a Hurthle cell morphology all confined to lobe with a clear margin and without any lymphovascular invasion (Figure 3).

Thyroid function test done one month post hemithyroidectomy showed her TSH remained elevated at 6.9nmol/L with normal FT4 and FT3. Interestingly, two months post-operation, her TSH had doubled to 12nmol/L but FT4 and FT3levels were still within the normal range.

Her case was discussed at the thyroid cancer multidisciplinary meeting in July 2023. Given she has well differentiated cancer, it was decided that she is not for radioactive iodine treatment but rather continue the observation of the contralateral lobe with serial ultrasound and thyroglobulin level. As her TSH concentration was rising, the outcome of meeting was to commence on levothyroxine therapy.

Table 1: Trend of thyroid function test including FT3

	20/10/20	11/06/21	06/07/21	06/12/21	17/10/22	13/12/22	24/01/23	14/04/23 (Post hemithyroidectomy 15/03/23)	30/06/23 (Post hemithyroidectomy, 15/03/23)	Reference range
TSH	5.5	6.9	4.6	5.5	6.08	5.6	7.01	6.9	12	**0.3-3.5mU/L**
FT4	17.7	16.1	18.8	20.1	26.1	21.6	23.8	17.7	14.6	**10-20pmol/L**
FT3	5.9	6.2	6.1	6.8	8.1	7.2	9.7	5.2	5.3	**0.3-3.5pmol/L**

Table 2: Laboratory results including trend of thyroid antibodies

	Oct 2020	Dec 2020	Jul 2021	Oct 2022	Reference range
Peroxidase Ab	<1	<1	<3	<1/0	<5.6IU/ml
Thyroglobulin Ab	1	1	<3	1.1	<4.1IU/ml
TRAB	<0.9	<0.9	<0.9	<0.9	<1.8IU/L
TSI	<0.1				<0.55IU/L

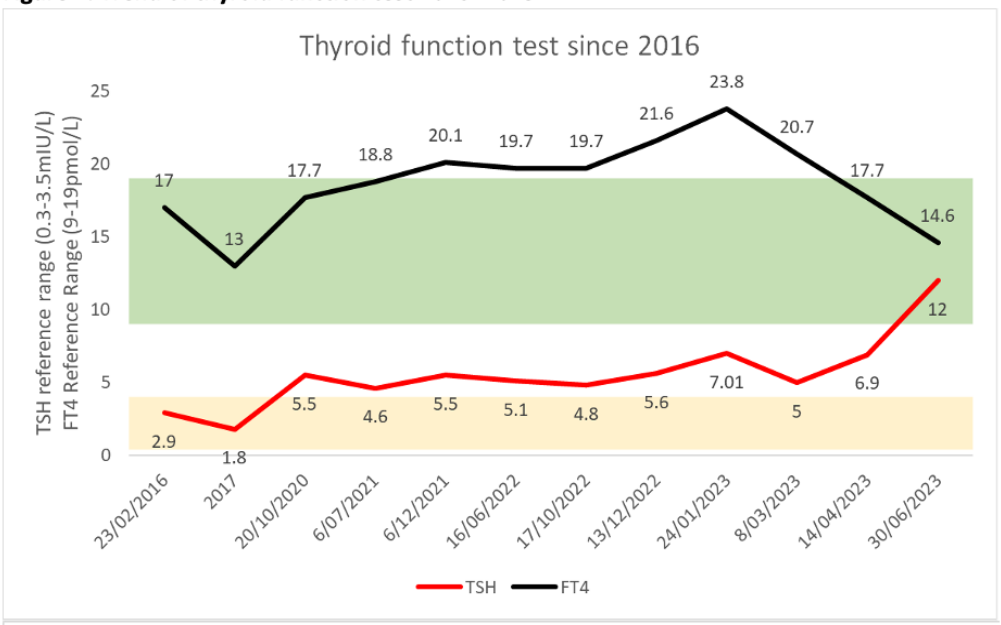
Table 3: Thyrotropin-releasing hormone (TRH) stimulation test (04/05/2023)

	TSH (Atellica)	Reference
Baseline	**21.52**	0.55-4.78mU/L
30mins + TRH given	**28.67**	0.55-4.78mU/L
60min+TRH given	**24.05**	0.55-4.78mU/L

Table 4: Pituitary panel, free alpha subunit and SHBG

Investigations	Results		Reference Range
FSH	57IU/L		Menopausal 20-140 IU/L Luteal 1-10 IU/L Mid- Cycle 7-24 IU/L Follicular 2-10 IU/L
LH	27IU/L		Menopausal 10-65IU/L Luteal 1-9 IU/L Mid- Cycle 9-74 IU/L Follicular 2-7IU/L
Oestradiol	107pmol/L		Menopausal <200 pmol/L Luteal 180-840 pmol/L Mid- Cycle 550-1650 pmol/L Follicular 110-180pmol/L
Progesterone	<0.5nmol/L		Menopausal <2.2nmol/L Luteal 12-90nmol/L Mid- Cycle 2.5-12.0nmol/L Follicular <0.5-2.5nmol/L
IGF-1	8 nmol/L		10-32nmol/L
Prolactin	600mIU/L	665mIU/L	<500mIU/L
ACTH	34 ng/L		9-51ng/L
Cortisol	328 nmol/L		100-535nmol/L
Free alpha glycoprotein subunit	5.35IU/L		Premenopausal: 0.00-0.60IU/L Postmenopausal: 0.00-1.30IU/L
SHBG test	58nmol/L		20-110nmol/L

Figure 1: Trend of thyroid function test 2016- 2023



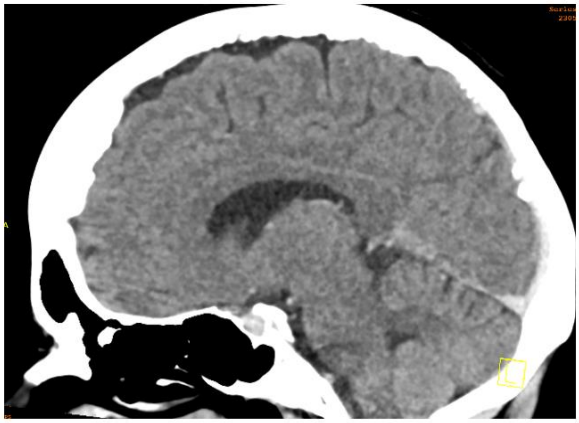


Figure 2: CT pituitary; pituitary gland appears normal in size with no evidence of pituitary macroadenoma

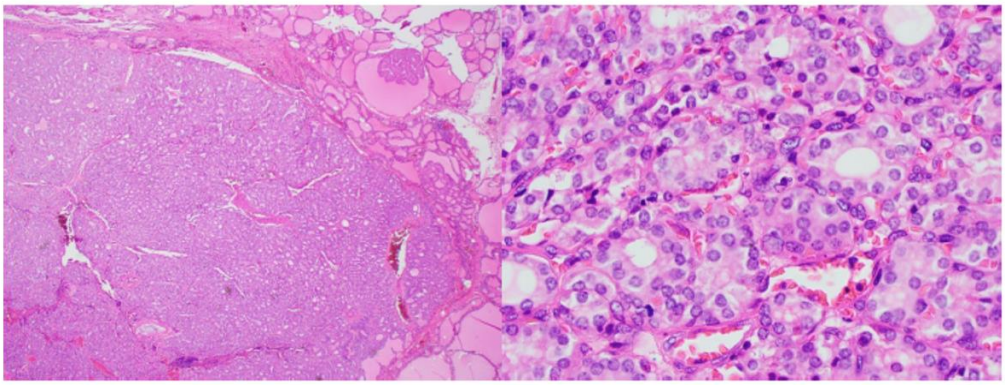


Figure 3: Histopathology of one of the nodules showing papillary carcinoma with hurthle morphology, 15/03/2023

This challenging case illustrates the long history of hyperthyrotropinemia with inappropriately elevated FT4 and FT3 with a proposed diagnosis of TSHoma and a concurrent diagnosis of differentiated thyroid cancer. However, the absence of radiological evidence of pituitary macroadenoma and difficulty in our patient getting MR pituitary pose a challenge in localising the TSHoma.

Inappropriately elevated TSH concentration and coexistence of elevated thyroid hormone is rare but seen in cases of TSHoma, intermittent administration of levothyroxine and RTH. First step that clinicians should consider is exclusion of methodological interferences. Several diagnostic steps can be carried out to differentiate the TSHoma from RTH including radiological and biochemical investigations. MR pituitary is the favoured modality for the visualisation of TSHoma as it identifies about 80% cases of adenomas (1). However, microadenomas not well visualised in MRI and ectopic tumours in the pharyngeal region have been reported in case reports (2) . Other feature of TSHoma includes suppression of hypothalamic-pituitary-adrenal due to the mass effect. High alpha-glycoprotein subunit/TSH ratios are also typically seen in patients with TSHoma. TRH test is helpful in differentiating as blunted TSH response to TRH stimulation are seen in 90% of patients with TSHomas (1). RTH is a rare autosomal dominant hereditary disorder with 90% mutations are due to the thyroid hormone receptor beta (TRb) gene (3). In this case, negative family history, the past record of normal thyroid function test, negative mutation TRb gene postulated RTH unlikely. Blunted TRH response seen in our case with elevated free alpha glycoprotein were suggestive less likely of RTH.

There are reported cases of differentiated thyroid cancers in patients with TSHomas or RTH. Authors postulated that uncontrolled and persistently high TSH leading to secondary hyperthyroidism may promote thyroid cell proliferation. Therefore, long-term exposure to high TSH may results in thyroid cell hyperplasia and contribute to oncogenesis. Studies have shown a positive association between TSH levels and thyroid cancers diagnosis with papillary thyroid carcinomas being the most common type (4, 5).

Another issue we may face in this case is inability for TSH suppression which is needed as a part of the management of papillary thyroid cancer. The suppression of TSH for differentiated thyroid cancer with pre-existing hyperthyrotropinaemia secondary to RTH or TSHoma is reported to be challenging and there is no agreed consensus regarding this long-term management. In cases with RTH, increasing the dose of levothyroxine can lead to thyrotoxicosis without TSH suppression. It is suggested that the optimal TSH level can be achieved by monitoring of the symptoms of excessive thyroid hormone effect including tachycardia. Other mechanisms suggested by authors was to consider of b-blocker to reduce the tachycardia to tolerate higher dose of levothyroxine however this may exacerbate osteoporosis. In cases of TSHoma, the first line treatment is surgical resection of TSHoma followed by consideration of somatostatin analogues and/or radiotherapy. (3, 6)

This is an interesting scenario in which case a differentiated thyroid carcinoma had been treated with hemithyroidectomy and persistently raised TSH provides a challenging situation about further long-term monitoring for cancer surveillance in the remaining lobe. We note that hyperthyroidism was resolved post hemithyroidectomy but TSH level further increased. It seems unlikely that TSH suppression would be achievable in this patient who already has a history of inappropriately high TSH levels with further rise in TSH level post hemithyroidectomy. This leaves an ongoing challenging situation about further monitoring for cancer surveillance of the remaining lobe and localisation of source of TSH hypersecretion. Furthermore, the localisation of the source of TSH hypersecretion is also challenging given difficulty in getting MR pituitary.

Take home messages

1. Conundrum of abnormal thyroid function tests with TSHoma and RTH is still clinically challenging and requires multiple tests to distinguish between the two conditions.
2. Differentiated thyroid cancer (DTC) coexisting with hyperthyrotropinaemia are rare but seen in the literature. Persistently raised TSH secretion might be a risk factor for the development of DTC.
3. Management of DTC with the primary aim to keep TSH suppressed is difficult when it coexists with RTH and TSHoma.
4. Localisation and management of TSHoma in reference to this case is challenging.

References

1. Beck-Peccoz P, Lania A, Beckers A, Chatterjee K, Wemeau JL. 2013 European thyroid association guidelines for the diagnosis and treatment of thyrotropin-secreting pituitary tumors. Eur Thyroid J. 2013;2(2):76-82.
2. Kumar S, Phang CA, Ni H, Diamond T. A patient with an ectopic sphenoid bone TSH secretory adenoma: Case report and review of the literature. Frontiers in Endocrinology. 2022;13.
3. Fang Y, Liu T, Hou H, Wang Z, Shan Z, Cao Y, et al. Resistance to thyroid hormone beta coexisting with papillary thyroid carcinoma—two case reports of a thyroid hormone receptor beta gene mutation and a literature review. Frontiers in Genetics. 2022;13.
4. Yoon JH, Choi W, Park JY, Hong AR, Kim SS, Kim HK, et al. A challenging TSH/GH co-secreting pituitary adenoma with concomitant thyroid cancer; a case report and literature review. BMC Endocrine Disorders. 2021;21(1):177.
5. Perticone F, Pigliaru F, Mariotti S, Deiana L, Furlani L, Mortini P, et al. Is the incidence of differentiated thyroid cancer increased in patients with thyrotropin-secreting adenomas? Report of three cases from a large consecutive series. Thyroid. 2015;25(4):417-24.
6. Ünlütürk U, Sriphrapradang C, Erdoğan MF, Emral R, Güldiken S, Refetoff S, et al. Management of Differentiated Thyroid Cancer in the Presence of Resistance to Thyroid Hormone and TSH-Secreting Adenomas: A Report of Four Cases and Review of the Literature. The Journal of Clinical Endocrinology & Metabolism. 2013;98(6):2210-7.

Refractory hypercalcaemia secondary to metastatic parathyroid carcinoma treated with immunotherapy

Brenda Ta[1, 2], **Michael Bennett**[1, 2]

1. University of New South Wales, Sydney, Australia

2. Prince of Wales Hospital, Randwick, NSW

Parathyroid carcinoma (PC) is a rare endocrine malignancy with an incidence of 6 cases per 10 million population (1). It accounts for 0.5-2% of cases of primary hyperparathyroidism (2). Metastatic PC carries a guarded prognosis with a median survival of 36 months (5) with refractory hypercalcaemia being the leading cause of mortality. We describe a case of refractory hypercalcaemia secondary to progressive metastatic PC with a dramatic biochemical and clinical response to nivolumab therapy.

A 64-year-old retiree presented with dysphonia in late-2021 and was referred to an ENT surgeon for assessment. She was diagnosed with left vocal cord palsy secondary to a nodular lesion arising from the left lobe of her thyroid gland identified on CT. Notably, she had primary hyperparathyroidism (PTH 16.8 pmol/L, RI 1.6-6.9) and mild hypercalcaemia (corrected calcium 2.76 mmol/L, RI 2.10-2.60). Parathyroid scintigraphy identified an autonomous left inferior parathyroid nodule.

Her medical history included a parathyroidectomy (2005) for a histologically benign left inferior parathyroid adenoma, and breast cancer (2012) treated with lumpectomy and three years of adjuvant tamoxifen treatment. She was diagnosed with osteoporosis in 2021, (total hip T-score of -2.0 SD and lumbar spine T-score of -2.7 SD) and received one dose of zoledronic acid 5 mg intravenously. She was functionally independent with an ECOG score of 0.

A fine needle aspirate of the nodule was consistent with a parathyroid neoplasm with positive staining for PTH, MNF116, GATA 3 and negative TTF1, CD3, CD5 and CD117. She underwent left hemithyroidectomy and left central neck dissection. Histopathology revealed a PTH-positive, high-grade carcinoma (Ki67 80%) with evidence of capsular, perineural and lymphovascular invasion. One of seven lymph nodes were positive. Her calcium and PTH normalised following surgery. A post-operative PET scan demonstrated moderate metabolic activity in the left thyroid bed and a left supraclavicular lymph node. External beam radiotherapy was administered to the thyroid bed. No germline mutations were identified on genetic testing.

Six months later, blood tests revealed PTH-dependent hypercalcaemia (corrected calcium 2.74 mmol/L, PTH 14.5 pmol/L). Subsequent MRI identified multiple new cerebral metastases and a PET-CT demonstrated an avid lesion in her right ilium. Cinacalcet was commenced and further radiotherapy was administered to sites of metastatic disease. MDT discussions did not identify any systemic treatment options. Molecular genomic studies revealed low microsatellite instability, however there was a high tumour mutation burden suggesting a possible role for immunotherapy.

Over the subsequent five months, the patient developed worsening hypercalcaemia, refractory to escalating doses of zoledronic acid and denosumab. Anti-resorptive treatment was initially given intermittently for hypercalcaemia before intensifying to fortnightly alternating zoledronic acid 4mg intravenously and denosumab 120 mg subcutaneously. She required two hospital admissions with a peak corrected calcium of 3.82 mmol/L and PTH levels consistently above 100 pmol/L. Cinacalcet was ceased due to persistent nausea and anorexia. The patient briefly trialled temozolomide, based on a favourable case report. Despite this, significant disease progression ensued with new nodal, pulmonary, and skeletal metastases. The patient opted to self-fund nivolumab, an anti-PD1 monoclonal antibody that inhibits immune checkpoint, thus upregulating T cell recognition of malignant cells. Less than 4 weeks after commencing nivolumab and one day prior to her second cycle, her PTH declined from 163.8 pmol/L to 5.3 pmol/L and corrected calcium from 3.90 mmol/L to 2.13 mmol/L. Her nausea, lethargy, and constipation resolved. She was commenced on calcium carbonate 600mg daily with close monitoring of her calcium levels as she had received denosumab two weeks earlier and zoledronic acid four weeks earlier. PTH was at its lowest level since her initial surgical resection 18 months ago. FDG PET-CT is planned to assess radiological response to treatment in 2 months.

Discussion

PC is a rare malignancy that may occur sporadically (90%) or in association with inherited cancer syndromes (10%) including hyperparathyroidism-jaw tumour syndrome, MEN1,or MEN2A (9). The loss of tumour suppressor gene CDC73 accounts for 70% of sporadic cases. This mutation is rarely found in benign pituitary adenomas, suggesting PC may arise de novo (9).

Challenges exist for both diagnosis and management of PC. PC can be difficult to distinguish from benign parathyroid lesions, both of which cause primary hyperparathyroidism, and definitive diagnosis requires histological analysis. Clinical features thought to favour PC include ALP > 285 IU/L, ionised calcium >1.77 mmol/L, parathyroid lesions > 3cm or PTH levels more than three times the upper limit of normal (9). Fine needle aspiration is generally not recommended due to the risk of malignant parathyromatosis.

PC carries a poor porgnosis and there are limited treatment options beyond primary resection. While antiresorptives and calcimimetics can provide initial short-term control of hypercalcaemia, disease progression often leads to severe refractory hypercalcaemia. Surgery remains the mainstay of treatment of localised disease, however the optimal surgical approach (localised excision vs en block resection) remains unclear (4).

There is limited evidence for radiotherapy, systemic chemotherapy or immunotherapy with PC generally considered to be “radioresistant” and “chemoresistant,”. A systematic review (5) of chemotherapy-treated patients with PC, reported a progression-free survival of 10-months with the combined use of fluorouracil, cyclophosphamide and dacarbazine in four patients. Patients who received other chemotherapy regimens experience partial or no response (5).

Evidence for the use of immunotherapy is sparse. There have been three case reports from 2004 that investigated PTH immunisation, a novel form of immunotherapy to induce antibody formation against human PTH. One Japanese patient died due to disease progression while receiving treatment (6); the other two case reports described 24 months and 12 years progression free survival, respectively (7, 8). These treatments have been largely superseded by modern immunotherapeutic agents.

There has been one case report of checkpoint immunotherapy for the treatment of metastatic PC (3). Pembrolizumab (anti-PD-1 antibody), administered over four months (five cycles), achieved a partial radiological response (60% reduction in pulmonary metastases) in a patient with Lynch Syndrome. There was a complete biochemical response (normalisation of serum calcium and PTH) before it was discontinued due to severe immune-related colitis. Despite cessation, disease volume and biochemistry remained stable 24 months later (3, 5).

To our knowledge, this is the first case report to detail the use of nivolumab for the treatment of metastatic PC. Immunotherapy may be an emerging treatment option, informed by individual cancer genomics.

Take home messages:

- PC is a rare endocrine malignancy that causes PTH-mediated hypercalcaemia.
- Consider PC as a diagnosis in cases of primary hyperparathyroidism with: ALP > 285 IU/L, ionised calcium >1.77 nmol/L, parathyroid lesions > 3cm, or PTH > 3x ULN.
- Limited treatment options exist, particularly for metastatic disease which carries a guarded prognosis.
- Management of hypercalcaemia is with antiresorptives and calcimimetics however break-through hypercalcaemia will invariably occur with disease progression.
- Immunotherapy may be a treatment option depending on cancer genomics.

References

1. Hundahl SA, Fleming ID, Fremgen AM, Menck HR. Two hundred eighty-six cases of parathyroid carcinoma treated in the U.S. between 1985-1995: A national cancer data base report. Cancer (1999) 86:538–44. doi: 10.1002/(SICI)1097-0142(19990801)86:33.0.CO;2-K
2. Ozolins, A., Narbuts, Z., Vanags, A., Simtniece, Z., Visnevska, Z., Akca, A., ... & Goretzki, P. E. (2016). Evaluation of malignant parathyroid tumours in two European cohorts of patients with sporadic primary hyperparathyroidism. Langenbeck's Archives of Surgery, 401, 943-951.
3. Park, D., Airi, R., & Sherman, M. (2020). Microsatellite instability driven metastatic parathyroid carcinoma managed with the anti-PD1 immunotherapy, pembrolizumab. BMJ Case Reports CP, 13(9), e235293.
4. McInerney, N. J., Moran, T. D., & O'Duffy, F. (2023). Parathyroid carcinoma: Current management and outcomes–A systematic review. American Journal of Otolaryngology, 103843.
5. Alberti, A., Smussi, D., Zamparini, M., Turla, A., Laini, L., Marchiselli, C., ... & Berruti, A. (2022). Treatment and outcome of metastatic parathyroid carcinoma: A systematic review and pooled analysis of published cases. Frontiers in oncology, 12, 997009.
6. Horie, I., Ando, T., Inokuchi, N., Mihara, Y., Miura, S., Imaizumi, M., ... & EGUCHI, K. (2010). First Japanese patient treated with parathyroid hormone peptide immunization for refractory hypercalcemia caused by metastatic parathyroid carcinoma. Endocrine journal, 57(4), 287-292.
7. Sarquis, M., Marx, S. J., Beckers, A., Bradwell, A. R., Simonds, W. F., Bicalho, M. A. C., ... & De Marco, L. (2020). Long-term remission of disseminated parathyroid cancer following immunotherapy. Endocrine, 67, 204-208.
8. Betea, D., Bradwell, A. R., Harvey, T. C., Mead, G. P., Schmidt-Gayk, H., Ghaye, B., ... & Beckers, A. (2004). Hormonal and biochemical normalization and tumor shrinkage induced by anti-parathyroid hormone immunotherapy in a patient with metastatic parathyroid carcinoma. The Journal of Clinical Endocrinology & Metabolism, 89(7), 3413-3420.
9. Cetani, F., Pardi, E., & Marcocci, C. (2019). Parathyroid carcinoma. Parathyroid disorders, 51, 63-76.

Juggling the highs and lows – a case of congenital hyperinsulinism and gestational diabetes

Jia Yong Tan[1], Ana McCarthy[1], Anjana Radhakutty[1]

1. Northern Adelaide Local Health Network, Elizabeth Park, SA, Australia

Case

A 29-year-old female was referred to the High-risk Pregnancy Clinic with a new diagnosis of gestational diabetes (GD) following a positive 75g oral glucose tolerance test done at 13 weeks of gestation (fasting glucose 5.1 mmol/L, 1-hour 12.9 mmol/L, 2-hours 13.4 mmol/L). Her HbA1c was 5.2% at 14 weeks and BMI 24kg/m^2.

At the age of 5, she had been diagnosed with congenital hyperinsulinism (CH) after confirmatory testing following a prolonged fast due to episodes of suspected symptomatic hypoglycaemia. After 16 hours of fasting, she had symptomatic hypoglycaemia (2.5 mmol/L) with high-normal insulin level (9 mU/L). A C-peptide level was not available for review. Her serum cortisol was appropriately elevated with normal lactate and ammonia. She had a younger brother with a severe form of CH, requiring near-total pancreatectomy shortly after diagnosis. No other family members were affected with CH. There was a strong family history of diabetes – her maternal grandfather had Type 1 diabetes, while her mother and maternal aunt had Type 2 diabetes.

During her childhood years, she had frequent episodes of symptomatic hypoglycaemia, needing to eat every 3-4 hours. These episodes reduced to 1-2 times per week when she reached adulthood, with preserved hypoglycaemia awareness.

Now with her first pregnancy and new diagnosis of gestational diabetes, the conundrum of balancing diabetes treatment and risk of hypoglycaemia presented itself. While some of her fasting and 2-hour post-prandial blood glucose levels were above GD targets, there were also occasional instances of mild symptomatic hypoglycaemia, with levels ranging from 3 to 4mmol/L.

She was first managed with dietary modification, with specific advice for low glycaemic index meals and snacks. She subsequently required commencement of metformin, as capillary blood glucose levels remained elevated 2-hours post-prandially. Acarbose was later used with high carbohydrate meals due to its minimal risk of hypoglycaemia. However, she reported an increase in frequency of symptomatic hypoglycaemia. Following education, she then acquired and utilised the Freestyle Libre 2 continuous glucose monitor (CGM) which was then compared with capillary blood glucose readings. (Figure 1, 2 and 3). Whilst her capillary readings (done as per local protocol) demonstrated hyperglycaemia in 2-hour post-prandial readings which would normally prompt escalation of diabetes treatment, her CGM revealed that in fact she had 17% time in hypoglycaemia, with 1% in severe hypoglycaemia range of <3.0mmol/L. These episodes seemed to occur overnight (fasting) or multiple hours post-meal, which are outside the recommended testing times for gestational diabetes. Given this, she continued to utilise the CGM as the main monitoring tool for her blood glucose.

17/04/2023 Mon	08:44 4.5		12:11 5.4		16:13 4.4		21:39 8.6	
18/04/2023 Tue	07:52 4.5		10:13 7.3	11:46 3.6	15:13 5.1		21:46 7.2	
19/04/2023 Wed	08:48 4.3				14:14 4.2		21:44 6.9	12:02 3.8
20/04/2023 Thu	06:49 3.9		10:13 5.6		14:22 6.9		21:39 7.0	16:40 3.6
21/04/2023 Fri	08:59 3.7		11:33 6.3		14:41 6.6			
22/04/2023 Sat	08:46 3.9		11:54 6.2		15:30 6.5		21:42 7.4	
23/04/2023 Sun	09:46 4.1		12:50 4.2		15:59 7.3		23:05 6.3	
24/04/2023 Mon	07:48 3.8		10:04 5.1		16:46 9.3		22:21 6.2	
25/04/2023 Tue	09:29 3.7		10:55 4.7		14:37 6.1		21:31 7.4	
26/04/2023 Wed	07:46 3.9		10:06 5.3		16:00 8.2		21:54 6.7	18:01 11.3
27/04/2023 Thu	07:56 4.0		10:04 4.9					

Figure 1 demonstrates the readings of capillary blood glucose measurements as per local guidelines, performed before breakfast and 2-hours after each main meal

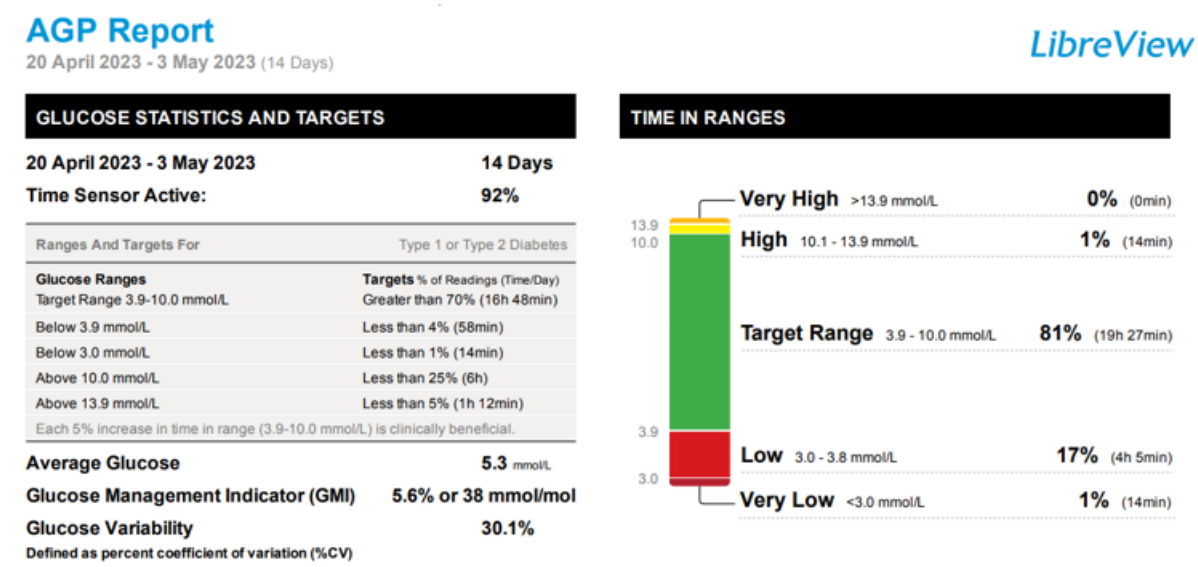


Figure 2 demonstrates the Ambulatory Glucose Profile (AGP) report when our patient first commenced on continuous glucose monitoring (CGM), corresponding to the same period of capillary blood glucose monitoring as in Figure 1

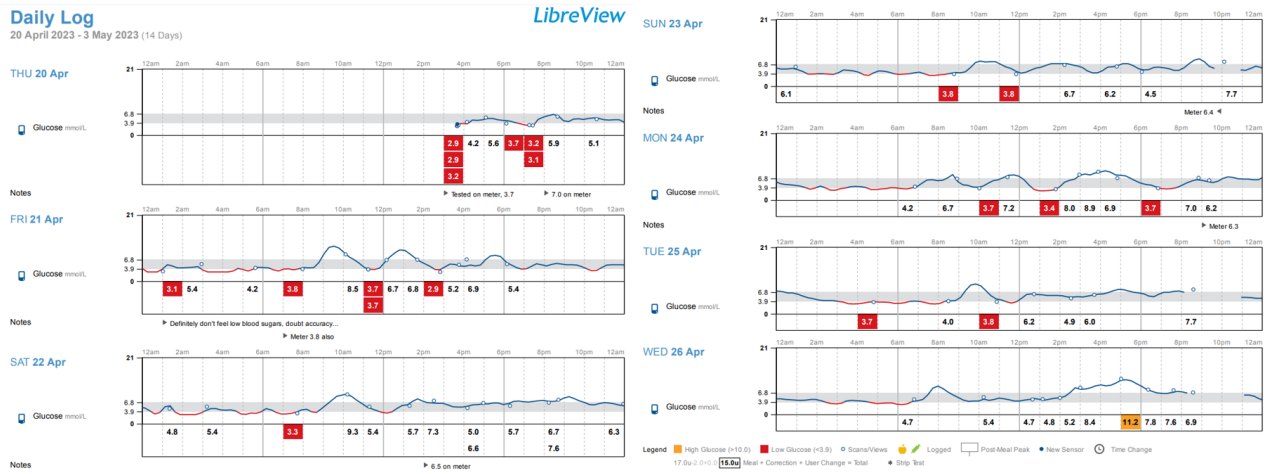


Figure 3 demonstrates the daily log from the CGM, which can be compared with capillary blood glucose monitoring for same days in Figure 1

Subsequently, she was commenced on prandial insulin due to progressive worsening of postprandial hyperglycaemia. This proved difficult to juggle with her hypoglycaemia time increasing to 22% (1% severe hypoglycaemia) despite her blood glucose otherwise being in target range. There was evidence of variability in effect on blood glucose based on erratic carbohydrate intake, hence she adopted a personalised insulin-to-carbohydrate ratio (ICR) with frequent remote diabetes clinician and dietitian contact.

Her insulin requirements continued to increase as she progressed through the third trimester. The hypoglycaemic episodes became less frequent, occurring less than 5% of total time (0% in severe hypoglycaemia).

Her obstetric ultrasound scans up to now have showed normal fetal growth where she is approaching 36 weeks of gestation. Close attention will be paid to her foetus, as there is a heightened risk of hypoglycaemia. It is possible that her foetus may inherit CH mutation as several variants have an autosomal dominant inheritance pattern. Macrosomia is also associated with some CH variants, in addition to GD. Currently, she has been referred for genetic analysis.

Discussion

This case presented the rare conundrum of balancing the management of gestational diabetes and congenital hyperinsulinism. Congenital hyperinsulinism describes the genetic form of hyperinsulinaemic hypoglycaemia in infants and is rare, with an estimated incidence of 1 in 27000-50000 live births [1,2]. Neonates with CH often present with severe hypoglycaemia and have a high risk of hypoglycaemic brain injury [2,3]. It can also present as late as adulthood, usually in a more subtle manner. Up to twelve genetic defects have been described in literature, each encoding key proteins involved in regulating insulin release from pancreatic beta cells. Depending on the genetic defect, the mode of inheritance can be either autosomal dominant or recessive. The severity of the phenotype is also variable.

Whilst there are no current diagnostic criteria, key clinical or biochemical features of CH include [3]:

- Low plasma glucose <3mmol/L, with
- Detectable serum insulin levels
- Detectable C-peptide level, often superior to insulin as more stable in blood

CH can also be classified based on histological involvement of the pancreas, either diffuse or focal, which can be estimated by specialised positron emission tomography/computed tomography (PET/CT) [3,4,5].

The management of congenital hyperinsulinism is dependent on its severity [2,3,4,5]. For milder phenotypes, individuals can mitigate hypoglycaemia by ensuring frequent feeding and education around strategies in managing hypoglycaemia. If this is inadequate, pharmacotherapy such as diazoxide and somatostatin analogues may be utilised. In more severe forms, surgery including near-total and total pancreatectomy is required.

To date, there are no case reports of individuals with CH developing GD. However, cases of individuals with CH who develop diabetes later in life have been reported [6,7,8]. The two mutations described thus far include hepatocyte nuclear factor 4 alpha (HNF4A) and ATP binding cassette subfamily C member 8 (ABCC8) mutations. HNF4A mutations are known to cause monogenic diabetes. However, there are observations of a “biphasic phenotype” in some with heterozygous mutations, which causes congenital hyperinsulinism and subsequently impaired glucose tolerance or diabetes. Macrosomia is also a consistent finding. The mechanism for this phenomenon is not known, but thought to be related to beta cell exhaustion after initial hypersecretory phase due to islet cell hyperplasia and macrosomia in the neonatal period.

On the other hand, ABCC8 is involved in formation of the sulfonylurea receptor 1 (SUR1) protein with gain-of-function mutations resulting in monogenic diabetes, while loss-of-function mutations result in hyperinsulinism [3]. There are fewer known cases with a biphasic phenomenon, unlike HNF4A variants. However, in known cases, the postulated mechanism is similar [7]. Progression to diabetes was especially seen in those with reduced insulin sensitivity [7,8].

The conundrum of managing such individuals with GD lies in the heightened risk of maternal hypoglycaemia. In GD, management is dietary modification, insulin and/or metformin [10]. Traditional dietary advice for GD will have to be balanced with that for CH, where individuals would be used to having frequent feeding. The use of CGM may be useful to provide additional information outside traditional BGL monitoring, despite its evidence of benefit being limited to Type 1 diabetes [10].

Finally, given that several CH variants are inherited through an autosomal dominant pattern, there is a chance of the fetus inheriting CH which increases the risk of neonatal hypoglycaemia. As such, close monitoring is required in the post-natal period.

Take-home messages

Congenital hyperinsulinism is a rare and heterogenous condition with subtypes that can predispose to future development of diabetes.

Evidence around management of CH with gestational diabetes is lacking. However, a personalised approach should be undertaken, particularly minimising hypoglycaemia.

Genetics analysis is also vital in determining management and prognosis for both mother and fetus.

1. Männistö JME, Jääskeläinen J, Otonkoski T, Huopio H. Long-Term Outcome and Treatment in Persistent and Transient Congenital Hyperinsulinism: A Finnish Population-Based Study. J Clin Endocrinol Metab. 2021 Mar 25;106(4):e1542-e1551. doi: 10.1210/clinem/dgab024. PMID: 33475139; PMCID: PMC7993590.
2. Banerjee I, Salomon-Estebanez M, Shah P, Nicholson J, Cosgrove KE, Dunne MJ. Therapies and outcomes of congenital hyperinsulinism-induced hypoglycaemia. Diabet Med. 2019 Jan;36(1):9-21. doi: 10.1111/dme.13823. Epub 2018 Oct 8. PMID: 30246418; PMCID: PMC6585719.
3. Demirbilek H, Hussain K. Congenital Hyperinsulinism: Diagnosis and Treatment Update. J Clin Res Pediatr Endocrinol. 2017 Dec 30;9(Suppl 2):69-87. doi: 10.4274/jcrpe.2017.S007. Epub 2017 Dec 27. PMID: 29280746; PMCID: PMC5790328.
4. Thornton PS, Stanley CA, De Leon DD. Congenital Hyperinsulinism: An Historical Perspective. Horm Res Paediatr. 2022;95(6):631-637. doi: 10.1159/000526442. Epub 2022 Nov 29. PMID: 36446321.
5. Banerjee I, Raskin J, Arnoux JB, De Leon DD, Weinzimer SA, Hammer M, Kendall DM, Thornton PS. Congenital hyperinsulinism in infancy and childhood: challenges, unmet needs and the perspective of patients and families. Orphanet J Rare Dis. 2022 Feb 19;17(1):61. doi: 10.1186/s13023-022-02214-y. Erratum in: Orphanet J Rare Dis. 2022 May 18;17(1):205. PMID: 35183224; PMCID: PMC8858501.
6. Bacon S, Kyithar MP, Condron EM, Vizzard N, Burke M, Byrne MM. Prolonged episodes of hypoglycaemia in HNF4A-MODY mutation carriers with IGT. Evidence of persistent hyperinsulinism into early adulthood. Acta Diabetol. 2016 Dec;53(6):965-972. doi: 10.1007/s00592-016-0890-9. Epub 2016 Aug 23. PMID: 27552834.Diabet Med. 2014 Jan;31(1):e1-5. doi: 10.1111/dme.12259. PMID: 23796040.
7. Huopio H, Otonkoski T, Vauhkonen I, Reimann F, Ashcroft FM, Laakso M. A new subtype of autosomal dominant diabetes attributable to a mutation in the gene for sulfonylurea receptor 1. Lancet. 2003 Jan 25;361(9354):301-7. doi: 10.1016/S0140-6736(03)12325-2. PMID: 12559865.
8. Kapoor RR, Flanagan SE, James CT, McKiernan J, Thomas AM, Harmer SC, Shield JP, Tinker A, Ellard S, Hussain K. Hyperinsulinaemic hypoglycaemia and diabetes mellitus due to dominant ABCC8/KCNJ11 mutations. Diabetologia. 2011 Oct;54(10):2575-83. doi: 10.1007/s00125-011-2207-4. Epub 2011 Jun 15. PMID: 21674179; PMCID: PMC3168751.
9. Plows JF, Stanley JL, Baker PN, Reynolds CM, Vickers MH. The Pathophysiology of Gestational Diabetes Mellitus. Int J Mol Sci. 2018 Oct 26;19(11):3342. doi: 10.3390/ijms19113342. PMID: 30373146; PMCID: PMC6274679.
10. ElSayed NA, Aleppo G, Aroda VR, Bannuru RR, Brown FM, Bruemmer D, Collins BS, Hilliard ME, Isaacs D, Johnson EL, Kahan S, Khunti K, Leon J, Lyons SK, Perry ML, Prahalad P, Pratley RE, Jeffrie Seley J, Stanton RC, Gabbay RA, on behalf of the American Diabetes Association. 15. Management of Diabetes in Pregnancy: Standards of Care in Diabetes-2023. Diabetes Care. 2023 Jan 1;46(Suppl 1):S254-S266. doi: 10.2337/dc23-S015. PMID: 36507645; PMCID: PMC9810465.

A Y that makes all the difference

Laura Careless[1], Tony Huynh[2], Brendan Nolan[1]

1. Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, Queensland, Australia

2. Department of Endocrinology and Diabetes,, Queensland Children's Hospital, Brisbane, Queensland, Australia

Case Presentation

D was referred to general paediatric clinic at age 15 for investigation of short stature, primary amenorrhea, and gender incongruence. He was born via spontaneous vaginal delivery at 40+2 weeks after an uncomplicated pregnancy with normal antenatal scans and no genetic testing done in utero. His assigned sex at birth was female based on external genitalia. Birth weight, length and head circumference were within healthy centile ranges. D was the first child born to non-consanguineous parents, he had two younger sisters aged 13 and 14yo with no health conditions and an 8yo half-brother with dyslexia. He had a background of attention deficit hyperactivity disorder and was on no regular medications.

His growth had begun to slow at 8 years of age and had been under the 3rd centile for several years prior to referral. Mid parental height was between the 10th and 25th centiles. His weight remained around the 50th centile. He had experienced pubertal arrest with tanner stage II adrenarche and thelarche at age 15 and was yet to undergo menarche. From the age of 11 he identified as male and had started wearing restrictor vests.

On examination he was wearing glasses for myopia, he had a wide carrying angle of his arms and was barrel chested. No external genitalia examination was performed but no virilisation had been reported.

Baseline pathology revealed primary ovarian insufficiency, with an undetectable serum estradiol concentration on immunoassay (<43 pmol/L) and elevated gonadotropins (LH 37 IU/L, FSH 155 IU/L). Repeat testing 12months later showed a serum estradiol concentration of 15pmol/L using LC-MS. Serum testosterone concentration was within normal range for female sex. Molecular karyotyping revealed mosaic Turner Syndrome with 45XO/46XY with an isodicentric Y. Y chromosome material was present in 60% of cells, monosomy X was in 40%. The structurally abnormal Y chromosome contained a terminal deletion at yp11.32 encompassing the entire sex determining region Y gene.

Ultrasound pelvis showed an infantile unicornuate uterus of 1.6mL in which endometrial thickness could not be identified. There was an oval structure to the right thought to be an ovary but the left ovary was not identified. MRI pelvis revealed a small uterus, again, with a possible right sided ovary. Tumour markers including AFP and LDH were normal. HCG was slightly elevated 1.8 U/L (RR 0.1-0.5), thought to be the pituitary form.

D was referred to paediatric endocrinology at this stage. Height was on the 75th centile on Turner specific charts so growth hormone was not prescribed. D was commenced on intramuscular testosterone enantate replacement, following a comprehensive psychiatry evaluation who deemed he had capacity to consent to testosterone replacement and the support of both parents. He underwent laparoscopic surgery revealing bilateral streak gonads with visualisation of the uterus and uterine tubes which were noted to be grossly normal. The right streak gonad was excised, the left gonad was left in situ. Histology from the right gonad showed a nodule of tissue comprising of ovarian like stroma with occasional tubules lined by simple columnar epithelium. No germ cells, seminiferous tubules or ovarian follicles were identified. Ovarian-like-stroma was ER negative with focal weak PR positivity. Prominent hyperplastic Leydig cells were identified but findings were not sufficient for a Leydig cell tumour, importantly no gonadoblastoma/invasive malignancy was identified.

He was then referred to adult endocrinology outpatient clinic as he commenced transition to the adult service. At this stage he was satisfied with secondary sexual characteristic development. Serum testosterone concentration measured 15nmol/L. He transitioned to Testogel 1% four pumps daily and has been referred for left gonadectomy in the context of gonadoblastoma risk with consideration for concurrent hysterectomy which is his preference. Repeat tumour markers remain within normal range.

Discussion

Turner syndrome affects phenotypical females and involves either complete or partial loss of one X chromosome. It is associated with delayed puberty and primary ovarian insufficiency, short stature, congenital malformations of the heart as well as multiple other comorbidities[1]. The prevalence of Turner syndrome (TS) is 1:2500-3000 live births and involves numerous genotypes. Typical monosomy X karyotype occurs in ~81.6%, mosaicism is present in ~16.8%[2]. The frequency of Y chromosome material in TS is around 5-12%[1,3], phenotype varies depending on the percentage distribution of mosaicism, phenotypical males and those with ambiguous genitalia are currently excluded from the diagnosis of TS[1].

Isodicentric Y chromosomes were first described in the 1960s and are one of the most common reported structural abnormalities of the Y chromosome. It involves breakage and fusion of the Y chromosome resulting in gain and loss of material[4]. Most cases are chromosomal mosaics generally including a 45XO cell line. Phenotypes vary from male, female and ambiguous sexual characteristics and is dependent on the location of breakpoints as well as proportion of each cell line[4]. Yp11.32 is a common breakpoint in iscodicentric Y chromosomes and contains the short stature homeobox gene as well as the sex determining Y region gene[4].

Gonadal dysgenesis and gonadoblastoma risk

Gonadal dysgenesis describes a condition in which gonadal development is interrupted leading to dysfunction, external and internal reproductive anatomy can be variable[5]. It can be complete in which no gonadal development occurs, leading to a female phenotype or partial in which Y chromosome material is present causing incomplete testis determination. External phenotype is dependent on degree of testicular function. The most common karyotype is 45XO/46XY[5].

The presence of Y chromosome material in TS is associated with a risk of gonadoblastoma which is a benign tumour with malignant potential of around 50-60%[5]. The rate of gonadoblastoma in those with TS with Y is probably around 10%[1,3] acknowledging substantial variation in frequency quoted across various studies[5]. Gonadoblastoma generally occurs in the 2nd decade of life but can occur earlier[5]. A 2017 International Turner Syndrome Guideline recommended gonadectomy for all

individuals with TS, but acknowledged this was based upon low quality evidence and no recommendations on timing were proposed[1]. A 2016 consensus statement by members of the Global DSD Update Consortium have recommended bilateral gonadectomy at diagnosis in phenotypical females with 45XO/46XY gonadal dysgenesis[6].

Gender incongruence in DSD

Most individuals with DSD identify with their gender assigned at birth but rates of gender incongruence are higher in this population. Gender incongruence rates have been quoted as 8.5-20% depending on the DSD[7]. Given the current definition of TS includes only those who are phenotypically female at birth regardless of the presence of Y material, they are invariably raised as female. A European cross-sectional study including 196 adults with TS reported 3 (1.5%) individuals with gender incongruence, though none had undergone gender affirming hormone therapy[8]. This proportion is lower than in other DSDs but is higher than rates of gender incongruence in the general population (0.1-2%)[7].

Take home messages

1. Mosaicism is present in around 16% of people with Turner syndrome
2. Isodicentric Y chromosome is the most common reported structural abnormality of the Y chromosome and mostly occurs in mosaic Turner syndrome
3. Y chromosome material in Turner Syndrome is associated with gonadoblastoma risk, it is currently recommended gonadectomy be offered at diagnosis
4. Rates of gender incongruence are higher in those with DSDs including Turner syndrome, gender contentment is an important aspect of clinical review

Mild Androgen Insensitivity Syndrome - considerations in management

Ruby Chang[1], Ravind Pandher[2], Veena Jayadev[2], David J Handelsman[2]

1. Endocrinology, Concord Hospital, Sydney

2. Andrology, Concord Hospital, Sydney

Case:

M, a 13-year-old male presented with bilateral gynecomastia and micropenis. He was in good general health and the product of an uncomplicated pregnancy with normal childhood development. He was at the 50th centile for height and weight for his age. By age 13, he had not experienced deepening of his voice but reported erections. At presentation, he had no facial hair, a hairless trunk and no axillary or pubic hair. He had 3cm true gynecomastia and a stretched phallus length of 3cm, <2.5th centile. There was no hypospadias and testis volumes were 8ml bilaterally. Serum testosterone measured 24.8 nmol/L (10-30 nmol/L), serum LH 5.4 IU/L (RR 1.7-8.6 IU/L), serum FSH 8.5 IU/L (1.5 – 12.4 IU/L) and serum SHBG 91.6 nmol/L (RR 15-80 nmol/L).

His maternal uncle MDM had a diagnosis of Mild Androgen Insensitivity Syndrome (MAIS) by genetic testing in 1998. The uncle (now aged 45) had presented to a paediatrician at age 16 with delayed puberty, bilateral gynecomastia, micropenis (stretched penile length 7cm, 3rd centile), 10th centile for height and weight and in early to mid-puberty. He reported erections but no ejaculations. Karyotype was 46 XY.

MDM, his mother and two sisters all underwent genetic testing in 1998. Sanger sequencing detected a missense nucleotide variation **a**ga à **g**ga in exon 8 of the androgen receptor (AR) gene resulting in a p.R872G (arginine to glycine) mutation [1]. This mutation in the AR ligand binding domain increases androgen dissociation from its receptor. MDM was hemizygous and his mother and one sister (the mother of M) were carriers with the other sister a non-carrier. The only previous report of this mutation was his first cousin (maternal) in Canada who also presented with gynaecomastia and male infertility (Figure 1) [2].

MDM underwent bilateral mastectomies at age 16 and high dose testosterone treatment for micropenis at the age of 18 years and 5 months with weekly 250mg testosterone enanthate injections. After 12 months, testes and penis size remained unchanged, but he reported more ejaculatory fluid, increased hair growth in upper lip, abdomen, and pubic region. He was then switched to testosterone implants also at twice the usual replacement dose (8x200mg per 4 months). He was maintained on testosterone implants for 2 years associated with height growth of 7cm, deepening of his voice and increased body hair. His testes and penis size remained unchanged. He cryostored sperm at age 22 but after two easily conceived children (at age 32 & 34 years) he discarded the stored sperm and underwent vasectomy.

M, the propositus, was referred for genotyping, bilateral mastectomies and a trial of topical dihydrotestosterone gel for micropenis.

Figure 1. Pedigree

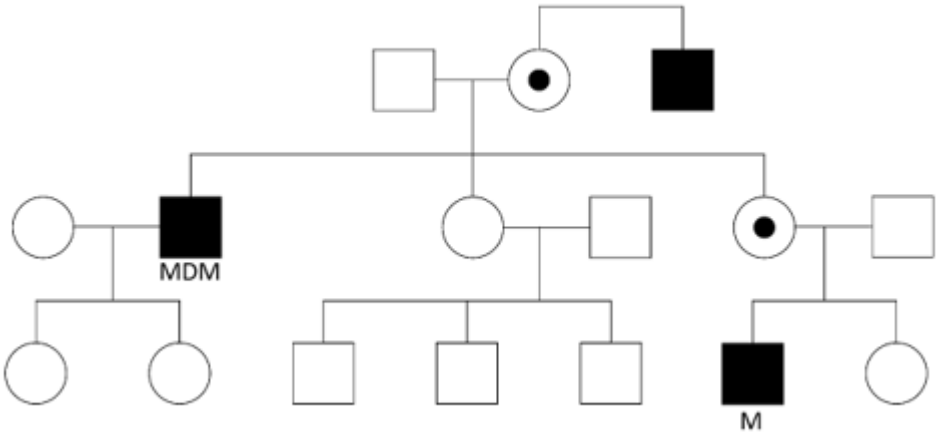


Table 1. Hormone levels before, during and after high dose testosterone therapy for MDM. **Bold** indicates abnormal value. Shading indicates time of high dose testosterone treatment. RR indicates reference range for that analyte.

Age (years & month)	Serum Testosterone (nmol/L) RR 11.0-35.0	Serum LH (IU/L) RR 1-10	Serum FSH (IU/L) RR 1-8.5	Serum SHBG (nmol/L) RR 10-50	Estradiol (pmol/L) RR 55-165	PSA (ng/mL) RR 0.2-0.5
17 11	**68.3**	8.4	1.7	**69.6**	105	< 0.2
18 5	**76.3**	3.6	**0.7**	46.9		
19 0	**102.0**	7.7	**0.8**	**60.3**		< 0.2
20 1	**36.5**	5.6	**0.5**	**57.8**		0.5
21 2	**39.2**	8.0	1.2	**75.5**		0.1
23 10	**43.8**	**12.5**	1.8	**77.8**		
26 2	**47.5**	**13.4**	2.1	**81.2**		0.2

Table 2. Semen analysis post high dose testosterone therapy

Age (years & month	Semen Volume (mL) RR >2.0	Sperm concentration (M/mL) RR > 14	Total sperm Count (M) RR > 39	Motility (%) RR >50%	Morphology Normal (%) RR >30
22 2	0.9	2.9	2.6	7	11
23 10	1.8	17.5	31.5	34	11
26 2	1.4	23.0	32.2	30	4
28 4	1.2	37.0	44.4	41	8

Discussion:

Androgen insensitivity syndrome is the most common disorder of sex development in 46 XY individuals. Mutations arise primarily in the androgen receptor gene on chromosome X which lead to end organ resistance to androgens, with over 600 genetic mutations described [3,4].

Individuals manifest varying phenotypes secondary to their degree of androgen responsiveness. Complete androgen insensitivity syndrome individuals typically have a female somatic phenotype, external genitalia and breasts but no menses and are unable to conceive or bear children. Individuals with partial androgen insensitivity present most commonly as severely undervirilized males, sometimes with ambiguous genitalia [3,5]. Hypospadias is the most sensitive phenotypic feature of androgen insensitivity.

Mild androgen insensitivity (MAIS) is likely an under-diagnosed condition. Individuals are male but may have reduced virilization, notably micropenis and gynecomastia, but phenotypes vary [5]. Individuals may present with gynaecomastia as an adolescent, but more commonly present later in life with infertility. Generally, genital abnormalities are not present. Hypospadias are characteristic when androgen insensitivity is more severe [4]. Individuals with the same mutation can present with varying phenotypes as seen in the kindred we described. Whilst both MDM and M had gynaecomastia and micropenis, MDM was shorter at the 10th centile whereas M was at the 50th centile for height. MDM also did not suffer from infertility, but M's maternal uncle was infertile and adopted children. Lack of correlation between genotype-phenotype is not completely understood but has been postulated to be secondary to AR coactivators, variations in 5a-reductase type 2 activity and/or somatic mosaicism [4,5].

Management of MAIS can include surgical therapy, hormonal replacement, fertility management, psychological and genetic counselling. Cosmetic surgery for gynecomastia may be valuable for the mental wellbeing of affected adolescents [4,5]. Whilst testosterone therapy is the standard of care for treatment of micropenis in MAIS, no guidelines exist to guide dosage, method, or duration of treatment [4,6, 7]. Results vary and may be best if initiated early in puberty. Despite high doses and high serum testosterone levels, adverse effects are rarely reported. There is literature describing temporary acceleration in growth rate and advances in bone age maturation, but no clear effect on final height has been established [6,8].

MDM was treated with high dose testosterone therapy for 3 years during the ages of 18 to 21 for micropenis. Despite receiving twice the standard testosterone dose, no effect on penile growth was achieved. However, he did experience increased ejaculate fluid, statural growth and increased virilization. No long-term harmful effects of prolonged high dose testosterone therapy were noted, and it is likely that his underlying androgen insensitivity may have been protective. In keeping with androgen insensitivity, large doses of testosterone therapy did not suppress MDM's LH and SHBG levels (Table 1). MDM possibly experienced spermatogenic suppression with testosterone treatment but this was reversed after treatment cessation and there was no appreciable impact on his fertility (Table 2).

Androgen action is vital for spermatogenesis and reproduction although not for life itself. Infertility is almost universal in MAIS and is frequently the presenting factor for individuals [5]. Case reports detailing spontaneous or induced fertility have only been noted in individuals with AR variants in the ligand binding domain, such as that seen in our kindred [5]. Semen analyses in MAIS is varied, with oligospermia or azoospermia reported in some cases. Patient MDM did not experience impaired fertility or spermatogenesis

with his phenotype of MAIS, nor was it negatively impacted by long term high dose testosterone therapy. In other individuals with MAIS, high dose testosterone therapy has been used successfully to temporarily induce spermatogenesis, leading to fertility [5].

Take Home Messages:

1. Mild androgen insensitivity should be considered in males with gynaecosmastia or infertility
2. Phenotypic differences exist within individuals with same MAIS genotype
3. High dose androgen therapy in individuals with MAIS does not appear harmful and may improve virilization, but its effect on micropenis may be limited especially if not started until late in puberty or thereafter
4. Genetic testing for offspring of an affected individual or carrier allows for unaffected individuals to be unburdened from the fertility concerns associated with MAIS and for early diagnosis and appropriate management of affected individuals

References

1. Shkolny, D.L., Beitel, L.K., Ginsberg, J., Pekeles, G., Arbour, L., Pinsky, L. and Trifiro, M.A., 1999. Discordant measures of androgen-binding kinetics in two mutant androgen receptors causing mild or partial androgen insensitivity, respectively. The Journal of Clinical Endocrinology & Metabolism, 84(2), pp.805-810
2. Gottlieb, B., Beitel, L.K., Wu, J.H. and Trifiro, M., 2004. The androgen receptor gene mutations database (ARDB): 2004 update. Human mutation, 23(6), pp.527-533.
3. Batista, R.L., Costa, E.M.F., Rodrigues, A.D.S., Gomes, N.L., Faria Jr, J.A., Nishi, M.Y., Arnhold, I.J.P., Domenice, S. and Mendonca, B.B.D., 2018. Androgen insensitivity syndrome: a review. Archives of endocrinology and metabolism, 62, pp.227-235.
4. Hughes, I.A., Davies, J.D., Bunch, T.I., Pasterski, V., Mastroyannopoulou, K. and MacDougall, J., 2012. Androgen insensitivity syndrome. The Lancet, 380(9851), pp.1419-1428.
5. Batista, R.L., Craveiro, F.L., Ramos, R.M. and Mendonca, B.B., 2022. Mild androgen insensitivity syndrome: the current landscape. Endocrine Practice, 28(9), pp.911-917.
6. Stancampiano, M.R., Suzuki, K., O'Toole, S., Russo, G., Yamada, G. and Faisal Ahmed, S., 2022. Congenital micropenis: etiology and management. Journal of the Endocrine Society, 6(2), p.bvab172.
7. Holzman Gottlieb B, Trifiro MA. Androgen Insensitivity Syndrome. In: Literature Cited. University of Washington, Seattle, Seattle (WA); 1993. PMID: 20301602.
8. Holzman, S.A., Davis-Dao, C.A. and Khoury, A.E., 2022. From Conception to Adulthood: The Impact of Androgens on Abnormalities of Male Genital Development and Size. Androgens: Clinical Research and Therapeutics, 3(1), pp.80-84.