Seeking a basic solution for a complex case of periostitis

Michael Bennett[1, 2], **Jerry Greenfield**[1, 3, 2], **Jackie Center**[1, 3, 2]

1. Department of Endocrinology, St Vincent's Hospital , Darlinghurst, NSW, Australia

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

3. Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

A 68-year-old woman presented to the emergency department with a four-month history of progressive lower limb weakness and lethargy associated with multiple small and large joint pains, worst in the bilateral shoulders, hips, elbows, and hands. She had lost 12 kilograms (17% body weight) over 12 months, and was no longer able to mobilise or perform her activities of daily living without assistance.

Her medical history included a single left lung transplant in August 2018 for pulmonary fibrosis and emphysema. Transplantation was complicated by *Lomentospora prolificans* colonisation with mycetoma formation in the right lung requiring long-term treatment with voriconazole and terbinafine. She was diagnosed with osteoporosis in August 2017 (femoral neck T-score -3.0SD) and had been receiving denosumab 60mg six monthly since diagnosis. She had no history of fragility fracture. She was diagnosed with type two diabetes and primary hypothyroidism in 2011, which were well controlled.

Her regular medications included: immunosuppressants (tacrolimus, mycophenolate, and prednisone 7.5mg daily), antimicrobials (azithromycin, trimethoprim/sulfamethoxazole, valganciclovir, terbinafine, and voriconazole 300mg TDS), colecalciferol, levothyroxine, and metformin. She had ceased calcium supplements one-month prior on the advice of her transplant team.

Initial investigations (Table 1) revealed hypophosphataemia (0.58 mmol/L) and secondary hyperparathyroidism (PTH 25.6 pmol/L, corrected calcium 2.30 mmol/L), normal 25-hydroxyvitamin D, renal function and magnesium. Despite treatment with denosumab her bone turnover markers were unsuppressed (P1NP 104 ug/L, CTX 307 ng/L). Voriconazole concentration was supratherapeutic (6.2 mg/L, target 3-4mg/L). 24-hour urine collection demonstrated renal calcium conservation and phosphate wasting.

Table 1: Initial investigations including serum (left) and urine (right) results

Parameter	2/5/20	8/5/20	20/5/20	Reference
Creatinine		72		45-90 umol/L
eGFR		74		>60 mL/min/1.73m^2
Urea		7.3		3.5-8 mmol/L
Corrected calcium		2.30		2.1-2.6 mmol/L
Phosphate		**0.58**		0.7-1.5 mmol/L
PTH	**25.6**			2-9 pmol/L
25(OH)vit D		65		50-150 nmol/L
1,25(OH)$_2$vit D			93	60-200 pmol/L
Albumin		**28**		33-48 g/L
ALP		107		30-110 U/L
GGT		70		0-35 U/L
AST		17		0-30 U/L
ALT		6		0-35 U/L
CTX		307		50-800 ng/L
P1NP		**104**		8-84 ug/L
FGF23		**189**		23.2-95.4 ng/L
Voriconazole	**6.2** mg/L on 15/5/20			

24h Urine	7/5/20	Reference
Volume	1.459	L
Calcium conc	0.4	mmol/L
Calcium excr	**0.6**	2.5-8 mmol/day
Phosphate conc	20.7	mmol/L
Phosphate excr	30.2	mmol/day
Creatinine conc	3.3	mmol/L
Creatinine excr	4.4	mmol/day
TmP/GFR	**0.116**	0.8-1.35 mmol/L

Urine metabolic screen (03/7/20): no abnormality
Urine glucose: 0

Skeletal x-rays showed widespread ill-defined calcific deposits consistent with thick periosteal reactions in the hands, forearms, shoulders, and hips (Image 1 and 2). Radionuclide bone scan demonstrated multiple areas of abnormal tracer uptake corresponding to radiological areas of periosteal new bone formation (Image 3). FDG-PET, DOTATATE-PET, and sestamibi parathyroid scintigraphy were normal (Image 3).

Image 1: X-ray images of right hand, left shoulder, hips and pelvis, right elbow, and lumbar spine

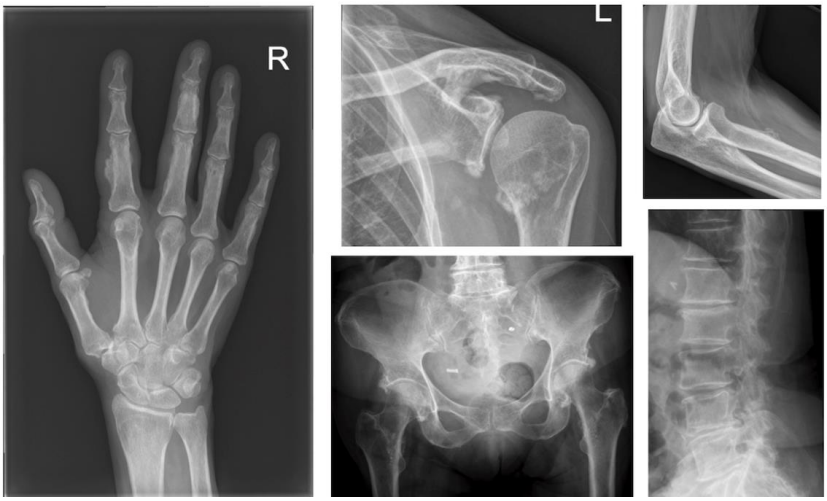


Image 2: Evolution of radiological changes in the left shoulder

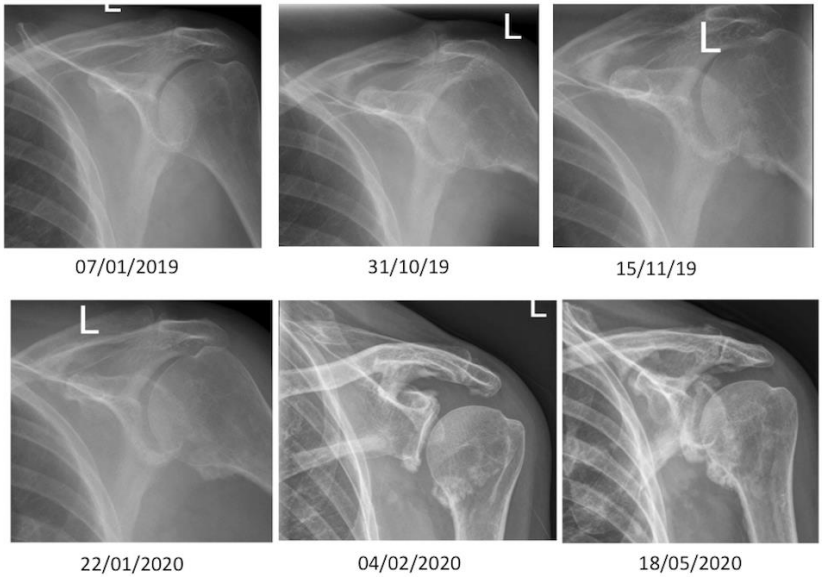
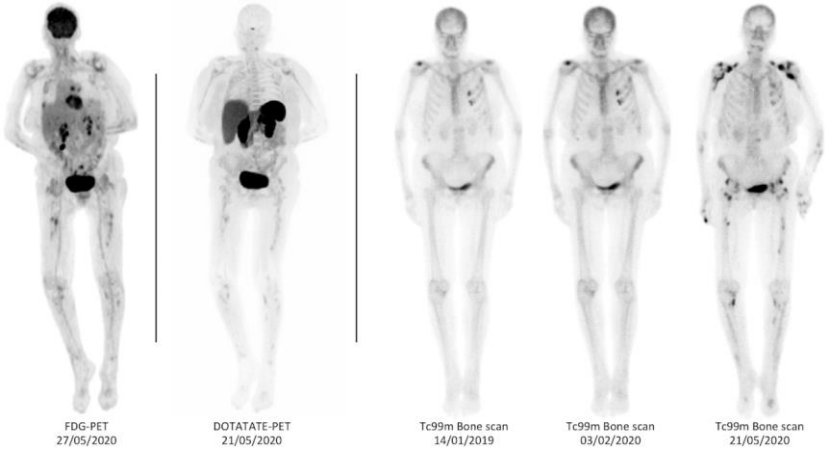


Image 3: Nuclear medicine studies (left to right): FDG-PET, DOTATATE-PET, and serial Tc99m Bone scans



DXA demonstrated normal T-scores in the lumbar spine and osteopenic T-scores in the right femoral neck (-2.0SD). Right total hip bone density had increased 25% over 3 years.

The emergence of widespread nodular periostitis and secondary hyperparathyroidism after transplantation suggested an acquired cause. The skeletal distribution and lack of digital clubbing argued against hypertrophic pulmonary osteoarthropathy. Imaging studies did not indicate a malignant source.

Voriconazole-related periostitis was suspected secondary to fluoride toxicity. This was confirmed by a plasma fluoride concentration (31 nmol/L, reference range 0.5-1.5 nmol/L).

Denosumab was ceased and calcium and calcitriol were commenced. *Lomentospora* was isolated from bronchoalveolar washings that was sensitive only to combination voriconazole and terbinafine. Access was sought to a novel orotomide antifungal (F901318) that was undergoing phase III clinic trials and was effective against *Lomentosporta*, however the manufacturer declined. To promote fluoride excretion, sodium bicarbonate was commenced for urinary alkalinisation (target pH >7.5).

Over the next five weeks, serum fluoride concentration slowly reduced to a nadir of 13 umol/L (Figure 2). With intensive rehabilitation her weight increased 3.6kg and mobility improved so that she was able to stand unassisted and mobilise with a four wheeled walker prior to discharge (Table 2).

Graph 1: Plasma fluoride concentration and voriconazole dose versus time

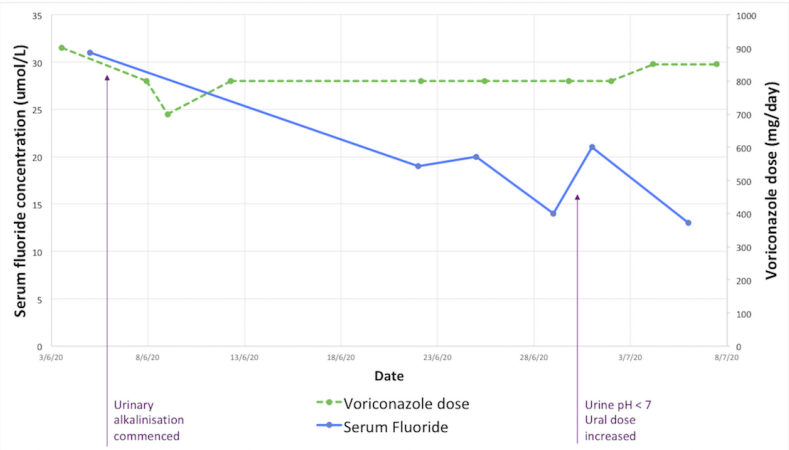


Table 2: Progress following calcium and calcitriol treatment and urinary alkalinisation

Parameter	6/7/20	4/6/20	2/5/20	Reference
Creatinine	67	74	69	45-90 umol/L
eGFR	81	72	78	>60 mL/min/1.73m^2
Corrected calcium	2.45	2.41	2.19	2.1-2.6 mmol/L
Phosphate	0.93	0.91	0.55	0.7-1.5 mmol/L
PTH	6.2	7.7	25.6	2-9 pmol/L
ALP	208	254	103	30-110 U/L
Voroconazole	1.0	3.4	0.4	mg/L
Calcium dose	500mg	500mg	Nil	/day
Phosphate dose	Nil	Nil	1000mg	/day
Voriconazole dose	850mg	900mg	1200mg	/day
Calcitriol dose	0.5mcg			/day
Mobility: STS	Standby assist	Moderate assist	Full assist	
Mobility: walking	Independent 4WW	Standby assist with 4WW	Full assist	
Weight	62.5	61	58.7	kg

Four weeks later, she was readmitted with deteriorating respiratory function, acute kidney injury and skeletal pain. After careful consideration and discussion with her treating physicians and family she decided to discontinue active treatment and receive terminal care. She died of respiratory failure six days later.

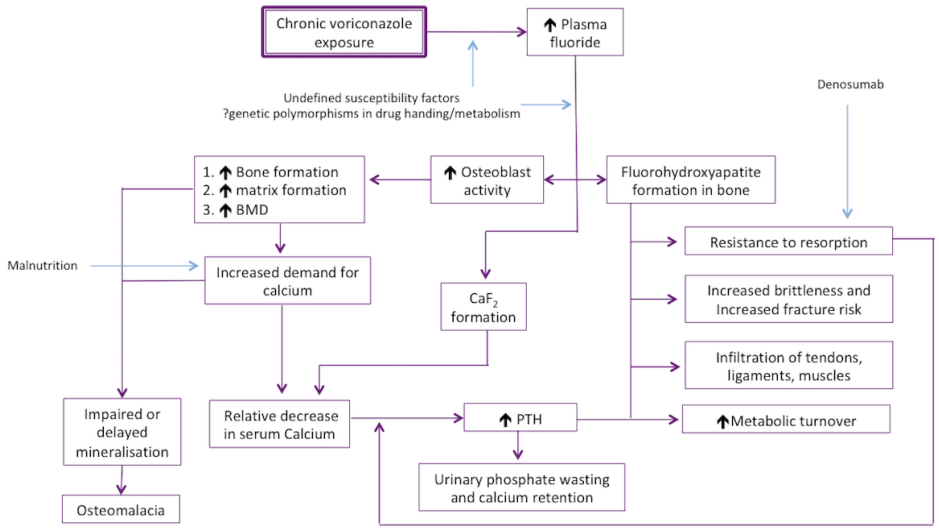
LITERATURE REVIEW

Voriconazole is a broad-spectrum triazole antifungal agent with activity against *Lomentospora*, *Aspergillus*, *Candida*, and *Fusarium* species(1). It is widely used in the post-transplant setting where fungal colonisation and invasive fungal infections are common. Since the first description of the condition among a series of lung transplant recipients in 2009, several other case series' have been published(2-6).

The condition is characterised by diffuse, irregular, periostitis and exostosis on x-ray, raised ALP, generalised bone and joint pain, and multifocal uptake on radionuclide bone scintigraphy(3). The clinical features are indistinguishable from subacute fluoride intoxication, which lead authors to conclude that these conditions have a common aetiology. This theory is supported by several observations: (a) significantly elevated plasma fluoride concentrations are universally observed among cases, (b) each voriconazole molecule contains three fluoride atoms and five precent of the dose is metabolised to fluorine, and (c) discontinuation of voriconazole results in a rapid normalisation of serum fluoride concentration that coincides with resolution of clinical and radiological features(3-5).

Excess fluoride exposure has deleterious cellular, mineral, matrix, and hormonal effects on bone (Image 4). Fluoride ions have a similar size and electrical charge to hydroxide ions but greater affinity for calcium(7). They replace hydroxide ions within the hydroxyapatite lattice forming fluoroapatite(8). This alters the physical and mechanical properties of the lattice increasing stability and density but making it brittle and more resistant to resorption(7, 8). Increased skeletal demand for calcium combined with resistance to resorption results in secondary hyperparathyroidism, which may lead to urinary phosphate wasting(8). Fluoride also has anabolic effects, stimulating osteoblasts to make excess new, poor quality, unmineralised bone (osteomalacia)(8). Furthermore, it accumulates most readily in the periosteal and endosteal regions of bone, explaining the pronounced radiological changes in these areas(9).

Image 4: Proposed pathophysiological model incorporating case-specific factors (denosumab & malnutiriton)



While the incidence of voriconazole-related periostitis is unknown, a retrospective review of 242 haematopoietic stem cell recipients receiving voriconazole at the Mayo Clinic found 32 (13.2%) patients had serum fluoride measurement performed to investigate musculoskeletal pain, of which 29 (93%) were elevated(10). Pain associated with voriconazole use was observed in 15.3% of patients after one year of treatment and 35.7% of patients after two years of treatment(10). No significant association between serum voriconazole concentration and plasma fluoride was observed(10).

Those who develop periostitis have significantly higher serum fluoride and ALP and cumulative voriconazole doses, compared with those who do not develop skeletal disease(4). Mean serum fluoride concentrations >8 umol/L are associated with skeletal toxicity and elevated fluoride concentrations with bony pain in the setting of voriconazole use is highly suggestive of periosotitis(3, 4) .

Pharmacogenomic variation in drug metabolising enzymes (particularly CYP2C19) is thought to account for individual susceptibility to this adverse effect(1, 3). Despite fluoride being predominantly renally excreted, renal function was shown not to predict serum fluoride levels among transplant patients taking voriconazole(3).

Discontinuation of voriconazole is the only curative treatment for voriconazole-related periosotitis. In a series of ten cases, all experienced normalisation of ALP and resolution of symptoms within two months of cessation(3). In cases where cessation is not possible, supportive care involves analgesia, correction of malnutrition, and treatment of hyperparathyroidism with calcium and calcitriol. As fluoride absorption, distribution, and excretion are pH-dependent, and not homeostatically regulated, we attempted urinary alkalinisation to increase free fluoride excretion(9). While this appeared to reduce serum fluoride concentrations in the short term, the long-term efficacy of this approach in preventing disease progression requires further investigation.

LEARNING POINTS

1. Chronic voriconazole exposure is associated with skeletal toxicity in a subset of individuals.
2. Clinical features include: diffuse, irregular periostitis and exostosis on x-ray, raised ALP, generalised bone and joint pain, and multifocal uptake on radionuclide bone scintigraphy.
3. All reported cases have been associated with high serum fluoride concentrations.
4. Discontinuation of voriconazole results in normalisation of serum fluoride concentration and resolution of signs and symptoms.
5. If it is not possible to cease voriconazole, supportive therapy involves calcium and calcitriol supplementation and correction of any malnutrition.
6. Urinary alkalinisation may increase fluoride excretion, although further studies are required.

REFERENCES

1. Scholz I, Oberwittler H, Riedel KD, Burhenne J, Weiss J, Haefeli WE, et al. Pharmacokinetics, metabolism and bioavailability of the triazole antifungal agent voriconazole in relation to CYP2C19 genotype. British journal of clinical pharmacology. 2009;68(6):906-15.
2. Ayub A, Kenney CV, McKiernan FE. Multifocal nodular periostitis associated with prolonged voriconazole therapy in a lung transplant recipient. JCR: Journal of Clinical Rheumatology. 2011;17(2):73-5.
3. Wermers RA, Cooper K, Razonable RR, Deziel PJ, Whitford GM, Kremers WK, et al. Fluoride excess and periostitis in transplant patients receiving long-term voriconazole therapy. Clinical Infectious Diseases. 2011;52(5):604-11.
4. Moon WJ, Scheller EL, Suneja A, Livermore JA, Malani AN, Moudgal V, et al. Plasma fluoride level as a predictor of voriconazole-induced periostitis in patients with skeletal pain. Clinical Infectious Diseases. 2014;59(9):1237-45.
5. Chen L, Mulligan ME. Medication-induced periostitis in lung transplant patients: periostitis deformans revisited. Skeletal radiology. 2011;40(2):143-8.
6. Wang T, Wang T, Altman R, Eshaghian P, Lynch III J, Ross D, et al. Periostitis secondary to prolonged voriconazole therapy in lung transplant recipients. American Journal of Transplantation. 2009;9(12):2845-50.
7. Johnston NR, Strobel SA. Principles of fluoride toxicity and the cellular response: a review. Archives of Toxicology. 2020:1-19.
8. Teotia S, Teotia M, Singh K, editors. Highlights of forty years of research on endemic skeletal fluorosis in India. 4th International workshop on fluorosis Prevention and Defluoridation of water; 2004.
9. Buzalaf MAR, Whitford GM. Fluoride metabolism. Fluoride and the oral environment. 22: Karger Publishers; 2011. p. 20-36.
10. Barajas MR, McCullough KB, Merten JA, Dierkhising RA, Bartoo GT, Hashmi SK, et al. Correlation of pain and fluoride concentration in allogeneic hematopoietic stem cell transplant recipients on voriconazole. Biology of Blood and Marrow Transplantation. 2016;22(3):579-83.

Not So Idiopathic SIADH

Rebecca Foskey[1], Emma Boehm[2], Andra Desra[3], Cherie Chiang[1, 3], John Wentworth[1]

1. Diabetes & Endocrinology, Royal Melbourne Hospital, Melbourne, VIC, Australia

2. Endocrinology and Diabetes, Western Health, Sunshine, Victoria, Australia

3. Department of Internal Medicine, Peter MacCallum Cancer Institute, Parkville, VIC, Australia

A 45-year-old male presented to the emergency department with acute malaise and fatigue. He had a past medical history of depression, anxiety and childhood bladder surgery for atonic bladder and was not prescribed any regular medications. He was clinically euvolaemic and his serum sodium was 121 mmol/L. Full blood examination, liver function tests, other electrolytes, creatinine, urea and tests for secondary causes of hyponatraemia were normal. Paired serum and urine osmolality were 252 mOsm/kg and 261 mOsm/kg respectively, with urine sodium 143 mmol/L. CT brain was unremarkable and a CT chest revealed occasional intraparenchymal knots and subpleural lung nodules which were classified as low risk and not in need for further investigation according to Fleischner lung nodule guidelines.

A diagnosis of idiopathic syndrome of inappropriate ADH (SIADH) was made and the patient was treated with a 750 mL fluid restriction which, over the subsequent year, maintained serum sodium between 128 and 134 mmol/L.

Fifteen months after the initial presentation with hyponatraemia, the patient presented to his general practitioner with back pain after a bicycle accident. Imaging revealed extensive lytic lesions throughout the humerus and spine, with widespread lymphadenopathy above and below the diaphragm. On GATATE PET scan, the spinal metastases were intensely avid but there was no significant uptake in the lymphadenopathy. Conversely, FDG PET revealed uptake in both the osseous metastases and the lymphadenopathy. Neither scan showed any focal avidity to suggest the location of a primary tumour. An excisional lymph node biopsy was performed. Histology revealed a Grade 3 metastatic neuroendocrine tumour of unknown primary, with Ki67 20.4% (<1/10 mitoses/HPF), strong and diffuse staining for synaptophysin and chromogranin, and focal but weak positive staining for cytokeratin AE1 and AE3. A research-grade polyclonal antibody raised against the first 100 amino acids of the vasopressin-neurophysin 2-copeptin precursor identified nests of immunoreactive tumour cells, confirming their ability to produce ADH prohormone. Copeptin concentrations in stored serum samples were dramatically elevated (>5000 pmol/L; no reference range for hyponatraemia; for non-water deprived, non-fasting adults normal <16.3 pmol/L), suggesting the efficiency of prohormone processing to ADH and copeptin by the tumour was very low.

The NET was initially treated with external beam radiotherapy to the spine, carboplatin/etoposide and zoledronic acid. Disease progression prompted a change to second line capecitabine/temozolamide in addition to a left femoral internal fixation for prophylaxis against pathological fracture.

The fluid load associated with this procedure precipitated severe symptomatic hyponatraemia (nadir sodium 113 mmol/L), treated with intravenous 3% saline and 500mL fluid restriction. The sodium concentration become progressively more difficult to maintain over subsequent months despite adherence to the fluid restriction. Further hospital admissions for hyponatraemia were precipitated by pain crises related to bony metastases. FOLFIRI chemotherapy and nivolumab monotherapy were trialled to halt disease progression without effect and daily tolvaptan was prescribed to maintain a safe sodium concentration.

Discussion

Hyponatraemia, defined as a serum sodium of less than 135mmol/L, is a common finding in the inpatient oncology setting, with one centre reporting a prevalence of 47% among inpatients with a diagnosis of cancer[1]. SIADH is the most common cause of euvolaemic, hypo-osmolar hyponatraemia. Inappropriate hypothalamic production and release of ADH from the posterior pituitary can occur as a result of numerous non-malignant and malignant disorders[2].

Paraneoplastic SIADH is due to ectopic secretion of ADH from tumour cells. The persistent unregulated expression of ADH in paraneoplastic SIADH leads to excessive dilution of free sodium, the primary aetiology of the observed hyponatraemia. SIADH has been reported in 11-15% of patients with small cell lung carcinoma and 3% of patients with head and neck cancer[3-4]. However, the condition is rare in non-small cell neuroendocrine tumours. There are only two other reported cases of paraneoplastic SIADH in these tumours confirmed by either elevated serum ADH or positive ADH immunohistochemistry staining of tumour specimen[5,6].

Historically, confirmation of ectopic ADH secretion has been challenging because ADH has a short half-life and readily degrades ex vivo[7]. The advent of copeptin sandwich immunoassay circumvents these issues. Copeptin is the more stable 39-amino-acid protein cleaved from the c-terminal end of pre-provasopressin released in stoichiometric equivalence with ADH[7,8].

Our report describes a male in his fifth decade initially diagnosed with idiopathic SIADH with a subsequent revision of the diagnosis after investigations revealed a slowly progressive neuroendocrine tumour of unclear primary that stained positive for the vasopressin-neurophysin 2-copeptin prohormone. The striking elevation in copeptin suggests his circulating ADH levels were also extremely high, which would explain the refractory nature of his SIADH. Had this degree of copeptin elevation been measured when he first developed hyponatraemia, it may have prompted more extensive investigation for occult tumour by PET scanning. Instead, the patient's disease progressed and his prognosis was poor at the time of diagnosis. As his symptoms worsened, the prescription of tolvaptan improved his quality of life by allowing a more permissive fluid restriction and reducing the severity of hyponatraemia during pain or periods of relative fluid loading, such as during surgery.

This case is novel given it is the first report of a patient with confirmed ectopic ADH secretion from a non-small cell neuroendocrine tumour with both positive ADH immunohistochemistry and elevated copeptin.

The implications of this clinical experience are numerous. Firstly, syndrome of inappropriate ADH should only be labelled "idiopathic" after extensive investigation. Our findings suggest that a serum copeptin level should now be considered for inclusion in this workup, particularly in young patients without significant comorbidities. There is value in making this diagnosis as it prognosticates for the persistence of the individual patient's predisposition to hyponatraemia. It provides impetus for early exploration of other treatment modalities, such as urea or tolvaptan, to relieve the burden of strict fluid balance and potentially prevent hospital admission with relapse[9,10]. Finally, in this setting copeptin could be used alongside PET imagining as a marker for tumour differentiation. This is an area that requires further study.

1. Fenske W, Sandner B, Christ-Crain M. A copeptin-based classification of the osmoregulatory defects in the syndrome of inappropriate antidiuresis. Best Pract Res Clin Endocrinol Metab. 2016;30(2):219-233. doi:10.1016/j.beem.2016.02.013
2. Doshi SM, Shah P, Lei X, Lahoti A, Salahudeen AK. Hyponatremia in hospitalized cancer patients and its impact on clinical outcomes. Am J Kidney Dis. 2012;59(2):222-228. doi:10.1053/j.ajkd.2011.08.029
3. Sorensen, J. B., M. K. Andersen, and H. H. Hansen. 1995. Syndrome of inappropriate secretion of antidiuretic hormone (SIADH) in malignant disease. J. Intern. Med. 238:97–110.
4. Talmi, Y. P., H. T. Hoffman, and B. F. McCabe. 1992. Syndrome of inappropriate secretion of arginine vasopressin in patients with cancer of the head and neck. Ann. Otol. Rhinol. Laryngol. 101:946–949.
5. Yarmohammadi H, Erinjeri JP, Brown KT. Embolization of metastatic neuroendocrine tumor resulting in clinical manifestations of syndrome of inappropriate secretion of antidiuretic hormone. J Vasc Interv Radiol. 2015;26(4):533-537. doi:10.1016/j.jvir.2014.11.032
6. Alshaikh OM, Yoon JY, Chan BA, et al. Pancreatic Neuroendocrine Tumor Producing Insulin and Vasopressin. Endocr Pathol. 2018;29(1):15-20. doi:10.1007/s12022-017-9492-5
7. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem. 2006;52(1):112-119. doi:10.1373/clinchem.2005.060038
8. Morgenthaler NG, Müller B, Struck J, Bergmann A, Redl H, Christ-Crain M. Copeptin, a stable peptide of the arginine vasopressin precursor, is elevated in hemorrhagic and septic shock. Shock. 2007;28(2):219-226. doi:10.1097/SHK.0b013e318033e5da
9. Nervo A, D'Angelo V, Rosso D, et al. Urea in cancer patients with chronic SIAD-induced hyponatremia: Old drug, new evidence. Clin Endocrinol (Oxf). 2019;90(6):842-848. doi:10.1111/cen.13966
10. Kai K, Tominaga N, Koitabashi K, Ichikawa D, Shibagaki Y. Tolvaptan corrects hyponatremia and relieves the burden of fluid/dietary restriction and hospitalization in hyponatremic patients with terminal lung cancer: a report of two cases. CEN Case Rep. 2019;8(2):112-118. doi:10.1007/s13730-019-00375-7

Not your typical atypical fracture

Annabelle M Warren[2, 1], Peter R Ebeling[3, 4], Vivian Grill[5], Ego Seeman[2, 6], Shoshana Sztal-Mazer[7, 1]

1. Department of Endocrinology, The Alfred Hospital, Melbourne, VIC, Australia

2. Department of Endocrinology, The Austin Hospital, Heidelberg, VIC, Australia

3. Department of Medicine, Monash University, Clayton, VIC, Australia

4. Department of Endocrinology, Monash Health, Clayton, VIC, Australia

5. Department of Endocrinology, Western Health, Sunshine, VIC, Australia

6. Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia

7. Women's Health Research Program, School of Public Health and Preventative Medicine, Monash University, Melbourne, VIC, Australia

Introduction

Hypophosphatasia (HPP) is a rare but under-recognised genetic defect of bone mineralisation often misdiagnosed as osteoporosis. Patients with hypophosphatasia can present with fragility fractures and have an increased risk of atypical femoral fracture (AFF) after bisphosphonate therapy. We diagnosed a case of adult onset hypophosphatasia in a 40-year-old woman presenting with bilateral atypical femoral fractures after 4 years of denosumab therapy. A low serum alkaline phosphatase (ALP) level is an important clue in the diagnosis of hypophosphatasia. Clinicians should be aware that hypophosphatasia can be misdiagnosed as antiresorptive therapy-related AFF and that both bisphosphonates and denosumab are contraindicated in this condition.

Case

A 40-year-old female from rural Victoria was referred to the Alfred Hospital metabolic bone clinic after sustaining bilateral AFFs 10 months prior. At the time of referral, she was mobilising with crutches and required a wheelchair over longer distances.

Her first fracture was sustained in 2005 at the age of 25 when she suffered left navicula, 3rd and 5th metatarsal fractures after a fall from standing height. She experienced delayed fracture healing for many years and a diagnosis of complex regional pain syndrome was made. On assessment of her delayed healing, a computed tomography (CT) scan of her foot was performed in 2013, which reported radiographic osteopenia, later confirmed on Dual Energy X-ray Absorptiometry (DXA). At that time the patient was commenced on a weekly oral bisphosphonate by her general practitioner but experienced gastrointestinal intolerance and ceased after 4 months. Denosumab was commenced in 2014, which she received regularly every 6 months for 4 years.

Notable past history included a seizure at the age of 28 for which she was prescribed sodium valproate. She denied any developmental or pubertal delay, early dental loss or any periodontal pathology, or family history of fractures.

In December 2018, aged 39, the patient sustained bilateral atypical femoral fractures after a fall from standing height. A symptomatic complete right femoral diaphyseal fracture was surgically fixed on 28 December 2018. The pre-operative x-ray showed features of an AFF with beaking of the lateral femoral periosteum at the fracture site. Imaging of the contralateral left femur demonstrated a nondisplaced asymptomatic partial AFF which was conservatively managed. Both fractures fulfilled 2014 ASBMR criteria for AFF. Denosumab was discontinued with no further doses administered after June 2018. DXA in February 2019 showed bone mineral density (BMD) at the lumbar spine 1.150 g/cm2 (T score +0.8, Z score +0.9) and left total hip BMD 0.730 g/cm2 (T score -1.7, Z score -1.4). (Z score represents number of standard deviations below age-and sex-matched mean).

Upon presentation to our bone clinic in October 2019, the patient's height was 153.1 cm and weight 69.2 kg, with body mass index 29.5 kg/m^2. She did not have obvious craniofacial abnormality.

Investigation in October 2019: Serum calcium 2.31 mmol/L (2.10 – 2.60), parathyroid hormone 2.9 pmol/L (1.1 – 6.0) and renal function (eGFR > 90 mL/min/1.73m^2) were normal. Serum phosphate was just above the upper limit of normal 1.55mmol/L (0.75-1.50. Serum alkaline phosphatase (ALP) was low at 11 U/L (30 – 110) which, on further review, had also been apparent in 2016 when it was 7 U/L (30 to 120) whilst the patient was on denosumab. Carboxy-terminal collagen crosslinks (CTX) were low at 127 ng/L (150-800) and *N*-terminal propeptide of type I procollagen (P1NP) was normal at 32 mcg/L (15-70). The serum 25-hydroxyvitamin D was sufficient at 96 nmol/L (50-100). Vitamin B6 (pyridoxine) was extremely elevated >2000 nmol/L (35 – 110). Cranial X-ray showed no evidence of craniosynostosis. Repeat femoral X-rays revealed non-union of the right AFF and near completion of the left AFF with the exception of an intact medial cortex. Knee and hip imaging demonstrated mild degenerative arthrosis of the left hip.

The clinical history, low ALP and elevated vitamin B6 supported a diagnosis of hypophosphatasia. ALPL gene testing performed by Sydney Children's Hospital Network Genetic Service revealed two pathogenic heterozygous ALPL variants (c.526G>A; 881 A>C), confirming the diagnosis of recessive hypophosphatasia.

In February 2020, 18 months of subcutaneous teriparatide 20mcg daily therapy was commenced via the Alfred Hospital's individual patient usage program. Compassionate use of asfotase alpha enzyme therapy will be sought in the future. Since commencement of teriparatide, there have been no further fractures but the patient continues to require a gait aid to mobilise. Serial femoral X-Rays are planned to monitor fracture healing.

Discussion

Hypophosphatasia is a rare genetic disorder affecting mineralisation of bone due to a defect in the ALPL gene leading to deficiency of alkaline phosphatase (ALP), causing osteomalacia. There is a wide spectrum of phenotypic presentation with a severe childhood-onset form occurring in approximately 1/100,000 births manifesting skeletal deformity and even neonatal death. Less severe forms detected in adulthood are more frequent, up to 1/2500, and are increasingly being recognised in adults misdiagnosed with osteoporosis (1). Other manifestations include delayed fracture healing, stress fractures, pyrophosphate arthropathy and vitamin B6-responsive seizures. Epilepsy occurs as ALP also plays a role in vitamin B6

metabolism, with ALP deficiency leading to reduced neuronal production of inhibitory GABA neurotransmitter, and accumulation in serum of its activated vitamin B6 precursor.

There are four previously-reported cases of AFF following bisphosphonate use in patients with hypophosphatasia (2), and an increased risk of AFF is also thought possible with denosumab (3). In support we identified two further cases of AFF in hypophosphatasia following denosumab treatment from the literature, one of whom had also received bisphosphonate therapy (4, 5). Our patient's short four-month course of bisphosphonate therapy and 4-year interval between bisphosphonate exposure and fractures would suggest denosumab as the greater contributor in her case.

Hypophosphatasia caused impaired bone mineralisation and osteomalacia as reduced tissue non-specific ALP (TNSALP) impairs hydroxyapatite crystal deposition, leading to accumulation of the inhibitory substrate inorganic pyrophosphate (PPi). In healthy bone, microscopic cracks that may predispose to fracture are resorbed by osteoclasts through targeted bone remodelling. Denosumab inhibits RANK ligand which is responsible for osteoclast recruitment and action. Impaired resorption of damaged bone coupled with the predisposition for impaired mineralisation of new bone is thought to explain increased risk for AFF in patients with hypophosphatasia using antiresoprtive therapy (2).

Targeted enzyme therapy with asfotasfe alfa is optimal treatment for hypophosphatasia, and has been shown to promote fracture healing in patients with hypophosphatasia (6). Access to Asfostase alfa is limited as its cost is prohibitive (>$1 million AUD per year). Teriparatide may be an alternative treatment that has not been observed to increase fracture and may theoretically improve healing while halting ongoing fracture propagation, but does not provide a sustained response in hypophosphatasia (7). Preliminary results suggest antisclerostin therapy may have a role (8).

Clinicians should be alert to the possible diagnosis of HPP in patients with fragility fractures and low serum ALP. In addition, both bisphosphates and denosumab can increase the risk of AFF in these patients and are therefore contraindicated. Hypophosphatasia is one of several monogenic bone diseases that can result in AFF (9).

1. Mornet E, Yvard A, Taillandier A, Fauvert D, Simon-Bouy B. A molecular-based estimation of the prevalence of hypophosphatasia in the European population. Ann Hum Genet. 2011;75(3):439-45.
2. Marini F, Brandi ML. Atypical femur fractures: a distinctive tract of adult hypophosphatasia. Clin Cases Miner Bone Metab. 2017;14(3):324-8.
3. Shapiro JR, Lewiecki EM. Hypophosphatasia in Adults: Clinical Assessment and Treatment Considerations. J Bone Miner Res. 2017;32(10):1977-80.
4. Lefever E, Witters P, Gielen E, Vanclooster A, Meersseman W, Morava E, et al. Hypophosphatasia in Adults: Clinical Spectrum and Its Association With Genetics and Metabolic Substrates. J Clin Densitom. 2018.
5. Magdaleno AL, Singh S, Venkataraman S, Perilli GA, Lee YY. Adult-Onset Hypophosphatasia: Before and After Treatment with Asfotase Alfa. AACE Clin Case Rep. 2019;5(6):e344-e8.
6. Klidaras P, Severt J, Aggers D, Payne J, Miller PD, Ing SW. Fracture Healing in Two Adult Patients With Hypophosphatasia After Asfotase Alfa Therapy. JBMR Plus. 2018;2(5):304-7.
7. Gagnon C, Sims NA, Mumm S, McAuley SA, Jung C, Poulton IJ, et al. Lack of sustained response to teriparatide in a patient with adult hypophosphatasia. J Clin Endocrinol Metab. 2010;95(3):1007-12.
8. Seefried L, Baumann J, Hemsley S, Hofmann C, Kunstmann E, Kiese B, et al. Efficacy of anti-sclerostin monoclonal antibody BPS804 in adult patients with hypophosphatasia. J Clin Invest. 2017;127(6):2148-58.
9. Nguyen HH, van de Laarschot DM, Verkerk AJMH, Milat F, Zillikens MC, Ebeling PR. Genetic Risk Factors for Atypical Femoral Fractures (AFFs): A Systematic Review. JBMR Plus. 2018;2(1):1-11.

A novel approach to the treatment of ectopic ACTH-dependent Cushing's

Kate Flentje[1, 2], Anna Cunliffe[1], Anindita Chakrabarti[1, 3], Adam Roberts[1, 2, 4]

1. University Hospital Geelong, East Geelong, Victoria, Australia

2. Barwon Endocrinology, Geelong, Victoria, Australia

3. Geelong Endocrinology and Diabetes, Geelong, Victoria, Australia

4. Epworth Geelong, Geelong, Victoria, Australia

Case Presentation

BC is a 75 year old man on chemotherapy for metastatic prostate small cell carcinoma who presented with profound hypokalaemia , hypertension and new onset hyperglycaemia. Very high cortisol levels were associated with non-specific cushingoid features including generalised weakness and debility, lower limb oedema, central adiposity and low mood. ACTH was also found to be very high raising suspicion of a paraneoplastic cause in the absence of a pituitary tumour. Metastatic deposits were widespread and included liver, lung, and skeletal involvement.

His background medical conditions also included hypertension, hyperlipidaemia and atrial fibrillation. There was no personal or family history of endocrine disorders. Regular medications included atorvastatin 20mg daily, apixaban 5mg twice daily, bicalutamide 50mg daily, and 4 monthly doses of leuprorelin. He was on carboplatin and etoposide chemotherapy. He had no drug allergies.

Investigations

Initial investigations showed hypokalaemia with a metabolic alkalosis and hyperglycaemia.

Table 1. Baseline haematology and biochemistry

Analyte	Result	Reference range
Hb (g/L)	130	125-175
WCC	**3.1 x10(9)/L**	4.0-11.0
Na (mmol/L)	142	135-145
K (mmol/L)	**2.4**	3.5-5.0
Bicarb mmol/L)	**37**	20-32
Glucose (mmol/L)	**18.1**	4.0-7.9
PSA	0.09	<6.51
ALP (U/L)	90	35-110
GGT (U/L)	**141**	5-50
AST (U/L)	36	10-40
ALT (U/L)	58	5-40
Albumin (g/L)	29	34-45

Further endocrine evaluation revealed the following:

Table 2: Baseline endocrine biochemistry

Analyte	Result	Reference range
Cortisol AM (nmol/L)	**1141**	133-537
Cortisol PM (nmol/L)	**1150**	68-327
24hr urinary free cortisol (nmol/d)	**10,653**	<130
Plasma ACTH (pmol/L)	**58**	1.6-13.9
Aldosterone (pmol/L)	**68**	100-950
Renin (mU/L)	32	3.3-41
ARR (pmol/L /mU/L)	2	<70
TSH (mU/L)	**0.11**	0.5-6.0
FT4 (pmol/L)	19.8	11.0-22.0
GH (ug/L)	0.2	
IGF-1 (nmol/L)	11	7-28
Prolactin (mIU/L)	377	90-400
Testosterone (nmol/L)	**1.7**	19-76
SHBG (nmol/L)	**15**	19-76
Free Testosterone (pmol/L)	**56**	130/570

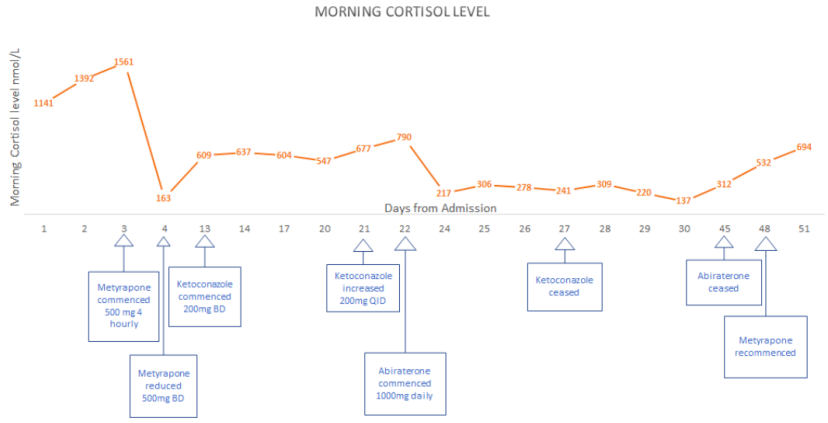


Figure 1: Timeline of treatments and morning cortisol level trend

The patient was immediately commenced on cortisol blocking therapy. Ketoconazole was not immediately available so treatment was initiated with metyrapone 500mg every four hours, the less preferred option due to patient expense. A repeat morning cortisol level within 24 hours of therapy was dramatically reduced at 163nmol/L. Shortly thereafter, E. coli urosepsis lead to an ICU admission for haemodynamic support, metyrapone dosing was subsequently reduced to 500mg twice daily and he was commenced on IV hydrocortisone under a block-and-replacement rationale.

When it became available metyrapone was replaced with ketoconazole 200mg twice daily. Hydrocortisone was changed to tapering doses of prednisolone. Pantoprazole was started for acid suppression and spironolactone was instituted to counteract mineralocorticoid excess and potassium deficits. As the patient improved, ketoconazole was incremented over a fortnight to 200mg six hourly, with monitoring for hepatotoxicity. However, morning cortisol levels remained elevated, from 575-790nmol/L.

At this time there was a discussion between the treating oncologist and endocrinologist about alternative treatment options. The novel idea of abiraterone, a CYP17 inhibitor used to treat metastatic prostate cancer, was put forth with potential dual benefit in treatment for cancer and hypercortisolaemia. Abiraterone was commenced at 1000mg daily and the following morning a cortisol level of 217nmol/L indicated good effect. Ketoconazole was able to be ceased.

Repeat morning cortisol level, five days later, demonstrated a decrease to 167nmol/L and was associated with a significant postural drop. Dexamethasone 2mg daily was commenced to treat potential adrenal insufficiency. He continued to improve and was weaned off potassium infusions. His blood pressure stabilised, blood glucose levels normalised without the need for insulin and he was able to be transferred to rehabilitation.

One week later routine monitoring showed recurrent hypercortisolism with a morning cortisol of 694nmol/L, an ACTH of 109pmol/L, with associated worsening hypertension and hypokalaemia. Liver function tests were deranged requiring cessation of abiraterone. Due to disease progression, BC was transferred to palliative care and passed away two months after initial diagnosis of CS.

Discussion:

This case demonstrates the treatment challenges of this disease as well as the novel use of an established oncology therapy for ACTH dependent Cushing's syndrome. The following points will form the basis of discussion:

1. Prostate small cell cancer is a rare cause of Cushing's syndrome. CS due to ectopic production of ACTH is associated with a large range of tumours including small cell lung cancer and bronchial carcinoid most commonly. Prostate cancer-causing CS is rare with less than 30 cases published worldwide1.

2. Suppressed aldosterone despite apparent mineralocorticoid excess in this case may be accounted for by: Profound hypercortisolism resulting in overwhelmed capacity of 11B-HSD2 enzyme which usually protects renal tubular mineralocorticoid receptors from cortisol2; Excessive mineralocorticoid receptor stimulation and downregulation of aldosterone[2;] Direct inhibition of 11B-HSD2 by ACTH,2 and/or Downregulation of the renin-angiotensin pathway as the patient was receiving intravenous potassium with normal saline at the time of blood collection.

3. Treatment of hypercortisolism associated with ectopic ACTH secretion includes resection of the primary tumour and nodes as first line therapy3. Second line therapies include bilateral adrenalectomy as well as medical therapies3. No optimal order of medications has been established for the management of severe hypercortisolism[3]. Potential therapies include metyrapone and ketoconazole which were used in this case for their quick onset of action. These medications work at different sites of the steroid synthesis pathway (figure 1). Ketoconazole is no longer registered for use in Australia but is available via the special access scheme. Treatment requires close monitoring for severe hepatitis and drug-drug interactions. Furthermore, absorption requires an acidic environment which may be the cause of treatment failure in our patient who was taking concurrent pantoprazole.

4. The mechanism of action of these adrenal blocking therapies warrants consideration. Metyrapone and ketoconazole inhibit multiple steps in cortisol biosynthesis (figure 2). Both agents have similar efficacy measured in retrospective multicenter trials, ranging from 53-88% for ketoconazole5 and 43-76% for metyrapone6. Other options include mitotane which is generally reserved for treatment of adrenocortical cancer and has toxic side effects, etomidate which is only available in an intravenous formulation and mifepristone, a glucocorticoid receptor antagonist. This agent is difficult to monitor and prohibitively expensive for prolonged use in Australia. A recent phase III trial of osilodrostat (LC1699 in figure 1), an inhibitor of CYP11B1 showed this is an effective new treatment option for endogenous Cushing's disease with 86% complete response at 34 weeks7, however this is not yet available for use in Australia. Close monitoring is required with all agents to assess efficacy of adrenal blockade, detect hypoadrenalism and toxicities.

5. Abiraterone as a novel treatment. It irreversibly inhibits CYP17 (17 alpha hydroxylase) which is required for glucocorticoid and androgen biosynthesis. It is commonly used to treat relapsed and metastatic prostate cancer, in addition to standard androgen deprivation therapy, with improved progression free survival8. In prostate cancer it is usually administered with glucocorticoid to overcome hypocortisolaemia and to suppress pit-ACTH mediated mineralocorticoid production. In this case,

abiraterone was used to ameliorate hypercortisolism and block androgen production in the management of his prostate cancer. To our knowledge, this is the first time abiraterone has been used for this dual purpose.

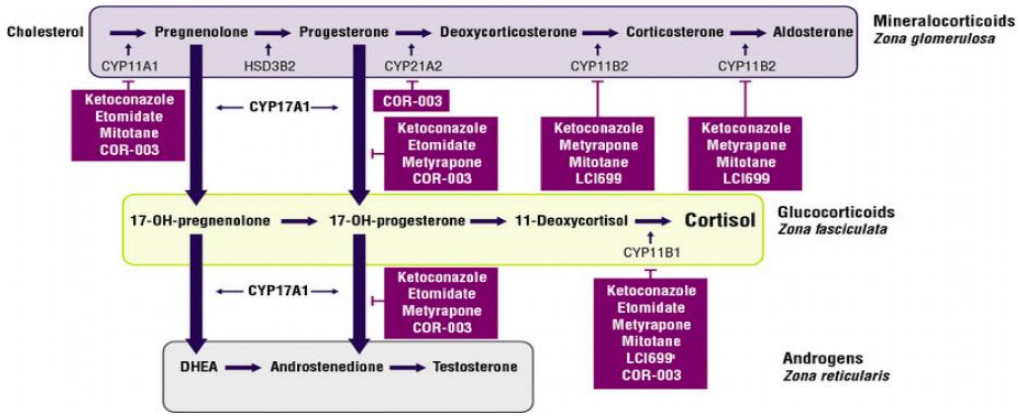


Figure 2: Steroid synthesis pathway and sites of steroidogenesis inhibitor action4.

Key Learning Points:

- ACTH dependant Cushing's syndrome in dedifferentiated prostate small cell cancer is very rare
- While surgery is the preferred treatment for ectopic ACTH secretion, medical therapies for inoperable cases is limited and determining best therapy can be difficult
- Abiraterone could become a novel treatment option for paraneoplastic ACTH secretion in prostate cancer associated hypercortisolaemia offering dual inhibition of androgen and steroid production

1. Elston et al. Severe Cushing's syndrome due to small cell prostate carcinoma: a case and review of literature. Endocrine Connections, 2017, R80–R86
2. Torpy, D. J., Mullen, N., Ilias, I., & Nieman, L. K. (2002). Association of hypertension and hypokalemia with Cushing's syndrome caused by ectopic ACTH secretion: A series of 58 cases. Annals of the New York Academy of Sciences, 970, 134–144. https://doi.org/10.1111/j.1749-6632.2002.tb04419.x.
3. Nieman et al Guidelines on Cushing's Syndrome Treatment J Clin Endocrinol Metab. August 2015, 100(8): 2807-2831
4. Fleseriu, Maria & Castinetti, Frederic. (2016). Updates on the role of adrenal steroidogenesis inhibitors in Cushing's syndrome: a focus on novel therapies. Pituitary. 19. 10.1007/s11102-016-0742-1
5. Castinetti F et al, (2014) Ketoconazole in Cushing's disease: is it worth a try? J Clin Endocrinol Metab 99:1623–1630
6. Daniel E et al. (2015) Effectiveness of metyrapone in treating Cushing's syndrome: a retrospective multicenter study in 195 patients. J Clin Endocrinol Metab 100:4146–4154
7. Pivonello et al (2020) Efficacy and safety of osilodrostat in patients with Cushing's disease (LINC 3): a multicentre phase III study with a double-blind, randomised withdrawal phase. Lancet Diabetes and Endocrinology, Volume 8, Issue 9, p748-761 748-761
8. James, N. D.,et al. (2017). Abiraterone for Prostate Cancer Not Previously Treated with Hormone Therapy. New England Journal of Medicine, 377(4), 338–351. https://doi.org/10.1056/NEJMoa1702900

5

Thyroid immune related adverse events following immune checkpoint inhibitor treatment: a multi-centre, retrospective cohort study

Christopher A Muir[1, 2], Venessa HM Tsang[3, 1], Georgina V Long[4, 1, 5], Roderick Clifton-Bligh[3, 1, 2], Alexander M Menzies[4, 1, 5]

1. Faculty of Medicine and Health, University of Sydney, Camperdown, NSW, Australia

2. Cancer Genetics, Kolling Institute of Medical Research, St. Leonard's, NSW, Australia

3. Endocrinology, Royal North Shore Hospital, St. Leonard's, NSW, Australia

4. Medical Oncology, Royal North Shore Hospital, St. Leonard's, NSW, Australia

5. Medical Oncology, Melanoma Institute Australia, Wollstonecraft, NSW, Australia

Background: Thyroid toxicity is common following treatment with CTLA-4 and PD-1 immune checkpoint inhibitors (ICIs). Published studies estimate the incidence at 10-20%, although rates vary widely between different ICIs. The aetiology of ICI-associated thyroid immune related adverse events (irAEs) is unknown and onset of thyroid dysfunction is highly variable with not all patients developing the classic presentation of transient hyperthyroidism followed by a hypothyroid phase. The current study aimed to fully characterize thyroid irAEs in a large cohort of patients with melanoma.

Methods: We reviewed outcomes in 1246 adult patients undergoing ICI treatment for advanced or metastatic melanoma from a prospectively maintained database.

Results: Immune related adverse events (irAEs) affecting the thyroid occurred in 518 (42%) patients. Over a median follow-up of 11.3 months, multiple patterns of thyroid-irAEs were observed. Primary hyperthyroidism developed in 31% of treated patients and comprised 75% of total thyroid-irAE cases. Primary hypothyroidism occurred in 8% of subjects, with euthyroid hyperthyroxinemia and hypothyroxinemia observed in 10 (<1%) and 6 (<1%) subjects respectively. Non-thyroidal illness (isolated low FT3) occurred in 14 (1%) participants. Thyroid irAEs were most frequent following combination CTLA-4/PD-1 inhibitor treatment (56%). CTLA-4 and PD-1 inhibitor monotherapy resulted in lower rates of thyroid irAEs, 25% and 38% respectively. Severity of thyroid irAEs differed by ICI, with higher rates of overt thyroid dysfunction following combination ICI treatment (45%) relative to PD-1 inhibitor (34%) and CTLA-4 inhibitor (17%) monotherapies.

Conclusions: Thyroid irAEs affect >40% of ICI-treated patients and multiple clinical phenotypes of thyroid dysfunction occur. Higher incidence and more severe thyroid irAEs occur following combination CTLA-4 + PD-1 inhibitor treatment relative to either CTLA-4 or PD-1 inhibitor monotherapy.

1. Muir CA, Menzies AM, Clifton-Bligh R, Tsang VHM. Thyroid toxicity following immune checkpoint inhibitor treatment in advanced cancer. Thyroid, 2020; (online ahead of print).

6

The copeptin response and adverse event profile to hypertonic saline in healthy volunteers - the effect of nausea and vomiting

Emily K Brooks[2, 1], Caroline Bachmeier[3], Juanita Vorster[2], Jane Sorbello[2], Faseeha Peer[2], Viral Chikani[2, 1], Goce Dimeski[4], Jacobus Ungerer[3], Carel Pretorius[3], Warrick Inder[2, 1]

1. Faculty of Medicine, University of Queensland, Brisbane, QLD, Australia

2. Department of Endocrinology, Princess Alexandra Hospital, Brisbane, QLD, Australia

3. Chemical Pathology, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia

4. Pathology Queensland, Princess Alexandra Hospital, Brisbane, QLD, Australia

Objective: Measurement of hypertonic saline-stimulated copeptin has recently been described for the differentiation of polyuria-polydipsia syndrome. This study aims to determine the copeptin response to intravenous 3% hypertonic saline, including evaluation of adverse effects, in a local cohort of healthy adults >18 years in Australia.

Design: Prospective clinical study

Methods: Twenty healthy volunteers (10 males and 10 females) were recruited. Participants underwent infusion of 3% hypertonic saline via a previously described standardised protocol (1,2), until the plasma sodium was ≥150 mmol/L, with measurement of plasma copeptin.

Results: Median peak sodium was 152 mmol/L (range 150 - 155) with osmolality 316 mmol/kg (range 306 - 320). Mean volume of hypertonic saline infused to reach target sodium ≥150 mmol/L was 1645 mL (range 1230 - 2220 mL). Median rate of plasma sodium rise was 5.6 mmol/L/hour (range 4.0 - 8.7 mmol/L/hour). Hypertonic saline-stimulated copeptin was normally distributed with mean of 48.0 pmol/L (median 29.1 pmol/L; range 9.6 - 167.4). Overall median symptom burden was 6/10 (range 3/10-9/10). Copeptin was significantly higher for those who experienced nausea and/or vomiting (n=13) (median 39.0 pmol/L; IQR 32.5, 90; range 25.0 - 67.4), compared to those participants who did not experience either (median 20.0 pmol/L; IQR 13.0 - 31.0; range 10.0 - 33.0) (p = 0.001). There were no serious adverse events.

Conclusions: Hypertonic saline-stimulated copeptin measurements were similar in our population compared to previously reported reference intervals in healthy volunteers (1). There is a wide range of stimulated copeptin measurements in the healthy population. Nausea and vomiting are common adverse effects and are associated with a significantly enhanced copeptin response. Significant nausea and/or vomiting could lead to possible false negative hypertonic saline-stimulated copeptin results in patients with central DI.

1. Fenske WK, Schnyder I, Koch G, Walti C, Pfister M, Kopp P, et al. Release and Decay Kinetics of Copeptin vs AVP in Response to Osmotic Alterations in Healthy Volunteers. J Clin Endocrinol Metab. 2018;103(2):505-13.
2. Fenske W, Refardt J, Chifu I, Schnyder I, Winzeler B, Drummond J, et al. A Copeptin-Based Approach in the Diagnosis of Diabetes Insipidus. N Engl J Med. 2018;379(5):428-39.

7

A Pragmatic Lifestyle Intervention in Obese Pregnant Women to Limit Gestational Weight Gain

Rebecca F Goldstein[1, 2], Jacqueline A Boyle[1, 3], Cheryce L Harrison[1, 2], Helena J Teede[1, 2]

1. School of Public Health and Preventive Medicine, Monash University, Clayton, VIC, Australia

2. Diabetes and Vascular Medicine Unit , Monash Health, Clayton, VIC, Australia

3. Department of Obstetrics and Gynaecology, Monash Health, Clayton, VIC, Australia

Background: Excess gestational weight gain (GWG) is common and has adverse maternal and infant outcomes including increased gestational diabetes. This trial aimed to demonstrate effectiveness of lifestyle intervention on reducing excess GWG and to explore implementation across intervention uptake and adherence in routine care.

Methods: This pragmatic controlled implantation-effectiveness trial was conducted in a tertiary hospital maternity service. Participants included women with pre-pregnancy BMI of 35-43kg/m² at risk of excess GWG, recruited before the 23rd week of gestation. The intervention group attended routine antenatal care with an integrated behavioural lifestyle intervention delivered by a health coach and an endocrinologist in a total of five visits. Women who developed endocrine complications (gestational diabetes, thyroid disease) were treated within the service, simplifying their treatment plan. The comparison group was existing standard of care.

Results: 277 women were studied: 157 in intervention, 120 in standard care. The intervention did not result in a difference in proportion of women who gained excessive GWG above IOM recommendations, 32% of intervention and 33% of standard care exceeding (p=0.91). There was a reduction in total GWG (-1.4 (95% CI -2.6,-0.1),p=0.04 adjusted bootstrap), also significant when excluding for women who developed GDM (-2.0 (95% CI -4.0,-0.2),p=0.03 adjusted). Mean GWG/week was also lower in intervention group (0.32kg/wk vs 0.37 kg/wk, p=0.02, adjusted). Intervention uptake was 95% and 87% attended 4 of 5 sessions.

Conclusion: Lifestyle intervention embedded in routine antenatal care for obese women lowered total GWG and GWG/week, although the proportion of pregnancies with excessive GWG was similar in both groups. Intervention uptake and engagement rates were high. This pragmatic trial of lifestyle implementation into pregnancy care is important in the context of new national guidelines recommending GWG control. Lower intensity intervention studies for non-obese women are underway across multiple services, states and countries, as are cost-effectiveness analyses.

8

Changes in radioactive iodine management in thyroid cancer; a quaternary centre experience.

Ayanthi A Wijewardene[1, 2], Matti Gild[1, 2], Adam Aniss[2], Diana Learoyd[1], Bruce Robinson[1, 2], Lyndal Tacon[1, 2], Roderick Clifton-Bligh[1, 2]

1. University of Sydney, Sydney, NSW, Australia

2. Department of Endocrinology , Royal North Shore Hospital, St Leonards

Background: Radioactive iodine (RAI) is often used in patients with thyroid cancer, however the optimal dosage of RAI has yet to be established. Adjuvant RAI reduces recurrence in moderate-high risk thyroid cancer [1]. In low risk disease, RAI is often employed to ablate remnant thyroid tissue. Changes to clinical practice over the past decade, influenced by the release of HiLO and ESTIMABLI studies in 2012 and ATA guidelines in 2015([1-3], have seen a shift to lower doses of RAI.

Method: Retrospective analysis of 1348 patients who received RAI at a quaternary centre in Australia between 2008 and 2018. Prospectively collected data included age, gender, histology, and AJCC stage (7th ed). ATA risk was calculated retrospectively. Statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) version 26 (SPSS Inc IL, USA).

Results: The median dose of RAI decreased from 4.21 GBq in 2008 to 3.9 GBq in 2018 (<0.0001). The principal driver of this change was an increased use of 1GBq dose from 1.2% in 2008-2011 to 20.4% in 2017-2018. In binomial regression analysis, factors significantly associated with low dose RAI (<1.3 GBq) were females (OR 1.67, p=0.037 [CI 1.03-2.72]), low ATA risk (OR 6.61, p <0.001 [CI 2.2.66- 16.39]) and stage 1 AJCC (OR 4.84, p=0.004 [CI 1.64-14.31]). Confining the analysis to 2017-2018, only low ATA risk (OR 6.82, p=0.007 [1.70-27.36]) were associated with use of low RAI dose. For patients assessed as low risk of recurrence by ATA criteria in 2017-2018, 42.3% received a low dose with a median dose of 1.95GBq.

Conclusions: RAI doses have significantly reduced over the past decade at our institution. Nevertheless, some patients assessed as low risk of recurrence by ATA criteria are still receiving high doses of RAI.

9

Skin glucocorticoid metabolism in burn injury: novel approaches to reduce scarring

Kevin Hung-Yueh Tsai[1, 2], Roxanne Janine Parungao[2], Huaikai Shi[2], Sina Naficy[3], Ying Hui Fung[2], Josephine Ivy Malcolm[2], Xiaosuo Wang[4], Zhe Li[5], Andrea C Issler-Fisher[5], Peter Maitz[2, 5], Mark Cooper[1], Yiwei Wang[2]

1. Adrenal Steroid Group, ANZAC Research Institute, Sydney, NSW, Australia

2. Burns & Reconstructive Surgery Research Group, ANZAC Research Institute, Sydney, NSW, Australia

3. School of Chemical and Biomolecular Engineering, The University of Sydney, Sydney, NSW, Australia

4. Bosch Mass Spectrometery Facility, Bosch Institute, Sydney, NSW, Australia

5. Burns & Reconstructive Surgery Unit, Concord Hospital, Sydney, NSW, Australia

The development of excessive scarring and fibrosis have become the most severe and common complications of burn injury. Current treatments have limited effect on postburn scarring. Prolonged exposure to high levels of glucocorticoids (Cushing's syndrome) detrimentally impacts on skin, leading to skin thinning and impaired wound healing. A major source of glucocorticoids in skin is local production by 11β-hydroxysteroid dehydrogenase type 1 enzyme (11βHSD1). We hypothesised that skin glucocorticoid metabolism by 11βHSD1 is an important regulator of wound healing, fibrosis and scarring after burn injury. We additionally proposed that pharmacological manipulation of this system would improve outcomes of burn wound healing.

We examined glucocorticoid metabolism in burn and non-burn skin from burn injury patients (n=14) and mouse models of burn injury (1cm^2 full thickness burn in C57Bl/6 mice). We utilised mice with genetic or pharmacological deletion of 11βHSD1 in skin to evaluate the effects of 11βHSD1 on burn injury healing and wound fibrosis. We also developed slow release scaffolds containing therapeutic agents including inactive glucocorticoids (prednisone) that are selectively reactivated in skin cells expressing 11βHSD1.

Expression of 11βHSD1 in human and mouse skin increased substantially after burn injury (7.1±1.8-fold increase on day 4-9 compared to non-burn skin, p<0.05). Mice with 11βHSD1 deletion experienced faster wound healing post burn (17% reduced wound area at day 7 compared to wildtype, p<0.0001) but healed wounds had excessive collagen density, myofibroblast accumulation and skin stiffness and strength (359±57 kPa tensile strength wildtype compared to 513±88 kPa KO, p<0.05). In wildtype mice application of scaffolds loaded with inactive glucocorticoid (prednisone) significantly reduced scar size, myofibroblast differentiation and collagen production, demonstrating feasibility of using enzyme substrates to improve wound outcomes.

The findings demonstrate the importance of skin 11βHSD1 in wound healing and scarring after burn injury and indicates ways in which excessive scarring might be prevented.

10

Using patient-derived models to systematically identify synergistic drug combinations for advanced prostate cancer

Nicholas Choo[1], Laura Porter[1], Birunthi Niranjan[1], Jennii Luu[2], Susanne Ramm[2], Kaylene Simpson[2], Mitchell Lawrence[1], Renea Taylor[3], Gail Risbridger[1, 4]

1. Monash Biomedicine Discovery Institute Cancer Program, Prostate Cancer Research Group, Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia

2. Victorian Centre for Functional Genomics, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

3. Monash Biomedicine Discovery Institute Cancer Program, Prostate Cancer Research Group, Department of Physiology, Monash University, Clayton, VIC, Australia

4. Melbourne Urological Research Alliance, Monash Biomedicine Discovery Institute Cancer Program, Monash University, Clayton, VIC, Australia

Advanced prostate cancer is typically treated sequentially with monotherapies, usually targeting the androgen receptor (AR). However, this inevitably produces more aggressive, drug-resistant tumours with varying aberrations in the AR pathway or AR loss altogether. Rational combination therapies may improve treatment of diverse phenotypes of prostate cancer. Yet, preclinical testing of new drug combinations is constrained by the paucity of patient-derived models spanning the diversity of prostate cancer, and the lack of methods for using them to assess drug synergy.

To identify effective combination treatments for AR-positive and AR-negative prostate cancer, we aimed to establish new methods for measuring drug synergy with patient-derived models.

We developed conditions to grow cells from patient-derived xenografts (PDXs) from the Melbourne Urological Research Alliance as organoids - 3D cultures embedded in Matrigel. Next, we used organoids to test drug synergy with a novel combination of talazoparib, a PARP inhibitor, and CX-5461, a small molecule that induces DNA damage. Combination therapy significantly decreased organoid viability based on overall metabolic activity. The effect was synergistic as shown with CompuSyn software. Combination therapy also enhanced DNA damage, marked by γH2AX, in AR-positive and AR-negative organoids.

To reveal the full complexity of drug synergy, we devised a novel, automated assay to measure the growth and composition of individual organoids. With segmentation-based analysis of confocal and brightfield microscopy, we showed that talazoparib and CX-5461 consistently and synergistically reduced the area, cellular density and uniformity of organoids.

In conclusion, talazoparib and CX-5461 synergistically inhibit the growth of advanced prostate cancer, regardless of AR expression. These results informed the design of a phase 1 clinical trial. For the first time, this shows that prostate cancer organoids can reveal drug synergy in high-throughput assays. This increases the scale and scope of organoid experiments, accelerating translation of new treatments for prostate cancer.

11

GDF15 over-expression in brainstem increases brown fat thermogenesis, raises sensitivity to aversion and reduces preference for sweet taste

Nikita Bajaj[1], Catherine Makdsi[1], Craig A Harrison[1], Kelly L Walton[1], Zane B Andrews[1], Sarah H Lockie[1]

1. Department of Physiology, Monash University, Melbourne, Victoria, Australia

Animals utilize distinct neural circuits for body weight maintenance via integration of hormonal and metabolic signals to balance energy expenditure and food intake. Brown Adipose Tissue (BAT) maintains metabolic balance via diet-induced thermogenesis. Growth Differentiation Factor 15 (GDF15) drives weight loss and reduces food consumption, but its impact on energy expenditure is unknown. GDF15 is elevated in a range of conditions with increased nausea and feeding aversion such as cachexia and pregnancy. GDF15 signals through its receptor GFRAL, expressed solely in the brainstem areas, the area postrema (AP) and nucleus of the solitary tract (NTS). Evidence suggests GFRAL activation may activate the lateral parabrachial nucleus and central amygdala, areas of brain associated with fear and feeding aversion.

To investigate how GDF15 signalling impacts on BAT thermogenesis and innate and learned responses to tastes, C57black/6 mice were stereotaxically injected into the NTS with an AAV to overexpress GDF15. Following this, mice were assessed for anxiety-like behaviour, conditioned taste aversion (CTA) to lithium chloride and saccharin preference. Mice were also implanted with radio-telemeters into the interscapular BAT for continuous monitoring of BAT temperature and locomotor activity during baseline and metabolic manipulations.

GDF15 overexpression resulted in a failure to adaptively decrease thermogenesis during fasting, and a suppression of food intake upon refeeding. Complete behavioural categorization of all time spent in a baited open field shows mice behave similarly during the test. GDF15-overexpressing mice have increased sensitivity to a CTA associated lithium chloride. They also show decreased preference for sweet taste (0.1% saccharin or water; two bottle choice) compared to controls, however this normalises after three days of exposure to the paradigm. This indicates increased sensitivity to aversive responses to taste, and decreased sensitivity to pleasurable taste – supporting the idea that elevated GDF15 may shift negatively impact emotional responses to feeding stimuli.

12

The role of adrenal glucocorticoids in circadian resynchronisation and stability in mice

Pureum Kim[1], Warrick Inder[2, 3], Oliver Rawashdeh[1]

1. School of Biomedical Sciences, University of Queensland, Brisbane, QLD, Australia

2. Faculty of Medicine, University of Queensland, Brisbane, QLD, Australia

3. Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Brisbane, QLD, Australia

The circadian system is a 24-h timing system that generates rhythms in behaviour and physiology and maintains their proper alignment to geophysical time. Adrenal glucocorticoids are important peripheral endocrine hormones that are rhythmically synthesised and released by the adrenals under the regulation of the central master circadian clock. The daily-timed release of glucocorticoids (corticosterone in rodents) suggests that the adrenals transmit information about daytime to its many targets within the body, and thus, maintaining the proper alignment of physiology and behaviour. One core clock gene, *Period1* (*Per1*), is involved in the regulation of corticosterone release. Its induction in the adrenal cortex results in rapid release of corticosterone when light is abruptly introduced. *Per1* also plays an important role in circadian clock resetting by light. Using a *Per1*-knockout mouse model, we aimed to investigate the role of *Per1* in glucocorticoids regulation, specifically, its role in circadian clock resetting of central-controlled rhythms (e.g. sleep/wake rhythms and metabolism) by subjecting mice to an experimental jet-lag paradigm. *Per1*-deficient mice showed normal rhythmic corticosterone secretion under standard 12:12 hour light:dark conditions but demonstrated prominently accelerated resynchronisation of both behaviour and corticosterone rhythm in response to the 12hr jet-lag. To further clarify whether rapid circadian clock resetting in *Per1*-deficient mice is attributed to corticosterone rhythm, we studied mice after bilateral adrenalectomy. Corticosterone deficiency further facilitated a more rapid resynchronisation of the circadian system in both wild-type and *Per1*-deficient mice. These data suggest that the absence of Per1 in the adrenals does not contribute the accelerated central clock resetting but rhythmic *Per1* and glucocorticoids reinforce the resistance of the circadian system to abrupt changes in photoperiod and enhance circadian stability. Extrapolating to humans, shift workers and international travellers may adapt to work roster/time zone changes more rapidly in the presence of reduced *Per1* and glucocorticoid activity.

13

ACE2 and SARS-CoV-2

Louise Burrell[1]

1. University of Melbourne, Melbourne, VIC, Australia

Content not available

14

The Year of the Rat (2020) in Clinical Diabetes

Jenny Gunton[1, 2, 3]

1. Garvan Institute, Darlinghurst, NSW, Australia

2. University of Sydney, Westmead, NSW, Australia

3. The Westmead Millennium Institute for Medical Research, The University of Sydney, Westmead Hospital, Division of Medicine, The Garvan Institute of Medical Research, Sydney

Content not available

15

Year in Thyroid

Don McLeod[1]

1. QIMR Berghofer Medical Research Institute, Herston, QLD

Content not available.

16

Paediatric Endocrinology during the *Covid-19 Pandemic*

Catherine Choong[1]

1. Princess Margaret Hospital, Subiaco, WA, Australia

Our understanding of susceptibility and effects of COVID – 19 infections continues to evolve as this pandemic continues. The spectrum of acute illness varies with age and children may have milder disease and deaths are rare. Some present with gastro intestinal symptoms rather than respiratory symptoms, others develop a systemic inflammatory syndrome temporally associated with SARS –CoV2 infection. SARS-CoV-2 utilises the angiotensin-converting enzyme 2 (ACE2) as a receptor for entry into host cells. This raises the possibility of internalisation into endocrine organs known to express ACE2. This includes pancreas, thyroid, testis, ovary, adrenal glands and pituitary. How COVID-19 infection affects the function and growth of these organs in children and neonates remains unknown. Early reports detailing the endocrine effects of COVID-19 infection in children in addition to advances in Paediatric Endocrine Research during 2019-2020 will be presented here.

17

Measuring glucose levels during an Insulin tolerance test: Can we improve the accuracy of results?

Maresa M Derbyshire[1]

1. St Vincent's Public Hospital, Melbourne, Fitzroy, VIC, Australia

In 2019 the Endocrine Testing Area (ETA) at our tertiary hospital, had seen a significant increase in referrals for the Insulin Tolerance Test (ITT), to assess adult pituitary hormone reserve, for the application of growth hormone (GH). The ITT requires an intravenous dose of insulin to reduce the blood glucose level to below 2.5mmol/l, while observing hypoglycaemic symptoms. A room temperature fluoride glucose tube is recommended for collection. Accuracy of results was questioned, when examining the laboratory glucose results compared to the lack of symptoms observed by some patient's. Senior scientists suggested a process known as glycolysis could result in a lower laboratory glucose level, and suggested an ice-water slurry or citrate tube to improve the accuracy as per the American Diabetes Association (ADA) guidelines.

The "Gold Standard" Yellow Springs Instrument Biochemical Analyser (YSI) was used to compare glucose results following the hospital and ADA guidelines for glucose tube handling and equipment:

- Fluoride tube at room temperature
- Fluoride tube in ice-water slurry
- Citrate tube at room temperature

Blood samples were collected at the same time point, from one syringe.

Due to significant patient insulin resistance, 16 time points were collected. Comparison of the YSI result, with the closest laboratory result, demonstrated the citrate tube, at room temperature was the most accurate at 76%, Fluoride tube on ice, 50% and fluoride tube at room temperature 0% with a difference of between 0.4-0.7mmol/l from the YSI value.

This small trial demonstrated the use of the fluoride tubes at room temperature as per the hospital guidelines provide the least accurate results. Larger studies need to be developed to assess the potential need for change in practice, handling and processing of glucose tubes to improve accuracy.

1. Potter, J., Hickman, P., Oakman, C., Woods, C. and Nolan, C., 2020. Strict Preanalytical Oral Glucose Tolerance Test Blood Sample Handling Is Essential for Diagnosing Gestational Diabetes Mellitus. Diabetes Care, 43(7), pp.1438-1441.
2. McAuley, S., Horsburgh, J., Ward, G., La Gerche, A., Gooley, J., Jenkins, A., MacIsaac, R. and O'Neal, D., 2016. Insulin pump basal adjustment for exercise in type 1 diabetes: a randomised crossover study. Diabetologia, 59(8), pp.1636-1644.
3. Department of Endocrinology and Diabetes, Endocrine Testing Area Protocol Book, St Vincent's Public Hospital Melbourne, G Drive, 2020
4. Bonetti, G., Carta, M., Montagnana, M., Lo Cascio, C., Bonfigli, A., Mosca, A. and Testa, R., 2016. Effectiveness of citrate buffer-fluoride mixture in Terumo tubes as an inhibitor of in vitro glycolysis. Biochemia Medica, pp.68-76.
5. Dimeski, G., Yow, K. and Brown, N., 2015. What is the most suitable blood collection tube for glucose estimation? Annals of Clinical Biochemistry, 52(2), pp.270-275.

18

Crisis Situation: A story of Addison's and pregnancy

Sarah Louise Fostekew[1]

1. Clinical Nurse Specialist, Endocrinology, Waikato Hospital, New Zealand

Primary adrenal insufficiency or Addison's disease is a rare chronic condition with an incidence of 4 -11/100,000 people. The management of Addison's disease during pregnancy is extremely difficult and rarely encountered by endocrinologists and obstetricians. Dysregulation of the maternal hypothalamic-pituitary-adrenal (HPA) axis determines foetal exposure to stress hormones influencing development and birth outcomes. In healthy pregnant women cortisol, ACTH, CRH and CBG increase throughout pregnancy whilst the placenta itself can produce active CRH and ACTH stimulating the maternal pituitary and adrenal glands, in Addison's it takes careful consideration to mimic the normal hormonal changes in pregnancy. Historically Addison's disease was associated with a high maternal mortality and women were strongly discouraged from becoming pregnant. Modern glucocorticoid replacement therapy and improved obstetric care, have reduced both maternal and foetal morbidity and mortality.

Addisonian crises are life threatening events in pregnant women. They can be precipitated by pregnancy specific stressors such as hyperemesis gravidrum, labour and surgery, as well as the same precipitants that occur outside of pregnancy.

This presentation describes the case of Miss B, a 24 year old woman. Following her diagnosis with Addison's disease. The first 18 months after her diagnosis and subsequent pregnancy will be explored, and the issues our team faced with her complex care needs.

Addison's disease in pregnancy requires a high degree of self-management to prevent Addisonian crises. The endocrine clinical nurse specialist (CNS) is vital to ensure the patient has the knowledge and support they need to continue pregnancy safely. The role of a CNS is to provide clinical expertise and support for patients. This case highlights the CNS role within the multidisciplinary team and the ongoing support we were able to provide within this complex case.

19

Newly appointed clinical nurse practitioner highlighting her role with case studies

Sinead Archbold[1]

1. Queensland Paediatric Endocrine Group, Runcorn, ACT, Australia

Content not available

20

Circadian, clock genes, and general metabolism

Oliver Rawashdeh[1]

1. Faculty of Medicine The University of Queensland, St Lucia, QLD, Australia

Content not available

21

Dynamic hormone diagnostics: Novel methods and new insights

Thomas Upton[1]

1. University of Bristol, Bristol, United Kingdom

Single point measurements in endocrinology are often very difficult to interpret. This is because hormone secretion is dynamic with variability across the day, within and between individuals. Diagnosis and management of endocrine disease could be greatly enhanced by the ability to easily capture and understand the dynamic information encoded in daily hormone profiles. Through the ULTRADIAN Dynamic Hormone Diagnostics trial (NCT02934399) we have developed a method of 'at home' high frequency, ambulatory sampling of multiple hormones over 24-hours, without the need for blood. Using features identified in the dynamic data, mathematical algorithms can be used to discriminate healthy from pathological states. Examples of the technique used in patients with endocrine disease (Cushing's and primary aldosteronism) will be presented. Integration of such ambulatory dynamic hormone data with outputs from wearable sensors is now a focus of my research which I hope will lead to a greater understanding of adaptive human physiology and the opportunity to provide more personal and individualised care.

22

Exploring the effect of general anaesthesia on patients post-operative sleep and circadian rhythms

Guy Warman[1], James F cheeseman[1], Nicola M Ludin[1], Diana Grieve[1], Alan F Merry[1], Andrew Kennedy-Smith[2], Carl MuthuKumaraswamy[2], Matthew DM Pawley[3]

1. The University of Auckland, Auckland, New Zealand

2. Department of Urology, Capital and Coast DHB, Wellington, New Zealand

3. Institute of Natural and Mathematical Sciences, Massey University, Auckland, New Zealand

Exploring the effect of general anaesthesia on patients post-operative sleep and circadian rhythms

Our research focusses on how general anaesthesia (GA) causes sleep and circadian disruption in patients, and what might be done to minimise this disruption and improve post-operate recovery. The overarching goal of this work is to: (1) understand *how* GA affects the circadian clock and sleep post-operatively using animal models and (2) develop ways to *combat* post-operative sleep and circadian disruption in patients using the principles of chronobiology. In this talk I will discuss the findings from our animal studies and our clinical trials on donor nephrectomy patients, and will summarize our current understanding of the disruptive effect of GA on the clock and sleep and what might be done about it.

Using the honey bee we have previously shown that GA (2% isoflurane for six hours) can effectively cause 'jet lag', shifting the circadian clock to a different time zone by acting on the expression of the core genes (*period* and *cryptochrome*) that drive daily rhythms (1). Furthermore, we have shown that the shifting effects of GA can be ameliorated by the administration of bright light during anaesthesia (2). These findings led us to investigate the same effect in more mainstream model organisms: transgenic circadian clock-reporter flies and mice.

Initial clinical studies on the extent of post-operative circadian and sleep disruption were hindered by the fact that disease itself causes sleep and clock disruption. We have recently completed a trial with a unique patient population—40 donor nephrectomy patients—to examine the "real world" effects of anaesthesia and surgery on the clock and sleep. In this patient population we have shown that anaesthesia and surgery cause substantial sleep and clock disruption to patients, and that "clock shifting" (blue) light, administered intraoperatively can reduce post-operative clock disruption (as measured by core body temperature rhythms). Patients receiving blue light show a delay in core temperature rhythms of 1.5h post-operatively compared to 3.4 h in the placebo group. Furthermore, patients receiving "clock shifting light" also show a reduced post-operative hospital stay (on average 3.55 days) compared to those receiving placebo light (3.95 days).

Our immediate aims are to conduct a larger multicentre trial to investigate this effect in larger and more diverse patient population and to further investigate the mechanisms underlying this effect in our laboratory studies.

References:

(1) Cheeseman, J.F., Winnebeck, E.C., Millar, C.D., Kirkland, L.S., Sleigh, J., Goodwin, M., Pawley, M.D.M., Bloch G., Lehmann, K., Menzel, R., and Warman, G.R. (2016). General anaesthesia alters time perception by phase shifting the circadian clock. *Proc Natl Acad Sci U S A.* 109 (18): 7061-6.

(2) Ludin, N.M., Cheeseman, J.F., Merry, A.F., Millar C.D., Warman, G.R. (2016). The effects of the general anaesthetic isoflurane on the honey bee (Apis mellifera) circadian clock *Chronobiology International* 33 (1): 128-133

23

Adrenal steroids and circadian clock signalling in the heart

Morag Young[1]

1. Baker Institute, Melbourne, VIC, Australia

The mineralocorticoid receptor (MR) has a direct role in cardiac physiology and disease and is a useful therapeutic target for patients with heart failure. However, significant side effects from mineralocorticoid receptor antagonist (MRA) treatment in up to 10% of patients has prompted efforts to identify new tissue specific mechanisms that may allow for selective MR therapies. Recent studies have identified the circadian clock as a novel, reciprocal interacting partner of the MR in the heart. While the closely related glucocorticoid receptor (GR) and its ligand, cortisol (corticosterone in rodents), are established regulators of the circadian clock, new data from this laboratory suggest that the MR can also regulate circadian clock gene expression and timing. Our recent data have revealed that MR signalling is modified by circadian transcription factors CLOCK and BMAL and thus by circadian time *in vivo.* Moreover, mRNA levels for the MR also follow a circadian pattern of expression. Taken together these data demonstrate a set of novel mechanisms whereby the always occupied MR may variations control of transcriptional activity. Moreover, defining the role of the MR and its ligands for the regulation of the molecular clock in the heart and other tissues has important implications for understanding dysregulation of these systems for cardiac disease progression, and for MR activation more broadly.

24

Recovery of Male Reproductive Function Following Prolonged Injectable Testosterone Undecanoate Treatment

Nandini Shankara Narayana[1], Reena Desai[2], Ann Conway[1], Bronwyn GA Stuckey[3], Warwick Inder[4], Mathis Grossmann[5], Bu B Yeap[6], Robert McLachlan[7], Lam P Ly[2, 1], Karen Bracken[8], Gary A Wittert[9], David J Handelsman[2, 1]

1. Andrology, Concord Repatriation General Hospital, Concord, NSW

2. Andrology, ANZAC Research Institute, Concord, NSW, Australia

3. Department of Endocrinology and Diabetes, Keogh Institute for Medical Research, Sir Charles Gairdner Hospital and University of Western Australia, Perth, Western Australia, Australia

4. Department of Endocrinology , Princess Alexandra Hospital and University of Queensland, , Brisbane, Queensland, Australia

5. Department of Endocrinology , The Austin Hospital and University of Melbourne, Melbourne, Victoria, Australia

6. Department of Endocrinology and Diabetes, Fiona Stanley Hospital, Medical School, University of Western Australia, , Perth, Western Australia, Australia

7. Endocrinology and Metabolism, Clinical Andrology , Hudson Institute of Medical Research, Melbourne, Victoria, Australia

8. NHMRC Clinical Trials Centre, University of Sydney, Sydney, NSW, Australia

9. Freemasons Foundation Centre for Men's Health, University of Adelaide, Adelaide, South Australia, Australia

Background: Exogenous androgen treatment suppresses the hypothalamo-pituitary testicular (HPT) axis causing reduced serum LH, FSH and testosterone (T). The Runoff Study [after Testosterone for Diabetes Mellitus (T4DM) Study] investigated the rate and extent of reproductive hormone recovery after 2 years of regular T undecanoate (TU) injections.

Methods: T4DM participants without pathological hypogonadism (n=1007) were randomised to TU or placebo (P) injections every 3 months for 2 years with 303 subsequently entering the Runoff study at 12 weeks after last injection. Before T4DM study unblinding, they provided blood samples and validated sexual function questionnaires (PDQ, IIEF-15) at entry, 6, 12, 18, 24, 40 and 52 weeks later. Serum steroid profile (T, DHT, E_2, E_1) was measured batchwise by LCMS and serum LH, FSH and SHBG by immunoassays.

Results: Runoff study participants in both groups were similar and no different from all T4DM participants. As expected, at Runoff study entry serum T was higher in TU-treated men but at all timepoints from 12 weeks onwards serum T and SHBG remained consistently 11% and 12%, respectively, lower in TU treated than in P treated men. Similarly, at Runoff entry sexual function scores were higher in TU-treated men but subsequently no different from P-treated men. Mean serum LH and FSH recovered gradually to reach mean baseline levels at 36 weeks after last injection with the median time to recover to their own pre-treatment baseline of 52.7 weeks (serum LH) and 51.1 weeks (serum FSH) after last injection.

Conclusion: After stopping 2 years of standard dose TU treatment in men without pathological hypogonadism, HPT function recovers slowly, taking 9 to >12 months since last dose, but is eventually complete. Persisting mild, proportionate reduction in serum SHBG and T reflect lasting effects of androgen treatment on hepatic SHBG secretion, but not androgen deficiency.

25

Metabolic syndrome in pregnancy and its association with child telomere length

Dale McAninch[1], Tina Bianco-Miotto[1, 2], Kathy Gatford[3], Shalem Leemaqz[3, 4], Prabha Andraweera[1], Amy Garrett[3], Michelle Plummer[3], Gus Dekker[1, 5], Claire Roberts[3, 4], Lisa Smithers[1, 6], Jessica A Grieger[3]

1. Robinson Research Institute, School of Medicine, University of Adelaide, Adelaide, SA, Australia

2. Waite Research Institute, University of Adelaide, Adelaide, SA, Australia

3. Adelaide Health and Medical Sciences and Robinson Research Institute, University of Adelaide, Adelaide, SA, Australia

4. Flinders Health and Medical Research Institute, Flinders University, Adelaide, SA, Australia

5. Department of Obstetrics and Gynaecology, Lyell McEwin Hospital, Elizabeth, Adelaide, SA, Australia

6. School of Public Health, University of Adelaide, Adelaide, SA, Australia

Aims: To determine whether maternal metabolic syndrome (MetS) in pregnancy associates with child telomere length or child anthropometry (weight, BMI) and blood pressure, measured at 10 y of age. **Methods:** The SCOPE study was a multi-centre, international prospective cohort of nulliparous pregnant women recruited from Australia, New Zealand, Ireland, and the UK (*n*=5628). The current analysis is a 10 year follow-up of SCOPE pregnant women and her children, from the Australian cohort. Clinical data collected at 14-16 weeks' gestation during the SCOPE study were used to diagnose MetS using International Diabetes Federation criteria. Telomere length, a biomarker of aging, was assessed by qPCR from child saliva collected at 10 y of age. **Results:** In women who completed follow up (*n*=255), 20% had MetS in pregnancy. After adjusting for a range of confounders, children of mothers who had MetS in pregnancy had 14% shorter telomeres than children of mothers without MetS (mean difference; 95% CI: -0.36; -0.74, 0.01). **Conclusion:** Children of mothers who had MetS in pregnancy have shorter telomeres, a biomarker of accelerated aging. Further studies in larger cohorts of children are warranted, as well as investigating whether telomere length measured in cord blood also associates with telomere length in childhood.

Proteomic signatures in early pregnancy to detect women at increased risk of gestational diabetes

Natassia Rodrigo[1, 2, 3], **Mark Larance**[4], **Sarah Glastras**[1, 2, 3]

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

2. University of Sydney, Sydney, NSW, Australia

3. Kolling Institute of Medical Researh, Sydney, NSW, Australia

4. Charles Perkins Centre, University of Sydney, Sydney, NSW, Australia

Background and Aims:

Gestational diabetes mellitus (GDM) increases pregnancy-related complications, obstetric interventions and adult-onset diabetes, in both mother and child. Established clinical risk factors for GDM lack specificity for its development. The addition of biomarkers may improve the prediction of GDM from early pregnancy. We aimed to determine if serum biomarkers would improve GDM prediction when added to established clinical risk factors.

Materials and Methods:

Pregnant women with at least one clinical risk factor for GDM (e.g. ethnicity, BMI) were recruited, a fasting blood sample collected, and a 75g oral glucose tolerance test performed at 12-18 weeks (blinded) and 24-28 weeks gestation. Metabolic and lipid profiles were analysed by standard laboratory measures and discovery proteomic profiles were obtained using mass spectrometry. ROC curve analysis (using probability scores) was carried out to determine the utility of adding metabolic markers to predict GDM from early pregnancy.

Results: 23/93 (24.7%) women developed GDM. Using logistic regression (LR), independent clinical risk factors conferring risk of GDM were BMI>30, ethnicity, past history of GDM ($P<0.05$). Independent metabolic markers at 12-18 weeks associated with GDM included fasting insulin, fasting glucose, 60min glucose on OGTT, and HDL ($P<0.01$). ROC curve analysis using clinical risk factors alone showed moderate predictability for GDM (AUC 0.735). Addition of these serum markers to the model improved risk prediction markedly (AUC 0.958). Discovery proteomic analyses demonstrate predictive potential for 7 protein markers, including adiponectin and matrix metalloproteinase-3 (MMP3).

Conclusions: Metabolic and proteomic serum markers at 12-18 weeks gestation were distinctly different and predictive of GDM development. Both adiponectin and MMP3 have known roles in diabetes pathogenesis. Further studies are needed to determine if these novel proteomic signatures have clinical utility in stratifying women most likely to develop GDM and benefit from early intervention.

Effect of Testosterone treatment on bone microarchitecture and bone mineral density in men: a two-year randomised clinical trial

Mark Ng Tang Fui[1, 2], Rudolf Hoermann[2], Karen Bracken[3], David J Handelsman[4, 5], Warrick J Inder[6, 7], Bronwyn GA Stuckey[8, 9], Bu B Yeap[10, 11], Ali Ghasem-Zadeh[2], Rob McLachlan[12], Kristy P Robledo[3], David Jesudason[13, 14], Jeffrey D Zajac[1, 2], Gary A Wittert[13, 14], Mathis Grossmann[1, 2]

1. Austin Health, Heidelberg, Victoria, Australia

2. Medicine, University of Melbourne, Heidelberg, Victoria, Australia

3. NHMRC Clinical Trials Centre, University of Sydney, Sydney, New South Wales, Australia

4. ANZAC Research Institute, University of Sydney , Sydney, New South Wales, Australia

5. Department of Andrology, Concord Hospital, Concord, New South Wales, Australia

6. University of Queensland, St Lucia, Queensland, Australia

7. Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Woolloongabba, Queensland, Australia

8. Keogh Institute for Medical Research, University of Western Australia, Nedlands, Western Australia, Australia

9. Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia

10. Medical School, University of Western Australia, Nedlands, Western Australia, Australia

11. Department of Endocrinology and Diabetes, Fiona Stanley Hospital , Perth, Western Australia, Australia

12. Hudson Institute of Medical Research, Clayton, Victoria, Australia

13. Freemasons Foundation Centre for Men's Health, University of Adelaide, Adelaide, South Australia, Australia

14. The Queen Elizabeth Hospital, Woodville South, South Australia, Australia

Importance: Testosterone (T) treatment increases bone mineral density (BMD) in men, but its effects on bone microarchitecture, a determinant of fracture risk, are unknown.

Objective: To determine the effect of T treatment on bone microarchitecture using high resolution-peripheral quantitative computed tomography (HR-pQCT).

Methods: Testosterone for Bone (T4Bone) is a sub-study of the Testosterone for the Prevention of Diabetes Mellitus (T4DM), a 2-year randomised placebo-controlled multicentre trial of injectable T undecanoate (TU) in men aged >50 years at high risk of diabetes.

Interventions: TU or placebo injections 3-monthly over 2 years on the background of a lifestyle program.

Main outcomes: Primary endpoint was cortical volumetric BMD (vBMD) at the distal tibia in 177 men in one centre. Secondary endpoints including other HR-pQCT parameters and bone remodelling markers. Areal BMD (aBMD) was measured by dual energy X-ray absorptiometry (DXA) in 601 men in five centres. Using a linear mixed model for repeated measures, the treatment effects were defined as mean adjusted differences (MAD [95% CI] over 2 years between groups.

Results: Baseline age was 60.2years, BMI 35.4kg/m^2 and total T 14.2nmol/L. At the tibia, T treatment increased cortical vBMD by 9.33mgHA/cm^3 [3.96;14.71], p<0.001 or 3.1% [1.2;5.0], total vBMD by 4.16mgHA/cm^3 [2.14;6.19], p<0.001 or 1.3% [0.6;1.9]). T treatment also increased cortical area and thickness but effects on trabecular architecture were minor. Results at the radius were similar. T treatment reduced CTX -48.1ng/L [-81.1;-15.1] p<0.001 and P1NP -6.8mg/L [-10.9;-2.7], p<0.001). T treatment increased aBMD at the lumbar spine (0.04g/cm^2 [0.03;0.05], p<0.001 or 3.3% [2.7;3.9]), and the total hip (0.01g/cm^2 [0.01;0.02], p<0.001 or 1.9% [1.2;2.7]). The treatment effect was not dependent on baseline T or oestradiol.

Conclusion: In men, T treatment for 2 years increased volumetric bone density, predominantly via effects on cortical bone. The implications for fracture risk reduction require further study.

28

Fractures in T2DM independently predicted by insulin use and vascular complications (FIELD study)

Angela Sheu[1, 2, 3], Rachel O'Connell[4], Alicia Jenkins[4], Jackie Center[1, 2, 3], Christopher White[1, 3, 5], Tony Keech[4]

1. Bone Division, The Garvan Institute, Sydney, NSW, Australia

2. Endocrinology Department, St Vincent's Hospital, Sydney, NSW, Australia

3. University of New South Wales Faculty of Medicine, University of New South Wales , Sydney

4. NHMRC Clinical Trials Centre, University of Sydney, Sydney

5. Department of Endocrinology and Metabolism, Prince of Wales Hospital, Sydney

Background: Type 2 diabetes mellitus (T2DM) is associated with increased risk of some fractures although the exact mechanisms are unclear. Bone fragility may be associated with T2DM severity (microvascular complications, longer duration, insulin use), although prospective studies evaluating their independent contributions are lacking.

Aims: To determine whether baseline micro- or macrovascular disease predict incident fractures in T2DM, and whether T2DM medications or clinical characteristics independently contribute to fracture risk.

Methods: Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) was a randomised controlled trial of fenofibrate therapy in T2DM patients (aged 50-75), with fractures collected as adverse events. In this post-hoc analysis, cox proportional hazards models were used to determine fracture predictors from baseline data.

Results: Over 49,470 person-years, 137/6138 men and 143/3657 women suffered a fracture. Men with fracture (vs without) were older (63.7±7.5 vs 62.4±6.8 years, p=0.03), more likely to have macrovascular disease (32.9% vs 23.4%, p=0.01), use insulin (21.9% vs 13.6%, p=0.005) and had longer T2DM duration (8.1±6.9 vs 6.9±6.2 years, p=0.03). Women with fractures had more neuropathy (9.8% vs 4.3%, p=0.002) and greater insulin use (22.4% vs 13.3%, p=0.002). Age was similar in women with and without fracture (61.8±6.8 vs 62.9±7.5 years, p=0.06). Overall, mean HbA1c was 7.1%.

In men, significant univariate predictors for fracture included age, macrovascular disease, T2DM duration, insulin use and serum triglycerides, but only insulin use (HR 1.69 (1.12-2.54), p=0.01) and macrovascular disease (HR 1.47 (1.02-2.12), p=0.04) remained significant in multivariable modelling.

In women, significant univariate predictors included age, neuropathy, insulin use and serum creatinine, but only neuropathy (HR 2.16 (1.23-3.80), p=0.007) and insulin use (HR 1.65 (1.11-2.47, p=0.01) remained in multivariable modelling.

Conclusions: Insulin use and gender-specific vascular complications (macrovascular disease in men and neuropathy in women) predict fractures in T2DM. Studies evaluating insulin use and T2DM fracture risk are warranted.

29

Androgen action via the androgen receptor in bone marrow progenitor cells confers protection from diet-induced obesity in male mice.

Varun S Venkatesh[1], Patricia K Russell[1], Barbara C Fam[1], Suzanne Golub[1], Christian Haralambous[1], Sofianas Andrikopoulos[1], Jeffrey D Zajac[1], Rachel A Davey[1]

1. Department of Medicine, Austin Health,, University of Melbourne, Heidelberg, Victoria, Australia

Publish consent withheld

30

Plasticity of gastric satiety signals during pregnancy and in response to pregnancy hormones.

Georgia S Clarke[3, 1, 2], Hui Li[3, 1], Stewart Christie[3, 1], Sharon R Ladyman[4], Stephen J Kentish[3, 1], Richard L Young[3, 1], Kathryn L Gatford[3, 1, 2], Amanda J Page[3, 1]

1. Nutrition, Diabetes & Gut Health, Lifelong Health Theme, South Australian Health and Medical Research Institue, adelaide, South Australia, Australia

2. Robinson Research Institute, University of Adelaide, adelaide, South Australia, Australia

3. University of Adelaide, Adelaide, SOUTH AUSTRALIA, Australia

4. Centre for Neuroendocrinlogy and Department of Anatomy, University of Otago, Dunedin, New Zealand

Background: Gastric vagal afferents (GVAs) sense food related mechanical stimuli and signal to the central nervous system, to integrate control of meal termination. Maternal food intake increases during pregnancy to ensure normal fetal growth and to maximise progeny survival and health.

Aims: This study evaluated changes in GVA function during pregnancy and in response to pregnancy-related hormones

Methods: Female C57BL/6J mice (10-12wk) were mated and randomised to non-pregnant, early (6.5 day (d), N=10), mid (12.5d, N=10) or late-pregnancy (17.5d, N=11) groups. Mice were individually housed in Promethion metabolic cages throughout pregnancy, then the mechanosensitivity of GVAs was determined using an *in vitro* tissue preparation. Effects of the pregnancy hormones oestradiol (10-1000pM), progesterone (30-300nM), prolactin (30-300ng/ml), and growth hormone (3-300ng/ml) on GVA mechanosensitivity were determined separately in 8-week-old female C57BL/6 non-pregnant mice.

Results: Pregnant mice were heavier from d7 ($P<0.05$) and ate more by mid-pregnancy, predominantly due to increased meal size ($P<0.05$). The mechanosensitivity of GVAs to stretch (0.5-5g) decreased as pregnancy progressed ($P<0.001$), and correlated negatively with meal size ($P=0.032$). Oestradiol increased ($P < 0.05$), growth hormone decreased ($P< 0.05$) and prolactin and progesterone had no effect on GVA responses to stretch (3g) in non-pregnant mice.

Conclusions: The mechanosensitivity of GVAs is attenuated during pregnancy and associated with increased food intake. These GVA adaptations are likely to support increases in food intake to meet the energy demands of the growing fetus, and may be driven by increases in circulating levels of growth hormone during pregnancy.

31

Novel mineralocorticoid receptor signalling mechanisms and the regulation of inflammation

Gregory S Ong[2, 1], Timothy J Cole[3], Gregory H Tesch[4], Jennifer H Dowling[5], James Morgan[1], Ashley Mansell[6], Peter J Fuller[1], Morag J Young[1, 7]

1. Centre for Endocrinology and Metabolism, Husdon Institute of Medical Research, Clayton, VIC, Australia

2. Fiona Stanley Hospital, Murdoch, WA, Australia

3. Molecular and Translational Sciences, Monash University, Clayton, VIC, Australia

4. Medicine (Monash Health), Monash University, Clayton, VIC, Australia

5. School of Pharmacy and Biomolecular Sciences, Royal College of Surgeons Ireland, Dublin, Ireland

6. Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, VIC, Australia

7. Baker Heart & Diabetes Institute, Melbourne, VIC, Australia

The mineralocorticoid receptor (MR) is expressed in numerous cell types which do not participate in its classically described function of salt homeostasis. Chronic excess MR activation in cardiovascular cells results in inflammation and fibrosis, manifesting as an excess risk of cardiovascular disease in primary hyperaldosteronism compared to blood pressure-matched controls. In immune cells such as macrophages, MR signalling influences the response to injury. Mice with MR-null macrophages exposed to excess mineralocorticoids are relatively protected from cardiac fibrosis and dysfunction compared to wild type controls. MR-null tissue macrophages tend to exhibit more anti-inflammatory and pro-reparative behaviours compared to wild type macrophages. MR exerts effects on macrophages via two broad mechanisms - canonical MR signalling as a transcription factor and direct regulator of gene expression, or non-canonical MR signalling via intracellular “second messenger” cascades. The former is critical for renal fluid and electrolyte handling. However, the importance of different MR signalling mechanisms on macrophage behaviour and inflammation is not known. To investigate this, we developed novel macrophage cell lines and transgenic mice with a mutant macrophage MR incapable of DNA binding. Canonical MR action was responsible for direct mineralocorticoid-induced gene transcription or modulating the Phorbol 12-myristate 13-acetate (PMA)-induced transcription of inflammatory cytokines and fibrotic factors. Non-canonical MR action was important for lipopolysaccharide (LPS-), but not PMA-induced JNK activation. Interestingly, the macrophage transcriptional response to LPS required intact non-canonical MR signalling irrespective of the presence of mineralocorticoid. Analysis of the MR target gene and profibrotic factor *Mmp12* identified promoter elements that are regulated by combined MR/MAPK/JNK signalling. An 8-day deoxycorticosterone/salt challenge of uninephrectomised male mice found that non-canonical MR signalling is sufficient for development of cardiac inflammation. Overall, MR signalling regulates pro-inflammatory macrophage behaviour via canonical and non-canonical mechanisms. Further, there is evidence of a novel link between LPS, MR and MAPK.

1. Ong, G., Cole, T. J., Tesch, G. H., Morgan, J., Dowling, J. K., Mansell, A., Fuller, P. J., & Young, M. J. (2020). Novel mineralocorticoid receptor mechanisms regulate cardiac tissue inflammation in male mice. The Journal of Endocrinology, 246(2), 123–134.

32

TFAP-2β interacts with AR to determine the phenotype and growth of molecular apocrine breast cancer

Ebtihal Mustafa[1], Richard Iggo[1, 2], Jean Winter[1], Wayne Tilley[1], Theresa Hickey[1]

1. Dame Roma Mitchell Cancer Research Laboratories, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia

2. Institut Bergonié, University of Bordeaux, Bordeaux, France

Molecular apocrine (MA) breast cancers are a highly aggressive, estrogen receptor-α (ERα) negative subtype that have poor prognosis and limited therapeutic options. MA tumours express high levels of the androgen receptor (AR) and genes known to be regulated by AR in prostate cancers. However, the role of AR in promoting growth of MA breast cancer remains equivocal, with preclinical data suggesting both proliferative and anti-proliferative effects. We hypothesised that differential AR-mediated growth effects are driven by differential interactions with key co-regulatory factors in the transcription complex that convert AR from an inhibitor to a transactivator of critical growth regulatory genes. Two MA breast cancer cell lines were investigated: MDA-MB-453 and MFM223. MDA-MB-453 cells are growth stimulated and MFM223 cells growth inhibited by treatment with the most potent natural androgen, 5α-dihydrotestosterone (DHT). Using an unbiased proteomic approach to detect AR interacting proteins, we identified the transcription factor activating protein-2β (TFAP-2β) as a candidate factor of interest. TFAP-2β protein levels were highly expressed in MDA-MB-453 cells but were not detectable in MFM-223 cells. Using several proteomic approaches, we validated the interaction between AR and TFAP-2β in MDA-MB-453 cells, which was enhanced by treatment with DHT. Silencing TFAP-2β induced apoptosis in MDA-MB-453 cells and decreased protein levels of the oncogenes *MYC* and *HER2*. Conversely, ectopic overexpression of TFAP-2β in MFM223 cells reversed DHT-induced growth inhibition. We also showed that *in vivo* growth of genetically defined organoids established from human mammary epithelial cells transformed with TFAP-2β and classic mammary oncogenes have apocrine histology and are growth stimulated by androgenic treatment *in vivo*. Our findings suggest that TFAP-2β is a critical regulator of the MA breast cancer phenotype and a determinant of oncogenic AR signalling. Hence, TFAP-2β may be a useful biomarker to determine whether patients with MA breast cancer would benefit from anti-androgen therapy.

33

Dissecting the interplay between diet and PCOS on gut microbiota in a hyperandrogenic PCOS mouse model

Valentina Rodriguez Paris[1], Nadeem O Kaakoush[2], Samantha M Solon-Biet[3], Melissa C Edwards[4, 1], William L Ledger[1], Robert B Gilchrist[1], Stephen J Simpson[3], David J Handelsman[4], Kirsty A Walters[4, 1]

1. School of Women's & Children's Health, University of New South Wales, Sydney, NSW 2052, Australia

2. School of Medical Sciences , University of New South Wales, Kensington, NSW, Australia

3. Charles Perkins Centre, University of Sydney , Sydney, NSW 2006, Australia

4. ANZAC Research Institute, University of Sydney , Sydney, NSW 2139, Australia

The gut microbiome has been implicated in the development of metabolic disorders, and recently polycystic ovary syndrome (PCOS). PCOS is a disorder with reproductive, endocrine and metabolic irregularities, and several studies report that PCOS causes a decrease in microbial diversity and composition. Diet is an important regulator of the gut microbiome, as alterations in macronutrient balance impact gut microbial communities which correlate with different metabolic health outcomes (1). We identified that macronutrient balance impacts the development of PCOS traits. Therefore, to investigate the interplay between macronutrient balance and PCOS on the gut microbiome, we analyzed the intestinal microbiome from fecal pellets of control and DHT-induced PCOS mice exposed to 10 different diets that varied in protein (P), carbohydrate (C) and fat (F) content. The amount of dietary P, C and F consumed significantly altered alpha and beta diversity of the gut microbiota of pooled control and PCOS mice ($P<0.0001$). Alpha diversity between control and PCOS mice on the same diet did not differ significantly, hence was only affected by diet composition. However, beta diversity was significantly altered between control and PCOS mice ($P<0.05$). DESeq2 analysis identified an operational taxonomic unit (OTU) within *Bacteroides* (OTU3) to be the most differentially abundant OTU between control and PCOS mice, with a significant decrease in PCOS mice ($P<0.0001$). The consensus sequence of *Bacteroides* OTU3 shares 99.2% similarity to *Bacteroides acidifaciens*, which is associated with obesity as it has been reported to protect its host against obesity and improve insulin sensitivity (2). Overall, these findings demonstrate that diet exerts a stronger influence over the gut microbiome than PCOS pathology. However, PCOS did lead to a specific decrease in an obesity-associated *Bacteroides* species in PCOS mice and supports further exploration of the potential preventative role of *Bacteroides acidifaciens* in the development of PCOS traits.

1. (1) Holmes et al., Cell Metabolism. 2017; 25(1): 140–151
2. (2) Yang et al., Mucosal Immunology. 2017, 10 (1), 104-116

34

Effect of high-intensity interval training on glycaemic control in adults with type 1 diabetes and overweight or obesity

Angela S Lee[1, 2], Nathan A Johnson[3, 4], Marg McGill[1, 2], Jane Overland[1, 2], Connie Luo[1], Callum J Baker[2], Sergio Martinez-Huenchullan[2, 5], Jencia Wong[1, 2], J R Flack[6, 7, 8], S M Twigg[1, 2]

1. Department of Endocrinology, Diabetes Centre, Royal Prince Alfred Hospital, Sydney, NSW, Australia

2. Central Clinical School, Charles Perkins Centre, Faculty of Medicine and Health, University of Sydney, Sydney, NSW, Australia

3. Faculty of Health Sciences, University of Sydney, Sydney, NSW, Australia

4. The Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders , University of Sydney, Sydney, NSW, Australia

5. School of Physical Therapy, Faculty of Medicine, Universidad Austral de Chile, Valdivia, Chile

6. Diabetes Centre, Bankstown-Lidcombe Hospital, Sydney, NSW, Australia

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

8. School of Medicine, Western Sydney University, Sydney NSW, Australia

Background and Aims: High-intensity interval training (HIIT) is associated with a lower risk of acute exercise-related hypoglycaemia in people with type 1 diabetes (T1D) compared with traditional moderate-intensity exercise. Effects of HIIT on glycaemic control have not been adequately studied, especially in those with T1D and increased body-weight. We examined the effect of 12 weeks of HIIT on HbA1c in adults with type 1 diabetes and overweight or obesity.

Methods: Thirty inactive adults with T1D, BMI≥25kg/m^2 and HbA1c≥7.5%, were randomized to 12 weeks of either: HIIT exercise intervention consisting of HIIT performed thrice weekly, or usual care control. In a partial cross-over design, the control group subsequently performed the 12-week HIIT intervention, while the intervention group continued HIIT for a total of 24 weeks. The primary endpoint was the change in HbA1c from baseline to 12 weeks. Glycaemic and cardiometabolic outcomes were measured at 0, 12, and 24 weeks.

Results: Participants were aged 44±10 years, with diabetes duration 19±11 years, and BMI 30.1±3.1 kg/m^2. HbA1c decreased from 8.63 ± 0.66% at baseline to 8.10 ± 1.04% at 12 weeks in the HIIT intervention group (p=0.01). This change was not significantly different from the control group (HIIT -0.53 ± 0.61%, control -0.14 ± 0.48%, p=0.08). In participants who undertook at least 50% of the prescribed HIIT intervention, the HbA1c reduction was significantly greater than control (HIIT -0.64 ± 0.64% (n=9), control -0.14 ± 0.48% (n=15), p=0.04). There were no differences in insulin dose or hypoglycaemia on continuous glucose monitoring between groups. After 24 weeks of HIIT, there were improvements in HbA1c, body composition, aerobic fitness and muscular strength.

Conclusions: Overall, there was no significant reduction in HbA1c with a 12-week HIIT intervention in adults with T1D. However, glycaemic control may improve for people who undertake HIIT with at least modest adherence.

35

A mouse hospital for identifying new combination therapies for prostate cancer

Mitchell G Lawrence[2, 1, 3, 4], Laura H Porter[1], Ashlee Clarke[1], Luc Furic[2, 1, 3], Renea A Taylor[2, 3, 4, 5], Gail P Risbridger[2, 1, 3, 4], Melbourne Urological Research Alliance[4]

1. Monash Biomedicine Discovery Institute Cancer Program, Prostate Cancer Research Group, Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC 3800, Australia

2. Peter MacCallum Cancer Centre, Melbourne, VIC 3000, Australia

3. Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, VIC 3010, Australia

4. Melbourne Urological Research Alliance (MURAL), Biomedicine Discovery Institute Cancer Program, Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC 3800, Australia

5. Monash Biomedicine Discovery Institute Cancer Program, Prostate Cancer Research Group, Department of Physiology, Monash University, Clayton, VIC 3800, Australia

The search for new cancer treatments is slow – fewer drugs for cancer reach the clinic than for other diseases. The high failure rate is due to the paucity of effective experimental models for selecting the most promising drugs for clinical trials. The models are particularly poor for prostate cancer, one of the most common cancers in Australia, yet also one of the most difficult to grow in the laboratory. To address these challenges, we established a new collection of patient-derived xenografts (PDXs) of prostate cancer and used them to identify effective combination treatments.

The Melbourne Urological Research Alliance collection of PDXs currently includes 58 tumours spanning the clinical, pathological, and genomic spectrum of prostate cancer, from treatment-naïve primary tumours to metastases from men who failed current systemic therapies. The PDXs have diverse mechanisms of resistance to androgen receptor (AR)-directed therapies, including mutations, amplifications, and structural rearrangements of the *AR* gene, expression of AR variants, and transformation into aggressive AR-null phenotypes. To identify new therapies for these heterogeneous tumours, we treated PDXs with different combinations of compounds targeting the DNA damage repair pathway. This showed that the combination of talazoparib, a PARP inhibitor, and CX-5461, a small molecule inhibitor of RNA polymerase I, synergistically inhibits the growth of PDXs with diverse phenotypes of advanced prostate cancer. A Phase 1 trial of this combination therapy will commence in 2021 for men with metastatic castration-resistant prostate cancer.

In summary, our collection of contemporary preclinical models provides diverse tumours for testing new treatments for prostate cancer, such as the combination of talazoparib and CX-5461. This highlights the potential of patient-derived models to prioritise treatment strategies for clinical translation.

36

Inpatient hyponatraemia: beyond fluid restriction

Nicholas Russell[1]

1. Austin Health, Heidelberg, VIC, Australia

Endocrinologists are frequently asked to consult on inpatients with non-hypovolaemic hyponatraemia for whom initial fluid restriction has failed. This case-based talk will explore the second-line management options in these common, but frequently complex cases. There will be a critical focus on whether there is a routine hospital role for tolvaptan, the only TGA-approved vasopressin V2-receptor antagonist.

37

Dilemmas in the diagnosis and management of primary aldosteronism

Jun Yang[1]

1. Endocrine Hypertension Group, Hudson Institute of Medical Research, Clayton, VIC, Australia

Primary aldosteronism (PA) is the most common endocrine cause of hypertension that affects ~5-10% of hypertensive patients in the primary care setting. Traditionally associated with hypokalemia, serum potassium actually lies in the normal range in the majority of patients. Hence, PA is rarely identified unless tested for specifically, with aldosterone and renin levels (expressed as a ratio, the ARR). At present, the ARR is not part of the routine hypertension work-up, so PA is frequently missed. Even when screening is performed, confounding factors such as antihypertensive medications and phase of menstrual cycle can interfere with the result. Further dilemmas lie in the interpretation of confirmatory tests and adrenal vein sampling results, and optimal management of patients with unilateral or bilateral forms of the disease. These issues will be discussed in the context of perplexing clinical cases.

38

Congenital Adrenal Hyperplasia (CAH)

Margaret Zacharin[1]

1. Murdoch Children's Research Institute, Parkville, VIC, Australia

Congenital adrenal hyperplasia (CAH) is the commonest adrenal disorder of childhood, with autosomal recessive inheritance and most commonly due to 21 hydroxylase deficiency. Clinical features vary depending on degree of enzyme deficiency and sex of the child.

Presentation and management in infancy and childhood will be briefly outlined, to provide understanding of past treatments including surgery and to give background context for later adolescent and adult management. Rare disorders will only be mentioned where important in less severe forms that may present late or where specific problems such as hypertension are important.

Non classical CAH presents in later childhood, adolescence or sometimes in adulthood, accounting for 10-25%of all girls who he oligomenorrhoea, anovulatory infertility and a PCOS like picture. Diagnosis often requires synacthen testing for confirmation. Treatment with glucocorticoid is usually not recommended. Management of NCCAH is outlined for females and males, with particular reference to testicular adrenal rest tissue (TARTs) and complex fertility issues.

Adult treatment of CAH differs from that of other adrenal insufficiency, requiring androgen suppression as well as GC and mineralocorticoid replacement. Controversy regarding optimal management including bilateral adrenalectomy, remains, despite many attempts to adjust regimes using modified release GC, with little evidence for improved outcomes, reduced risk for adrenal crisis, quality of life or better fertility. Illustrative cases will be used to facilitate discussion.

Congenital adrenal hyperplasia (CAH) is the commonest adrenal disorder of childhood, with autosomal recessive inheritance and most commonly due to 21 hydroxylase deficiency. Clinical features vary depending on degree of enzyme deficiency and sex of the child.

Presentation and management in infancy and childhood will be briefly outlined, to provide understanding of past treatments including surgery and to give background context for later adolescent and adult management. Rare disorders will only be mentioned where important in less severe forms that may present late or where specific problems such as hypertension are important.

Non classical CAH presents in later childhood, adolescence or sometimes in adulthood, accounting for 10-25%of all girls who he oligomenorrhoea, anovulatory infertility and a PCOS like picture. Diagnosis often requires synacthen testing for confirmation. Treatment with glucocorticoid is usually not recommended. Management of NCCAH is outlined for females and males, with particular reference to testicular adrenal rest tissue (TARTs) and complex fertility issues.

Adult treatment of CAH differs from that of other adrenal insufficiency, requiring androgen suppression as well as GC and mineralocorticoid replacement. Controversy regarding optimal management including bilateral adrenalectomy, remains, despite many attempts to adjust regimes using modified release GC, with little evidence for improved outcomes, reduced risk for adrenal crisis, quality of life or better fertility. Illustrative cases will be used to facilitate discussion.

Congenital adrenal hyperplasia (CAH) is the commonest adrenal disorder of childhood, with autosomal recessive inheritance and most commonly due to 21 hydroxylase deficiency. Clinical features vary depending on degree of enzyme deficiency and sex of the child.

Presentation and management in infancy and childhood will be briefly outlined, to provide understanding of past treatments including surgery and to give background context for later adolescent and adult management. Rare disorders will only be mentioned where important in less severe forms that may present late or where specific problems such as hypertension are important.

Non classical CAH presents in later childhood, adolescence or sometimes in adulthood, accounting for 10-25%of all girls who he oligomenorrhoea, anovulatory infertility and a PCOS like picture. Diagnosis often requires synacthen testing for confirmation. Treatment with glucocorticoid is usually not recommended. Management of NCCAH is outlined for females and males, with particular reference to testicular adrenal rest tissue (TARTs) and complex fertility issues.

Adult treatment of CAH differs from that of other adrenal insufficiency, requiring androgen suppression as well as GC and mineralocorticoid replacement. Controversy regarding optimal management including bilateral adrenalectomy, remains, despite many attempts to adjust regimes using modified release GC, with little evidence for improved outcomes, reduced risk for adrenal crisis, quality of life or better fertility. Illustrative cases will be used to facilitate discussion. Questions regarding pregnancy in patients with CAH are common . These will be addressed.

39

Nuclear Receptors, Circadian Rhythms, and Metabolism

Mitchell A Lazar[1]

1. University of Pennsylvania, Philadelphia, PA, United States

The panepidemics of diabetes and obesity have a strong genetic basis, but their inexorable rise is mainly due to the impact of environmental factors such as fattening diets, insufficient physical activity, and exposure to light around the clock. These challenges promote metabolic diseases by overwhelming homeostatic mechanisms that control normal physiology, in large part through alterations in gene transcription. Nuclear receptors are transcription factors that regulate the functional output of the genome by altering the epigenome in a tissue-specific manner that is influenced both by environmental factors, including hormones and drugs, and by natural genetic variation that influences their interaction with the genome. PPARg is a nuclear receptor that is a major regulator of adipocyte biology, and the target of antidiabetic drugs. REV-ERB nuclear receptors are transcriptional repressors that function both as core components of endogenous circadian clocks and as regulators of metabolism. Recent work highlights the function demonstrates the mechanisms by which these nuclear receptors link the environment to the genome the context of physiology and metabolic disorders.

40

The holy triad

Caroline Bachmeier[1, 2], Kate Hawke[2], Jacobus Ungerer[1], Michael D'Emden[2]

1. Department of Chemical Pathology, Pathology Queensland, Brisbane, QLD, Australia

2. Department of Endocrinology, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia

Background

Insulin autoimmune syndrome (IAS) is characterised by hyperinsulinaemic hypoglycaemia, elevated insulin autoantibodies without prior exposure to insulin, and normal pancreas anatomy.

Case

A 43 year-old Caucasian woman presented with an 8-week history of spontaneous, postprandial hypoglycaemia on a background of a sleeve gastrectomy nine years prior. Past medical history included migraines, insomnia, asthma and post-traumatic stress disorder. She did not take any medications causative of hypoglycaemia and appeared clinically well.

Serum cortisol, thyroid function, hepatic and renal function were normal. Urine drug screen did not detect oral hypoglycaemic agents. Despite multiple low point-of-care blood glucose values, automated biochemistry did not reveal hypoglycaemia despite significantly elevated fasting insulin and c-peptide levels (**table 1**). Computer tomogram showed a head of pancreas lesion measuring 30mm at largest diameter, magnetic resonance imaging showed a non-specific 9x6mm lesion in the pancreatic body only. 68-Gallium-Exenedin-4 positron emission tomography did not confirm abnormal uptake in the pancreas.

After referral to endocrinology, a modified mixed meal test was performed, which was unremarkable. Insulin and c-peptide were analysed on two alternative platforms and yielded similarly elevated results, therefore the suspicion of abnormally bound insulin was raised. Subsequently, insulin antibodies were measured on two platforms, both yielding significantly elevated results. Further interference testing using polyethylenglycol (PEG) precipitation and affinity chromatography using Protein G columns confirmed abnormally bound insulin: decreasing from 124 mU/L to 29.17 mU/L after PEG, and demonstrating an unbound (free) fraction of 31.70 mU/L after column separation (**table 2 & 3**).

Conclusion

IAS is rare in insulin-naïve, Caucasian patients. Post gastric bypass hypoglycaemia, insulinoma or nesidioblastosis initially seemed more plausible, and this case highlights the importance of considering a wide range of differentials when investigating hypoglycaemia.

41

Risk of bias against premenopausal breast cancer patients in gene expression-based precision medicine

Sarah M Bernhardt[2, 1], Pallave Dasari[2, 1], Danielle J Glynn[2, 1], Joseph Wrin[2, 1], Lucy Woolford[3], Wendy Raymond[4], Lachlan M Moldenhauer[2], Suzanne Edwards[5], David Walsh[1], Amanda R Townsend[6, 1], Timothy J Price[6, 1], Wendy V Ingman[2, 1]

1. Adelaide Medical School, The University of Adelaide, Woodville, SA, Australia

2. The Robinson Research Institute, The University of Adelaide, Adelaide, SA, Australia

3. School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, SA, Australia

4. Flinders Medical Centre, Flinders University of South Australia and Clinpath Laboratories, Adelaide, SA, Australia

5. School of Public Health, The University of Adelaide, Adelaide, SA, Australia

6. Department of Medical Oncology, The Queen Elizabeth Hospital, Woodville, SA, Australia

Background: Gene expression-based algorithms that guide treatment decisions for breast cancer patients are at the forefront of a new era of precision medicine. However, they were developed and validated using datasets predominantly comprised of postmenopausal women. In premenopausal women, fluctuations in estrogen and progesterone during the menstrual cycle impact gene expression in hormone-responsive cancers. However, the extent to which menstrual cycling affects gene expression-based algorithms remains unclear. Here, we use mouse models and human breast cancer samples to demonstrate that the clinically-employed Oncotype DX 21-gene algorithm is critically affected by the menstrual cycle.

Methods: RNA was extracted from 25 pairs of formalin-fixed paraffin-embedded, invasive hormone receptor (HR)-positive breast cancer samples that had been collected approximately 2 weeks apart. Additionally, RNA was extracted from HR-positive mammary tumours dissected from naturally cycling *Mmtv-Pymt* mice at different ovarian cycle stages (n=53). A 21-gene signature analogous to the Oncotype DX platform was assessed through quantitative real time PCR and experimental recurrence scores (RS) were calculated.

Results: There was a significant inverse association between patient age and discordance in RS. For every one-year decrease in age, discordance in RS between paired samples increased by 0.08 units (95% CI: -0.14, -0.01; p=0.017). Discordances were driven primarily by proliferation and HER2-associated genes. In mice, RS were significantly increased in mammary tumours collected at diestrus, driven by genes associated with proliferation and HER2, compared to tumours dissected at estrus. Clustering analysis revealed a relationship between ovarian cycle stage and tumour gene expression.

Conclusions: These results suggest that gene expression-based algorithms are critically affected by menstrual cycle stage at the time of tissue collection. Caution in the adoption of gene expression-based algorithms is required, as their use in informing treatment decisions for premenopausal breast cancer patients could lead to unnecessary or suboptimal therapy.

42

Environmental contamination by a widespread endocrine-disrupting steroid alters reproductive behaviour in aquatic wildlife

Michael G Bertram[1, 2], Patrick Tomkins[1], Minna Saaristo[1, 3], Jake M Martin[1], Marcus Michelangeli[1, 4], Raymond B Tomkins[5], Bob BM Wong[1]

1. Monash University, Melbourne, VICTORIA, Australia

2. Swedish University of Agricultural Sciences, Umeå, VäSTERBOTTEN, Sweden

3. Åbo Akademi University, Turku, Finland

4. University of California, Davis, Davis, California, United States

5. Department of Environment, Land, Water and Planning, Melbourne, Victoria, Australia

Endocrine-disrupting chemicals are accumulating in environments globally. This includes trenbolone, a potent growth-promoting steroid that enters waterways in agricultural run-off. However, whether and how endocrine disruptors like trenbolone impact complex behaviours in wildlife remain largely unknown. We exposed male guppies (*Poecilia reticulata*) to trenbolone and compared the response of exposed and unexposed males to sequentially presented large and small females. Due to a positive size-fecundity relationship, larger females are generally expected to be preferred by males. While we found no evidence that the size of a previously encountered female affected the amount of mating behaviour performed by males during the second presentation, males from both exposure treatments conducted more frequent courting events towards larger females during both presentations, suggesting an absolute preference for greater female size. Further, across both presentations, trenbolone exposure caused a shift in male mating strategy towards increased 'sneaking' behaviour, with major potential implications for reproductive dynamics in exposed populations. Taken together, our findings contribute to a growing understanding of the impacts of endocrine disruptors on complex reproductive behaviours in wildlife.

43

Mice with muscle stem cell (satellite cell) deletion of the vitamin D receptor (sVDR) are weak and have small myocytes

Jennifer Chen[1], Christian M Girgis[2, 3, 1], Jessie Zhou[1], Jenny E Gunton[2, 3, 1]

1. The Westmead Institute for Medical Research, University of Sydney, Westmead, NSW, Australia

2. Faculty of Health and Medicine, University of Sydney, Sydney, NSW, Australia

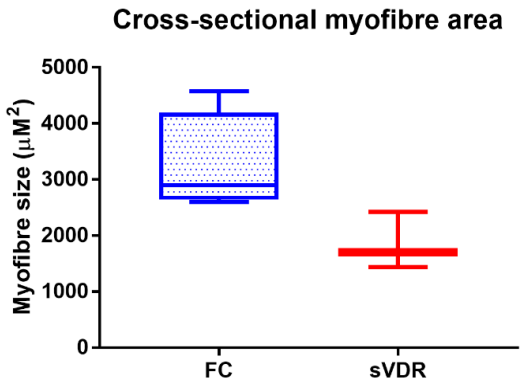
3. Department of Diabetes and Endocrinology, Westmead Hospital, Westmead, NSW, Australia

Vitamin D deficiency is prevalent, especially amongst older persons and some ethic groups. It is associated with an increased risk of falls and sarcopenia. Our group demonstrated mice with myocyte-specific deletion of the vitamin D receptor (mVDR) are weak with small muscles and have fewer, larger myocytes. The large myocytes contrast findings in whole-body VDR knockout mice which have smaller myocytes. This study aimed to determine the effect of deleting VDR in satellite cells on muscle strength and myocyte size.

Floxed VDR mice were crossed with paired box 7 (Pax7)-Cre mice to generate sVDR mice. Muscle development during the embryonic stages is predominantly Pax3 driven; Pax7 has a greater postnatal role. Thus, initial myocytes are expected to be normal, and only newer myocytes should derive from $Pax7^{+}$ VDR-null cells. Muscle function was examined using forelimb grip strength tests and treadmill endurance tests in which mice were encouraged to run until fatigued. At sacrifice, muscles were collected and myofibre histology was quantified.

While body weight was similar between the two groups, sVDR mice displayed consistently weaker grip strength compared to their floxed control siblings as they aged (P<0.01 by ANOVA with repeated measures for both sexes). The time and distance spent running on the treadmill before the onset of fatigue was similar in both genotypes. At cull, wet muscle mass in the sVDR mice was not different to their control group siblings. However, the average myofiber cross-sectional area was smaller in sVDR mice (P<0.01).

These results suggest that satellite cell-specific VDR deletion has different effects on muscle function and physiology in contrast to skeletal myocyte deletion. Future experiments will test whether there are any changes in muscle fibre typing, and measure the expression of genes in apoptotic and cell cycle pathways.



44

Impact of high fat diet on human islet function

Charmaine Cheung[1], Heather Burns[1], Rebecca Stokes[1], Jenny Gunton[1]

1. The Westmead Institute for Medical Research, Westmead, NSW, Australia

Obesity is a well-known risk factor for type 2 diabetes. Diet is therefore a critical aspect, but the effect of different diets on human islet function has yet to be determined.

AIM: This research used "humanised mice" which are diabetic mice transplanted with human islets so that human islets control the mouse's blood glucose levels (BGL). Mice were fed chow or high-fat diet (HFD) to assess effects on human islets.

METHODS: Female immunodeficient RAG1-null mice (C57Bl/6 background) were used as transplant recipients as this avoids the issue of immune-mediated islet rejection. Prior to transplant, diabetes was induced by alloxan. Mice (N=10) each received 2000IEQ human islets under the kidney capsule from human donors with normal glucose tolerance. Donor pancreases did not yield sufficient islets for human transplantation, and the donors were consented for research use of the islets.

Eight weeks after transplantation, 2 mice which did not have post-transplant resolution of diabetes were excluded. Mice with functioning grafts (n=8) were placed on high-fat diet (HFD, 45% of calories from fat) or continued on normal chow (n=2 for each diet for each of 2 human donors). Glucose tolerance testing (GTT) was performed before and 16 weeks after this diet change.

RESULTS: Mice fed HFD gained significant weight (≥3g) and random-fed BGL were higher (2-3mmol/L) than chow-fed mice with transplants from the same human donors . Formal GTT showed that mice fed HFD had deterioration of their glucose tolerance compared to chow-fed animals and to their pre-diet-change GTTs. Deterioration was more pronounced with donor 282 than 281but glucose tolerance deteriorated with HFD for both donor's islets ($p=<0.01$ by 2-way ANOVA). **CONCLUSION**: Consumption of HFD caused detrimental effects on human islets even though the original human donors had normal glucose tolerance. These results support the lipotoxicity hypothesis for human islets.

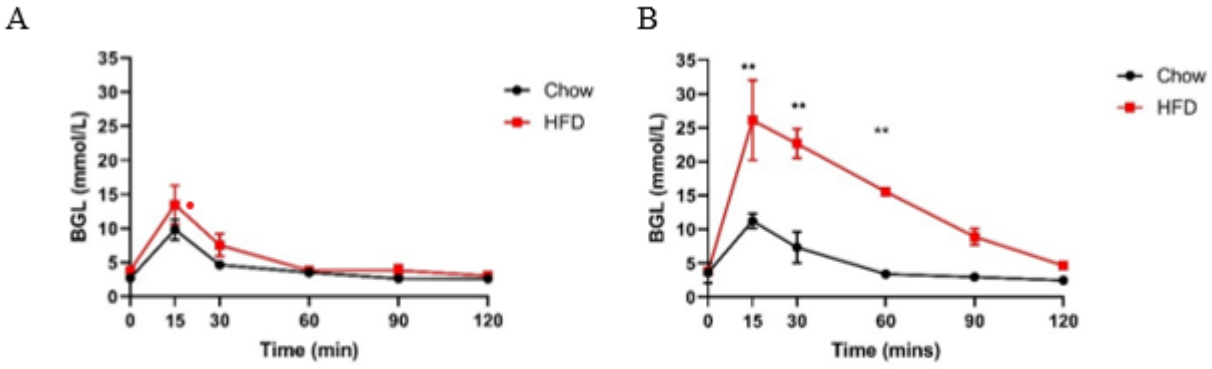


Figure 1: GTT results Chow vs HFD (n=2 per group per donor) after 16 weeks of diet for human donor H281(**Figure A**) and H282(**Figure B**). Data show mean ± SEM. ** = $p<0.05$ versus chow mice.

45

Development of a high throughput total inhibin assay using mass spectrometry

Abby AC Choo[1], Simon SC Chu[1], Trang TN Nguyen[1], Peter PF Fuller[1]

1. Centre of Endocrine and Metabolism, Hudson Institute, Clayton, Victoria, Australia

Inhibins are glycoprotein hormones, composed of an α-subunit and either a βA or a βB subunit. They play a major role in the hypothalamus-pituitary-gonad feedback system to negatively regulate FSH secretion, thereby regulating spermatogenesis and folliculogenesis. Total inhibin is a useful detection marker for some early-stage ovarian cancers; in particular, it is an excellent biomarker for granulosa cell tumours (GCT). GCTs have a tendency for late recurrence, often many years after initial diagnosis. Thus, measuring inhibin is useful for long term monitoring for recurrent GCT. Currently, an efficient and cost-effective total inhibin assay is not available, highlighting a need to develop a high-throughput total inhibin assay. Introduction of mass spectrometry in clinical endocrinology has played a tremendous role in improving the management of numerous endocrine diseases. Our aim is to establish a methodology using selective reaction monitoring (SRM)-based targeted proteomics using liquid chromatography-mass spectrometry (LC-MS) to detect total inhibin with enhanced sensitivity in serum. Using conditioned cell culture media from HEK293 cells overexpressing inhibin B, we successfully detected inhibin α peptides using a NanoLC/Q Exactive Orbitrap mass spectrometer. We observed that an inhibin a peptide sequence (STPLMSWPWSPSALR) was one of the top 8 detectable proteins in the conditioned media. Using the MASCOT analysis software, this peptide (observed M.W. of 1714.85) had a significant MASCOT score (74.26) with a retention time of 2142.1156 seconds corresponding to the MS/MS spectrum. These parameters are now being used in conjunction with a suitable enrichment of low abundant proteins in serum, in order to detect this inhibin α peptide in serum from GCT patients. The implications of this study will allow women with ovarian cancer to gain access to a highly specific and sensitive diagnostic assay for α-inhibin, subsequently predicting relapse and/or monitoring recurrence to achieve better overall survival rate.

46

The impact of pubertal adipose tissue deposition on mammary cancer development during adulthood

Amita G Ghadge[1, 2], Pallave Dasari[1, 2], David J Sharkey[2], Rebecca L Robker[2], Wendy V Ingman[1, 2]

1. Discipline of Surgery, Adelaide Medical School, The Queen Elizabeth Hospital, University of Adelaide, Woodville, Adelaide, SA, Australia

2. Robinson Research Institute, University of Adelaide, Adelaide, SA, Australia

Epidemiological studies demonstrate that high body mass index during puberty is associated with reduced lifetime breast cancer risk. It is suggested that increased abundance of adipose tissue in pubertal girls affects future breast cancer risk through reducing mammographic density, which is a major breast cancer risk factor. However, causal relationships are yet to be established. This project aimed to investigate the impact of increased pubertal adipose tissue deposition on mammary density and cancer development using the *Alms* null mouse model of adiposity and the *Mmtv-Pymt* transgenic model of mammary cancer.

Alms$^{-/-}$ mice overeat and exhibit increased weight gain by 5 weeks of age when fed a normal mouse diet. These mice were fed ad libitum until 7 weeks and then calorie-matched with wildtype mice thereafter such that adult *Alms*$^{-/-}$ mice weight was comparable to wildtype. Mammary glands were dissected from *Alms*$^{-/-}$and wildtype *Alms*$^{+/+}$ female mice during puberty (6 weeks; n=10/group) and adulthood (12 weeks; n=10/group) and fixed for histological assessment or frozen for cytokine assessment by Luminex. Mammary tumours were dissected from *Alms* mice crossed with *Mmtv-Pymt* mice (18 weeks; n=15/group).

At puberty, *Alms*$^{-/-}$mice exhibited larger adipocytes than wildtype, and increased number of terminal end buds and proliferating epithelial cells. At adulthood, *Alms*$^{-/-}$ mice exhibited a 55% decrease in percent fibroglandular density, accompanied by elevated interleukin 6 and tumour necrosis factor alpha, compared to wildtype. At 18 weeks, *Alms*$^{-/-}$x*Mmtv-PyMT* mice exhibited a 60% decrease in tumour burden and tumour development was delayed compared to *Alms*$^{+/+}$x*Mmtv-PyMT* controls.

Together with epidemiological studies, these findings provide strong support for the notion that increased pubertal adipose tissue deposition is a key determinant of adult breast cancer risk through altering mammographic density. We propose that puberty is a critical time for breast development which establishes breast cancer risk for the life course.

47

Suppression of hyperinsulinemia by diazoxide restores insulin-growth hormone balance and improves substrate and energy metabolism in obese mice

Zhengxiang Huang[1], Xuehan Lu[1], Lili Huang[1], Yang Chen[1], Chunhong Zhang[1], Johannes D Veldhuis[2], Chen Chen[1]

1. School of Biomedical Sciences, The University of Queensland, Brisbane, Queensland, Australia

2. Department of Medicine, Mayo School of Graduate Medical Education, Rochester, MN, USA

The well-balanced secretion between insulin and growth hormone (GH) is essential in regulating substrate metabolism, energy metabolism and body composition. Hyperinsulinemia and reduced GH secretion are often observed in obese individuals, leading to reduced energy expenditure and further fat accumulation. Although suppression of hyperinsulinemia has been proposed as a treatment for obesity, changes in GH secretion following the suppression of hyperinsulinemia in obesity are unknown. This leaves unexplained observations, such as unchanged lean mass following insulin reduction. Besides, the energy metabolism following the suppression of hyperinsulinemia in obesity has not been thoroughly investigated. In this study, high-fat diet-induced obese (DIO) and normal chow-fed lean mice on a C57BL/6J background were treated for 7 weeks with diazoxide (1250 mg/kg in food), a K_{ATP} channel opener that suppressed insulin secretion. Diazoxide treatment for 10 days was sufficient to increase pulsatile GH secretion in DIO mice prior to any significant body weight change. The restored insulin-GH balance in DIO mice was followed by improvement in substrate and energy metabolism in a prolonged treatment period (4-6 weeks), including reduced fat mass, increased lipid oxidation and energy expenditure, as well as improved insulin sensitivity and metabolic flexibility. These metabolic benefits occurred along with the changes in the expression level of genes regulated by the insulin-GH balance. When applying diazoxide to normal chow-fed non-hyperinsulinemic lean mice, none of the above metabolic effects was observed, suggesting that the metabolic changes following diazoxide treatment were mediated through the suppression of hyperinsulinemia. Therefore, suppression of hyperinsulinemia by diazoxide restores insulin-GH balance followed by improvement in substrate and energy metabolism in DIO mice.

48

Heteromerisation of the angiotensin II type 1 and type 2 receptor

Elizabeth KM Johnstone[2, 1, 3], Kevin DG Pfleger[2, 4, 1, 3]

1. Harry Perkins Institute of Medical Research, Nedlands, WA, Australia

2. Centre for Medical Research, The University of Western Australia, Crawley, WA, Australia

3. Australian Research Council Centre for Personalised Therapeutics Technologies, Australia

4. Dimerix Limited, Nedlands, WA, Australia

Angiotensin II (AngII) is a vital hormone that is involved in the regulation of many physiological processes, including the maintenance of blood pressure, inflammation and proliferation. AngII exerts its actions via two G protein-coupled receptors (GPCRs), the AT_1 and the AT_2 receptor. The AT_1 receptor mediates most of the well-established actions of AngII, such as vasoconstriction and inflammation. In contrast, the AT_2 receptor often exerts countervailing effects, such as vasodilation and anti-inflammation. In addition, it is also well established that the AT_2 receptor is able to antagonize the functions of the AT_1 receptor, with one suggested mechanism for this being heteromerisation of the receptors. This phenomenon was first described by AbdAlla et al (1), who demonstrated the proximity between the two receptors and showed that the AT_2 receptor inhibited AT_1 receptor signaling. In our studies, we have previously uncovered a potential mechanism for the antagonism of the AT_1 receptor by the AT_2 receptor, showing that although the heteromer recruits the regulatory protein β-arrestin, it is unable to subsequently internalise (2).

We have further investigated the pharmacology of the AT_1-AT_2 heteromer using novel bioluminescence resonance energy transfer (BRET) assays. Firstly, we adapted our BRET ligand binding assay (3), enabling us to confirm the close proximity of the receptors. Additionally, this assay revealed no evidence for binding cooperativity between the two receptors in this system. We also used our BRET trafficking assay (4) to thoroughly investigate the cellular trafficking of the heteromer following agonist stimulation. In accordance with our earlier study (2), we found no evidence for AngII-induced internalization of the heteromer. However, some AngII-induced trafficking was observed, such as an increase in surface expression and movement out of various intracellular compartments. Overall, these results provide further evidence of the existence of the AT_1-AT_2 heteromer and reveal more of its novel pharmacology.

1. AbdAlla et al. (2001) Journal of Biological. Chemistry 276(43), 39721–39726.
2. Porrello et al. (2011) Cellular Signalling 23(11): 1767-1776.
3. Stoddart et al. (2015) Nature Methods 12(7): 661 663.
4. Tiulpakov et al. (2016) Molecular Endocrinology 30(8): 889 904.

49

Time of day regulates renal mineralocorticoid receptor transcriptional control of electrolyte balance

Monica Kanki[1, 2], James Morgan[3], Peter Fuller[3], Morag Young[1, 2, 4]

1. Cardiovascular Endocrinology Laboratory, Baker Heart & Diabetes Institute, Melbourne, Victoria, Australia

2. Department of Molecular & Translational Science, Monash University, Clayton, Victoria, Australia

3. Steroid Receptor Biology Laboratory, Hudson Institute of Medical Research, Clayton, Victoria, Australia

4. Department of Cardiometabolic Health, University of Melbourne, Melbourne, Victoria, Australia

Publish consent withheld

50

Exposing the mechanistic link between hypothyroidism in pregnancy and gestational diabetes mellitus

Nykola L Kent[1], Sharat C Atluri[1], James SM Cuffe[1]

1. The University of Queensland, St Lucia, QLD, Australia

Hypothyroidism affects approximately 3% of pregnant women and has been linked to gestational diabetes mellitus (GDM). Few studies have investigated the mechanisms by which thyroid dysfunction in pregnancy may contribute to the development of GDM. This study used a model of hypothyroidism in pregnancy to explore disruption to key pathways that have previously been implicated in GDM.

Female Sprague-Dawley rats were exposed to either 0.02% (severe hypothyroidism, SEV) or 0.005% (moderate hypothyroidism, MOD) methimazole in their drinking water for seven days prior to mating and throughout pregnancy. On embryonic day (E) 16, pregnant dams were fasted prior to an intraperitoneal glucose tolerance test. Animals were culled on E20 for collection of maternal blood, tissues, and placentas for subsequent analysis.

This study is the first to provide novel evidence linking hypothyroidism to the pathogenesis of GDM. On E16, both MOD and SEV dams were glucose intolerant, and had a significant reduction in fasting plasma insulin and placental lactogen (rPL). Placental junctional zone expression of *Prl3d1* and *Prl3b1*, two key placental peptides implicated in GDM, were not affected, however maternal plasma rPL remained decreased at E20. Pancreatic expression of *Nkx6-1*, a critical gene required for beta-cell expansion, was significantly reduced in MOD and SEV dams. This suggests that inefficient placental production of rPL may be failing to facilitate appropriate beta-cell expansion during pregnancy in hypothyroid dams, reducing insulin synthesis and secretion. Within maternal skeletal muscle, there was a significant reduction in *Irs1* and GLUT4 in both MOD and SEV dams. In combination with changes in the pancreas, this suggests that hypothyroidism in pregnancy induces reduced insulin secretion and peripheral insulin resistance, culminating in a classic GDM-like phenotype. Further investigation into other pathways within the liver and pancreas that may contribute to dysregulated maternal glucose homeostasis in pregnancies affected by hypothyroidism are underway.

51

Proteomic profiling of hormone-dependent prostate cancer identifies LOXL2 as a potential therapeutic target in the tumour microenvironment.

Natalie L Lister[2, 1], Elizabeth V Nguyen[3], Brooke A Pereira[4], Mitchell G Lawrence[2, 5, 1], Thomas R Cox[4, 6], Gail P Risbridger[2, 5, 1], Renea A Taylor[2, 5, 1], Roger J Daly[3, 1]

1. Cancer Program, Biomedicine Discovery Institute, Monash University, Clayton, Victoria, Australia

2. Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia

3. Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

4. Cancer Division, The Garvan Institute of Medical Research and The Kinghorn Cancer Centre, Sydney, NSW, Australia

5. Sir Peter MacCallum , Department of Oncology, The University of Melbourne , Parkville, Melbourne, Australia

6. Faculty of Medicine, St Vincent's Clinical School, UNSW Sydney, Sydney , NSW, Australia

Introduction: The prostate tumour microenvironment plays a key role in prostate cancer disease progression. Although current therapies primarily target the tumour epithelium, resolving how tumour cells interact with their surrounding microenvironment may offer novel therapeutic targets. Cancer-associated fibroblasts (CAFs) are found within or near tumour regions and have been shown to promote prostate cancer progression compared to fibroblasts from non-malignant regions of the prostate (NPFs).

Methods: Four pairs of patient-matched CAF and NPF stromal cells were isolated from patients undergoing radical prostatectomy for primary, castrate-sensitive prostate cancer. To identify potential target proteins within the tumour microenvironment, the proteome and phosphoproteome of CAF/NPF populations were characterised by liquid chromatography-tandem mass spectrometry (LC-MS/MS) with a hyper-reaction monitoring data-independent acquisition (HRM-DIA) workflow.

Results: STRING analysis of the CAF proteome revealed a prominent protein interaction hub associated with collagen synthesis, modification, and signaling within the extracellular matrix (ECM). CAFs expressed multiple proteins that regulate a pro-tumourigenic extracellular matrix, including lysyl oxidase-like 2 (LOXL2), which promotes collagen crosslinking and tumour 'stiffness'. Importantly, our data demonstrates that pharmacological inhibition of LOXL2 in CAF perturbed extracellular matrix (ECM) organization and impaired the motility of prostate tumor cells in a co-culture assay.

Conclusion: CAF-derived LOXL2 is an important mediator of intercellular communication within the prostate tumor microenvironment and a potential therapeutic target. Next generation selective inhibitors of LOXL2 show promise for therapeutic treatment of solid tumours.

52

Targeting central feeding circuits to improve outcomes in cancer cachexia

Kelly L Walton[1], Phuong Silvie Bui Hoang[1], Swati Kharoud[1], Bronia Harding-Davis[1], Craig Harrison[1], Zane B Andrews[1], Sarah H Lockie[1]

1. Department of Physiology, Monash University, Clayton

Cachexia is a progressive loss of body weight, accompanied by loss of appetite, which affects as many as 80% of cancer patients. The current focus of anti-cachexia research is blockade of tumour-derived circulating factors at the level of fat and muscle. However, given that the brain is the master regulator of metabolic control, targeting the brain to alter metabolic outcomes in cachexia is an attractive idea. Ghrelin is a peptide hormone which rises in response to fasting, drives eating behaviour, decreased energy expenditure and growth hormone release. The primary target neurons of ghrelin are the Agouti related peptide/neuropeptide Y-containing neurons in the arcuate nucleus of the hypothalamus (AgRP neurons). Therapies using ghrelin analogues have been trialled as a way to target the brain to treat cachexia. We used a mouse model of pancreatic ductal adenocarcinoma (PDAC) to assess ghrelin action *in vivo*. After the onset of PDAC-induced anorexia, PDAC-carrying mice ate significantly less than control mice in response to injected ghrelin, indicating ghrelin resistance is present in cancer cachexia even before noticeable wasting has occurred.

To circumvent the observed ghrelin resistance, we used targeted chemogenetics (DREADDs) to chronically artificially activate AgRP neurons during cancer cachexia in PDAC-bearing mice. AgRP neuronal activation rescued fat and skeletal muscle mass loss, decreased brown fat thermogenesis and slightly but significantly increasing locomotor activity in PDAC mice. We measured circulating levels of the pro-cachexia factors, activin A and B. PDAC-bearing mice with or without AgRP neuronal activation showed a similar, significant elevation in activin A and B levels, compared to non-PDAC bearing mice. Importantly, AgRP neuronal activation protected mice from the wasting effects of elevated activins, as the levels seen in this model are sufficient to drive significant wasting. This provides early evidence that targeting central feeding circuits may improve outcomes in cancer cachexia.

53

NMN supplementation rescues fertility and bone strength in chemotherapy-treated mice

Maria B Marinova[1, 2], Wing-Hong Jonathan Ho[3, 1], Michael J Bertoldo[1, 2], Vedran Lovric[4], Kaisa Selesniemi[5], William R Walsh[4], David A Sinclair[5], Kirsty Walters[2], Robert B Gilchrist[2], Lindsay E Wu[1]

1. School of Medical Sciences, UNSW Sydney, Sydney, NSW

2. School of Women's and Children's Health, UNSW Sydney, Sydney, NSW

3. Garvan Institute of Medical Research, Sydney

4. Prince of Wales Clinical School, UNSW Sydney, Sydney, NSW

5. Department of Genetics, Harvard Medical School, Boston, MA

Cancer survivors who have undergone chemotherapy often face infertility. Chemotherapy drugs such as cisplatin trigger a cascade of events ultimately causing exhaustion of the follicular reserve and endocrine disruption. Apart from infertility, this can have severe consequences on women's health such as early-onset menopause and a decline in bone health.

For girls with cancer, ovarian tissue cryopreservation is the only available option for fertility preservation, which while providing options for future parenting, does not protect from endocrine failure and osteoporosis.

We hypothesise that the NAD+ booster nicotinamide mononucleotide (NMN) protects the ovarian reserve from the chemotherapy. The aim of this study is to assess NMN's effects on fertility and late-life bone health.

Female mice at 7-days of age were treated with cisplatin (2mg/kg) and then 2 weeks later NMN (2g/L) delivered in drinking water. NMN treatment was sustained until animals were sacrificed at 24 months of age. Animals were bred at 6 weeks of age. NMN increased litter size, total pups per mouse 5-fold ($p=0.015$), and the cumulative rate of pregnancy compared to non-NMN, chemo-treated animals. Aside from improving fertility, we sought to determine the impacts of these interventions on bone strength, related to loss of ovarian function. Bones were subject to mechanical and structural analysis to determine changes in bone function, which is related to the increased risk of osteoporosis observed following ovarian failure and menopause. NMN treatment rescued cisplatin-induced reductions in cortical bone thickness (femur diaphysis -$p<0.0001$, femur metaphysis - $p=0.0081$, and tibia - $p=0.0072$), volume (femur - $p=0.0015$, and tibia – $p=0.0102$), and density (femur - $p=0.0463$) to control levels, as well as the increased physical strength of tibia ($p=0.0024$). These results infer that long-term NMN treatment protects against chemotherapy-induced bone fragility by preventing premature ovarian failure and maintaining estrogen levels sufficient to support healthy bone maintenance.

54

Gene expression profiling of PPARγ:RXRα activation in a human ovarian granulosa cell line using RNA-seq transcriptome analysis

Trang Nguyen[1], Maria Alexiadis[1], Peter Fuller[1], Simon Chu[1]

1. Hudson Institute of Medical Research, Clayton, VIC, Australia

The peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptors involved in metabolic processes comprising lipid and glucose metabolism, energy homeostasis, cell proliferation and differentiation. Granulosa cells (GC) of developing ovarian follicles have been shown to predominantly express PPARgamma (PPARγ). However, its role is unclear in normal ovarian biology. Thiazolidinediones (TZD) are a class of exogeneous PPARγ agonists that include the anti-diabetic drug rosiglitazone (RGZ). This study investigated the underlying molecular mechanisms of PPARγ activation which may affect ovarian function.

A transformed non-luteinised human GC cell line, hGrC1, was treated with either vehicle or RGZ and retinoic acid (the ligand for PPARγ heterodimeric partner RXRα). RNA was extracted to generate RNA-seq libraries, then sequenced (average of 50 million reads/sample). For data analysis, we utilised the RNASik pipeline (Monash Bioinformatics Platform), followed by DEGUST analysis to establish transcriptomic profiles.

Preliminary analysis identified 350 differentially expressed genes in PPARγ-activated GC cells, 67% of which were upregulated (FDR 0.05, fold change>1.5). The following genes were of interest: CREB3L3 (6-fold increase), IL1B (4-fold), PDGFB (2-fold), VEGFA (1.7-fold), ID1 (2-fold) and PTGER2 (2-fold). KEGG pathway analysis revealed these genes contributed to critical signalling pathways: AMPK, PI3K-Akt, MAPK, Ras, Rap1 and cAMP, all of which are key regulators of basic cellular functions such as proliferation, differentiation and/or cell energy homeostasis. Furthermore, regulation of lipid metabolism including steroid hormone biosynthesis (CYP11A1, CYP26B1 and HSD3B1 genes, >2-fold); suggests that activation of PPARγ plays a key transcriptional link between energy metabolic and signal transduction functions in normal ovarian GC biology.

Transcriptomic data can provide valuable insight into how PPARγ modulation may impact normal basal ovarian physiology. Given its almost exclusive expression in GC, disruption in PPARγ could potentially effect GC development and normal oocyte maturation, which may have functional implications in ovarian pathology.

55

Preconception weight modulation better optimises maternal metabolic outcomes compared to intrapartum weight modulation in late gestation mice.

Natassia Rodrigo[1, 2, 3], Hui Chen[4], Carol Pollock[2, 5, 3], Sarah Glastras[1, 2, 3]

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

2. University of Sydney, Sydney, NSW, Australia

3. Kolling Institute of Medical Researh, Sydney, NSW, Australia

4. University of Technology, Sydney, Sydney, NSW, Australia

5. Renal Department, Royal North Shore Hospital, Sydney, NSW, Australia

Background and aims:

Maternal obesity affects 20% of pregnant women and negatively impacts metabolic health in mothers and offspring. Maternal complications include gestational diabetes, fatty liver disease and increased rates of cardiovascular disease, while offspring are at increased risk of obesity and diabetes. Intrapartum diet modification has shown limited efficacy in improving health outcome in mother and offspring. To date, no studies have addressed whether pre-conception maternal weight loss improves outcomes in obese mothers and offspring. We aimed to determine if weight loss prior to pregnancy by diet modification, improves maternal and offspring weight and metabolic outcomes compared to intrapartum diet modification.

Methods:

C57BL/6 dams were fed a high fat diet (HFD) versus chow diet for 8 weeks. Then, HFD-fed dams were continued on HFD, switched to chow pre-pregnancy, or switched to chow once pregnancy was confirmed. Pregnancy rates were observed after male co-housing. Maternal anthropometriy, glucose tolerance and metabolic markers were measured before and after diet change, and at late gestation. Dams were sacrificed during late pregnancy, or delivered their offspring and the offspring's anthropometry, and glucose tolerance assessed at postnatal week 12.

Results:

HFD-fed dams had greater weights and reduced glucose tolerance compared to control (both $p<0.0001$). Following preconception diet modification, insulin resistance and body weight were reduced ($p<0.001$), with improved fecundity. In late gestation, glucose tolerance was optimised with preconception diet change, compared to intrapartum diet change (area under the curve 81.97 vs 100.1mmol/L/min, $p=0.0008$). Offspring of obese mothers with preconception weight loss had lower body weight ($p<0.001$) and improved glucose tolerance ($p<0.01$).

Conclusions:

Preconception dietary change to induce weight loss improves maternal weight and metabolic outcomes in pregnancy compared to intrapartum dietary change , with transgenerational benefits to offspring. Therefore, obese women should be targeted for pre-conception weight loss to improve intergenerational metabolic outcomes.

56

Microglia as the central regulators of circadian rhythms

Luba Sominsky[1], Tamara Dangel[2], Sajida Malik[1], Simone N De Luca[1], Nicolas Singewald[2], Sarah J Spencer[1, 3]

1. School of Health and Biomedical Sciences, RMIT University, Melbourne, VIC, Australia

2. Dept. of Pharmacology and Toxicology, Institute of Pharmacy and CMBI, University of Innsbruck, Insbruk, Austria

3. ARC Centre of Excellence for Nanoscale Biophotonics , RMIT University, Melbourne, VIC, Australia

Microglia, the major brain immune cells, have multifaceted roles not only in maintaining immune homeostasis, but also in driving neurodevelopment, regulating satiety, promoting memory, and contributing to other non-immune functions. Many of these microglia-mediated functions are affected by circadian rhythms, fluctuating throughout the course of the day. Emerging evidence suggests microglia contribute to this circadian rhythmicity. To test the role of microglia in regulating circadian rhythms, we used our *Cx3cr1-Dtr* transgenic Wistar rat model to acutely deplete microglia and examined if this could lead to a disruption in circadian temperature, metabolism and activity measures. We also examined if shifts in these physiological rhythms correspond with changes in the expression of key circadian rhythm-regulating genes and proteins. Our data show that microglial depletion leads to a profound disruption of circadian rhythms in several domains consistent with a shift towards the inactive phase throughout the day and night. These shifts in circadian rhythmicity are accompanied by changes in the expression of central circadian rhythm-regulating genes and proteins. These findings indicate microglia are potential dynamic regulators of circadian rhythms in the CNS and indicate an exciting possibility to manipulate these cells to restore disrupted circadian rhythms such as with shift-work or jet-lag.

57

Development of long-acting human growth hormone antagonists by solid-phase site-specific PEGylation

Yue Wang[1], Ries Langley[2], Kyle Tomshen[3], Julia Harms[1, 4], Heather Maynard[3], Stephen Jamieson[4], Jo Perry[1]

1. Liggins Institution, The University of Auckland, Auckland

2. Department of Molecular Medicine and Pathology, The University of Auckland, Auckland

3. Department of Chemistry and Biochemistry, University of California, Los Angeles

4. Auckland Cancer Society Research Centre, The University of Auckland, Auckland

Growth hormone (GH) mediates actions through binding to the GH receptor (GHR), activating key signalling pathways including the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway [1]. Excessive GH secretion leads to acromegaly and tumoral expression has been implicated in cancer progression [2,3]. GHR antagonist B2036 effectively inhibits GH signalling. B2036 is a biological agent based on GH; a single mutation in binding site 2 of the hormone converts it from an agonist into an antagonist. Conjugation of 4-6 of 5 kDa polyethylene glycol (PEG) to B2036 generates the clinically used agent, pegvisomant [4]. PEGylation considerably extends the serum half-life of the antagonist but inevitable leads to a loss in bioactivity. In addition, the Ghr from rodents has very low affinity for pegvisomant and as a consequence very high doses are required for cancer xenograft studies (60-250 mg/kg/day) [5]. This drug is therefore not suitable for routine preclinical studies. To generate an antagonist for *in vivo* study, B2036 was site-specifically conjugated to 20, 30, or 40 kDa PEG maleimide through an introduced cysteine at amino acid 144 (S144C). A codon optimised B2036-S144C was generated by gene synthesis and recombinantly engineered by gene fusion with thioredoxin. Recombinant B2036-S144C was produced from *E. coli* and was PEGylated using cysteine-specific conjugation chemistry. *In vitro* bioactivity of these conjugates was significantly improved compared with amine PEGylated B2036. The circulating half-life of the 20, 30, and 40 kDa PEG conjugates was 16.4, 18.6 and 58.3 h in mice, respectively. Administration of 40 kDa PEG conjugates (10 mg/kg/day) reduced serum IGF-I concentrations by 50.6% in mice. This *in vivo* reduction in serum IGF-I was at a considerably lower dose compared to the higher doses required to observe comparable activity in studies with pegvisomant. Future studies will investigate their efficacy in cancer xenograft studies.

1. Lu, M.; Flanagan, J. U.; Langley, R. J.; Hay, M. P.; Perry, J. K. Targeting Growth Hormone Function: Strategies and Therapeutic Applications. Signal Transduct. Target. Ther. 2019, 4 (1), 3. https://doi.org/10.1038/s41392-019-0036-y.
2. Wang, Y.; Langley, R. J.; Tamshen, K.; Jamieson, S. M.; Lu, M.; Maynard, H. D.; Perry, J. K. Long-Acting Human Growth Hormone Receptor Antagonists Produced in E. Coli and Conjugated with Polyethylene Glycol. Bioconjug. Chem. 2020, 31 (6), 1651–1660. https://doi.org/10.1021/acs.bioconjchem.0c00208.

58

Bone Health Among Indigenous Population Post Renal Transplant in Central Australia

Sin Dee Yap[1, 2], Audrey Khaing[1], Basant Pawar[3], Elna Ellis[1]

1. Department of Medicine, Alice Springs Hospital, The Gap, NT, Australia

2. Department of Medicine, Monash Health, Clayton, VIC, Australia

3. Department of Nephrology, Alice Springs Hospital, The Gap, NT, Australia

Objective: To assess the bone health of indigenous Australians who received renal transplant compared to non-indigenous Australians in Central Australia, between 2009 and 2019.

Background:

A study shown that bone mineral density (BMD) in Aboriginal and/or Torres Strait Islander Australians is higher than Caucasian Australian reference ranges and these differences still remained significant in men after adjustment for lean mass, but there are few published BMD data in this population[4].

In the general population, several studies have shown that biochemical abnormalities of bone minerals and metabolites improved following successful renal transplant. However, there are insufficient data describing bone health following transplantation among indigenous population.

Method:

List of patients who received renal transplant between 2000 and 2019 in Alice Springs was collated. Baseline characteristic of patients were collected from hospital database. Serum bone turnover markers including total alkaline phosphatase (ALP), serum calcium, phosphate, Vitamin D and parathyroid hormone (PTH) levels and dual-energy bone densitometry (if applicable) were entered into Excel spreadsheet.

Results:

31 patients received deceased organ after circulatory death (DCD) kidney transplant between 2009 and 2019. 24 patients were indigenous and 7 were non-indigenous.

Baseline serum corrected calcium, phosphate, PTH levels were similar between the groups, except total ALP levels. Following renal transplant, the differences in serum corrected calcium, phosphate; PTH level changes were significant.

The baseline bone density was not significant between the two groups. Following transplantation, the reduction in bone density at the hip was statistically significant in the indigenous group. The rate of bone loss did not differ between groups in both sites.

Conclusion: Baseline, post-transplant bone density at the lumbar spine and the rate of bone loss were similar between indigenous and non-indigenous patients who received renal transplant over 10 years in Central Australia. However, the changes in bone density at the hip were statistically significant.

59

Determining Vitamin D Status: Analytical Variability Between Available Assays

Azni Abdul-Wahab[1], Marion Black[1], Jeff Pope[1], Hans-Gerhard Schneider[1]

1. Clinical Biochemistry , Alfred Health, Melbourne, VIC

Clinical interest to evaluate serum 25-hydroxyvitamin D[25(OH)D] to assess Vitamin D status in health risks continue to increase. Variability of 25(OH)D measurements remains controversial despite the international initiative Vitamin D Standardization Program (VDSP) to standardise the assays. The aim of this study was to examine the correlation of 25(OH)D concentrations measured by different assays. We measured 25(OH)D using the new Abbott Alinity and DiaSorin LiaisonXL chemiluminescence immunoassays against the National Institute of Standards and Technology (NIST)-traceable liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The immunoassay method were compared with the LC-MS/MS using Passing-Bablok regression and Bland-Altman analysis. Common 25(OH)D cut-point for classification of vitamin D deficiency was used for assay comparison.

125 adult serum samples were randomly selected and measured 25(OH)D levels ranged between <10 nmol/L to 290 nmol/L as determined by LC-MS/MS. Compared to the LC-MS/MS, both immunoassays demonstrated strong positive relationship based on Passing-Bablok regression analysis. The results were as follows: Abbott Alinity = 0.85*x* + 1.29nmol/L, 95% CI: -2.39 to 5.03 (r=0.94); DiaSorin LiaisonXL= 0.74*x* + 2.54nmol/L, 95% CI: -2.53 to 6.18 (r=0.91).

Despite good correlation, the overall mean bias was -12.6% for Abbott Alinity and -24.4% for DiaSorin LiaisonXL assays. A higher percentage of patients were classified as vitamin D deficient (25(OH)D <50 nmol/L) using DiaSorin LiaisonXL (53%) followed by Abbott Alinity (49%), when compared with LC-MS/MS (34%). Using 25(OH)D ≥ 50nmol/L as “adequate” determined by LC-MS/MS method, 22% (18/82) and 28% (23/82) of patients were classified as “deficient” when analysed on Abbott Alinity and DiaSorin LiaisonXL respectively.

Clinician should be aware of the inter-method variability among different Vitamin D assays despite standardization efforts. These differences could be due to cross-reactivity with $25(OH)D_2$ and vitamin D metabolites, including $24,25(OH)_2D$ and epimeric forms. It is advisable to monitor serum 25(OH)D level following treatment in the same laboratory.

Ampullary gangliocytic paraganglioma: a rare but important entity

Minoli V Abeysekera[1, 2, 3], Varun S Manoharan[1, 2, 4], Amir Ashrafy[5], Emily Hibbert[1, 2]

1. Department of Endocrinology, Nepean Hospital, Penrith, NSW, Australia

2. Nepean Clinical School, Faculty of Medicine and Health, The University of Sydney, Penrith, NSW, Australia

3. Griffith Medical School, Griffith University, Gold Coast, QLD, Australia

4. South Western Sydney Clinical School, University of New South Wales, Liverpool, NSW, Australia

5. Department of Anatomical Pathology, Nepean Hospital, Penrith, NSW, Australia

A 67-year-old man presented with a 12-month history of epigastric discomfort and low normal iron stores for at least 3 years (ferritin 32-42ug/L), but no weight loss. He had a history of hypertension, hypercholesterolaemia and chronic obstructive pulmonary disease. Gastroscopy revealed a large ampullary polypoid mass without bleeding, and endoscopic ultrasound demonstrated a submucosal lesion 30x12mm in the ampullary/periampullary region. Cytology revealed a malignant cell pattern of unclear differentiation. Staging FDG-PET scan revealed intense uptake in the periampullary mass, with uptake in pulmonary hilar/mediastinal lymph nodes and caecum, though no mass lesions were present on colonoscopy. Staging laparoscopy revealed no peritoneal disease, and a Whipple's pancreatoduodenectomy with cholecystectomy was performed. Histopathology revealed a well-differentiated ampullary gangliocytic paraganglioma (GP) with clear margins and no lymph node involvement. There was no loss of staining for SDHB, SDHA and fumarate hydratase.

Duodenal GPs are rare tumours with only 236 cases reported between 1957 and 2018 (1). They are reported in people aged 16 to 92 years with a male predominance (1.8:1) (2,3). Ninety percent arise from the second part of the duodenum (4). Whilst frequently asymptomatic, common presenting symptoms include abdominal pain and melaena (4,5). Histologically, GPs consist of 3 cell types: spindle, epithelial and ganglion (5). They can be misdiagnosed as Grade 1 neuroendocrine tumors (NET) (6). However, accurate diagnosis is important, as GPs have a better prognosis than NETs and other ampullary tumours (4,6). Nonetheless, though generally considered benign, distant metastasis and rarely, death have been reported (1,7). Optimal treatment remains unclear due to low case numbers, but includes options of endoscopic resection or surgical resection +/- adjuvant radiotherapy (3,8). Recurrence post-resection is rare but has been reported up to 11 years post-operatively (5). Follow up is imperative as no prognostic biomarkers are available (9,10).

1. Ebi, M., Ochiai, T., Sugiyama, T., Yamamoto, K., Adachi, K., Yamaguchi, Y., Tamura, Y., Izawa, S., Hijikata, Y., Yamamoto, S., Funaki, Y., Ogasawara, N., Sasaki, M., Kasugai, K. Endoscopic resection of gangliocytic paraganglioma of the duodenum: a case report. Clinical Journal of Gastroenterology 2020; 13: 203–208.
2. Matsubayashi, H., Ishiwatari, H., Matsui, T., Fujie, S., Uesaka, K., Sugiura, T., Okamura, Y., Yamamoto, Y., Ashida, R., Ito, T., Sasaki, K., Ono, H. Gangliocytic Paraganglioma of the Minor Papilla of the Duodenum. Internal medicine (Tokyo, Japan) 2017; 56(9): 1029–1035.
3. Yang, J., Choi, J., Lee, G., Kim, B., Moon, S., Kang, M., Ahn, H. A case of ampullary gangliocytic paraganglioma. The Korean Journal of Internal Medicine 2014; 29(3): 375-378.
4. Kwon, J., Lee, S., Kang, M., Jang, J., Kim, S. A case of gangliocytic paraganglioma in the ampulla of Vater. World Journal of Surgical Oncology 2010; 8: 1-4.
5. Loftus, T., Kresak, J., Gonzalo, D., Sarosi, G., Behrns, K. Duodenal gangliocytic paraganglioma: A case report and literature review. International Journal of Surgery Case Reports 2015; 8: 5-8.
6. Chiang, C., Shyr, B., Chen, S., Shyr, Y., Wang, S. Periampullary Gangliocytic Paraganglioma. Journal of Gastrointestinal Surgery 2019; 23(11): 2247–2254.
7. Li, B., Li, Y., Tian, X., Luo, B., Li, Z. Malignant gangliocytic paraganglioma of the duodenum with distant metastases and a lethal course. World Journal of Gastroenterology 2014; 20(41): 15454–15461.
8. Adams, L., Friedman, T., Shaver, T., Younan, G. Duodenal Gangliocytic Paraganglioma Requiring a Pancreaticoduodenectomy: A Case Report and Review of the Literature. Case Reports in Surgery 2018; 2018: 1-4.
9. Reis, D., Damião, F., Noronha Ferreira, C., Cruz, R., Vitorino, E., Carrilho Ribeiro, L., Tato Marinho, R. Duodenal Gangliocytic Paraganglioma: A Unique Cause of Abdominal Pain. ACG case reports journal 2019; 7(1): 1-2.
10. Wang, B., Zou, Y., Zhang, H., Xu, L., Jiang, X., Sun, K. Duodenal gangliocytic paraganglioma: report of two cases and review of literature. International journal of clinical and experimental pathology 2015; 8(9): 9752–9759.

61

A rare neuroendocrine cause for severe and refractory hypercalcaemia

Laura E Adams[1], King Wei Yong[1], Kingsley Nirmalaraj[1], Jeremy Rossaak[1]

1. Bay of plenty disctrict health board, Mount Maunganui, Tauranga, BAY OF PLENTY, New Zealand

Primary PTH-rp secreting neuroendocrine tumours are a rare cause of humeral hypercalcaemia of malignancy. Significant elevation in PTH-rp levels is often associated with extensive metastatic disease, and associated uncontrolled hypercalcaemia leads to substantial morbidity and eventual mortality.

A 66 year old female presented with symptomatic hypercalcaemia resulting from a large PTH-rp hypersecreting pancreatic neuroendocrine tumour. The patient had marked hypercalcaemia at 4.22 mmol/L (Normal range (NR)2.1-2.55), in association with highly elevated PTH-rp levels of 55.4pmol/L (NR 0-1.5), with an 11cm pancreatic tail mass identified on imaging, without evidence of metastatic disease. Calcium levels and symptoms improved on standard therapy with bisphosphonate, calcitonin and saline hydration, however the effect was not sustained. The patient re-presented with symptomatic hypercalcaemia, this time responding only to the addition of a somatostatin analogue. This effect was also short-lived and the patient presented with hypercalcaemia refractory to saline hydration, calcitonin, octreotide, and calcium continued to rise to a maximum of 5.14 mmol/L despite haemofiltration.

Progressive symptoms lead to the need for emergent surgery, with eventual distal pancreatectomy, splenectomy and en bloc colonic resection to control calcium levels.

There is significant heterogeneity in the behaviour of PTH-rp secreting NETs, without apparent correlation between PTH-rp and calcium levels. Initial management is centred on medical intervention including hydration, bisphosphonates, calcitonin and somatostatin analogues, but ultimately surgical resection and reduction of tumour bulk is required to achieve adequate and sustained control of calcium levels. There are many mechanisms involved in escape of hypercalcaemia from standard therapy, demonstrated within this case with significant hypercalcaemia developing over several symptomatic periods with progressive resistance to treatment over a short time interval.

62

Endocrine Adverse Events Associated with Immune Checkpoint Blockade used for treatment of solid tumours

TALIB A PROF AL-JUMAILY[1], JASOTHA DR SANMUGARAJAH[1]

1. GOLD COAST UNIVERSITY HOSPITAL, SOUTHPROT, QLD, Australia

ABSRACT

Objectives: To estimate the prevalence and the types of immune endocrine dysfunction associated with use of immune check point inhibitors used for treatment of solid tumours.

Methods: This retrospective observational study reviews the types and frequency of the endocrinopathies associated with immune checkpoint inhibitors available to date among a large cohort of patients attending a local tertiary oncology centre.

Results: A total of 234 patients undergoing treatment with a check-point inhibitor treatment were recruited, most patients 214 (91.4%) received single therapy whilst only 20 (8.6%) received combination therapy. The overall prevalence of adverse events in this group was 15.0 per 100 with a 95% confidence interval of (11.0-21.1). Subclinical thyroid disorders, including subclinical hypothyroid and subclinical hyperthyroid, occurred for 6.4 and 12.4% of patients, respectively. Among our cohort, thyroid disorders including both hyperthyroid and hypothyroid was the most common endocrine adverse event with prevalence of 7.7% and 5.1%, with a 95% confidence interval of (4.9-11.9) and (3.0-8.7) respectively.

Conclusion: Despite its considerable benefit in patients with cancer, immune checkpoint blockade can be limited by the occurrence of irAEs that can be life threatening. Although certain endocrine adverse events are frequent during cancer immune-therapy, their management is relatively uncomplicated. Thyroid dysfunction and hypophysitis are the most common abnormalities.

Practice Implications: In our view, clinical and biochemical screening of endocrine toxicity would improve our knowledge of physio-pathological mechanisms as well as help modify our management and in preventing severe events.

KEYWORDS:

Adverse events, Endocrine toxicity, Checkpoint inhibitors, Immunotherapy, Thyroid dysfunction, Hypophysitis, Type 1 diabetes mellitus, and Adrenal insufficiency

63

The decline of TSH Receptor Autoantibody (TRAb) levels post Total Thyroidectomy in Graves Ophthalmopathy: A meta-analysis and systematic review

Arsalan Anees[1], Guy Eslick[1], Senarath Edirimanne[1]

1. University of Sydney, Penrith, NSW, Australia

Background: TSH Receptor Autoantibodies (TRAbs) are largely pathognomonic for Grave's Disease and are also thought to underly the pathogenesis of Grave's Ophthalmopathy (GO). Total Thyroidectomy (TTx) is one of the oldest approaches for GO management, and it is thought to work by removing the target tissue for intrathyroidal autoantibodies. A decline in TRAb levels has been documented post Total Thyroidectomy (TTx) in GO patients, however with conflicting correlations with disease outcome. Hence, we aimed to determine whether the reduction in TRAb levels post-TTx could improve or stabilize GO.

Methods: We conducted a systematic review and meta-analysis using six publicly available electronic databases (Medline, Embase, Scopus, Cochrane, CINAHL and Web of Science). Our inclusion criteria identified GO patients undergoing TTx with measurements of both TRAb levels and progression of the disease using a validated scoring system. The random-effects model was used to calculate the pooled odds ratio (OR) and 95% confidence interval to compare the number of patients with normalized TRAb levels and the progression of GO between TTx and other interventions.

Results: A total of 13 studies encompassing data from 1050 GO patients met our eligibility criteria and were included in our systematic review. Furthermore, 5 of these studies (4 RCTs, 1 Cohort study) had comparable data that were suitable for a meta-analysis. The meta-analysis included 1 study with radioiodine ablation (RAI) group, 2 studies with subtotal thyroidectomy (STx) groups, and 2 studies with RAI post-TTx groups. We found that significantly more patients had normalized TRAb levels post-TTx as compared to other interventions (p=0.035). However, there was no significant difference in improvement, unchanging or worsening outcomes of GO post-TTx as compared with other intervention groups.

Conclusion: These results suggest that while TRAb levels decline more post-TTx, they may not predict added improvements to GO progression.

64

Relapse of ketosis prone diabetes mellitus: a case report

Kenrick Blaker[1], Roger Chen[1]

1. St Vincent's Hospital, Darlinghurst, NSW, Australia

There has been increasing recognition of an atypical entity of ketosis-prone diabetes, initially called Flatbush Diabetes, a heterogeneous syndrome characterised by patients initially developing diabetic ketoacidosis but who are then able to discontinue insulin therapy and remain in near-normoglycaemic remission, even without therapy, for months to years. The pathogenesis and if this atypical diabetes is a unique type of diabetes or a subset of more severe type 2 diabetes are unclear. A 47-year-old Colombian male was admitted with diabetic ketoacidosis in 2011. His blood glucose was 30mmol/L, ketones 3.6 mmol/L, bicarbonate 23 mmol/L and venous blood gas pH 7.25. Islet cell and GAD antibodies were negative. He was initially treated with insulin for a short period and then changed to metformin and gliclazide, with gliclazide subsequently ceased. He had excellent control of his diabetes with a HbA1c between 5-6%. In February 2020 he developed symptoms of hyperglycaemia, with pathology performed confirming an elevated BGL of 19.1 mmol/L, HbA1c of 13.3% and C-peptide of 0.2 nmol/L. He had no other significant medical history and reported no particular stressors. He was taking metformin 500mg BD. There was a positive family history of type 2 diabetes in his father. On assessment, his capillary BGLwas 16.5 mmol/L with ketones of 1.1 mmol/L. His weight was 72kgs and BMI 22.7. His abdomen was soft and non-tender. A CT of the chest, abdomen and pelvis revealed no evidence of a pancreatic lesion. Metformin was increased to 1000mg BD and he was commenced on basal bolus insulin. One month after initial review, glycaemic control had improved with only basal insulin required and metformin. This case demonstrates the importance of recognition of ketosis prone diabetes mellitus and its possible recurrence, which may have significant treatment and employment implications.

1. Smiley D, Chandra P, Umpierrez GE. Update on diagnosis, pathogenesis and management of ketosis-prone Type 2 diabetes mellitus. Diabetes Manag (Lond). 2011;1(6):589-600.

65

Prospective screening for Primary Aldosteronism in patients with Suspected Obstructive Sleep Apnoea

Min Ru Chee[1], Jesse Hoo[1], Renata Libianto[2], Stella Gwini[1], Om Narayan[2], Garun Hamilton [2], Jun Yang[2]

1. Monash Universtiy , Clayton

2. Monash Health , Clayton

Several studies have demonstrated a potential for a bi-directional relationship between obstructive sleep apnoea (OSA) and primary aldosteronism (PA), however many of these studies are limited to subsets of patients diagnosed with hypertension or existing PA. We recruited 85 patients who were attending a diagnostic sleep study, otherwise unselected, for suspected OSA and screened for PA measuring serum aldosterone, direct renin concentration and aldosterone:renin ratio (ARR). OSA was diagnosed via diagnostic overnight polysomnography and neck and calf circumferences were measured before and after polysomnography. Blood pressure was measured using a 24-hour ambulatory blood pressure monitor (ABPM) in 58 participants. 2 out of 85 participants were identified to have likely PA (2.4%) based on elevated ARR or suppressed renin on blood test results despite being on anti-hypertensives known to elevate renin, together with CT evidence of an adrenal adenoma. Another 10 were identified to have possible PA (11.8%) due to evidence of suppressed renin while on interfering anti-hypertensive medications. In participants with both OSA and hypertension (n=41), the prevalence of likely or possible PA increases to 29.3%. Differences in the awake diastolic blood pressure load were significant across ARR tertiles (p=0.035). No correlation was observed between aldosterone, renin or ARR and the apnoea-hypopnea index (AHI) using multiple regression analysis despite adjusting for interfering medications and hypertension status. In conclusion, we were unable to confirm a relationship between OSA and PA but found that the potential prevalence of PA amongst hypertensives with OSA is 29.3%. Our study results suggest that a diagnostic sleep study may be a good opportunity to screen for PA, given that hypertension is common in both OSA and PA.

66

Management of glucocorticoid-induced hyperglycaemia in in-patients with acute exacerbation of chronic obstructive pulmonary disease – a retrospective audit

Kay Hau Choy[1], Rena Hai Man Cao[1, 2], Lawrence Cai[3], Sibgat Al Saleheen[3], Dong Seok Yi[3], Tang Wong[1, 2], Sarah Abdo[1, 4], Jeff R Flack[1, 2, 4]

1. Diabetes Centre, Bankstown-Lidcombe Hospital, Bankstown, NSW

2. Faculty of Medicine, University of New South Wales, Randwick, NSW

3. Department of Respiratory Medicine, Bankstown-Lidcombe Hospital, Bankstown, NSW

4. School of Medicine, Western Sydney University, Campbelltown, NSW

Background: Hyperglycaemia is common amongst in-patients receiving glucocorticoids(GCs). Uncontrolled hyperglycaemia is associated with increased morbidity and mortality.

Aim: To review the prevalence and management of glucocorticoid-induced hyperglycaemia(GIH) among in-patients with acute exacerbation of chronic obstructive pulmonary disease(AECOPD) and to pilot a management guideline.

Methods: An initial retrospective audit was conducted on 30 randomly selected patients (cohort 1) admitted to Bankstown-Lidcombe Hospital with AECOPD, May-August 2017. Data on blood glucose (BGL) monitoring and GIH management were collected. The Respiratory team thence received education on a management guideline. A further audit was conducted for a similar cohort (cohort 2), June-August 2020.

Results: 30 files were reviewed in cohort 1. Most were treated with prednisolone and 80% received steroids for ≥3 days. 63% had no pre-existing diabetes, with almost 60% having ≤1 BGL test throughout their admission. None had an HbA1c done, 2 had ≥1 documented BGL≥12mmol/L, with no follow up action taken. Of those with pre-existing diabetes, only 55% had ≥4 BGL tests per day and 64% had significant hyperglycaemia. 24 patient files were reviewed in cohort 2. Most were treated with prednisolone. 63% had no pre-existing diabetes, of those, 53% had ≤1 BGL test during admission, and only 27% had HbA1c tested before commencing GC. None were commenced on insulin. Of those who had diabetes, almost 90% had ≥4 BGL tests per day with significant hyperglycaemia in 78% and two-thirds either commenced insulin or required insulin dose augmentation. Less than half of the patients were referred to the Endocrinology team.

Conclusion: Guideline education appears to have at least improved the management of patients with known diabetes given the increased proportion having more BGL tests, HbA1c assessment and insulin titration. However, GIH is poorly recognised and sub-optimally managed in this patient cohort. Extensive GIH management education and management guideline implementation are warranted.

67

Zoledronic acid-associated acute tubular necrosis

Kay Hau Choy[1]

1. South Western Sydney Local Health District, Sydney, NSW

Introduction: Zoledronic acid (ZA) is a bisphosphonate with high antiresorptive potency. Association of ZA with severe acute kidney injury (AKI) has been reported, albeit rare, and predisposing factors include pre-existing renal insufficiency, dehydration and concomitant use of nephrotoxic agents.

Methods: We present a case of severe AKI secondary to toxic acute tubular necrosis (ATN) following treatment with ZA for osteoporosis.

Results: A 70-year-old lady received her first ZA infusion for osteoporosis which was diagnosed after sustaining minimal trauma rib fractures. She had stage 3 chronic kidney disease secondary to diabetic nephropathy. She saw her general practitioner one week after her ZA infusion due to persistent myalgia which started one day after receiving ZA. Blood tests arranged by her GP showed AKI, with a rise in serum creatinine from a baseline of 100µmol/L to 700µmol/L. She was subsequently hospitalised. Apart from ZA, there were no new medications. There was no prior intravenous contrast material exposure. Infection was excluded. Her creatinine peaked at 1,165µmol/L. Her admission was complicated by anuria and fluid overload requiring renal replacement therapy (RRT). A kidney biopsy revealed ATN with mild acute interstitial nephritis (AIN). The close temporal relationship between ZA administration and the onset of AKI strongly implicated ZA as the causative agent in the development of ATN. She was treated with tapering course of prednisolone for AIN. Her renal function gradually improved, with a creatinine of 260µmol/L on hospital discharge. Further RRT was not required. ZA was permanently discontinued. At 4 weeks post-hospital discharge, her creatinine was 140µmol/L and had remained stable.

Conclusion: ZA provides substantial clinical benefits for patients with osteoporosis and has an established safety profile when used appropriately. Appropriate selection of patients and patient monitoring by clinicians can reduce the risk of ZA-associated adverse effects and help ensure the safe use of ZA.

68

FGF23-mediated hypophosphataemia following intravenous iron administration: a case series

Lucy Collins[1], **Rebecca J Foskey**[1], **Paul Wraight**[1], **Spiros Fourlanos**[1, 2]

1. Department of Diabetes and Endocrinology, Royal Melbourne Hospital, Melbourne, Victoria, Australia

2. Royal Melbourne Hospital Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia

Background

Severe and symptomatic hypophosphataemia is an increasingly recognised complication of intravenous iron infusion, affecting up to 40% of patients (1, 2). This is likely mediated by fibroblast growth factor 23 (FGF23), a peptide hormone synthesised by osteocytes and osteoblasts. FGF23 is a key regulator of phosphate homeostasis directly through its phosphaturic actions and indirectly by reducing activation of cholecalciferol to calcitriol causing secondary hyperparathyroidism (3).

As prescription of iron infusion increases in Australia, it is important to recognise this common but underappreciated complication (4).

Aim

To examine the clinical and biochemical characteristics of patients detected with hypophosphataemia following iron infusion, and describe its treatment and natural history.

Results

Thirteen patients were identified with hypophosphataemia following intravenous iron administration. Median age was 72.5 years (interquartile range [IQR] 66.5 to 91), three patients were male and iron preparation was ferric carboxymaltose in seven patients. Six patients had received concurrent Denosumab, a receptor activator of nuclear factor kappa-B (RANK) ligand inhibitor. The median time from iron infusion to detection of hypophosphataemia was 12 days (IQR 8 – 14.5). The median creatinine was 60 mmol/L (45-60) (IQR 52-67). The median nadir phosphate value was 0.3mmol/L (0.75-1.50) (IQR 0.27 – 0.36). The median urinary fractional excretion of phosphate was 74% (IQR 66.75 – 88) (>5% indicates renal phosphate wasting). Median PTH was 29.8 (1.7-10) (IQR 25.3-38.3). Serum FGF23 and calcitriol values continue to be acquired. Most patients were managed with intravenous followed by oral phosphate and calcitriol. There were no severe complications.

Conclusion

Clinicians should be aware of the potential complication of profound hypophosphataemia following iron administration. The risk may be potentiated by use of RANK ligand inhibitors, malnutrition and normal renal function. We recommend patients at risk of hypophosphataemia undergo measurement of serum phosphate at one- and two- weeks following iron infusion.

1. Zoller H, Schaefer B, Glodny B. Iron-induced hypophosphatemia. Current Opinion in Nephrology and Hypertension. 2017;26(4):266-275.
2. Adkinson N, Strauss W, Macdougall I, Bernard K, Auerbach M, Kaper R et al. Comparative safety of intravenous ferumoxytol versus ferric carboxymaltose in iron deficiency anemia: A randomized trial. American Journal of Hematology. 2018;93(5):683-690.
3. 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. Journal of Bone and Mineral Research. 2003; 19(3), 429-435.
4. Shand A, Bell J, Henry A, Grzeskowiak L, Kidson-Gerber G, Pearson S et al. Rapid increase in intravenous iron therapy for women of reproductive age in Australia. Medical Journal of Australia. 2020;213(2):85-86.

69

Glycaemic disorders complicating insulin-dextrose treatment of hyperkalaemia

Ljiljana Crnobrnja[1], Andy Lim[1, 2], Mauli Govinna[1], Cathy Jiang[1], Manogna Metlapalli[1]

1. Department of General Medicine, Monash Health, Melbourne, VIC, Australia

2. Department of Medicine,School of Clinical Sciences, Monash university, Clayton, Melbourne, VIC, Australia

Aim**:** Moderate to severe hyperkalaemia is a common problem for hospital inpatients which requires treatment with Insulin and Dextrose. We identified the incidence of hypoglycaemia related to this treatment, timing of glucose nadir and associated risk factors.

Methods**:** We conducted a retrospective, multi-site cohort study of all hospitalized adult patients with hyperkalaemia (serum potassium ≥ 6mmol/l) treated with intravenous insulin-dextrose from 1st January 2019 to 1st March 2020.

Results**:** A final number of patients included in the analysis was 421. The change in serum potassium post-treatment was 1.4 mmol/L (standard deviation,0.8 mmol/L) lower than pre-treatment and the degree of potassium lowering was not different based on hypoglycaemia status. The incidence of hypoglycaemia was 21.4%. Independently associated risk factors are identified from a multivariable logistic regression analysis. Risk factors associated with a higher risk of hypoglycaemia are eGFR < 60 ml/min/1.73m2 and location of treatment in the Emergency Department. Higher body mass index and pre-treatment glucose are associated with lower odds of hypoglycaemia. Most hypoglycaemia occurred in the second hour of treatment and peaked at around 90 minutes.

Conclusion**:** Our hospital's protocol for management of moderate to severe hyperkalaemia with insulin dextrose carries a significant risk of hypoglycaemia (21.4%). Our study supports the use of the current protocol to provide blood glucose monitoring for 6 h after a single insulin dextrose treatment. Patients with a higher body mass index and higher pre-treatment of blood glucose are at a lesser risk of hypoglycaemia unlike patients with chronic kidney disease (eGFR < 60 ml/min/1.73m2) who are at a higher risk. Further work is needed to understand the reasons behind why the Emergency Department appears to be a higher risk area for hypoglycaemia post insulin dextrose treatment.

70

Sarcoidosis with hypercalcaemia in a patient with multiple sclerosis previously treated with alemtuzumab

Jessica Deitch[1], Shoshana Sztal-Mazer[1]

1. Alfred Health, Melbourne, Victoria, Australia

Background

Hypercalcaemia is a common endocrinology issue that requires thorough investigation to identify its cause. Sarcoidosis, a multisystem granulomatous disorder of unknown aetiology, is an infrequent cause of hypercalcaemia. We present a case of PTH-independent hypercalcaemia complicated by an acute kidney injury, requiring extensive work-up to establish a diagnosis. Our patient had a significant background of multiple sclerosis previously managed with alemtuzumab, a monoclonal antibody against CD52.

Case

A 63 year old female with relapsing remitting multiple sclerosis previously managed with alemtuzumab presented with confusion and malaise. Assessment was consistent with hypovolaemia while investigations revealed hypercalcaemia (corrected calcium 3.34mmol/L) and an acute kidney injury (creatinine 153µmol/L, eGFR 33ml/min). Acute management included fluid resuscitation and intravenous pamidronate. PTH was 1.2pmol/L, Vitamin D was 54nmol/L, 1,25-dihydroxyvitamin D was elevated at 282pmol/L and serum ACE was elevated at 181units/L. CT imaging demonstrated extensive paratracheal and subcarinal lymphadenopathy. A renal biopsy demonstrated nephrocalcinosis. A bronchoscopy with endobronchial ultrasound guided transbronchial needle aspiration was performed with histopathology identifying non-necrotising granulomatous inflammation. Sarcoidosis was diagnosed, likely secondary to previous alemtuzumab treatment. The patient was commenced on oral prednisolone 10mg twice daily following a rise in serum calcium.

Discussion

Alemtuzumab is an important management option for relapsing remitting multiple sclerosis but its use is complicated by potentially serious adverse reactions, including thyroid autoimmunity.[1] Hypercalcaemia and sarcoidosis are not listed complications of alemtuzumab however, there have been five reported cases suggestive of this. Pulmonary and ocular manifestations of sarcoidosis have been documented in case reports in patients with multiple sclerosis and previous alemtuzumab treatment.[2,3] Respiratory symptoms and mild hypercalcaemia attributed to sarcoidosis have been documented in a patient with mycosis fungoides treated with alemtuzumab.[4] Our patient's presentation is highly suggestive of alemtuzumab precipitated sarcoidosis resulting in hypercalcaemia, with both radiological and pathological evidence of sarcoidosis.

1. Willis MD and Robertson NP. Alemtuzumab for multiple sclerosis. Curr Neurol Neurosci Rep 2016; 16(9):84.
2. Willis MD, Hope-Gill B, Flood-Page P et al. Sarcoidosis following alemtuzumab treatment for multiple sclerosis. Mult Scler 2018; 24: 1779-1782
3. Graf J, Ringelstein M, Lepka K et al. Acute sarcoidosis in a multiple sclerosis patient after alemtuzumab treatment. Mult Scler 2018; 24; 1776-1778
4. Thachil J, Jadhav V, Gautam M et al. The development of sarcoidosis with the use of alemtuzumab – Clues to T-cell immune reconstitution. Br J Haematol 2007; 138(4): 559-560.

71

Enhanced Recovery after Surgery (ERAS) protocol reduces length of stay in Type 2 Diabetes Mellitus patients undergoing elective colorectal surgery

Katrina Festejo[1]

1. Ateneo School of Medicine and Public Health, Paranaque, METRO MANILA, Philippines

Background: Enhanced Recovery After Surgery (ERAS), a multimodal surgical care protocol has been shown to to reduce length of stay after surgery and post-operative complications in colorectal surgery. However, this protocol has not been widely studied among patients with T2DM. It has not been established whether ERAS interventions confer the same benefits for patients with diabetes.
Objective: To determine if implementation of an Enhanced Recovery After Surgery (ERAS) pathway is associated with decreased length of stay and decreased adverse post-operative outcomes among patients with T2DM who underwent colorectal surgery
Methods: Among adults with diabetes who underwent elective colorectal surgery from July 2015 to October 31, 2017, we compared post-operative length of hospital stay (LOS) prior to ERAS pathway implementation (pre-ERAS) and after implementation of an ERAS pathway (ERAS). Secondary outcomes were 30 day in-patient mortality, post-operative complications, reoperations, pneumonia and wound infection. Using Stata SE version 13, we used independent t-test for continuous variables and Fisher's exact test for categorical variables and a level of significance of 5%.
Results: A total of 72 patients were included (36 in the pre ERAS group and 36 in the ERAS group). Mean post- operative length of hospital stay was 7.2 +/- 5.5 days in the pre ERAS group and 5.7 +/- 4.0 days in ERAS group (p=0.205). There was no statistically significant difference in length of stay, complications, reoperation rate, pneumonia or wound infection between the two groups.
Conclusion: ERAS protocol implementation in a single center was not associated with a significant decrease in length of stay or adverse post operative outcomes among diabetic patients who underwent colorectal surgery. Type 2 DM patients who underwent colorectal surgery under ERAS had 1.5 nights shorter average length of stay which was not statistically significant, but may be clinically significant with regard to impact on costs.

72

Pit-1: A useful discriminative marker in prolactin immunonegative pituitary adenomas associated with hyperprolactinaemia

Prishila Fookeerah[1, 2], **Meena Shingde**[3], **Katherine Benson**[4], **Mark Mclean**[1, 2]

1. Department of Diabetes and Endocrinology, Blacktown Hospital, Sydney, NSW, Australia

2. School of Medicine, Western Sydney University, Sydney, NSW, Australia

3. Tissue Pathology and Diagnostic Oncology, Westmead Hospital, Sydney, NSW, Australia

4. Endocrinology and Metabolism Department, Concord Repatriation General Hospital, Sydney, NSW, Australia

A 41-year-old man was diagnosed with a prolactinoma on account of a significantly elevated serum prolactin and a large pituitary lesion. He was managed with dopamine agonist therapy. Although serum prolactin had normalised, tumour shrinkage was not achieved, and he subsequently underwent transsphenoidal resection of the mass. An unexpected finding of negative prolactin immunostaining was noted on pathological evaluation of the tumour specimen. Postoperative serum prolactin remained in the normal range. Three years later, we performed further immunohistochemical analysis of the adenoma. The lack of prolactin expression was reconfirmed, but positive staining for the transcription factor Pit-1 was observed, supporting the initial diagnosis of a lactotroph adenoma. We draw attention to the fact that absence of tumour prolactin content in prolactin secreting macroadenomas is uncommon. If serum prolactin is only mildly or moderately elevated, distinguishing a prolactinoma from a non-functioning pituitary macroadenoma associated with stalk compression can be difficult. Determination of Pit-1 expression is therefore a crucial step in establishing a diagnosis in this scenario.

73

O-vary unusual case of metastatic thyroid cancer

Prishila Fookeerah[1], Teresa Lam[1]

1. Department of Diabetes and Endocrinology, Westmead Hospital, Sydney, NSW, Australia

A 24-year-old woman presented to hospital with severe abdominal pain secondary to a ruptured 17cm left ovarian cyst. Histopathological evaluation of the cyst showed a 12x10mm focus of follicular variant papillary thyroid carcinoma (PTC) arising from a mature cystic teratoma with no evidence of struma ovarii. CT staging did not demonstrate distant metastases, but laparoscopic staging was undertaken to exclude peritoneal seeding. Omental biopsy showed a 1mm nodule with microclusters of PTC measuring 0.2mm and <0.1mm. BRAF staining was negative. She subsequently underwent a total thyroidectomy which confirmed the absence of PTC in the thyroid gland. Postoperative thyroglobulin was undetectable. A 5.6 GBq dose of I 131 was administered after thyroxine withdrawal, in view of the omental micrometastases. Right ovarian tissue cryopreservation was performed prior to radioactive iodine for fertility preservation. Her post treatment course was complicated by a right tubo-ovarian abscess which responded well to antibiotics. Thyroglobulin levels have remained undetectable throughout and there has been no evidence of cancer recurrence at 12 months.

Metastatic PTC arising from the ovarian teratomas is rare and evidence to support treatment options is scarce.[1] Our case illustrates that PTC can be present without struma ovarii and highlights the utility of laparoscopic staging when investigating micrometastases following intraperitoneal rupture of ovarian cysts. Furthermore, concurrent pelvic infections can complicate assessment of response to therapy. Guidelines to standardise management of ovarian PTC would be beneficial.

1. 1. Makani S, Kim W, Gaba AR. Struma Ovarii with a focus of papillary thyroid cancer: a case report and review of the literature. Gynecologic Oncology. 2004;94(3):835-839.

74

Relative hypotension increases adrenal crisis detection in aging patients

Thomas Goubar[1], Cecilia Ostman[2], David Torpy[3], Shaun McGrath[2], Louise Rushworth[1]

1. The University of Notre Dame Australia, School of Medicine, Sydney, Darlinghurst, NSW, Australia

2. Endocrinology, John Hunter Hospital, Newcastle, NSW, Australia

3. Endocrine and Metabolic Unit, Royal Adelaide Hospital and University of Adelaide, Adelaide, South Australia, Australia

Background

Adrenal crises (AC) are episodes of severe adrenal insufficiency (AI) with hypotension. Cardiovascular compromise may not be detected in some patients leading to delays in emergency treatment.

Design

A retrospective study of paired systolic blood pressure (sBP) measurements in hospitalised patients with primary AI (PAI).

Patients

Patients with PAI and an acute medical illness admitted for urgent treatment between 2000 and 2017.

Measurements

A comparison between sBP on arrival at hospital and on discharge. Hypotension was classified as either absolute hypotension (sBP 100mg or lower) or relative hypotension (sBP over 100mg but at least 20mmHg lower than discharge sBP).

Results

There were 152 admissions with paired blood pressure measurements. Of these, 46 (30.3%) included a record of a medically diagnosed AC. Absolute hypotension was found in 38 (25.0%) records and a further 21 (13.8%) patients were classified as having relative hypotension. Patients aged 65 years and older had the lowest (14.8%, n=8) proportion with absolute hypotension but the highest (27.8%, n=15) with relative hypotension. Use of absolute and relative hypotension as the criterion for AC diagnosis increased the proportion of patients with an AC by 28.3% and the proportion of patients with an AC in the oldest age group by 130%.

Conclusions

Failure to detect cardiovascular compromise is common in older AI patients, underestimates the true rate of ACs in this group, and may result in delays in essential treatment. Relative hypotension should be assessed in all ill patients with AI.

75

Diagnosis of ovarian hyperthecosis by ovarian vein sampling with LCMS steroid profiling and treatment with a pure GnRH antagonist

David Handelsman[1, 2], Louise Goodall[3], Robert Schmidli[3], Simone Strasser[4], Bruce Cooper[5], Julie Hetherington[6], Reena Desai[1]

1. ANZAC Research Institute, Sydney, NSW, Australia

2. Andrology, Concord Hospital, Sydney

3. Endocrinology, Canberra Hospital, Canberra, ACT

4. Gastroenterology, Royal Prince Alfred Hospital, Sydney, NSW

5. Renal Medicine, Royal North Shore Hospital, Sydney, NSW

6. Endocrinology, Royal Prince Alfred Hospital, Sydney, NSW

A 58-year old women with a complex medical history (renal failure with 3rd transplant, post-Hep C cirrhosis, steroid-induced diabetes, hyperparathyroidism, uterine fibroid) presented with persistent, highly elevated (male range) serum testosterone concentrations with some limited virilisation. Serum testosterone was 15.7-31.0 nmol/L (normal female <1.8 nmol/L) on multiple occasions, confirmed by two different LCMS methods, menopausal serum LH (59-69 IU/L), FSH (62-64 IU/L), SHBG (76-96 nmol/L) and GFR 64 ml/min. No mass lesions in adrenal (ultrasound, MRI) or ovaries (MRI, CT). Adrenal and ovarian vein cannulation identified strong gradients in left ovarian vein (10-30 fold vs peripheral serum in 17OHP4, 17 OHP5, A_4, T, DHEA from 15 steroid LCMS profile), no adrenal vein gradients but right ovarian vein unable to be cannulated. A second ovarian vein cannulation confirmed the first study (18-fold gradient T, >60-fold gradient 17OHP4, 17OHP5, A_4, DHEA) but right ovarian vein cannulation again failed. Presumptive diagnosis was ovarian hyperthecosis with bilateral ovarian steroidogenesis of a wide range of precursors and metabolites of bioactive steroids driven by high (menopausal) serum gonadotrophins. The diagnosis was confirmed by a single dose of a pure GnRH antagonist (80 mg degarelix, Ferring) producing a complete and rapid (within 24 hr) suppression of all ovarian steroids as well as serum LH and FSH lasting for at least 4 weeks. Transient side-effects were injection site pain (2 days) and severe flushing in 2nd to 3rd week post-injection, alleviated by an estradiol patch but wearing off in 4th week. This case illustrates the utility (and limitations) of ovarian vein cannulation coupled with steroid LCMS profiling for diagnosis of severe hyperandrogenism in a postmenopausal woman without adrenal or ovarian tumour on imaging. This is the first reported use of a pure GnRH antagonist for diagnosis and treatment of ovarian hyperthecosis.

76

Adrenocortical carcinomas in Multiple Endocrine Neoplasia Type 1 - The Tasmanian Experience

Hikaru Hashimura[1], John Burgess[1]

1. Royal Hobart Hospital, Hobart, TAS, Australia

Background

Adrenocortical carcinomas (ACC) typically follow an aggressive clinical course. The incidence of ACC is 1-2/million/year with a minority of ACC potentially linked to Multiple Endocrine Neoplasia type 1 (MEN 1). The prevalence of benign adrenocortical tumours in MEN 1 ranges between 20-73%, mostly adenomas or hyperplasia. The prevalence of ACC is believed to be 1-4% in MEN 1.

Aim

To examine the prevalence and behaviour of ACC and adrenal lesions in a large Tasmanian cohort with MEN 1.

Methods

A retrospective analysis of 95 MEN 1 patients in Tasmania with a common MEN 1 gene mutation was performed. All patients underwent one or more of ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI); 63 had CT, 29 had ultrasound and three had MRI. Fluorodeoxyglucose positron emission tomography (FDG PET) was also performed in 68/95 patients.

Results

Of 95 patients, forty-three (27 females and 16 males) (45.3%) had adrenal lesions. The median age of diagnosis of adrenal lesion was 48 years. All adrenal lesions were asymptomatic and found incidentally on imaging. Adrenal lesion size ranged between 9-51mm (median 15mm) with Hounsfield units ranged between -21 to 40 (median 10HU). Of 26 patients with CT results, 19 (73.1%) exhibited benign features (14 adenomas, two hyperplasia and three bulky adrenals). The remaining seven lesions were not further characterised. Of patients with FDG PET imaging, six showed FDG-avid lesions (one ACC, two metastatic neuroendocrine tumours, one benign adenoma and two with mild FDG-uptake of unclear clinical significance).

Conclusion

Adrenal lesions are common in MEN 1, however ACC is rare. Routine abdominal imaging is useful in detecting adrenal lesions in patients with MEN 1, and FDG PET is capable of characterising malignant potential.

77

Objective reporting of neuroendocrine differentiation in hormone naïve prostate cancer correlates with poor outcomes: A systematic review and meta-analysis

Ashwini Kannan[1, 2], David Clouston[3], Mark Frydenberg[4], Dragan Ilic[1], Md Nazmul Karim[1], Sue Evans[1, 5], Roxanne Toivanen[2], Gail Risbridger[2], Renea Taylor[2]

1. Department of Epidemiology and Preventative Medicine, School of Public Health & Preventive Medicine, Monash University, Melbourne, Victoria, Australia

2. Department of Anatomy and Developmental Biology, Biomedicine Discovery Institute, Cancer Program, Monash University, Melbourne, Victoria, Australia

3. TissuPath, Mount Waverley, Melbourne, Victoria, Australia

4. Department of Surgery, Monash University, Melbourne, Victoria, Australia

5. Victorian Cancer Registry, Cancer Council Victoria, Melbourne, Victoria, Australia

Publish consent withheld

78

Atypical presentations of FHH– not so benign?

Albert Kim[2, 1], Ashley Crook[3], Michael Field[3], Mathilda Wilding[3], Lyndal Tacon[2, 1], Roderick Clifton-Bligh[2, 1]

1. Department of Endocrinology, Diabetes and Metabolism, Royal North Shore Hospital, St Leonards, NSW, Australia

2. Faculty of Medicine & Health, University of Sydney, NSW 2600, Australia

3. Department of Cancer Services, Northern Sydney Local Health District Familial Cancer Service, Royal North Shore Hospital, St Leonards, NSW, Australia

Familial Hypocalciuric Hypercalcaemia(FHH) is an inherited disorder of calcium homeostasis due to inactivating mutations of the *CASR* gene.FHH individuals typically have mild hypercalcaemia, hypocalciuria and normal parathyroid hormone(PTH) levels and no specific treatment is required[1].Atypical presentations with parathyroid adenomas have been previously reported[2].We describe two families in whom *CASR* variants were identified following presentation with hyperparathyroidism.

Case 1

48year-old female presented with hypercalcaemia, elevated PTH and fractional urinary excretion of calcium of0.014. She had previous nephrolithiasis and underwent resection of a parathyroid adenoma with normalisation of her serum calcium.Her eldest daughter was also diagnosed with primary hyperparathyroidism and 3-gland parathyroidectomy revealed hyperplasia. Her serum calcium also normalised post-operatively.Her father also has a history of multigland hyperparathyroidism and nephrolithiasis. A previously described heterozygous *CASR* variant c.2449G>A,p.Val817Ile)was detected by next-generation sequencing. All four of her children have been confirmed to carry this *CASR* variant and are under surveillance.

Case 2

45year-old male with long standing hypercalcaemia was diagnosed with primary hyperparathyroidism and underwent resection of an adenoma,with normalisation of calcium postoperatively. A novel likely pathogenic heterozygous CASR variant(c.205C>A, p.Arg69Ser)was detected by next-generation sequencing and further testing of his family members is in progress.

More than 130 different pathological variants of *CASR* have been reported with heterogeneous clinical manifestations[2]. FHH variants have been located in the transmembrane[3] and intracellular[4] domains leading to impaired intracellular signalling. *In-vitro* studies of CASR variants have demonstrated altered cell surface expression and shifts in the dose-response curve leading to impaired signalling activity[5],and this may be more severely impaired in the parathyroid relative to the kidneys[6]. This may explain the atypical findings of parathyroid adenomas,correction of hypercalcaemia following parathyroidectomy,hypercalciuria and nephrolithiasis observed in our cases. These cases highlight the heterogeneity of familial hypercalcaemic syndromes and the overlap in clinical features between FHH and primary hyperparathyroidism.

1. Hannan FM, Kallay E, Chang W, Brandi ML, Thakker RV. The calcium-sensing receptor in physiology and in calcitropic and noncalcitropic diseases. Nature Reviews Endocrinology. 2019 Jan;15(1):33-51.
2. Christensen SE, Nissen PH, Vestergaard P, Mosekilde L. Familial hypocalciuric hypercalcaemia: a review. Current Opinion in Endocrinology, Diabetes and Obesity. 2011 Dec 1;18(6):359-70.
3. Jap TS, Wu YC, Jenq SF, Won GS. A novel mutation in the calcium-sensing receptor gene in a Chinese subject with persistent hypercalcemia and hypocalciuria. The Journal of Clinical Endocrinology & Metabolism. 2001 Jan 1;86(1):13-5.
4. Mastromatteo E, Lamacchia O, Campo MR, Conserva A, Baorda F, Cinque L, Guarnieri V, Scillitani A, Cignarelli M. A novel mutation in calcium-sensing receptor gene associated to hypercalcemia and hypercalciuria. BMC endocrine disorders. 2014 Dec 1;14(1):81.
5. Leach K, Wen A, Davey AE, Sexton PM, Conigrave AD, Christopoulos A. Identification of molecular phenotypes and biased signaling induced by naturally occurring mutations of the human calcium-sensing receptor. Endocrinology. 2012 Sep 1;153(9):4304-16.
6. Carling T, Szabo E, Bai M, Ridefelt P, Westin G, Gustavsson P, Trivedi S, Hellman P, Brown EM, Dahl N, Rastad J. Familial hypercalcemia and hypercalciuria caused by a novel mutation in the cytoplasmic tail of the calcium receptor. The Journal of Clinical Endocrinology & Metabolism. 2000 May 1;85(5):2042-7.

Glucose Tolerance Test Induced Hypoglycaemia

Veli Kiriakova[1], John Burgess[1, 2]

1. Royal Hobart Hospital, Hobart, TAS, Australia

2. School of Medicine, University of Tasmania, Hobart, TAS, Australia

Background

The 75g oral glucose tolerance test (OGTT) is routinely used to screen for Gestational Diabetes Mellitus (GDM) and to diagnose Diabetes Mellitus (DM) and impaired glucose tolerance (1,2). Some patients develop biochemical hypoglycaemia during the OGTT (3), the significance of which is unclear. Potential mechanisms leading to hypoglycaemia include Beta cell dysfunction with increased insulin secretion relative to insulin resistance (4). There is, however, a paucity of information regarding the long-term prognosis of patients experiencing hypoglycaemia during OGTT.

Aim

To investigate the incidence, aetiology and outcomes for hypoglycaemia during OGTT.

Methods

Data was obtained from all patients (18-80yo) who underwent an OGTT at the Royal Hobart Hospital (1996-2017). Patients experiencing biochemical hypoglycaemia were identified and hospital medical records were interrogated to determine the long-term outcomes for these patients.

Results

A total of 10559 separate OGTT tests were assessed for biochemical hypoglycaemia (£ 3.9mmol/L) at 0hr, 1hr or 2hr. Most patients were female (n=9913, 93.9%) and 6632 (62.8%) of referrals for OGTT were in the context of antenatal care. Findings revealed 654 (6.2%) of OGTT episodes were associated with a 2hr Blood Glucose Level (BGL) of £ 3.9mmol/L (mean 3.4mmol/L, SD 0.5), with 150 (1.4%) having a 2hr BGL of £3.0mmol/L (mean 2.3mmol/L, SD 0.3). Of these patients 106 (70.7%) were antenatal. Patient with hypoglycaemia £3.0mmol/L (n=143) were examined further. Five were ultimately diagnosed with diabetes mellitus and eight were deceased. None were diagnosed with an insulinoma. Seven patients had further episodes of hypoglycaemia (2hr BGL £3.0) during subsequent OGTT. All of these were in the context of antenatal care. Four patients were found to have a 1hr and 2hr BGL £3.0, one was postpartum and breastfeeding, none were found to have an insulinoma. No patients with a 0hr (n=9) or 1hr (n=23) BGL £3.0mmol/L were diagnosed with DM or had an insulinoma.

Conclusion

Moderate to severe hypoglycaemia during OGTT is an infrequent but important response to OGTT. Our results show that hypoglycaemia is not commonly associated with any serious morbidity or underlying pathology, such as the development of diabetes mellitus or diagnosis of insulinoma.

1. Nankervis, A., McIntyre, H.D., Moses, R., Ross, G.P., Callaway, L., Porter, C., et al., 2014. ADIPS consensus guidelines for the testing and diagnosis of gestational diabetes mellitus in Australia. Modified June.
2. Welborn, T.A., 1996. ADS Position Statements: Screening for Non-Insulin Dependent Diabetes.
3. Parekh, S., Bodicoat, D.H., Brady, E., Webb, D., Mani, H., Mostafa, S., et al., 2014. Clinical characteristics of people experiencing biochemical hypoglycaemia during an oral glucose tolerance test: cross-sectional analyses from a UK multi-ethnic population. Diabetes research and clinical practice, 104(3), pp.427-434.
4. Cai, X., Han, X., Zhou, X., Zhou, L., Zhang, S. and Ji, L., 2017. Associated factors with biochemical hypoglycemia during an oral glucose tolerance test in a Chinese population. Journal of Diabetes Research, 2017.

80

Audit of the diagnosis and management of hypertriglycaedemia-induced pancreatitis

Sneha Krishna[1], Peter Davoren[1]

1. Gold Coast University Hospital, Southport, QLD, Australia

BACKGROUND: Hypertriglyceridaemia is an oft-forgotten cause of acute pancreatitis (AP), which can lead to delay in appropriate therapy, increased morbidity, and recurrent pancreatitis. Secondary hypertriglyceridaemia can be a result of uncontrolled diabetes mellitus, acute alcohol ingestion, and hypothyroidism.[1] There are no clear guidelines on the management, and there is limited evidence supporting current treatment methods of insulin infusion and apheresis.

OBJECTIVE: A two-centre retrospective audit was conducted of presentations with AP over a three-year period, and management of those with elevated triglyceride levels (defined as ≥6mmol/L) was reviewed.

RESULTS: There were 1628 presentations of AP between 1st January 2016 to 31st December 2018 to either Gold Coast University Hospital or Robina Hospital, Australia. 27 of these presentations (1.64%) from 20 patients are attributed to hypertriglyceridaemia due to recorded peak serum triglyceride level ≥6mmol/L during the admission (mean 67.4mmol/L). However, aetiology of 18.5% presentations was documented in electronic records as AP either due to acute alcohol binge or no cause listed, despite elevated triglyceride levels.

92.6% required admission into hospital with an average of 11.5 day stay. Of those those admitted, 36% had insulin infusion, 12% had apheresis, and 28% required intensive care admission. Surprisingly, only 84% were discharged on either statins and/or fibrates. There was a high recurrence rate, as 72% had either prior or future hypertriglyceridaemia-induced AP.

Pre-existing diabetes mellitus affected 44% of patients, however HbA1c was measured in only 63.6% of these presentations. Thyroid function tests were measured in 36%, with 11.1% yielding an abnormal result. Endocrinologist opinion was sought in 51.8% of presentations.

CONCLUSION: It can be derived that hypertriglyceridaemia-induced AP, whilst uncommon, is undertreated, undermanaged, and specialist opinion is often not sought. Patients are thus at risk of recurrent pancreatitis, which results in increased morbidity with recurrent abdominal pain and further hospital presentations.

1. Vipperla K, Somerville C, Furlan A, Koutroumpakis E. Clinical Profile and Natural Course in a Large Cohort of Patients With Hypertriglyceridemia and Pancreatitis. Journal of Clinical Gastroenterology. 2017;51(1):77-85.

81

Audit of the use of short synacthen test

Sneha Krishna[1], Peter Davoren[1]

1. Gold Coast University Hospital, Southport, QLD, Australia

BACKGROUND: The Short Synacthen Test (SST) is used to rule in or out adrenal insufficiency. Failure of stimulation by native ACTH in pituitary or hypothalamic disease can also result in a lack of response to Synacthen, but a normal Synachten test does not absolutely exclude central hypoadrenalism. A morning cortisol ± ACTH level may provide adequate information to avoid the need for a stimulation test.

AIM: To determine the appropriateness of the use of the SST in a single centre.

METHODS: A retrospective audit of all SSTs for one year was conducted. Indications, orderer, availability of a morning cortisol and/or or ACTH and results were recorded.

RESULTS: 27 SSTs were performed in adults. A morning cortisol was available before the SST in 12 patients. In 8 patients, a morning cortisol alone adequately confirmed normal adrenal and in a further 3, central hypodrenalism was not adequately assessed. In 13 patients with borderline morning cortisol levels, the laboratory comment did not consider central hypoadrenalism where the clinical picture might have suggested the same. In total, only 3 tests were regarded as completely appropriate.

DISCUSSION: The SST is commonly used without first measuring the morning cortisol and ACTH. Central hypoadrenalism does not seem to be considered by the order or pathology comment when the morning cortisol is borderline and a 'normal' response is demonstrated. Early morning low serum cortisol concentrations <80nmol/L has 100% specificity for detecting adrenal insufficiency.[1] A morning serum cortisol concentration greater than 415 nmol/L predicts a normal serum cortisol response to insulin-induced hypoglycaemia.[1]

1. Hägg E, Asplund K, Lithner F. Value of basal plasma cortisol assays in the assessment of pituitary-adrenal insufficiency. Clin Endocrinol (Oxf) 1987; 26:221.

82

The *Bee* Family, SDH*B*-related Pheochromocytoma, Paraganglioma and Pituitary Adenoma

Johanna Kuehn[1], Shamasunder Acharya[1, 2]

1. Department of Diabetes & Endocrinology, John Hunter Hospital, Newcastle, NSW, Australia

2. School of Medicine and Public Health, University of Newcastle, Newcastle, NSW

Case Reports:

A 46-year old male presented with bitemporal hemianopia in the context of known prolactinoma; initially diagnosed aged 31 years with vision failure, prolactin level of 13680 mIU/L and pituitary macroadenoma measuring 27x90x26mm, associated with hypogonadism and hypothyroidism. Initial treatment response had been satisfactory and pituitary function remained optimised on Cabergoline, Thyroxine and Reandron. Current MRI findings were consistent with stable traction hemianopia and did not show progression over time.

His mother, a 76-year old female, was subsequently found to have a right base of skull paraganglioma (PGL) measuring 31x21x30mm with intense DOTATATE avidity. She remained clinically asymptomatic without biochemical evidence of catecholamine hypersecretion. Considering her advanced age and the location of PGL conservative management was implemented.

His older brother, a 53-year old with history of longstanding hypertension and a recent diagnosis of Diabetes mellitus, was found to have metastatic pheochromocytoma (Pheo) originating as a large right bilobed adrenal mass (53 x 31 x 55mm). Plasma and urinary normetanephrines were elevated at 18-times the upper reference range and he was clinically symptomatic with palpitations, perspiration and 5 kg weight loss. Blood pressure was inadequately controlled on 5 antihypertensive agents. Post right radical adrenalectomy and retroperitoneal lymph node resection (pT3 pN1 Mx), he underwent biochemical and clinical remission.

Genetic testing confirmed a SDH*B* gene mutation in all three cases and further eight family members over 3 generations.

Discussion:

A combination of Pheo, PGL and pituitary adenomas (PA) is an uncommon occurrence, but has been described as 'The three P Association' and can be associated with SDH*B* gene mutation.[1-3] Whereas the occurrence of PA in SDH germline mutation carriers is rare; genetic testing is advisable in all patients or families with '3PA'; familial or early onset PAs, and/or Pheo/PGLs especially when presenting with multifocal, bilateral or metastatic disease.

1. Samuel M O'Toole, Judit Denes, Mercedes Robledo, Constantine A Stratakis, Marta Korbonits, The association of pituitary adenomas and phaeochromocytomas or paragangliomas, Endocrine-Related Cancer (2015) 22, T105–T122.
2. Xekouki P, Szarek E, Bullova P, Giubellino A, QuezadoM, Mastroyannis SA, Mastorakos P, Wassif CA, Raygada M & Rentia N, Pituitary adenoma with paraganglioma/pheochromocytoma (3PAs) and succinate dehydrogenase defects in human and mice. Journal of Clinical Endocrinology and Metabolism 100 E710–E719.
3. Denes J, Swords F, Rattenberry E, Stals K, Owens M, Cranston T, Xekouki P, Moran L, Kumar A,Wassif C et al., Heterogeneous genetic background of the association of pheochromocytoma/paraganglioma and pituitary adenoma – results from a large patient cohort. Journal of Clinical Endocrinology and Metabolism 100 E531–E541.

83

The impact of multifocality on the recurrence of papillary thyroid carcinoma

Hyungju Kwon[1]

1. Ewha Womans University, Yangcheon-gu, SEOUL, South Korea

Background

The incidence of thyroid cancer has dramatically increased over the last few decades, and up to 87% of patients have multifocal tumors. However, the prognostic impact of multifocality in patients with papillary thyroid carcinoma (PTC) remains controversial. In this study, we investigated the role of tumor multifocality in clinical outcomes of PTC.

Methods

Study subjects included 1937 patients who underwent thyroidectomy for PTC between March 2009 and December 2019. Data collected included patient demographics, pathologic features including multifocality, development of recurrence, and follow up period.

Results

Multifocality was found in 591 patients (30.4%) and mean follow up period of 5.2 ± 2.8 years. The 5-year recurrence free survival (RFS) rate was 96.4% in patients with multifocal tumors, whereas those with unifocal disease showed 98.3% of 5-year RFS ($p = 0.001$). Multivariate Cox regression analysis indicated that tumor size (HR 1.83, 95% CI 1.36-2.45), LN metastasis (HR for N1a 3.12, 95% CI 1.42-6.84; HR for N1b 4.24 95% CI 1.20-15.0), and multifocality (HR 2.11, 95% CI 1.14-3.89) were independent predictors of recurrence.

Conclusions

Our data suggest that multifocality increases the risk of recurrence in patients with PTC. Patients with multifocal PTCs require careful treatment and follow-up approaches.

84

The prognostic impact of multifocality in clinical outcomes of papillary thyroid carcinoma: a systematic review and meta-analysis

Hyungju Kwon[1]

1. Ewha Womans University, Yangcheon-gu, SEOUL, South Korea

Objectives: The incidence of thyroid cancer has dramatically increased over the last few decades, and up to 87% of patients have multifocal tumors. However, the prognostic impact of multifocality in patients with papillary thyroid carcinoma (PTC) remains unestablished and controversial.

Materials and methods: A systematic search was conducted using PubMed, SCOPUS, the Web of Science Core Collection, and the Cochrane Database of Systematic Reviews from their inception to June 30, 2020. The relevant studies compared recurrence rates or cancer-specific survival using the hazard ratio (HR) of multifocality; 26 studies of 34251 patients were identified and included.

Results: Recurrence rates for patients with multifocal disease were significantly higher than those with unifocal PTC (HR 1.81, 95% CI 1.52–2.14), while cancer-specific mortality was comparable between the groups (HR 1.19, 95% CI 0.85–1.68). In subgroup analyses, primary tumor size (HRs for PTC ≤1 cm and >1 cm were 1.81 and 1.90, respectively), number of tumor foci (HRs for two foci and more than 2 foci were 1.45 and 1.95, respectively), and patient age (HRs for pediatric and adult patients were 3.19 and 1.89, respectively) could affect the HRs of multifocality for recurrence.

Conclusions: Multifocality increases the risk of recurrence in patients with PTC. Differences in tumor size, number of tumor foci, and patient age should be considered when interpreting the impact of multifocality on the risk of recurrence.

85

Glucometric analysis of inpatient hypoglycaemia at Concord Hospital

Annabel Lee[1], **Benjamin Kwan**[1, 2]

1. Concord Repatriation General Hospital, Concord, NSW

2. University of Sydney, Sydney, NSW

Introduction and Aim:

Auditing and benchmarking of hospital glucometric outcomes has been advocated by Kyi and colleagues[1] from Melbourne to help improve the quality of inpatient diabetes care. Inpatient hypoglycaemia is a common adverse event among patients on insulin therapy. Its incidence is underestimated by DRG coding data. Live Glucose is an EMR-based solution that provides real-time informatics from point-of-care glucose data, allowing identification of all inpatient hypo- and hyperglycaemia. The aim of this study was to assess rates of inpatient hypoglycaemia at our hospital.

Method:

We used Live Glucose to prospectively identify all cases of hypoglycaemia (point-of-care glucose <4.0 mmol/L) among medical and surgical inpatients at Concord Repatriation General Hospital over a 10-week period (8 January–17 March 2020). Consecutive low glucose values were counted as part of the same hypoglycaemic episode.

Results:

During the study period, there were 711 admissions for 643 patients with diabetes (mean age 72.0years), totalling 5,996 patient-days. We identified 81 patients (mean age 72.6 years) with at least one episode of hypoglycaemia over 86 separate admissions. There were 211 unique episodes of hypoglycaemia during the study period, corresponding to 188 patient-days with hypoglycaemia. Most patients were on insulin therapy at admission (70%) and had type 2 diabetes (78%). Recurrent hypoglycaemia was observed in 50% of patients; level 2 hypoglycaemia (<3.0 mmol/L) in 47%. Hypoglycaemia incidence was comparable to Royal Melbourne Hospital for all hypoglycaemia (3.1% vs. 4.6%, patient-days) and level 2 hypoglycaemia (1.1% vs. 1.3%, patient-days) 1, and lower than international benchmarks.

Conclusion:

While rates of hypoglycaemia at our hospital are similar to local benchmarks, the incidence of recurrent hypoglycaemia is concerning. Live Glucose provides real-time glucose informatics and may enhance the ability of inpatient diabetes teams to identify, intervene, and prevent cases of recurrent hypoglycaemia

1. Kyi et al. Med J Aust. 2019;211(4):175-180

86

Inpatient hypoglycaemia management and follow-up care

Annabel Lee[1], Benjamin Kwan[1, 2]

1. Concord Repatriation General Hospital, Concord, NSW

2. University of Sydney, Sydney, NSW

Introduction and aim:

Inpatient hypoglycaemia is a serious complication of insulin therapy. Following hypoglycaemia, review of glucose-lowering therapy is often the responsibility of junior medical staff, with specialist diabetes team involvement determined by local referral pathways and treating team discretion. The aim of this study was to review the quality of hypoglycaemia management and follow-up care at our hospital and to identify potential interventions to minimise recurrent hypoglycaemia.

Method:

Inpatients with hypoglycaemia (point-of-care glucose < 4.0mmol/L), admitted to a medical or surgical ward at Concord Repatriation General Hospital over a 10-week period (8 January – 17 March 2020) were prospectively identified using Live Glucose, an EMR-based glucose informatics system. We collected patient and admission data, and quality measures relating to hypoglycaemia treatment, medical review, recurrence, and rates of inpatient diabetes team referral.

Results:

We identified 211 episodes of hypoglycaemia among 81 patients (Type 2 diabetes 78%; insulin therapy on admission 70%; mean ± SD age, 72.6 ± 15 years), over 86 separate admissions (medical 64%, surgical 36%). Half of these patients had recurrent hypoglycaemia within their admission. Following hypoglycaemia, most had appropriate documentation of acute treatment (92%) and adequate glucose monitoring over the next 48 hours (97%), but only 42% had immediate medical review. A specific management plan addressing hypoglycaemia (review of insulin doses, diabetes team referral, etc.) was not documented in a third of cases. In patients with recurrent hypoglycaemia, only 63% were referred to endocrinology, and only 61% had hypoglycaemia documented as a complication in discharge paperwork.

Conclusion:

There is significant room for improvement in hypoglycaemia management and follow-up care. Despite clear best practice standards at the local, district and state level, specialist diabetes services are underutilised, particularly following recurrent hypoglycaemia. EMR-based solutions such as Live Glucose would allow early proactive review by specialist teams following hypoglycaemia.

87

A Case of Ipilimumab and Nevolumab Induced Multiple Endocrinopathies

Bon Hyang Lee[1], Yong Mong Tan[1, 2], Kunwarjit Sangla[1, 2]

1. Department of Diabetes and Endocrinology, Townsville University Hospital, Townsville, Queensland, Australia

2. College of Medicine and Dentistry, James Cook University, Townsville, Queensland, Australia

Introduction

Immune check-point inhibitors have revolutionised cancer treatment by improving prognosis but are associated with adverse immune-related side effects (irSEs) such as thyroid dysfunction and hypophysitis. Other endocrine irSEs are less common.

Case Description

A 54-year-old male was started on Ipilimumab (anti-CTLA4 monoclonal antibody) and Nivolumab (PD-1 inhibitor) for metastatic melanoma. After 2 cycles of immunotherapy, he presented with non-ketotic hyperglycaemia and was diagnosed with insulin dependent diabetes. GAD, IA-2 and ZnT8 antibodies were not detected. His admission was complicated by atrial fibrillation with acute thyroiditis. He had mildly elevated thyroid peroxidase and thyroglobulin antibodies. Treatment with Prednisolone was complicated by diabetic ketoacidosis requiring hospital admission. PET CT after 2 cycles of immunotherapy showed reduction in all metastatic lesions. Due to the irSEs, Ipilimumab was ceased but Nivolumab continued. He developed postural dizziness, lethargy, abdominal discomfort with subnormal short Synacthen test, confirming adrenal insufficiency, requiring hydrocortisone replacement. Anti-adrenal antibodies were negative. Normal pituitary MRI and hormones excluded hypophysitis. His acute hyperthyroidism was followed by hypothyroidism requiring thyroxine replacement. After six cycles of immunotherapy with multiple irSEs, he had complete radiological response of metastatic melanoma.

Conclusion

Immune check-point inhibitor therapy is associated with endocrine irSEs; thyroid dysfunction and hypophysitis being the most common. This case demonstrates insulin-dependent diabetes and adrenal insufficiency, rarer forms of irSEs. Novel features included absence of diabetes autoantibodies and anti-adrenal antibodies.

Learning Points

1) Understand the mechanism of action of immune checkpoint inhibitors;
2) Identify endocrinopathies caused by immune checkpoint inhibitors;
3) Understand how to manage immune checkpoint inhibitor related endocrinopathies

88

A case of clinical and biochemical manifestations of undiagnosed Hashimoto's thyroiditis

Sarah Lewis[1], Daniel Fineberg[1]

1. Alfred Health, South Yarra, VIC, Australia

We report the case of a 69 year old female who presented with mild cognitive impairment and difficulty mobilising in the setting of profound hypothyroidism secondary to Hashimoto's thyroiditis with associated elevated creatine kinase (CK), hyponatraemia, anaemia, renal impairment, hypercholesterolaemia and hypertryglyceridaemia. On initial investigations the patient had a thyroid stimulating hormone (TSH) of 49 mU/L, free T4 <5.4 pmol/L, thyroid peroxidase (TPO) antibody positive, CK 1628 units/L, sodium 120 mmol/L, haemoglobin 87 g/L, creatinine 109 mcmol/L, total cholesterol 8.1 mmol/L and tryglycerides 4.7 mmol/L. On examination the patient had no features of myxoedema coma but was found to have delayed relaxation of tendon reflexes, puffy facies with loss of outer one third of eyebrows, coarse hair, brittle nails and slowing of speech and movement with obvious cold intolerance. There was no muscle weakness on examination to suggest myositis although the patient complained of generalised aches and lethargy. The patient was initially treated with 100mcg oral thyroxine daily however this was increased and oral liothyroxine introduced following an inadequate improvement. Eleven days post admission the TSH was 6.26 mU/L and the free T4 was 12.4pmol/L following a total of 1500mcg oral thyroxine replacement and 60mcg oral liothyronine replacement. The hyponatraemia improved with a strict fluid restriction of 500 millilitres daily to sodium 133 mmol/L and the renal function improved to a creatinine of 70 mcmol/L on discharge. Atorvastatin was withheld due to the elevated CK which improved to 370 units/L and the anaemia remained stable throughout the admission. Although the patient refused formal cognitive assessments her functional abilities improved with treatment. This case highlights the clinical and biochemical features of severe hypothyroidism in the setting of undiagnosed Hashimoto's thyroiditis.

89

A multi-centre study of neutrophil-to-lymphocyte ratio in primary aldosteronism

Renata Libianto[2, 1], Jinbo Hu[3], Min R Chee[1], Jesse Hoo[1], Yin Y Lim[1], Jimmy Shen[1, 4], Qifu Li[3], Morag J Young[1], Peter J Fuller[1, 4], Jun Yang[1, 4]

1. Hudson Institute of Medical Research, Clayton

2. Endocrinology, Monash Health, Clayton, VIC, Australia

3. The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

4. Monash Health, Clayton, VIC, Australia

Background: Hypertensive patients with primary aldosteronism (PA) have a higher risk of cardiovascular complications than those with blood pressure-matched essential hypertension. The excess cardiovascular consequences of PA can be attributed to the pro-inflammatory effect of excessive aldosterone and mineralocorticoid receptor activation. The neutrophil-to-lymphocyte ratio (NLR) is a widely available markers of inflammation which has been shown to predict cardiovascular outcome in the general population. This study aims to evaluate the use of NLR as a potential biomarker of PA and PA severity.

Methods: Patients with PA (n=355) were identified from two large PA databases in Australia and China, while controls (n=222) were patients with hypertension who were referred for assessment but did not meet the diagnostic criteria for PA. The NLR was retrospectively collected from routine full blood examination, prior to commencement of targeted treatment for PA.

Results: The NLR did not differ between PA patients and hypertensive controls (median 2.3 and 2.4, p=0.563). However, amongst patients with PA, the NLR was positively correlated with baseline and post-saline aldosterone levels (r=0.22 and p<0.001 for both) and negatively correlated with serum potassium (r=-0.15, p=0.006). Furthermore, in a logistic regression of patients with PA, the NLR predicted the presence of co-morbid CKD with odds ratio of 1.5 (p=0.003).

Conclusion: Whilst the NLR did not distinguish PA from controls, it was a marker of PA severity, being associated with aldosterone concentration as well as the presence of CKD. A prospective study is needed to further clarify the role of NLR in predicting end-organ damage associated with PA.

Dose-Dependent Response to Long-term Clomiphene Citrate in Male Functional Hypogonadotropic Hypogonadism: A Case study

Beryl Lin[1, 2], **Stephen M Twigg**[1, 2]

1. Endocrinology, Royal Prince Alfred Hospital, Sydney

2. Sydney Medical School, University of Sydney, Sydney

INTRODUCTION: Functional hypogonadotropic hypogonadism is a relatively common condition in middle-aged to elderly men that can significantly impair quality of life.[1,2] Although its aetiology is incompletely understood, the phenotype is characterized by clinical androgen deficiency in the absence of structural hypothalamic-pituitary pathology. In addition to lifestyle optimisation, androgen replacement remains the treatment mainstay; however it can cause azoospermia and testicular atrophy.[3]

Clomiphene citrate is a serum estrogen receptor modulator that acts centrally to increase testosterone production without affecting fertility. It has demonstrated efficacy in short-duration studies, but longer-term data is lacking.[3]

CASE REPORT: An otherwise well 42-year-old male presented with a 6-year history of generalised fatigue, reduced libido and insomnia. Hormonal profile demonstrated secondary hypogonadism: low total testosterone 8.5(9.5-28)nmol/L, low-normal LH 1.0(0.6-12)IU/L, low FSH 0.9(1.0-12)IU/L. Semen analysis was normal. A dedicated pituitary MRI did not identify any focal abnormality.

A biopsychosocial approach to management was employed. Due to patient preference, clomiphene citrate was trialled. After six weeks at 25mg daily, all symptoms including libido, erectile dysfunction and cognition improved. Blood total testosterone increased to the upper limit of normal 20.3nmol/L with corresponding increases in LH 1.7IU/L and FSH 3.5IU/L. Despite adherence to intensive lifestyle practices, clomiphene dose reductions below 15mg daily precipitated symptom recurrence. Thus, clomiphene was continued with dose titrations on regular follow up every 4-6 months.

Over five years to date, our patient has demonstrated a linear correlation between clomiphene dose and blood testosterone, oestradiol, LH and FSH levels ($p<0.001$). He has sustained an excellent clinical response with no known adverse effects.

CONCLUSION: Clomiphene induces a dose-dependent response in pituitary function and endogenous testosterone production. It has potential as a safe and efficacious longer-term treatment option for male functional hypogonadism. Further studies including randomized controlled trials comparing clomiphene to conventional androgen replacement are indicated to inform evidence-based therapy.

1. Mulligan T, Frick MF, Zuraw QC, Stemhagen A, McWhirter C. Prevalence of hypogonadism in males aged at least 45 years: the HIM study. International journal of clinical practice. 2006;60(7):762-9.
2. Araujo AB, Esche GR, Kupelian V, O'Donnell AB, Travison TG, Williams RE, Clark RV, McKinlay JB. Prevalence of symptomatic androgen deficiency in men. The Journal of Clinical Endocrinology & Metabolism. 2007;92(11):4241-7.
3. Wheeler KM, Sharma D, Kavoussi PK, Smith RP, Costabile R. Clomiphene citrate for the treatment of hypogonadism. Sexual medicine reviews. 2019;7(2):272-6.

Experience with sodium–glucose co-transporter 2 inhibitors initiation in a public hospital diabetes outpatient population- Extension of a previous study

MINA MOHAMMAD EBRAHIM[1], Nuttaradee Lojanapiwat[1], Nicola Hogan[1], Christopher Gilfillan[1]

1. Department of Diabetes and Endocrinology, Eastern Health, Box Hill, VIC , Australia

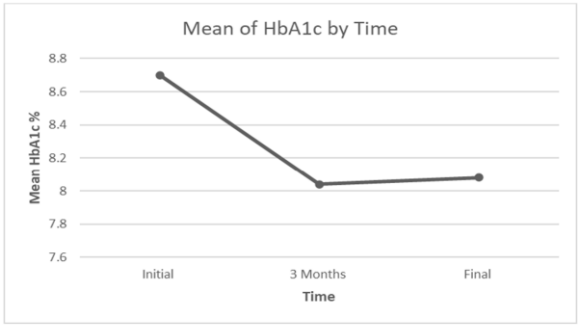


Figure 1. Changes in mean HbA1c over time in patients treated with SGLT2 inhibitors

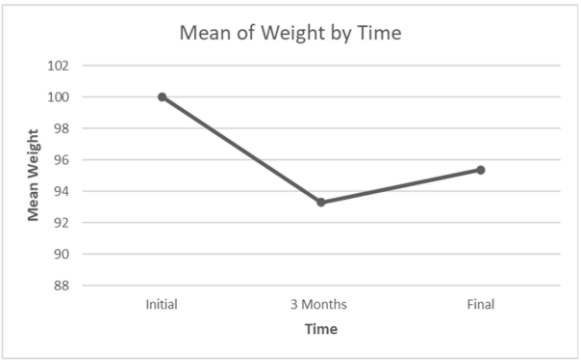


Figure 2. Changes in weight over time in patients treated with SGLT2 inhibitors

Table 1. Adverse events reported in patients treated with SGLT2 inhibitors

Adverse Events	Patients Affected by the Adverse Event	Adverse Event Led to Discontinuations of SGLT2 Inhibitor
Hypoglycaemia	18	0
Genital infection	15	8
Urinary frequency	9	3
Urinary tract infection	6	3
Diabetic foot ulcer	4	1
Adverse Cardiovascular Event	4	0
Worsening of renal function	2	2
Deep vein thrombosis	2	0
Gastrointestinal symptoms	3	2
Dizziness	1	0

Table 2. Outcomes -Paired Samples Test

	Initial- Mean	Final- Mean	Sig. (2-tailed)
Weight (kg)	100.03	95.37	**.000**
Systolic blood pressure (mm Hg)	136.15	127.34	**.003**
Diastolic blood pressure (mm Hg)	81.03	77.38	**.017**
HbA1c %	8.88	8.08	**.000**
Total Cholesterol (mmol/L)	4.02	4.05	.782
Triglyceride (mmol/L)	2.01	2.11	.456
LDL Cholesterol (mmol/L)	2.02	1.98	.725
HDL Cholesterol (mmol/L)	1.05	1.12	**.027**
Aspartate aminotransferase - AST (U/L)	25.95	25.04	.579
Alanine Aminotransferase - ALT (U/L)	34.52	29.55	**.017**
Gamma-glutamyl transferase- GGT (U/L)	49.70	41.34	**.004**
Alkaline Phosphatase- ALP (IU/L)	79.34	78.19	.576
Serum Creatinine (umol/L)	78.91	81.67	.081
Estimated glomerular filtration rate -eGFR (ml/min/1.73m2)	79.68	77.58	.058
Urine Albumin-Creatinine Ratio (mg/mmol)	16.70	16.47	.923

Background:

Sodium–glucose co-transporter 2 (SGLT2) inhibitors are group of medications that reduce plasma glucose by reduction of glucose reabsorption in kidney (1).

They have favorable effects on glycaemic control, weight and blood pressure (2-5). Uro-genital tract infections are the most common side effects reported in 4-5% of patients (6-7).

This study assesses the safety and efficacy of SGLT2 inhibitors in a real -world public diabetes outpatient setting.

Method:

This is an extension of a retrospective study on patients with type 2 diabetes who were treated with SGLT2 inhibitors in the diabetic clinics across Eastern Health, between 2014 and 2019.

All statistical computations were performed using SPSS version 23.0. Descriptive statistics were analysed by T tests, Pearsons and ANOVA correlations and Multivariate analysis.

Results:

One hundred patients were included in the study, 42% of them were women. The mean age was 58.5 years. Patients were treated with dapagliflozin or empagliflozin.

Adverse events noted in 50 patients which led to discontinuation of SGLT2 inhibitor in 19 of them (Table 1). No ketoacidosis was reported. The SGLT2 inhibitor was discontinued in 28 patients due to genital infection (8%), worsening of glycaemic control (8%), urinary tract infection (3%), urinary frequency (3%), weight gain (2%), price (2%) and worsening of renal function (2%).

Significant decrease noted from baseline in HbA1c (-0.61%), weight (-4.7 kg), systolic (-8.8 mm Hg) and diastolic blood pressure (-3.7 mm Hg), ALT and GGT. HDL cholesterol also increased significantly (Table 2). Changes in HbA1c and weight were more significant in the first 3 months of therapy (Figure 1,2).

Discussion:

Despite the significant benefits of SGLT2 inhibitors shown in this study in improving glycaemic control, weight, blood pressure, liver function tests and HDL cholesterol levels, the SGLT2 inhibitor was discontinued in nearly 1 out of 3 patients in real clinical setting.

1. Andre ´ J. Scheen. Pharmacodynamics, Efficacy and Safety of Sodium–Glucose Co-Transporter Type 2 (SGLT2) Inhibitors for the Treatment of Type 2 Diabetes Mellitus. Drugs. 2015;75:33–59.
2. Tikkanen I, Chilton R, et al. Potential role of sodium glucose cotransporter 2 inhibitors in the treatment of hypertension. Curr Opin Nephrol Hypertens. 2016;25:81–6.
3. Cefalu WT, Leiter LA, et al. Dapagliflozin's Effects on Glycemia and Cardiovascular Risk Factors in High-Risk Patients With Type 2 Diabetes: A 24-Week, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study With a 28-Week Extension. Diabetes care. 2015; 38(7):1218–27.
4. Zinman B, Wanner C, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N ENGL J MED. 2015;373:2117-28.
5. Mohammad Ebrahim M, Gilfillan C, et al. Experience with SGLT-2 initiation in a public hospital diabetes outpatient population. The Joint Annual Scientific Meetings of the Endocrine Society of Australia and the Society for Reproductive Biology 2018. (#252)
6. Kalra S. Sodium Glucose Co-Transporter-2 (SGLT2) Inhibitors: A Review of Their Basic and Clinical Pharmacology. Diabetes Ther. 2014; 5:355–366.
7. Johnsson KM, Ptaszynska, et al. Vulvovaginitis and balanitis in patients with diabetes treated with dapagliflozin. J Diabetes Complications. 2013;27(5):479-84.

92

Osteopetrosis with a potentially novel genetic mutation

Ashish Munsif[1], Chris Muir[1]

1. Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia

Background:

Osteopetrosis is a rare genetic disorder that causes abnormally dense bones, increasing fracture risk. Autosomal dominant forms affect approximately 1:20,000 people, whereas autosomal recessive cases affect 1:250,000 people. Rarely, X-linked inheritance can also occur. It is also associated with optic nerve compression and hearing loss due to sclerosis of the skull base, as well as odontomas and mandibular osteomyelitis.

Clinical case:

A 60 year old Caucasian gentleman with a background of renal calculi and Osgood-Schlatter disease presented with generalised bilateral knee pain that progressively worsened over 6 months prior to outpatient review. Examination revealed noticeable difficulty in sitting and standing manoeuvres, as well as limited range of motion on flexion and extension of the knees. X-ray imaging of the knees revealed severe tricompartmental degenerative changes and patchy areas of mixed sclerosis and lucency. BMD-DEXA values were markedly elevated globally; L2-L4 T-score +14.4 SD, L total hip T-score +16.9 SD, R total hip T-score +17.3 SD, TBS 1.44. NM bone scan displayed a classical 'superscan' pattern. Spinal imaging demonstrated a 'rugger-jersey' vertebrae appearance. Other imaging showed dense long bone cortices and increased skull base thickness. These features are clinically suggestive of CLCN7 autosomal dominant osteopetrosis (1), which would have significant clinical implications for his three children, who are currently well. Interestingly, on genetic testing no focal mutation was identified. This represents a case where a novel mutation may be contributory to the diagnosis, given over 95% of pathogenic variants causing osteopetrosis are routinely confirmed on testing of the CLCN7 gene otherwise (2). The patient is currently being managed symptomatically for pain and is limiting mobility on inclines given discomfort associated with this.

1. Cleiren, E., Bénichou, O., Van Hul, E., Gram, J., Bollerslev, J., Singer, F. R., ... & deVernejoul, M. C. (2001). Albers-Schönberg disease (autosomal dominant osteopetrosis, type II) results from mutations in the ClCN7 chloride channel gene. Human molecular genetics, 10(25), 2861-2867.
2. Del Fattore, A., Peruzzi, B., Rucci, N., Recchia, I., Cappariello, A., Longo, M., ... & Devito, R. (2006). Clinical, genetic, and cellular analysis of 49 osteopetrotic patients: implications for diagnosis and treatment. Journal of medical genetics, 43(4), 315-325.

93

Plasmapheresis induced hypocalcaemia in a cystic fibrosis patient with bilateral lung transplants

Ashish Munsif[1], Chris Muir[1]

1. Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia

Background:

Hypocalcaemia is a rare side-effect of plasmapheresis. It occurs secondary to the use of citrate as an anticoagulant, to maintain patency of plasmapheresis circuits. While the hypocalcaemia associated with this procedure is not typically critical, it can lead to tetany and arrhythmias (1). This is especially the case with pre-existing hypocalcaemia, which can lead to citrate toxicity.

Clinical case:

A 37 year old lady with bilateral sequential single lung transplants performed in 2008 for cystic fibrosis underwent plasmapheresis on four separate occasions over the last four years, including twice in 2020. The treatment was required for antibody mediated rejection. On each of these occasions, despite oral calcium and vitamin D supplementation, she developed hypocalcaemia as low as 1.80mmol/L. She was symptomatic with perioral tingling and paraesthesia the first time this occurred, and required intravenous calcium gluconate therapy.

Fortunately, no arrhythmias or tetany were identified with the hypocalcaemia. However, she did develop hypotension with her most recent course. Her calcium levels recovered post-plasmapheresis and were maintained at a mid-reference range level with her usual supplementation otherwise. Whilst undergoing plasmapheresis, she required oral calcium 4800mg/day. Pathology during her most recent course identified CKD with an eGFR varying between 40-50mL/min/1.73m2, PTH 7.9pmol/L, 25(OH) vitamin D 104nmol/L, phosphate 1.04mmol/L, magnesium 0.73mmol/L, TSH 0.82mIU/L, fT4 11.7pmol/L, albumin 35g/L and normal lipid studies and liver function tests.

This case demonstrates the importance of monitoring calcium levels closely in patients undergoing plasmapheresis, as patients can develop symptomatic hypocalcaemia. There may even be a role for pre-emptive treatment to reduce the risk of hypocalcaemia in patients with a known history of having such a response to plasmapheresis. Particular attention should be given to patients with a history of hypoparathyroidism (including surgical and medication induced, such as with PPIs) or pre-existing hypocalcaemia.

1. Philip J, Sarkar RS, Pathak A. Adverse events associated with apheresis procedures: Incidence and relative frequency. Asian journal of transfusion science. 2013 Jan;7(1):37.

94

Paraganglioma and GIST tumours (Carney-Stratakis syndrome) in a young man

Elisabeth Ng[1], John Zalcberg[2, 3], Duncan J Topliss[1, 4]

1. Department of Endocrinology & Diabetes, Alfred Health, Melbourne, Australia

2. Department of Medical Oncology, Alfred Health, Melbourne, Australia

3. School of Public Health, Monash University, Melbourne, Australia

4. Department of Medicine, Monash University, Melbourne, Australia

A 20 year-old male had presented at the age of 11 years with abdominal pain and anaemia, having previously been well, and was found to have a haemorrhagic retroperitoneal tumour requiring prompt excision. The tumour was a hormonally-inactive paraganglioma; a germline SDHB mutation was found. His father, paternal grandfather, four paternal aunts and uncles and two cousins were also found to carry the mutation, though our proband was the only one with an identified paraganglioma. He had recurrent episodes of adhesion-related small bowel obstruction during his teens. During a laparoscopic adhesiolysis at age 19 he was observed to have two solid liver lesions and two large gastrointestinal lesions. He was normotensive with normal range plasma metanephrines, but a Ga68-DOTATATE PET scan showed metastatic paraganglioma to the lung, retroperitoneum, spine, and frontal bone. The co-registered CT scan showed three low density gastrointestinal tract lesions and several liver lesions. On FDG-PET scanning the bowel and liver lesions showed uptake consistent with metastatic gastrointestinal stromal tumour (GIST) and metabolically active metastatic paraganglioma. With the primary abdominal mass measuring 90mm, along with liver masses measuring up to 28mm, a decision was made in consultation with medical oncology and the hepatopancreatobiliary surgeons to proceed with a distal gastrectomy and liver wedge resection. Histopathology confirmed metastatic GIST, and in the context of metastatic paraganglioma, the diagnosis of Carney-Stratakis syndrome was made. This autosomal dominant syndrome with incomplete penetrance is characterised by paraganglioma and GIST, recognised as occurring due to germline mutations in succinate dehydrogenase gene subunits, mainly SDHB, SDHC or SDHD. Loss of function of these tumour suppressor genes predisposes to the development of this dyad. We depict the early course of this young patient with Carney-Stratakis syndrome and a description of his management in the context of what is known about this rare condition.

95

Gestational diabetes and future cardiovascular disease risk in women: A systematic review and meta-analysis.

Maleesa Pathirana[1, 2], Zohra S Lassi[1, 2], Anna Ali[1, 3, 2], Margaret A Arstall[2, 4], Claire T Roberts[1, 2, 5], Prabha H Andraweera[1, 2, 4]

1. Robinson Research Institute, The University of Adelaide, Adelaide, SA, Australia

2. Adelaide Medical School, The University of Adelaide, Adelaide, SA, Australia

3. Basil Hetzel Institute, Woodville, SA, Australia

4. Department of Cardiology, Lyell McEwin Hospital, Elizabeth Vale, SA, Australia

5. Flinders Health and Medical Research Institute, Flinders University, Bedford Park, SA, 5042

Background/Aims: Gestational diabetes mellitus (GDM) affects 1 in 7 pregnancies. Emerging evidence suggests that women who experience GDM have a higher risk of cardiovascular disease (CVD) later in life. The primary aim of this systematic review and meta-analysis was to synthesize evidence on conventional cardiovascular risk factors among women who experience GDM.

Methods: Studies were found through an electronic search of PubMed, CINAHL and EMBASE. Studies reporting on conventional cardiovascular risk factors including blood pressure, lipids, blood glucose, fasting insulin, body mass index (BMI) and metabolic syndrome (MetS) among women who experienced GDM were selected. We included studies that defined GDM based on the International Association of Diabetes and Pregnancy Study Group definition or any previous definitions. MetS was defined using the National Cholesterol Education Program Adult Treatment Panel III/World Health Organization /International Diabetes Federation definitions.

Results: A total of 190 studies were included in the review, and 129 were included in the meta-analysis. Women diagnosed with MetS in early pregnancy have a significantly higher risk of developing GDM compared to those without MetS (RR 20.51, 95% CI 5.04 to 83.55) Women with previous GDM have a significant increase in all cardiovascular risk factors, with the exception of HDL which is lower, compared to women without a history of GDM. Subgroup analyses showed that metabolic syndrome, and elevated blood pressure, total cholesterol, triglycerides and glucose are seen in women with previous GDM as early as <1 year post-partum compared to those without previous GDM.

Conclusion: Women with previous GDM have a higher risk of cardiovascular disease based on a significant increase in risk factors compared to those with no history of GDM. Some risk factors are seen as early as <1 year post-partum. Therefore, women with GDM may benefit from early screening to identify modifiable CVD risk factors.

96

Clotting factors & its associations in patients with hyperparathyroidism – Evidence from a systematic review & meta-analysis.

Kavindra N Ratnaweera[1], Alexander J Rodriguez[2, 3]

1. School of Medicine, Griffith University, Southport, QLD, Australia

2. Bone and Muscle Health Research Group, School of Clinical Sciences, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Victoria, Australia

3. Disorders of Mineralisation Research Group, School of Medical and Health Sciences, Edith Cowan University, Perth, Western Australia, Australia

Calcium is critical in coagulation. Parathyroid hormone (PTH) maintains calcium homeostasis. Elevated PTH increases cardiovascular risk, but it is unclear if these patients are predisposed to thrombosis possibly related to PTH action on calcium. We aimed to determine if PTH was associated with increased clots or markers of coagulation in a systematic review. We searched MEDLINE and EMBASE (29 March, 2020) for studies that were non-randomized; directly measured PTH levels or specifically enrolled patients with hyper/hypo-parathyroidism; measured any clotting factor; or reported thromboembolic or haemorrhagic events. We excluded interventional studies, case reports, studies of surgical correction of hyperparathyroidism or therapies that interfere with mineral metabolism or haemostasis. Primary outcome was the association between PTH and clotting factors and the association of PTH with the incidence of thromboembolic or haemorrhagic events. Continuous data were meta-analysed if reported in at least 100 patients in more than one study. Random-effects models were fitted and reported as standardized mean difference (SMD) with 95% confidence intervals (95%CI). Heterogeneity was determined by the I^2 statistic. All data were computed using R (4.0.0). 2404 records were screened. Eight were eligible for inclusion. Seven studies were cross-sectional analyses of patients with primary (PHPT) or secondary (SHPT) hyperparathyroidism compared to controls. Study quality was poor. In pooled analyses comparing PHPT to controls, there was no statistical difference in fibrinogen [SMD=0.01 (-0.92–0.94); k=3 trials; n=133 patients; I^2=86%]; D-dimer [0.46 (0.03–0.97); 3; 133; 52]; PAI [0.01 (-0.54–0.57); 3; 181; 69]. Other outcomes were reported in less than 100 patients as were outcomes in studies involving SHPT patients. There was little evidence to support an association between PTH and increased coagulation. Prospective data are needed to understand what role if any, PTH plays in coagulation and if patients with elevated or reduced PTH are predisposed to clots or bleeds.

97

Parathyroid Localisation Imaging in Primary Hyperparathyroidism: A Tertiary Hospital Audit

Karen Rothacker[1], Gerard Chew[1]

1. Department of Endocrinology and Diabetes, Royal Perth Hospital, Perth, Western Australia, Australia

Background: Primary hyperparathyroidism (PHPT) is a biochemical diagnosis. Where clinically indicated, surgical removal of the culprit gland(s) offers potential cure. Parathyroid localisation imaging (PLI) is not used to diagnose PHPT, but can guide surgical planning, and so may be undertaken if (focused) parathyroidectomy is contemplated.

Objective: To determine if PLI is optimally utilised (surgical planning for established PHPT) in a tertiary hospital setting.

Methods: We audited retrospectively the reports of all imaging studies undertaken at Royal Perth Hospital over a one-year period containing the keyword "parathyroid", limited to PLI modalities in patients without end-stage renal failure (pre-dialysis/dialysis). We obtained additional clinical and biochemical data using electronic data sources.

Results: There were 58 PLI studies performed in 45 patients, but only 38 (84%) patients had an established biochemical PHPT diagnosis. Of those with PHPT, only 28 (74%) proceeded to parathyroidectomy. There were 21 PLI studies (36% of total) performed in patients without established PHPT, or in those with PHPT but who did not proceed to surgery. PLI studies were requested most frequently by Endocrine Surgeons (31 [53%]) and Endocrinologists (22 [38%]). Patients investigated by Endocrine Surgeons (71%) and Endocrinologists (67%) were similarly likely to proceed to parathyroidectomy, whereas none of the patients investigated by other clinicians proceeded to surgery.

Conclusion: Most PLI studies (37 [64%]) were performed in patients with established PHPT who proceeded to surgery, but a significant proportion were undertaken without clear indication or utility. Confirming a biochemical diagnosis of PHPT and the patient's suitability for surgery prior to requesting PLI could decrease the number of low-value investigations being performed.

98

Association of Vitamin D Deficiency with Erectile Dysfunction among Men with Type 2 Diabetes

Shahjada Dr Selim[1], Hafiza Dr Lona[2]

1. Endocrinology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh

2. Biochemistry, Medical College for Women's & Hospital, Dhaka, Bangladesh

Background and aims: Several recent studies found strong relationship of vitamin D deficiency (VDD) with erectile dysfunction (ED). The morbidity of men with diabetes is also becoming more increasingly recognized which has been taken to have association again with VDD. The aim of this study was to determine the association between vitamin D status and ED Bangladeshi adult men with type 2 diabetes. **Materials & Methods:** The nested case-control study included 2860 patients with type 2 diabetes mellitus (T2DM) who had ED (aging between 30 to 69 years). The patients who found to have normal vitamin D level, were categorized as control and those who had VDD, were grouped as the case. The study was conducted in eight diabetes care centers, one from each of the eight administrative divisional cities of Bangladesh. Socio-demographic and personal information were collected by face to face interview and disease-specific data were recorded from the patient's diabetic record book. Body weight, height, waist circumference, hip circumference and blood pressure were also recorded. Fasting blood sample was collected and serum levels of vitamin D, glucose, and free testosterone were measured. **Results:** The diabetes patients with ED has more severe VDD [(25 OH)D <10 ng/mL] than the controls (61.28% and 62.16%, respectively). The more severe form of ED found in the lowest level of serum vitamin D level. The multivariate logistic regression analysis found VDD has linear relationship with ED [OR 2.83, CI 2.36, 3.97]. **Conclusions:** Vitamin D deficiency is an independent risk factor of ED in men with type 2 diabetes mellitus and severity of ED is linearly associated with the degree of deficiency of vitamin D.

Duration of Action of Injectable Testosterone Undecanoate

Nandini Shankara-Narayana[1], Ly P Lam[1], Veena Jayadev[1], Carolyn Fennell[1], Sasha Savkovic[1], Ann J Conway[1], David J Handelsman[2, 1]

1. Andrology, Concord Repatriation General Hospital, Concord, NSW

2. Andrology, ANZAC Research Institute, Concord, NSW, Australia

Objective: The duration of action of injectable testosterone undecanoate (TU) in routine clinical practice outside clinical registration trials is not well defined.

Design and Methods: Prospective observational study of consecutive TU injections as testosterone replacement therapy for pathological hypogonadism, subject to individual dose titration to optimise achieve a stable replacement regimen. Participants had primary hypogonadism (PH, n=118), secondary hypogonadism (SH, n=85) or were female-to-male transgender (F2M, n=94). After first and 6-week 1000mg loading doses, initially 12-weekly injections were then subject to titration of injection interval based on individual's lead symptoms and trough serum testosterone, LH and FSH.

Results: Among 6300 injections given to 297 patients having at least three injections (median number of injections 14, interquartile range [IQR] 7-25), the mean inter-injection interval was 11.9 ± 0.02 (SEM) weeks (mode and median 12 weeks, IQR 11.0 – 12.4 weeks). The optimal stabilised injection interval was significantly influenced by age, body surface area (BSA) and serum SHBG but not diagnosis. Shorter (≤10 weeks) intervals in 23/297 (7.7%) patients were positively correlated with age and longer (≥14 weeks) intervals in 37/297 (12.5%) patients were positively correlated with age and BSA. Serum LH was fully suppressed in the PH group to levels comparable with SH, whereas in F2M serum LH was minimally suppressed (vs pre-titration) and significantly less than PH and SH groups despite higher dose/BSA. Serum FSH was markedly but not fully suppressed in men with PH while in F2M serum FSH was not suppressed.

Conclusion: After individual dose titration to optimise interval between TU injections, the approved 12-week duration of action was observed in 80% and influenced by age, serum SHBG and BSA but not diagnosis. Shorter and longer intervals in 20% of patients depended mainly on age and BSA.

Supported by the Genetic Hypogonadism Registry

Evaluation of maternal and neonatal outcomes in patients with gestational and pre-gestational diabetes receiving antenatal corticosteroids

Chelsea Tan[1], Debra Renouf[1]

1. Peninsula Health , Frankston, Victoria , Australia

Background

Patients with gestational and pre-gestational diabetes receiving antenatal corticosteroids are at an increased risk of developing hyperglycaemia, predisposing to neonatal hypoglycaemia.[1-3] The use of antenatal corticosteroids in this obstetric subpopulation has not been well studied.[4,5]

Methods

We conducted a single-centre retrospective analysis of 103 obstetric patients with gestational and pre-gestational diabetes who received antenatal corticosteroids. The primary aim of our study was to evaluate maternal and foetal outcomes in relation to antenatal corticosteroid use, with a focus on neonatal hypoglycaemia, to rationalise the use of antenatal corticosteroids.

Results

The major maternal complications were pre-eclampsia (11.7%), preterm premature rupture of membranes (10.7%) and postpartum haemorrhage (9.7%). There were 49 cases (43.8%) of neonatal hypoglycaemia. 26 cases (53.1%) required dextrose infusions. 60% of neonates born at 34 to 36^{+6} weeks had hypoglycaemia, of which 40% were emergency Caesarean sections. 33.4% of neonates born at 37 to 38^{+6} weeks had hypoglycaemia, whereby 89.3% were, conversely, elective Caesarean sections. Neonates with hypoglycaemia reached high APGAR scores at 5 minutes of birth at a slower rate ($p<0.01$). Comparisons between neonates with and without hypoglycaemia showed no significant difference in the rates of neonates with low birth weight ($p = 0.46$) and macrosomia ($p = 0.09$). Neonatal hypoglycaemia was associated with a significant increase in special care nursery admissions ($p<0.01$), and a trend towards increased length of stay ($p = 0.31$) and tertiary transfers ($p = 0.10$).

Conclusion

Neonatal hypoglycaemia remains a concerning potential adverse outcome of antenatal corticosteroid use. Larger studies are required to inform the use of antenatal corticosteroids in patients with gestational and pre-gestational diabetes, especially in late preterm and term gestations. At current, the use of antenatal corticosteroids in late preterm and term gestations in patients with diabetes should be individualised and should involve careful planning in the peripartum period.

1. Setji TL, Brown AJ, Feinglos MN. Gestational diabetes mellitus. Clin diabetes. 2005;23(1):17-24. doi: 10.2337/diaclin.23.1.17
2. González-Quintero VH, Istwan NB, Rhea DJ, Rodriguez LI, Cotter A, Carter J, et al. The impact of glycaemic control on neonatal outcome in singleton pregnancies complicated by gestational diabetes. Diabetes Care. 2007;30(3):467-70. doi: 10.2337/dc06-1875
3. The HAPO study cooperative research group. Hyperglycaemia and adverse pregnancy outcomes. New Engl J Med. 2008;358(19):1991-2002. doi: 10.1056/NEJMoa0707943
4. Amiya RM, Mlunde LB, Ota E, Swa T, Oladapo OT, Mori R. Antenatal corticosteroids for reducing maternal and child outcomes in special populations of women at risk of imminent preterm birth: a systematic review and meta-analysis. PLoS One. 2016;11(2):e0147604. doi: 10.1371/journal.pone.0147604
5. Antenatal Corticosteroid Clinical Practice Guidelines Panel. Antenatal corticosteroids given to women prior to birth to improve fetal, infant, child and adult health: clinical practice guidelines. Auckland: Liggins Institute; 2015.

101

"Oh, this is 20 years too late!": a qualitative evaluation of an integrated polycystic ovary syndrome service.

Chau Thien Tay[1, 2], Stephanie Pirotta[1], Helena Teede[1, 2], Lisa Moran[1], Tracy Rpbinson[3], Helen Skouteris[1], Anju Joham[1, 2], Siew Lim[1]

1. Monash Centre for Health Research and Implementation, Clayton, VIC, Australia

2. Department of Diabetes and Vascular Medicine, Monash Health, Clayton, VIC, Australia

3. 3School of Nursing, Midwifery & Indigenous Health, Charles Sturt University, Wagga Wagga, New South Wales, Australia

Objective: Women with polycystic ovary syndrome (PCOS) have complex needs which are unmet by currenlty fragmented healthcare services. This study evaluates an integrated PCOS service from the women's perspective.

Design and methods: A qualitative study, complimented by quantitative data, was conducted on 15 women who attended the Monash Health statewide integrated PCOS service. Semi-structured interviews and surveys were conducted between 11th March 2019 and 1st Oct 2019. Data was analysed using thematic approach and mapped into predetermined framework of appropriateness, effecticeness, efficiency, impact and future suggestions.

Results: Twelve women were satisfied and 14 women would recommend the service to another person. Integrated care, tailored treatments, education on PCOS, lifestyle support and laser dermal therapy were all valued elements of the service. Positive impacts were reported on improving PCOS symptom severity, risk perception, self-efficacy and general emotional well-being. Infrastructure, delayed access and inconsistent service delivery during the initial phase of service and the contradictions between evidence-based treatment and patient preference were reported negatively by women. Sensitive communication considering psychological impacts of PCOS was highlighted as being important by women.

Conclusion: A co-designed, integrated PCOS service which aligns with evidence-based practice and patients' priorities, appears from the user perspectives to be appropriate, beneficial and effective to meet women's needs. The efficiency domain could be further improved and sensitive communication where providers remain cognizant of the psychological aspects of PCOS is important for effective care. Further research, including longitudinal quantitative data, is needed to verify these results conclusively.

102

Relationship between the Hypothalmic-Pituitary-Adrenocortical axis activity and the characteristics of Aldosterone-producing adenomas

Moe Thuzar[1, 2], Yu-Chin Lo[1, 2], Zeng Guo[2], Warrick J Inder[1, 3], Michael Stowasser[2]

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

2. Endocrine Hypertension Research Centre, University of Queensland Diamantina Institute, Princess Alexandra Hospital, Brisbane, Queensland, Australia

3. Faculty of Medicine, University of Queensland, Brisbane, Queensland, Australia

Background: Aldosterone production can be regulated by adrenocorticotrophic hormone (ACTH) which normally controls cortisol secretion. Some cases of aldosterone-producing adenoma (APA) display features which may suggest increased sensitivity to the stimulatory effects of ACTH.

Aim: To investigate if there is any relationship between the hypothalamic-pituitary-adrenocortical axis (HPA) activity and the characteristics of APAs.

Methods: This is a retrospective review involving 41 histologically-confirmed APA cases which were characterised with regards to clinical, biochemical and somatic mutation status. HPA activity was assessed from morning plasma cortisol, ACTH and 1mg overnight dexamethasone (DEX) suppression test. Correlation was analysed by Pearson's correlation coefficient, and the HPA activity between APAs with *KCNJ5* mutation and those without was compared using Mann Whitney U-test.

Results: Twelve out of 41 patients (29.3%) were women, median age was 49 years and 85.4% were overweight/obese. All except 2 had somatic mutations within APA. Fourteen (34.1%) were *KCNJ5* mutation. Only one patient (not *KCNJ5*-mutated) had post-DEX cortisol >50 nmol/L. Upright morning plasma aldosterone concentration (PAC) correlated with tumour size (r=0.347, P=0.026), plasma cortisol (r=0.425, P=0.006) and plasma ACTH (r=0.446; P=0.056). Plasma ACTH and cortisol were positively correlated (r=0.511, P=0.025). Higher PAC, cortisol and ACTH concentrations were in turn associated with the need for higher defined-daily-dose of anti-hypertensives (r=0.466, P=0.002 for PAC; r=0.315, P=0.045 for cortisol; r=0.449, P=0.054 for ACTH). Higher PAC was also predictive of higher BMI (r=0.348, P=0.026). Plasma cortisol, ACTH, post-DEX cortisol, BMI and anti-hypertensives dose were not significantly different between those with *KCNJ5* mutation vs those without.

Conclusions: HPA activity correlated with clinico-biochemical characteristics in patients with APAs, but the prevalence of autonomous hypercortisolism was low. The findings suggest a potential role of ACTH in the pathophysiology of APAs. There was no significant difference in HPA activity between those with somatic *KCNJ5* mutation compared to those without.

103

Clival Prolactinoma Masquerading as a Chordoma

Quynh Truong[1], Simon J Ryder[2], Jennifer Gillespie[3], Donald S.A Mcleod[2]

1. Medicine, Royal Brisbane & Women's Hospital, Brisbane, QLD, Australia

2. Endocrinology, Royal Brisbane & Women's Hospital, Brisbane, QLD, Australia

3. Medical Imaging, Royal Brisbane & Women's Hospital, Brisbane, QLD, Australia

Ectopic pituitary adenomas (EPAs) are a rare clinical entity and are frequently mistaken for other base of skull lesions on imaging. We report the clinical presentation and management of a presenting with an ectopic prolactinoma located in the clivus. A 66-year-old female presented with a six-month history of headaches and light-headedness. Anatomical imaging demonstrated a clival lesion most suspicious for chordoma. Endocrinological assessment showed a modestly increased prolactin and gonadotrophins that were lower than expected for her age. Surgical resection confirmed an ectopic prolactinoma.

A skull base lesion in a patient with hormonal derangement should lend to a high clinical suspicion of an EPA as they may be treated with medications before surgery. There exists potential for a guideline to be created for the management of ectopic pituitary adenomas.

104

Cross-sectional determinants of hip and spine bone mineral density (BMD) and trabecular bone score (TBS) in a postpartum gestational diabetes (GDM) cohort.

Mawson Wang[1, 2], Allison Sigmund[1], Susan Hendon[1], Tien-Ming Hng[1, 2], Sue Lynn Lau[1, 2]

1. Department of Endocrinology, Blacktown Hospital , Blacktown, NSW, Australia

2. Blacktown Clinical School, School of Medicine, Western Sydney University, Sydney, NSW, Australia

BMD loss during the postpartum lactation period is driven by hypo-oestrogenism and calcium transfer into breastmilk. TBS is a parameter of bone microarchitecture, not previously studied in this population. Population studies demonstrate positive BMD correlation with BMI, diabetes status and calcium intake, while TBS negatively correlates with BMI and diabetes status. We explored traditional and novel determinants of BMD and TBS z-scores at 3 months post-GDM pregnancy.

205 women completed anthropometric measurements, DXA scan, oral glucose tolerance test (OGTT) and lifestyle questionnaires at a mean (±SD) 11.0±2.5 weeks postpartum.

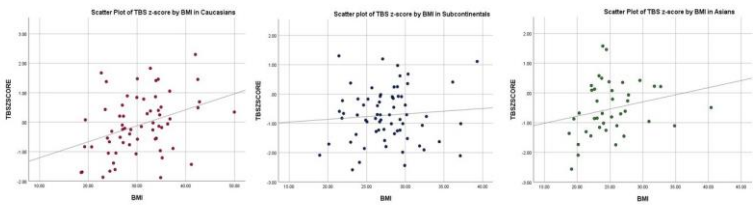
Mean z-scores and 95% confidence intervals for lumbar spine (LS) BMD (-0.58 [-0.71 to -0.44]), femoral neck (FN) BMD (-0.25 [-0.37 to -0.12]) and spine TBS (-0.36 [-0.51 to -0.22]) were below the expected age and weight-matched mean of zero. LS and FN BMD z-scores did not correlate with BMI and central fat percentage, whereas TBS z-score correlated with both BMI (r=0.31, p<0.01) and central fat (r=0.23, p<0.01).

BMD z-score did not differ between ethnicities. Subcontinental and Asian women exhibited lower TBS z-scores compared to Caucasian women (-0.72 vs. -0.54 vs. -0.09 respectively, p<0.01) but also had lower mean BMI (27.7 vs. 25.1 vs. 30.5 respectively, p<0.01). When analysed separately (Figure.1), the relationship between BMI and TBS z-score remained present in Caucasians (r=0.33, p<0.01) and almost reached significance in Asians (r=0.31, p=0.054).

There was no difference in LS BMD or TBS z-score between breastfeeding and formula-feeding women. BMD and TBS z-scores did not differ according to OGTT result, vitamin D status, dietary calcium intake or exercise intensity.

BMD and TBS z-scores were similarly low at 3 months postpartum. Ethnic differences in TBS z-scores have not been previously reported. Contrary to other studies, BMI positively associated with TBS but not BMD z-scores, partly modified by ethnicity. Other determinants of bone health were not identified.

Figure.1 Scatter Plots of TBS z-score by BMI in Different Ethnicities



a) Caucasians: r= 0.33, p<0.01 b) Subcontinentals: r=0.03, p=0.78 c) Asians: r=0.31, p=0.054

Ectopic insulin-secretion by a large cell neuroendocrine carcinoma of the cervix

Mawson Wang[1, 2], Quinlan Vasey[1], Winny Varikatt[3, 4], Mark Mclean[1, 2]

1. Department of Endocrinology, Blacktown Hospital , Blacktown, NSW, Australia

2. Blacktown Clinical School, School of Medicine, Western Sydney University, Sydney, NSW, Australia

3. Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, NSW, Australia

4. Westmead Clinical School, University of Sydney, Sydney, NSW, Australia

A 62 year-old postmenopausal female presented with postcoital bleeding, on a background of two vaginal childbirths and tubal ligation. Tumour cells from a cervical biopsy stained positively for p16, CD56 and synaptophysin, consistent with a neuroendocrine tumour. Staging scans confirmed a localised 3x4cm cervical mass without metastases, and a diagnosis of Stage IB2 neuroendocrine cervical carcinoma was made. She completed six cycles of carboplatin/etoposide, followed by radiotherapy and brachytherapy.

At one-year post-diagnosis, the patient presented with symptomatic hypoglycaemia (plasma glucose 1.7mmol/L) in the fasted state and not on antidiabetic treatment. She exhibited sinus tachycardia to 120bpm, diaphoresis and tremor. Symptoms were promptly corrected by glucose administration, fulfilling Whipple's triad. During an episode of hypoglycaemia to 2.2mmol/L, serum c-peptide was 2.33nmol/L [0.26-1.73] and serum insulin 19mIU/L [<9]. Computed tomography demonstrated pulmonary and hepatic metastases, and no pancreatic lesion. Retrospective immunohistochemistry on the cervical biopsy revealed positive insulin staining and negative glucagon staining, confirming the diagnosis of an insulin-secreting neuroendocrine carcinoma (NEC).

Intravenous dextrose and hydrocortisone were insufficient to maintain normoglycaemia. The somatostatin analogue octreotide was commenced at 200mcg SC q8hrly, allowing cessation of intravenous therapy and significantly improved hypoglycaemia. In view of progressing aggressive malignancy a palliative approach was decided, and the patient died on day 20 of admission.

Neuroendocrine neoplasms (NENs) are malignancies that arise from neuroendocrine cells and may produce and secrete peptide hormones[1]. Non-islet cell tumours secreting insulin are infrequently reported, and comprise 1-2% of insulinomas, most commonly arising in peripancreatic or periduodenal regions, but also in ectopic sites including kidney, liver, ovary and lung[2]. Positive insulin immunohistochemistry in the tumour, combined with elevated c-peptide and insulin levels suggests that the insulin-secreting tumour was the aetiology for the patient's hypoglycaemia. We are aware of only two previously reported cases of insulin-induced hypoglycaemia originating from a cervical NEN[3-4].

1. Rindi G., Klimstra D., Abedi-Ardekani B., et al. A common classification framework for neuroendocrine neoplasms: an International Agency for Research on Cancer (IARC) and World Health Organization (WHO) expert consensus proposal. Modern Pathology. 2018;31(12):1770-1786.
2. Ramkumar S., Dhingra A., Jyotsna V., et al. Ectopic insulin secreting neuroendocrine tumor of kidney with recurrent hypoglycemia: a diagnostic dilemma. BMC Endocrine Disorders. 2014; 14:36.
3. Seckl M., Mulholland P., Bishop A., et al. Hypoglycemia due to an insulin-secreting small-cell carcinoma of the cervix. New England Journal of Medicine. 1999;341(10):733-736.
4. Kiang D., Bauer G., Kennedy B. Immunoassayable insulin in carcinoma of the cervix associated with hypoglycemia. Cancer. 1973;31(4):801-805.

Drug Rash with Eosinophilia and Systemic Symptoms (DRESS) followed by thyrotoxicosis and eosinophilic myocarditis

Mawson Wang[1, 2], Ramy Bishay[1, 2]

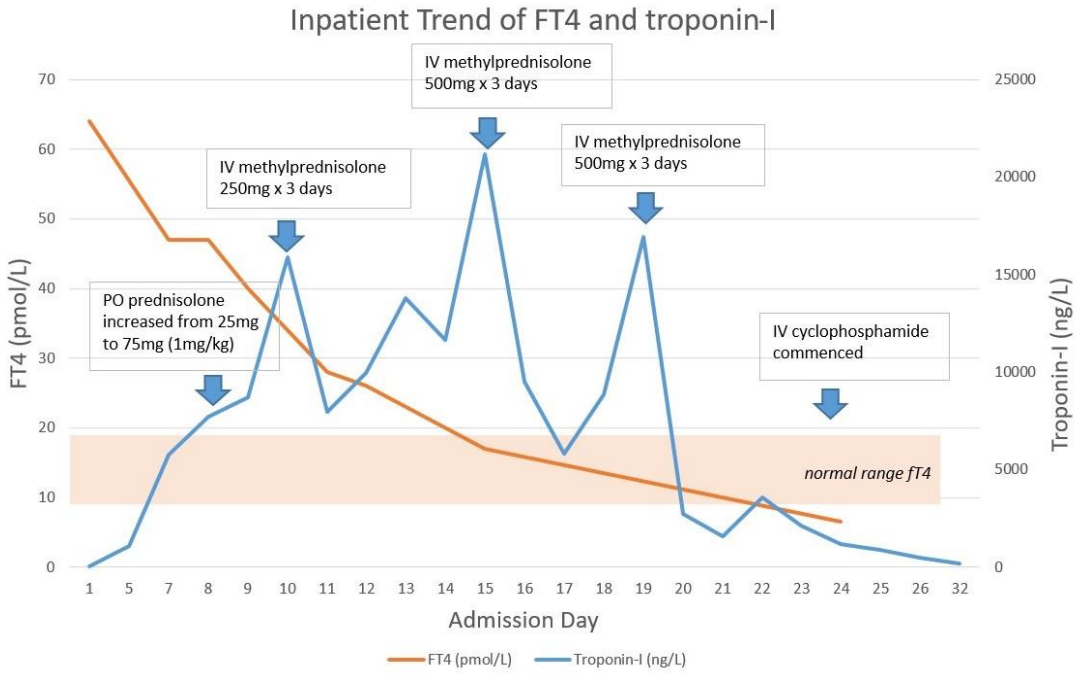
1. Department of Endocrinology, Blacktown Hospital , Blacktown, NSW, Australia

2. Blacktown Clinical School, School of Medicine, Western Sydney University, Sydney, NSW, Australia

A 58 year-old male with a history of localised prostate cancer commenced ciprofloxacin for presumed prostatitis. Two weeks later, he was referred to the dermatology service for generalised pruritic maculopapular and pustular eruption, associated with fever and liver function derangement. Based on skin biopsy findings of drug reaction with eosinophilia, fevers, liver involvement and peripheral eosinophilia, the patient fulfilled criteria for DRESS[1]. He was managed with high-dose oral and topical glucocorticoids and discharged on a weaning prednisolone course.

The patient represented after two weeks with exertional dyspnoea, sinus tachycardia (130 bpm) and heart failure with reduced left ventricular ejection fraction (LVEF) on echocardiogram of 33%. A CT pulmonary angiogram excluded pulmonary embolus. Troponin-I was elevated at 52ng/L (<50) accompanied by new-onset thyrotoxicosis, with a suppressed TSH <0.01 mIU/L (0.4-4.0), high fT4 >64pmol/L (9.0-19.0), fT3 22.3pmol/L (2.6-6.0) and negative TSH-receptor antibody. Thyroid ultrasound demonstrated normal vascularity and no focal lesion. Management consisted initially of carbimazole 15mg bd, which was later ceased due to suspicion of subacute thyroiditis. Gradual escalation of propranolol to 80mg tds for persistent tachycardia was titrated and transitioned to nebivolol. Serial TFTs demonstrated gradual improvement in thyrotoxicosis during admission[Fig.1]. Conversely, troponin-I increased from 52ng/L to a peak of 21,172ng/L on Day 14[Fig.1] without angina. Coronary angiogram excluded coronary artery disease, and a ventricular biopsy demonstrated eosinophilic myocarditis, satisfying two autoimmune sequelae of DRESS:subacute thyroiditis and eosinophilic myocarditis. He was managed with intravenous methylprednisolone and cyclophosphamide as a steroid-sparing agent. His heart failure, thyroid and cutaneous manifestations improved within 4-6 weeks.

The manifestations of DRESS and its autoimmune sequelae may be explained by dramatic expansions of functional T-regulatory cells during the acute phase, followed by gradual loss of function after resolution, triggering risk of autoimmune disease[2]. This is a rare but important condition with multi-system and endocrine manifestations.



1. Choudhary S, McLeod M., Torchia D., Romanelli P. Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) Syndrome. The Journal of clinical and aesthetic dermatology. 2013;6(6):31-37.
2. Takahashi R., Kano Y., Yamazaki Y., Kimishima M., Mizukawa Y., Shiohara T. Defective regulatory T cells in patients with severe drug eruptions: timing of the dysfunction is associated with the pathological phenotype and outcome. Journal of Immunology. 2009;182(12):8071-8079.

107

Correlating technetium-99m sestamibi thyroid scintigraphy with histopathology in amiodarone-induced thyrotoxicosis

Ray Wang[1], Nathan Better[3, 4, 2], Dinesh Sivaratnam[3, 4], Simon Forehan[1], David Pattison[5], Spiros Fourlanos[1, 2]

1. Diabetes and Endocrinology, Royal Melbourne Hospital, Parkville, Victoria, Australia

2. Medicine, Royal Melbourne Hospital, University of Melbourne, Parkville, VIC, Australia

3. Nuclear Medicine, Royal Melbourne Hospital, Parkville, Australia

4. Cardiology, Royal Melbourne Hospital, Parkville, VIC, Australia

5. Nuclear Medicine and Specialised PET Services, Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia

The two main types of amiodarone-induced thyrotoxicosis (AIT) present diagnostic challenges, and management strategies differ. Numerous investigation techniques have been suggested to differentiate between type 1 and type 2 AIT. To date, few have proven consistently reliable. Technetium-99m sestamibi thyroid scintigraphy (^{99m}Tc-STS) has been proposed as a diagnostic tool for differentiating types of AIT.

To examine correlation between ^{99m}Tc-STS diagnosis and thyroid histological diagnosis of AIT type, retrospective analysis was undertaken of all patients who have had ^{99m}Tc-STS for AIT at the Royal Melbourne Hospital, with subsequent thyroid surgery. Four patients were identified, all male, median age 63.5 years (range 56-75), median duration amiodarone treatment 26 months (range 10-39). All underwent total thyroidectomy for AIT (indication for surgery was agranulocytosis in one patient and medically-refractory thyrotoxicosis in the other three, including one who also had significant steroid myopathy). The ^{99m}Tc-STS diagnosis made by nuclear medicine physicians was type 2 AIT in all four patients, based on low thyroid-background ratio (TBR) for sestamibi uptake (median TBR 1.227 at 15 minutes; IQR 1.125-1.311; TBR reference range 1.90-2.55 for type 1 AIT, 1.23-1.52 for type 2 AIT), as well as qualitative assessment. The histological diagnosis made by pathologists was type 2 AIT in all four cases.

Our case series is the first to examine pathological correlation with ^{99m}Tc-STS nuclear medicine imaging findings. There was evidence of type 2 AIT related change in histology samples, consisting of follicular atrophy, lymphohistiocytic infiltrate and fibrosis, in all four patients who had a diagnosis of type 2 AIT based on ^{99m}Tc-STS, suggesting diagnostic accuracy of this imaging modality. ^{99m}Tc-STS could assist clinicians to more accurately determine the subtype of AIT which could influence decision-making in regards to the need for and duration of glucocorticoid treatment.

108

Harrowing hypoglycaemia: Diagnosing an IGF-2 secreting tumour

Lisa Ward[1, 2], Ashim Sinha[1]

1. Endocrinology department, Cairns Hospital, Cairns, QLD

2. Endocrinology department, Gold Coast University Hospital, Gold Coast, QLD

Background: Non-islet cell tumour hypoglycaemia is a rare paraneoplastic phenomenon associated with tumours secreting IGF-2 (termed IGF-2-oma). The recommended confirmatory test is the measurement of serum IGF-2: IGF-1 ratio, looking for normal to elevated IGF-2 and low IGF-1.

Clinical Case: A 51-year old woman presented with severe hypoglycaemia (glucose 1.6 mmol/L) with a six month history of episodic confusion, fatigue and altered level of consciousness. She had clubbing, arthalgia and wrist swelling consistent with hypertrophic pulmonary osteoarthropathy.

Biochemistry showed non-insulin mediated hypoglycaemia with a plasma glucose of 1.6 mmol/L, a suppressed insulin level 0.1 mU/L (2.0 – 23), C-peptide < 0.1 nmol/L (0.3 – 1.4), proinsulin <3.1 pmol/L (<13.3) and low normal IGF-1 8.1 (7.2 – 31). CT chest identified a 17.8 cm right hemithoracic mass. There were no Australian pathology sites identified to measure serum IGF-2.

She was successfully bridged with intravenous dextrose and 25mg daily prednisone prior to resection of a 2kg right solitary fibrous tumour. Immunohistochemistry staining for IGF-2 was strongly positive in keeping with an IGF-2-oma causing recurrent hypoglycaemia.

Non-islet cell tumor hypoglycemia is usually associated with large mesenchymal tumors that secrete excessive mature and incompletely processed forms of IGF-2. Abnormal IGF-2 exerts potent insulin-like activity contributing to hypoglycaemia. Within the literature, a review of 800 cases of solitary fibrous tumours documented a 5% rate of hypoglycaemia (1). The tumours are often found to be large in size; on average 20 cm in greatest dimension. Solitary fibrous tumours can be associated with hypokalaemia, acromegaloid features and hypertrophic osteoarthropathy. Here we describe a rare paraneoplastic phenomenon and simultaneously a rare cause for hypoglycaemia.

Conclusion:

This case highlights the logistical and diagnostic challenges of hypoglycaemia associated with IGF-2 secreting tumours.

1. 1. Kalebi A, Hale M, Wong M, Hoffman T, Murray J. Surgically cured hypoglycemia secondary to pleural solitary fibrous tumour: case report and update review on the Doege-Potter syndrome. Journal of Cardiothoracic Surgery 2009, 4:45.

109

A stormy course - Thyroid storm and plasma exchange

Lisa Ward[1, 2], Ashim Sinha[1]

1. Endocrinology department, Cairns Hospital, Cairns, QLD

2. Endocrinology department, Gold Coast University Hospital, Gold Coast, QLD

Background: Thyroid storm is a rare manifestation of a common endocrine condition. Plasma exchange is increasingly recognised as a bridge to total thyroidectomy for refractory thyrotoxicosis.

We present a case of a 59 year-old male who was in fulminant thyroxtoxicosis. He described a four day history of palpitations and peripheral oedema. On examination he had new onset atrial fibrillation, congestive heart failure and Graves' orbitopathy. Biochemistry showed TSH <0.05 mU/L (0.3 – 4.5), T4 73 pmol/L (7.0 – 17), T3 39 pmol/L (3.5 – 6.0), TSH receptor antibody 70 IU/L (<1.8).

Shortly during admission, he had fluctuating altered level of consciousness, requiring an intensive care admission for intubation and sedation. Burch-wartofsky point scale was highly suggestive for thyroid storm at 50 points. Treatment for thyroid storm was commenced: carbimazole 20mg TDS, via nasogastric route (NG), propranolol 80mg TDS NG, cholestyramine 4g QID NG and intravenous hydrocortisone 100mg TDS. His ICU stay was complicated with ventilator associated pneumonia, difficulty weaning from ventilation, anaemia, thrombocytopaenia and further aspiration pneumonia and shock requiring vasopressor support. His echocardiogram demonstrated moderate global systolic dysfunction.

Due to limited improvement with maximal medical therapy, plasma exchange was given in three sessions with succeeding rapid clinical improvement in neurological status. Plasma exchange was used to bridge to total thyroidectomy and tracheostomy insertion day 25 of admission. He was rehabilitated to functional independent status after three months.

Learning points:

1. Thyrotoxic patients with central nervous system dysfunction appear to derive the greatest benefit from aggressive treatment for thyroid storm (1).
2. Consider plasma exchange early if clinical improvement is not noted within 24 – 48 hours particularly in regards to tachycardia, high fever and altered consciousness (2).

1. Trevor E. Angell, Melissa G. Lechner, Caroline T. Nguyen, Victoria L. Salvato, John T. Nicoloff, Jonathan S. LoPresti, Clinical Features and Hospital Outcomes in Thyroid Storm: A Retrospective Cohort Study, The Journal of Clinical Endocrinology & Metabolism, Volume 100, Issue 2, 1 February 2015, Pages 451–459, https://doi.org/10.1210/jc.2014-2850
2. Satoh T, Isozaki O, Suzuki A, et al. 2016 Guidelines for the management of thyroid storm from The Japan Thyroid Association and Japan Endocrine Society (First edition). Endocr J. 2016;63(12):1025-1064. doi:10.1507/endocrj.EJ16-0336

110

Focal nesidioblastosis in an adult – a rare neuroendocrine presentation

Yu-Fang Wu[1], Tarama Preda[2, 3], Koroush Haghighi[2, 4], Veronica Preda[1]

1. Macquarie University Hospital, Macquarie University, NSW, Australia

2. Prince of Wales Private Hospital, Sydney, NSW, Australia

3. University of Notre Dame, Sydney, NSW, Australia

4. St Vincent's Hospital, Sydney, NSW, Australia

Persistent hyperinsulinaemic hypoglycaemia is usually caused by insulinoma in adults and nesidioblastosis in infancy. Nesidioblastosis is a functional beta cell disorder caused by the proliferation of both ductal elements and islet cells, with hypertrophy of beta cells in islets (1). In adults, the pathophysiology of nesidioblastosis remains unclear. There is increasing recognition of nesidioblastosis post gastric bypass surgeries as a cause for post prandial hypoglycaemia (2). The disease can be categorised histologically into diffuse or focal form. In adults, focal disease is exceedingly rare with only 4 cases reported in literature.

We present a case of a 50-year-old truck driver with six months history of unexplained fatigue, dizziness and loss of consciousness prior to driving. His symptoms were relieved by frequent oral intake which resulted in 15kg weight gain. There was documented hypoglycaemia of 2.7mmol/L at the time of loss of consciousness. Biochemical investigation revealed elevated serum fasting insulin of 24 mU/L (0-20 mU/L) and proinsulin of 25.7 pmol/L (< 13.3 pmol/L), and normal c-peptide of 1.4 nmol/L (0.4-1.5 nmol/L). Magnetic resonance imaging demonstrated hyperenhancement in the pancreatic head measuring 15x13mm abutting D2. Ga68 Dotatate positron emission tomography was inconclusive. Contrast enhanced endoscopic ultrasound (EUS) demonstrated a 20mm mass in the uncinate process of the pancreas with contrast uptake. Whipple procedure was successfully performed with resolution of symptoms. Histopathology confirmed focal nesidioblastosis with predominantly beta cells and interestingly a 2mm pancreatic neuroendocrine tumour (NET), grade 1 Ki67 < 2%.

The diagnostic work up is a conundrum. Contrast enhanced EUS was vital to localisation. Differential diagnoses include pancreatic NETs which are a heterogeneous group of islet-cell tumours with a tendency for hormonal production. Whilst insulinomas and proinsulin-secreting NETs have many parallels, focal nesidioblastosis is a discrete entity and should be considered in a patient with presumptive diagnosis of an insulinoma.

1. Whitley, E.M. (2014). Comparative Pancreatic Pathology. Pathobiology of Human Disease, 1101 - 1123.
2. Ceppa EP, Ceppa DP, Omotosho PA, Dickerson JA 2nd, Park CW, Portenier DD. Algorithm to diagnose etiology of hypoglycemia after Roux-en-Y gastric bypass for morbid obesity: case series and review of the literature. Surg Obes Relat Dis. 2012;8(5):641-647. doi:10.1016/j.soard.2011.08.008

111

Correlation between the aldosterone renin ratio and blood pressure in young adulthood - a longitudinal study

Jun Yang[1, 2], Stella Gwini[3], Michael Stowasser[4], Morag J Young[5], Peter J Fuller[1], Markus P Schlaich[6], Lawrence Beilin[6], Trevor Mori[6]

1. Centre for Endocrinology and Metabolism, Hudson Institute of Medical Research, Clayton, VIC, Australia

2. Medicine, Monash University, Clayton, VIC, Australia

3. University Hospital Geelong, Barwon Health, Geelong, Victoria, Australia

4. Faculty of Medicine, University of Queensland, St Lucia, QLD, Australia

5. Cardiovascular Endocrinology Group, Baker Heart and Diabetes Institute, Melbourne, VIC, Australia

6. Faculty of Health and Medical Sciences, University of Western Australia, Perth, WA, Australia

Background: Hypertension tracks throughout childhood into adulthood. Aldosterone excess, or primary aldosteronism, has been reported as the most common secondary cause of hypertension in adults. However, the relationship between aldosterone and blood pressure in childhood is unclear.

Objectives: To evaluate the relationship between aldosterone, renin and the aldosterone:renin ratio (ARR) and blood pressure (BP) at age 17 and 27 in a community-based population.

Design: Prospective pregnancy cohort study.

Participants: Young adult offspring of women enrolled during pregnancy in the Raine Study, who provided blood samples and BP measurements at 17 years (538 males and 335 females not on hormone contraception) and/or 27 years (507 males and 251 females).

Results: At age 17, females had significantly higher aldosterone (median 350 vs 345 pmol/L, p=0.013), lower renin (20.6 vs 25.2 mU/L, p<0.001) and higher ARR (18.4 vs 13.6, p<0.001), but lower systolic BP (108 vs 118 mmHg, p< 0.001) than males. The ARR correlated significantly with systolic BP in males, but not females, at age 17 when adjusted for alcohol consumption, physical activity, urinary sodium:creatinine ratio and body mass index (standardized β-coefficient 0.11, p=0.008). The ARR at age 17 was significantly associated with both systolic (standardized β-coefficient 0.26, p=0.005) and diastolic BP (standardized β-coefficient 0.31, p=0.001) among females, but not males, at age 27.

Conclusion: A robust relationship between ARR and BP exists from a young age, with distinct sex differences.

Hypoaldosteronism post-adrenalectomy for primary aldosteronism

Lachlan Angus[1, 2], Jun Yang[3, 4], Ada Cheung[1, 2]

1. Austin Health, Heidelberg, VIC, Australia

2. Medicine (Austin Health), University of Melbourne, Heidelberg, VIC

3. Department of Molecular and Translational Science, Hudson Institute, Clayton, VIC

4. Endocrinology & Diabetes, Monash Health, Clayton, VIC

Case details

A 76-year-old man was referred to Endocrinology Clinic with a 25-year history of treatment-refractory hypertension and suspected primary aldosteronism. Notably, he had a history of thiazide diuretic induced hypokalaemia and suboptimal blood pressure control despite four anti-hypertensive medications including olmesartan, atenolol, prazosin and moxonidine. To investigate for primary aldosteronism, these medications were switched to prazosin, verapamil and moxonidine to reduce interference with aldosterone and renin measurements. A seated saline suppression test confirmed the diagnosis of primary aldosteronism, with a baseline plasma aldosterone concentration of 533 pmol/L (100-950 pmol/L erect), direct renin concentration of 1.1 mU/L (3.3 – 4.1 mU/L erect) and aldosterone renin ratio of 490 (<70), and plasma aldosterone concentration of 290 pmol/L 4 hours following the administration of 2L 0.9% sodium chloride solution (normal response <170 pmol/L). A dedicated adrenal CT scan demonstrated a left adrenal adenoma measuring 12 x 7 mm with absolute contrast washout of 83.6% while the right adrenal gland was normal in size, shape and enhancement characteristics with no discrete lesion. However, adrenal vein sampling demonstrated right sided lateralisation (bilateral simultaneous adrenal vein sampling, pre-ACTH infusion: right adrenal vein aldosterone cortisol ratio to left adrenal vein aldosterone cortisol ratio 47.9; both adrenal veins were successfully cannulated with left and right adrenal vein to peripheral cortisol ratio >2). Laparoscopic right adrenalectomy was performed 8 weeks later with histology consistent with a benign adrenocortical adenoma. A mineralocorticoid receptor antagonist was not introduced prior to surgery. On day 1 post-adrenalectomy, the patient's serum sodium was 133 mmol/L (135 - 145 mmol/L), serum potassium 3.8 mmol/L (3.5 – 5.5 mmol/L) and blood pressure up to 170/90 mmHg. All anti-hypertensive medications were ceased with a plan to reintroduce if required.

The Endocrine Surgery team reviewed the patient 1 week post-operatively, noting ambulatory systolic blood pressure measurements up to 150 mmHg on moxonidine, which was changed to verapamil but no electrolyte monitoring was performed. The Endocrinology team reviewed the patient 12 weeks post-operatively, with a recorded blood pressure of 136/76 mmHg on verapamil modified release 240mg daily. Assessment of serum electrolytes at this time revealed severe hyponatraemia (Na 124 mmol/L) and mild hyperkalaemia (5.7 mmol/L). Due to initial concern of glucocorticoid and mineralocorticoid deficiency, hydrocortisone 10mg twice daily and fludrocortisone 50microg daily were commenced whilst other investigations were arranged. Subsequent investigation was consistent with isolated mineralocorticoid deficiency with low plasma aldosterone (74 pmol/L), minimally stimulated renin (27 mU/L) and a normal cortisol response to ACTH (peak cortisol 667 nmol/L at 60 minutes following administration of ACTH). Hydrocortisone was ceased and fludrocortisone 50 microg daily was continued with subsequent normalisation of serum sodium (139 mmol/L) and .

Discussion

Primary aldosteronism refers to a spectrum of conditions characterised by inappropriately high aldosterone production and suppressed renin, and can result in clinically significant hypertension, hypokalaemia, renal impairment and an increased risk of atrial fibrillation, stroke and myocardial infarction. (1) While primary aldosteronism may affect over 10% the hypertensive population, only a minority of eligible patients are screened in the primary care setting contributing to underdiagnosis and undertreatment. (1)

The diagnostic algorithm for primary aldosteronism involves screening, confirmatory testing and localisation studies to distinguish unilateral pathology which may be amenable to adrenalectomy from bilateral adrenal hyperplasia. Current Endocrine Society guidelines recommend dedicated adrenal imaging for all cases of primary aldosteronism and adrenal vein sampling in most cases to assist with localisation prior to surgery. (1) This is due to the high prevalence of non-functioning adrenal adenomas and poor correlation between CT finding and adrenal vein sampling: a review of 950 patients who underwent CT/MRI and adrenal vein sampling showed that CT/MRI accurately predicted unilateral or bilateral disease in only 62.2% of cases. (2)

Post-operative hyperkalaemia is an uncommon and usually transient phenomenon following adrenalectomy for primary aldosteronism, attributed to suppression of renin and therefore reduced aldosterone production from the contralateral adrenal gland. (3) Previously identified risk factors include age >50 years, duration of hypertension >10 years, pre-existing renal impairment, adrenal adenoma size >2cm (3, 4) and a contralateral suppression index <0.47 (calculated by dividing the aldosterone cortisol ratio of the non-dominant vein by the external iliac vein). (5)

The use of pre-operative mineralocorticoid receptor antagonists to stimulate renin activity has been suggested to decrease the risk of hypoaldosteronism post-adrenalectomy. While some studies have not demonstrated a benefit using this approach, this may be due to failure to achieve non-suppressed renin in patients prior to surgery. (3, 4) Post-operative hypoaldosteronism is conventionally managed with fludrocortisone. In the presence of relative contraindications to mineralocorticoid replacement such as significant heart failure or hypertension, alternatives such as furosemide, sodium bicarbonate or potassium binders may be considered as part of individualised therapy to manage hyperkalaemia.

Our case provides a pertinent example of primary aldosteronism due to a unilateral aldosterone-producing adenoma with discordant CT and adrenal vein sampling findings in an elderly man. Adrenalectomy cured primary aldosteronism and significantly improved hypertension, but was complicated by the delayed recognition of significant hyponatraemia and hyperkalaemia due to suppressed aldosterone production from the contralateral gland, which required fludrocortisone supplementation. Clinicians should be aware of the risk of post-operative hypoaldosteronism and hyperkalaemia following adrenalectomy and routinely measure electrolytes within the first 1 – 2 weeks post-adrenalectomy. Pre-operative mineralocorticoid antagonist therapy can improve control of blood pressure and hypokalaemia and may reduce the risk of hypoaldosteronism post-adrenalectomy if stimulation of renin is adequately achieved.

Table 1: Summary of key pre- and post-operative investigations

Test	3/10/19	15/10/19	11/3/20	4/6/20	9/6/20	18/6/20	Reference range
Sodium	3.8	140	**133**	**124**	135	139	135 – 145 mmol/L
Potassium		4.3	3.8	**5.7**	5.4	4.9	3.5 – 5.5 mmol/L
Urea		6.9	8.0	9.1	**10.3**	7.3	3.5 – 9.5 mmol/L
Creatinine		75	76	90	106	80	60 – 115 µmol/L
eGFR		85	85	71	59	83	>60 mL/min/1.73m^2
Aldosterone	533				**74**	**<50**	100 -950 pmol/L erect
Renin	**1.1**				**27**	**5.0**	3.3 – 4.1 mU/L erect
ARR	**490**				3	<10	<70

Comments:	Saline suppression test
3/10/19	Day 1 post- right adrenalectomy
11/3/20	Hyponatraemia and hyperkalaemia detected, 12 weeks post adrenalectomy
4/6/20	
9/6/20	Endocrine review: hydrocortisone 10mg twice daily and fludrocortisone 0.5mg daily commenced
16/6/20	ACTH stimulation test demonstrated normal cortisol response (662 nmol/L at 60 minutes).
23/6/20	Endocrine review: hydrocortisone ceased, fludrocortisone continued

Key messages

1. Adrenal vein sampling is an important investigation for the lateralisation of aldosterone excess, as the findings of adrenal imaging may be discordant
2. Clinically significant hyperkalaemia due to hypoaldosteronism may occur post-adrenalectomy for primary aldosteronism. Routine electrolyte monitoring is recommended for at least 1-2 weeks. While typically transient, there are cases of persistent hypoaldosteronism requiring ongoing mineralocorticoid replacement.
3. Risk factors include age >50 years, duration of hypertension >10 years, pre-existing renal impairment and adrenal adenoma size >2cm
4. Pre-operative use of a mineralocorticoid receptor antagonist to achieve non-suppressed renin may reduce the risk of post-operative hypoaldosteronism

References

1. 1. Funder JW, Carey RM, Mantero F, Murad MH, Reincke M, Shibata H, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. The Journal of Clinical Endocrinology & Metabolism. 2016;101(5):1889-916.
2. 2. Kempers MJ, Lenders JW, van Outheusden L, van der Wilt GJ, Schultze Kool LJ, Hermus AR, et al. Systematic review: diagnostic procedures to differentiate unilateral from bilateral adrenal abnormality in primary aldosteronism. Ann Intern Med. 2009;151(5):329-37.
3. 3. Fischer E, Hanslik G, Pallauf A, Degenhart C, Linsenmaier U, Beuschlein F, et al. Prolonged zona glomerulosa insufficiency causing hyperkalemia in primary aldosteronism after adrenalectomy. J Clin Endocrinol Metab. 2012;97(11):3965-73.
4. 4. Park KS, Kim JH, Ku EJ, Hong AR, Moon MK, Choi SH, et al. Clinical risk factors of postoperative hyperkalemia after adrenalectomy in patients with aldosterone-producing adenoma. Eur J Endocrinol. 2015;172(6):725-31.
5. 5. Shariq OA, Bancos I, Cronin PA, Farley DR, Richards ML, Thompson GB, et al. Contralateral suppression of aldosterone at adrenal venous sampling predicts hyperkalemia following adrenalectomy for primary aldosteronism. Surgery. 2018;163(1):183-90.

Mysteriously missing ACTH

Caroline Bachmeier[1], Emily Brooks[2], Jacobus Ungerer[1], Carel Pretorius[1], Warrick Inder[2]

1. Department of Chemical Pathology, Pathology Queensland, Brisbane, QLD, Australia

2. Department of Endocrinology, Princess Alexandra Hospital, Brisbane, QLD , Australia

Abstract

Determining the underlying cause of hypercortisolism requires an accurate measurement of plasma adrenocorticotropic hormone (ACTH). Most laboratories measure ACTH via immunoassays. Interference in immunoassays is rare but well described and if present can lead to false results which can become clinically significant when they lead to invasive testing and inappropriate treatment. We describe the case of a 69-year old man with bilateral adrenal masses and associated mild cushingoid features. Mild biochemical hypercortisolism was accompanied by repeatedly grossly elevated ACTH levels. No ectopic or pituitary source was identified on various imaging modalities. The inconsistency of clinical, imaging and biochemistry findings raised concerns of assay interference. Several laboratory tests to investigate this possibility were performed and indicated that interference in the ACTH assay resulted in falsely elevated ACTH concentrations. The man underwent an adrenalectomy which confirmed an adrenocortical tumour. The erroneous ACTH results could have led to unnecessary and inappropriate further investigations and treatment. Close collaboration of the laboratory and the clinical teams is important to avoid adverse patient outcomes especially if there are inconsistencies in laboratory and clinical findings.

Structure of case:

1) Discussion of initial investigations for hypercortisolism and caveats of each test.
2) Discrepancies between imaging findings and biochemistry.
3) What to do if both pituitary MR and adrenal CT show lesions.
4) Laboratory investigations for possible interference.
5) Dynamic tests to differentiate the source of ACTH.

Adrenals: Can't Live With Them, Can Live Without Them

Shananthan Balachandran[1], Richard Simpson[1], Christopher Gilfillan[1], Rosemary Wong[1]

1. Department of Endocrinology, Eastern Health, Box Hill, VIC, Australia

A 67-year-old woman undergoes treatment for an autonomously functioning thyroid nodule. Around this time, she begins to feel increasingly unwell with new hypertension, palpitations and dizziness. She is rendered euthyroid, however presents 2 months following radioiodine ablation with left-sided chest pain, dyspnoea, fatigue, peripheral oedema and productive cough for green sputum. She has also developed progressive proximal myopathy and noticeably thin skin with worsening ecchymoses. Biochemistry reveals intermittent but spontaneous hypernatraemia and hypokalaemia. CT chest reveals cavitating left-sided pneumonia. Stenotrophomonas maltophilia and aspergillus fumigatum are isolated. Lung Biopsy is consistent with pneumonia without features to suggest malignancy. She is treated with directed antimicrobial therapy and is left to continue a course of voriconazole for presumed aspergilloma. A course of prednisolone therapy is also prescribed for possible allergic bronchopulmonary aspergillosis, although testing for this later returned negative.

2 weeks into the subsequent rehabilitation stay, she returns to hospital with severe epigastric pain. Investigation reveals a 7cm pancreatic pseudocyst secondary to presumed pancreatitis however with a normal serum lipase. For ongoing epigastric pain and anorexia with early satiety, therapeutic aspiration under endoscopic ultrasound is performed. Cytology is negative for malignancy. Prednisolone is inadvertently but fortunately withheld during this readmission and serum cortisol is performed to exclude hypothalamic-pituitary-adrenal axis suppression. It is > 1750 nmol/L and leads to endocrinology involvement for probable Cushing's syndrome. Cushing's syndrome is confirmed with elevated 24 hr urine free cortisol and 1mg dexamethasone suppression test (DST) that fails to suppress, serum cortisol 1036 nmol/L. Cyclic Cushing's syndrome is demonstrated with four peaks and three troughs in cortisol production noted over a four-week period. Serum ACTH is elevated whilst hypercortisolaemic, confirming ACTH-dependent disease. 8mg DST fails to suppress the serum cortisol, 1088 nmol/L. Peripheral CRH stimulation test results in a 22% rise in serum cortisol with no rise in serum ACTH. Although the 8mg DST and CRH stimulation testing favour an ectopic source, MRI Pituitary however, reveals a 4.7mm pituitary microadenoma. Bilateral inferior petrosal sinus sampling achieved successful radiological catheterisation but fails to show an adequate prolactin gradient to verify pituitary venous effluent. Peak central-peripheral ACTH gradient measured 1.19 prior to administration of ACTH and only 1.25 following. The prolactin-normalised ACTH central-peripheral ratio was 1.23. Localisation studies are performed alongside the aforementioned investigations and are unable to reveal a source of potential ectopic ACTH. Investigation included but was not limited to DOTATATE PET/CT and FDG-PET imaging.

During the extensive work up, the patient experienced worsening features of Cushing's syndrome. Pertinent challenges were that of progressive myopathy, and hypokalaemia despite the use of spironolactone and oral potassium supplementation. In view of severe biochemical hypercortisolism, the rapidly advancing clinical phenotype and somewhat equivocal testing, ketoconazole was commenced with a view to bilateral adrenalectomy. Use of ketoconazole was met alternating adrenal insufficiency requiring glucocorticoid replacement and hypercortisolism due to extremely short peaks and troughs in cortisol production.

The patient has undergone bilateral adrenalectomy for definitive management of hypercortisolism. Surgery has been complicated by likely remnant adrenocortical tissue with biochemical evidence of endogenous cortisol secretion albeit less severe than previously. Clinical state remains preoccupied by a post-operative pancreatic leak requiring ongoing surgical intervention. Following a period of recovery, it is planned to recommence ketoconazole and consider the use of mitotane in further management of her Cushing's syndrome.

Discussion:

Cushing's Syndrome (CS) is associated with significantly increased mortality and morbidity. Majority of cases are ACTH-dependent with the bulk accounted for by Cushing's Disease (CD). Ectopic ACTH secretion (EAS) is by comparison far less common. Distinguishing the two is essential as optimal outcomes are achieved only with directed management. Transphenoidal resection of an ACTH-secreting pituitary adenoma is the treatment of choice in CD. EAS is managed with excision of a culprit tumour, however medical therapy or bilateral adrenalectomy may need consideration in patients with occult disease which may occur in up to 17%.

Distinguishing EAS from CD can be challenging. High dose DST, CRH stimulation, Desmopressin, Combined CRH-Desmopressin are all dynamic tests that may discriminate between the two but none of these tests is infallible. Bilateral inferior petrosal sinus sampling (BIPSS) is considered the gold standard to distinguish EAS from CD, however false negatives have been described in CD usually relating to anomalous venous drainage or lack of expertise. False positives may be seen with cyclic and CRH-secreting EAS. Radiologically successful catheterisation is established by demonstrating retrograde flow of contrast into the contralateral cavernous sinus. Prolactin (PRL) levels have also been measured to improve diagnostic accuracy of BIPSS. Baseline central-peripheral PRL gradient > 1.8, is considered to represent successful sampling. Venous anomalies however may account for the absence of a prolactin gradient, and there is data that a prolactin-normalised ACTH central-peripheral ratio may be helpful in such cases. Ratios > 1.3 have been suggestive for CD, < 0.8 for EAS and those in between being indeterminate, however further prospective study is required.

Cyclic CS may account for false positives at BIPSS, when sampling is performed during a period of spontaneous remission of hypercortisolism. EAS has been reported to account for 26% of cyclic CS indicating a higher prevalence in cyclic CS than in all

CS. Medical management of hypercortisolism in cyclic CS may encounter adrenal insufficiency during troughs in cortisol production, a challenging issue when cycle lengths are as short as in our case.

Bilateral adrenalectomy (BLA) is considered an effective and sometimes essential treatment option in selected patients with CS. It offers immediate control of hypercortisolism and is typically used in cases refractory to other medical options as it is not without significant risk. Median 30-day mortality has been reported as 3% with considerably higher rates in EAS than CD. Cardiovascular events and infection are leading causes of early death following BLA. Total mortality is also high with median mortality rate of 17% at 41 months follow up and again much higher rates are seen in EAS. Although laparoscopic approach is associated with improved morbidity, it has not demonstrated any mortality benefit. Despite the goal of serum cortisol levels falling to undetectable following BLA, persistent endogenous cortisol secretion is described in up to 34% of cases. Majority of these cases have adrenal remnants or ectopic adrenal tissue identifiable on imaging or further surgical exploration. Ectopic adrenal tissue has been described in retroperitoneal fat, gonads and mediastinum. Although persistent endogenous cortisol secretion may be encountered, few cases develop relapse of CS with clinical signs of hypercortisolism. Whilst BLA carries real risk, this must be weighed against the dismal prognosis of otherwise untreated CS.

CS poses significant diagnostic and management challenges. The rarity of the disease and particular aetiology such as EAS means that an individualised approach discussed in a multidisciplinary setting is essential to patient care. In some instances, definitive strategies for hypercortisolism will be required prior to extensive and sometimes time consuming investigation but the risks and benefits can only be considered on a case by case basis.

Take Home Messages:

- Determining aetiology of ACTH-depending CS may be challenging
- Prolactin-normalised ACTH central-peripheral ratios at BIPSS may be helpful in assisting diagnosis but further study is needed
- Cyclic CS is more commonly encountered in EAS
- Bilateral adrenalectomy has significant mortality and morbidity risk as well as rates of persistent endogenous cortisol secretion

1. Dekkers OM, Horváth-Puhó E, Jørgensen JO, Cannegieter SC, Ehrenstein V, Vandenbroucke JP, Pereira AM, Sørensen HT, Multisystem Morbidity and Mortality in Cushing's Syndrome: A Cohort Study, The Journal of Clinical Endocrinology & Metabolism, Volume 98, Issue 6, 1 June 2013, Pages 2277–2284
2. Meinardi JR, Wolffenbuttel B, Dullaart R. (2007). Cyclic Cushing's syndrome: a clinical challenge, European Journal of Endocrinology, 157(3), 245-254
3. Young J, Haissaguerre M, Viera-Pinto O, Chabre O, Baudin E, Tabarin A. (2020). MANAGEMENT OF ENDOCRINE DISEASE: Cushing's syndrome due to ectopic ACTH secretion: an expert operational opinion, European Journal of Endocrinology, 182(4), R29-R58
4. Sharma ST, Raff H, Nieman LK. Prolactin as a marker of successful catheterization during IPSS in patients with ACTH-dependent Cushing's syndrome. The Journal of Clinical Endocrinology & Metabolism. 2011;96(12):3687–3694
5. Findling JW, Kehoe ME, Raff H. Identification of Patients with Cushing's Disease with Negative Pituitary Adrenocorticotropin Gradients during Inferior Petrosal Sinus Sampling: Prolactin as an Index of Pituitary Venous Effluent, The Journal of Clinical Endocrinology & Metabolism, Volume 89, Issue 12, 1 December 2004, Pages 6005–6009
6. Ritzel K, Beuschlein F, Mickisch A, Osswald A, Schneider HJ, Schopohl J, Reincke M, Outcome of Bilateral Adrenalectomy in Cushing's Syndrome: A Systematic Review, The Journal of Clinical Endocrinology & Metabolism, Volume 98, Issue 10, 1 October 2013, Pages 3939–3948

Phaeochromocytoma-induced cardiomyopathy

Emma Boehm[1], Joshua Hawson[2], Mark Nolan[3], Shane Hamblin[1], Christopher J Yates[1, 4]

1. Endocrinology and Diabetes, Western Health, Melbourne, Vic, Australia

2. Cardiology, Melbourne Health, Melbourne, Vic, Australia

3. Cardiology, Western Health, Melbourne, Vic, Australia

4. Diabetes and Endocrinology, Melbourne Health, Melbourne, Vic, Australia

Case 1

A 50-year-old male was admitted to the Footscray campus of the Western Hospital with pneumonia and acute decompensated heart failure. This occurred on a background of recently diagnosed human immunodeficiency virus, type 2 diabetes mellitus managed with metformin, and fortnightly recreational inhaled methamphetamine use. There was no history of excessive alcohol consumption. Transthoracic echocardiography demonstrated a dilated cardiomyopathy with severe global systolic dysfunction and a left ventricular ejection fraction (LVEF) of 15% (normal >50%). CT coronary angiography revealed no significant coronary artery disease, however a 41x42mm right-sided heterogeneous hyper-enhancing adrenal mass was incidentally detected. Adrenal function tests were sent and the patient was referred for outpatient endocrinology follow-up. Prior to endocrinology review the patient was prescribed carvedilol, ramipril and ivabradine. The plasma normetanephrine returned significantly elevated at 10743pmol/L (<900pmol/L) and remained elevated despite a period of abstinence from methamphetamine (Table 1)

Test	Result	Reference
During inpatient stay		
Normetanephrine	10743 pmol/L	<900 pmol/L
Metanephrine	149 pmol/L	<500 pmol/L
Outpatient after methamphetamine abstinence		
Normetanephrine	7819 pmol/L	<900 pmol/L
Metanephrine	139 pmol/L	<500 pmol/L
3-methoxy tyramine	106 pmol/L	<110 pmol/L
Chromogranin A	255 ug/L	<94ug/L

Table 1: Case 1 results of inpatient and outpatient testing for catecholamine excess

The patient attended endocrinology clinic and immediately commenced phenoxybenzamine 10mg BD for treatment of a noradrenaline-secreting phaeochromocytoma. Phenoxybenzamine was uptitrated over 4 months to 30mg BD. A pre-operative repeat echocardiogram showed myocardial recovery with an LVEF of 40-50% and mild global systolic dysfunction. He tolerated a high salt and fluid diet for 7 days pre-operatively and his phenoxybenzamine was withheld the morning of surgery. He underwent open adrenalectomy and did not require inotropic support post-operatively. Histopathology confirmed a completely resected phaeochromocytoma, with a Ki67 of <1% and marked nuclear pleomorphism (PASS score: 2). He has been referred for genetic testing.

Case 2

A 39-year-old female presented to the Royal Melbourne Hospital Emergency Department with a severe, sudden-onset headache associated with vomiting. Initial biochemical investigations and CT brain were unremarkable. She was treated for a migraine with morphine, metoclopramide, chlorpromazine, prochlorperazine and transferred to the Emergency Short Stay unit for observation. Nine hours later she had an episode of syncope and chest pain. Electrocardiography demonstrated ST elevation in leads I and aVL, with reciprocal ST depression in leads II, III, aVF, V1, V3 and V4. A high sensitivity troponin was 7920 ng/L (<16ng/L). Urgent coronary angiography was performed showing normal coronary arteries. The left ventriculogram (Figure 1) and subsequent transthoracic echocardiogram demonstrated akinesis of basal and mid segments with only apical contraction preserved, consistent with reverse-takotsubo cardiomyopathy. The ejection fraction was estimated to be 5-10% (normal >50%).

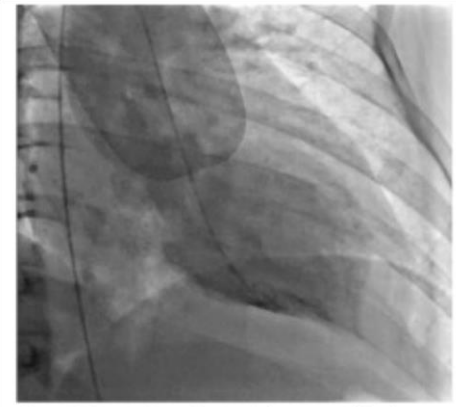


Figure 1: Left ventriculogram showing apical contraction with basal segment hypokinesis consistent with a "reverse takotsubo" pattern of cardiomyopathy. Left ventricular end diastolic pressure was 30mmHg (normal <15mmHg) signifying severe cardiac failure

Post-coronary angiography, the patient became febrile, confused, and hypoxaemic. She was transferred to the intensive care unit with cardiogenic shock that proved refractory to inotropic support and was commenced on extracorporeal membrane oxygenation (ECMO). Her haemodynamics were difficult to manage, having periods of hypotension alternating with hypertension and tachycardia requiring periods of noradrenaline and glyceryl trinitrate respectively. An abdominal ultrasound was performed to investigate an ischaemic right lower limb that had complicated ECMO cannulation, which incidentally found an 8cm mass abutting the upper pole of the right kidney. Endocrinology was consulted and plasma metanephrines and chromogranin A were sent (Table 2). It was suspected that the patient had a secretory phaeochomocytoma and alpha-adrenal blockade with phentolamine infusion was commenced. The patient was transferred to another quaternary centre given the concern about potential requirement for a ventricular assist device or cardiac transplantation. There, the patient was transitioned to phenoxybenzamine 10mg BD via nasogastric tube, uptitrated to 40mg QID. Metoprolol 25mg BD was later commenced and uptitrated to 100mg TDS. On day 9 of admission the patient was decannulated from ECMO and extubated. She was transferred to the ward 5 days later and a transthoracic echocardiogram showed complete recovery of left ventricular function. A CT abdomen identified a 7.7cm left adrenal mass. A DOTATATE PET/CT showed a large DOTATATE avid left adrenal mass with no evidence of locoregional nodal or distant metastasis (Figure 2). She underwent an elective left adrenalectomy 6 weeks later with histopathology confirming a phaeochromocytoma, 67mm in maximum dimension, clear of margins, with a PASS score of 0, favouring a benign tumour. On follow-up genetic testing there was no germline mutation conferring increased risk for pheochromocytoma found.

Test	Result	Reference
Normetanephrine	60780 pmol/L	<900 pmol/L
Metanephrine	13760 pmol/L	<500 pmol/L
3-methoxy tyramine	<90 pmol/L	<110 pmol/L
Chromogranin A	2190 ug/L	<94ug/L

Table 2: Case 2 results of biochemical screening for phaeochromocytoma

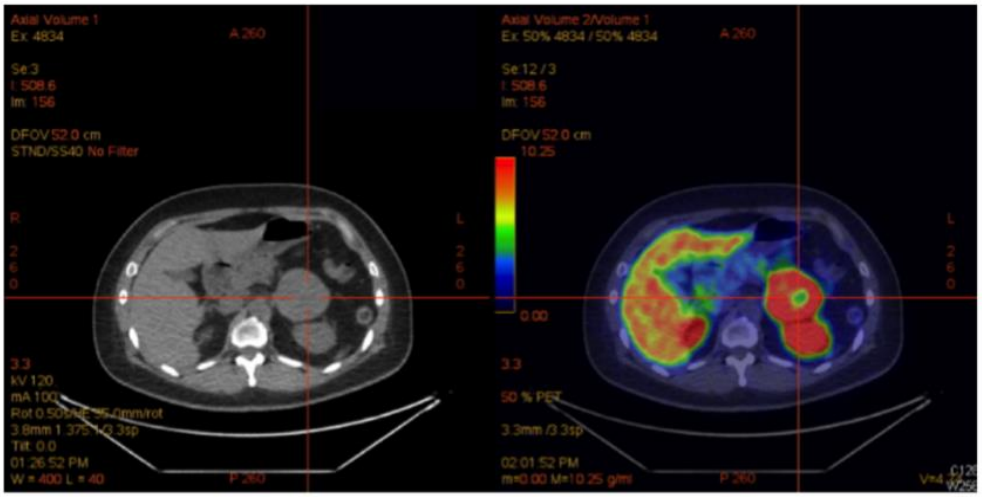


Figure 2: DOTATATE PET/CT images. Large DOTATATE-avid left adrenal mass.

Discussion

Acute catecholamine-mediated cardiomyopathy has been described in association with phaeochromocytoma, occurring in 11% of patients in one series[1]. Catecholamines initially have a positive inotropic effect on the myocardium. States of excess, however, lead to massive concentrations of calcium in the myocardial sarcoplasmic reticulum, reducing mitochondrial energy production resulting in stunning of the myocardial fibres[1]. Additionally, impaired myocardial oxygen supply due to coronary vasospasm leading to reduced perfusion has been hypothesised to contribute[1]. The classic manifestation of this is a takotsubo cardiomyopathy characterised by left ventricular apical akinesis and ballooning with hyperkinesis of the basal segments[1,2]. This cannot by differentiated from an acute coronary syndrome clinically, and the diagnosis of takotsubo cardiomyopathy is made at angiogram on the basis of patent coronary arteries and characteristic left ventriculogram appearance. In the second case, the intra-angiographic and echocardiographic appearance of apical myocardial contractility with basal stunning was the opposite of the classical pattern, hence the moniker of “reverse takotsubo” cardiomyopathy[2].

The first case is remarkable due to the dual catecholaminergic insults of methamphetamine as well as noradrenaline hypersecretion from the phaeochromocytoma. Methamphetamine is a sympathomimetic drug, causing release of noradrenaline, dopamine and serotonin from storage vesicles due to its structural similarity to monoamines[3]. Use of methamphetamine is known to cause both catecholamine-mediated cardiomyopathy; as well as elevated urinary catecholamine and plasma metanephrines, in a pattern that can mimic a noradrenaline or dopamine secreting phaeochromocytoma[3,4]. Methamphetamine can be detected in urine for up to 60 hours after use, making diagnosis of phaeochromocytoma in a person who uses methamphetamines challenging and reliant on a period of abstinence for reliable results as in our case[3]. Abstinence from methamphetamine and pharmacological alpha- and beta-adrenoceptor blockade lead to almost complete recovery of myocardial function.

The role that catecholamine excess played in the fulminant course of cardiac failure in the second case was multifactorial. Acute catecholamine excess, or “phaeo crisis”, potentially precipitated by medications including metoclopramide used to treat presumed migraine, lead to the aforementioned cardiomyopathy, severely reducing cardiac output. Additionally, the peripheral vasoconstriction associated with catecholamine agonism of alpha-adrenoceptors lead to an increase in peripheral resistance and a dramatic increase in afterload, further impairing cardiac output. This resulted in cardiac failure refractory to inotropic support and necessitated the use of extracorporeal membrane oxygenation (ECMO). ECMO lends itself as a solution to the unique haemodynamic challenges of providing alpha- and then beta-adrenoceptor blockade to a patient with cardiogenic shock[5].

The mechanical and pharmacological offloading of the left ventricle achieved was seen in the second case to result in resolution of the cardiomyopathy. This is typical of catecholaminergic cardiomyopathy wherein the echocardiographic features are estimated to resolve within months of cessation of the insult[2]. Prior to adrenalectomy it is imperative that all patients with phaeochromocytoma have adequate alpha and beta-adrenoceptor blockade to prevent life-threatening intra-operative cardiogenic shock.

Take home messages:

- Methamphetamines cause elevation of plasma noradrenaline and dopamine, which can lead to elevated plasma metanephrines and urinary catecholamines
- ECMO is potentially life-saving in the setting of a severe phaeochromocytoma crisis with acute catecholamine cardiomyopathy and peripheral vasoconstriction
- Alpha- and beta-adrenoceptor blockade improves myocardial recovery in patients with phaeochromocytoma and catecholamine-mediated cardiomyopathy prior to adrenalectomy
- Adequate alpha and beta-adrenoceptor blockade is required to prevent an intra-operative phaeochromocytoma crisis.

1. Kounatiadis, P., Kolettas, V., Megarisiotou, A. and Stiliadis, I. (2013). Cardiomyopathy due to pheochromocytoma. Herz, 40(1), pp.139-143.
2. Giavarini, A., Chedid, A., Bobrie, G., Plouin, P., Hagège, A. and Amar, L. (2013). Acute catecholamine cardiomyopathy in patients with phaeochromocytoma or functional paraganglioma. Heart, 99(19), pp.1438-1444.
3. Cruickshank, C. and Dyer, K., 2009. A review of the clinical pharmacology of methamphetamine. Addiction, 104(7), pp.1085-1099.
4. Pacak K, Tella SH. Pheochromocytoma and Paraganglioma. [Updated 2018 Jan 4]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK481899/
5. Banfi, C., Juthier, F., Ennezat, P., de Saint Denis, T., Carnaille, B., Leteurtre, E., Prat, A. and Vincentelli, A. (2012). Central Extracorporeal Life Support in Pheochromocytoma Crisis. The Annals of Thoracic Surgery, 93(4), pp.1303-1305.

An unusual cause of parathyroid hormone-independent hypercalcaemia and hypercalciuria

Emma Boehm[1], Catherine Luxford[2], Roderick Clifton-Bligh[3], Vivian Grill[1]

1. Dept Endocrinology and Diabetes, Western Health, Melbourne, Vic

2. Kolling Institute, Royal North Shore Hospital, Sydney, NSW

3. Dept Endocrinology, Royal North Shore Hospital, Sydney, NSW

Case

A 62-year-old postmenopausal woman was referred to the Metabolic Bone Disorders clinic for evaluation of hypercalcaemia noted during a recent hospitalisation for a subarachnoid haemorrhage from a posterior communicating artery aneurysm. She had a history of diet-controlled Type 2 Diabetes, fibromyalgia, anxiety and depression. The patient also had a distant history of nephrolithiasis, and no previous clinical minimal trauma fractures. She had partial dentures after unexplained teeth loss in her early adulthood. She took no regular medications.

Albumin corrected serum calcium was 2.96 mmol/L (2.15 – 2.65 mmol/L) (Albumin 37g/L), Phosphate 1.02 mmol/L (0.75 – 1.50 mmol/L), eGFR >90 mL/min, PTH 0.5 pmol/L (2.0 – 8.5 pmol/L) and 25(OH)D 76nmol/L. A 24 hour urinary calcium excretion was 10.3 mmol/24h (2.0-7.5 mmol). PTHrP was undetectable (<2 pmol/L). TSH was 1.27 mIU/L (0.50 – 4.00 mIU/L). Serum 1,25(OH)2D was 179 pmol/L (50 – 190 pmol/L). Angiotensin Converting Enzyme was 46.1 U/L (8.0 – 75.0 U/L). CT Chest was normal, as was a whole body bone scan.

Bone Mineral Density measurement showed T scores of -3.6 at L1-4, -1.8 at the Femoral Neck and -4.1 at the Distal Radius (Hologic Horizon). There were no vertebral fractures on spinal X-rays. There was no evidence of either nephrolithiasis or nephrocalcinosis on renal tract ultrasound.

A family history of hypercalcaemia in a sibling, persisting after excision of a parathyroid adenoma and unexplained despite extensive investigations came to light. The patient has one adult daughter following an uneventful pregnancy.

In the absence of available assays for 24,25 (OH)2D levels, Sanger sequencing of *CYP24A1* was performed at the Kolling Institute. Our patient was found to be homozygous for the pathogenic variant c.1186C>T, p.Arg396Trp (R396W). Cascade testing is being offered to her contactable siblings.

Discussion

Hypercalcaemia is a relatively common disorder most frequently attributable to either primary hyperparathyroidism or malignancy. Hypercalcemia due to a disorder of Vitamin D metabolism is less common, but neither a history of Vitamin D intoxication, nor a granulomatous disorder or a lymphoma were found in our patient, as an explanation for the relatively high 1,25(OH)D level associated with hypercalcemia and a suppressed PTH.

Inactivating mutations in the gene encoding CYP24A1 constitute a mechanism which can lead to a syndrome of hypercalcaemia, hypercalciuria and nephrolithiasis due to reduced catabolism of 1,25(OH)2D. The pathogenic nature of *CYP24A1* variants was first described in 2011, wherein five different variants in either homozygous or compound heterozygous form were associated with idiopathic infantile hypercalcaemia. Vitamin D supplementation exacerbates the hypercalcemia in this condition (1). Adult cases of *CYP24A1* mutation have subsequently been described in women with hypercalcemia identified during pregnancy (2,3), or in males presenting with hypercalcaemia and complications of hypercalciuria (4-8).

We describe a rare case of *CYP24A1* variant in a postmenopausal female presenting with hypercalcemia and hypercalciuria outside of pregnancy.

The R396W variant identified in our patient might be associated with a milder phenotype. This variant was described in a family of compound heterozygotes (8) and in a homozygous female with a normal serum calcium after 25 years of follow-up (3).

The impact of an inactivating *CYP24A1* variant on bone is not well understood. Osteopaenia, osteoporosis and normal Bone Mineral Density have been seen in adult males with *CYP24A1* variants (7,8). Animal studies of *Cyp24a1*$^{-/-}$ mice revealed defective mineralisation throughout the skeleton at birth (9). The mandible of infant mice is most severely affected, which is of interest given our patient's history of early dental loss.

Possible treatments to manage the risk of hypercalcaemia in patients with CYP24A1 deficiency include avoidance of vitamin D over-supplementation and consideration of inhibition of CYP450 enzymes with ketoconazole (4, 8) or rifampicin (10). The long-term safety of ketoconazole and rifampicin for this purpose, however, needs to be established.

Conclusion

This case illustrates that mutation of *CYP24A1* should be considered in cases of unexplained hypercalcemia associated with normal or elevated 1,25(OH)2D and kidney stones.

Take Home Messages

- Consider genetic disorders of vitamin D metabolism in the differential diagnosis of hypercalcaemia in patients of all ages
- Genetic testing is further developing our understanding of disease and should be further studied to de-label other "idiopathic" conditions

1. Schlingmann, K., Kaufmann, M., Weber, S., Irwin, A., Goos, C., John, U., Misselwitz, J., Klaus, G., Kuwertz-Broking, E., Fehernbach, H., Wingen, A., Guran, T., Hoenderop, J., Bindels, R., Prosser, D., Jones, G., Konrad, M., 2011. Mutations in CYP24A1 and Idiopathic Infantile Hypercalcaemia. The New England Journal of Medicine, 365:410-21
2. Arnold, N., O'Toole, V., Huynh, T., Smith, H., Luxford, C., Clifton-Bligh, R. and Eastman, C., 2019. Intractable hypercalcaemia during pregnancy and the postpartum secondary to pathogenic variants in CYP24A1. Endocrinology, Diabetes & Metabolism Case Reports, 2019.

3. McBride, L., Houlihan, C., Quinlan, C., Messazos, B., Stark, Z. and Crosthwaite, A., 2019. Outcomes Following Treatment of Maternal Hypercalcemia Due to CYP24A1 Pathogenic Variants. Kidney International Reports, 4(6), pp.888-892.
4. Dinour, D., Davidovits, M., Aviner, S., Ganon, L., Michael, L., Modan-Moses, D., Vered, I., Bibi, H., Frishberg, Y. and Holtzman, E., 2014. Maternal and infantile hypercalcemia caused by vitamin-D-hydroxylase mutations and vitamin D intake. Pediatric Nephrology, 30(1), pp.145-152.
5. Woods, G., Saitman, A., Gao, H., Clarke, N., Fitzgerald, R. and Chi, N., 2016. A Young Woman With Recurrent Gestational Hypercalcemia and Acute Pancreatitis Caused by CYP24A1 Deficiency. Journal of Bone and Mineral Research, 31(10), pp.1841-1844.
6. Molin, A., Baudoin, R., Kaufmann, M., Souberbielle, J., Ryckewaert, A., Vantyghem, M., Eckart, P., Bacchetta, J., Deschenes, G., Kesler-Roussey, G., Coudray, N., Richard, N., Wraich, M., Bonafiglia, Q., Tiulpakov, A., Jones, G. and Kottler, M., 2015. CYP24A1 Mutations in a Cohort of Hypercalcemic Patients: Evidence for a Recessive Trait. The Journal of Clinical Endocrinology & Metabolism, 100(10), pp.E1343-E1352.
7. Jacobs, T., Kaufman, M., Jones, G., Kumar, R., Schlingmann, K., Shapses, S. and Bilezikian, J., 2014. A Lifetime of Hypercalcemia and Hypercalciuria, Finally Explained. The Journal of Clinical Endocrinology & Metabolism, 99(3), pp.708-712.
8. O'Keeffe, D., Tebben, P., Kumar, R., Singh, R., Wu, Y. and Wermers, R., 2016. Clinical and biochemical phenotypes of adults with monoallelic and biallelic CYP24A1 mutations: evidence of gene dose effect. Osteoporosis International, 27(10), pp.3121-3125.
9. St-Arnaud, R., Arabian, A., Travers, R., Barletta, F., Raval-Pandya, M., Chapin, K., Depovere, J., Mathieu, C., Christakos, S., Demay, M. and Glorieux, F., 2000. Deficient Mineralization of Intramembranous Bone in Vitamin D-24-Hydroxylase-Ablated Mice Is Due to Elevated 1,25-Dihydroxyvitamin D and Not to the Absence of 24,25-Dihydroxyvitamin D1. Endocrinology, 141(7), pp.2658-2666.
10. Hawkes CP, Li D, Hakonarson H, Meyers KE, Thummel KE, Levine MA.J Clin Endocrinol Metab. 2017. CYP3A4 Induction by Rifampin: An Alternative Pathway for Vitamin D Inactivation in Patients With CYP24A1 Mutations. May 1;102(5):1440-1446. doi: 10.1210/jc.2016-4048.

Familial partial lipodystrophy associated with an unknown variant of the PTRF gene

Amelia R Fernandes[1, 2], **Samantha L Hocking**[1, 3]

1. Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW, Australia

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

3. Central Clinical School, Faculty of Medicine and Health, Sydney, NSW, Australia

We report a mother-daughter pair with clinical features suggestive of familial partial lipodystrophy (FPL). Our case discusses a young woman presenting with metabolic syndrome with a recent history of hypertriglyceridaemia-induced pancreatitis. Her mother has a similar body habitus, metabolic phenotype and recurrent episodes of hypertriglyceridaemia-induced pancreatitis with additional symptoms of a progressive neuromuscular disorder. Maternal genetic testing revealed two novel missense mutations in the *PTRF* gene of uncertain pathogenic significance. Mutations in the *PTRF* gene cause congenital generalized lipodystrophy type 4 (CGL4) which is associated with myopathy. There have been no reports of *PTRF* gene variants associated with FPL. This kindred may present with a novel gene variant causing FPL.

Background

The lipodystrophies are a rare heterogeneous group of disorders characterized by generalized or selective lipoatrophy of adipose tissue. FPL presents with selective loss of subcutaneous fat in the arms and legs with excess subcutaneous fat accumulation in other areas of the body, especially the neck and face resulting in a cushingoid appearance. Lipodystrophy is commonly associated with a variety of metabolic complications including dyslipidaemia, hypertriglyceridaemia, hyperglycaemia, insulin resistance, diabetes mellitus, hepatic steatosis, central adiposity and increased cardiovascular risk [1]. Mild to moderate myopathies, cardiomyopathies and conduction system abnormalities indicative of a multisystem dystrophy can occur in FPL.

Several genes are associated with FPL with five subtypes identified. In subtypes 2-5 the causative gene is known, however, the variant in FPL1 remains unknown and many novel FPL genes remain to be discovered [2,3,4].

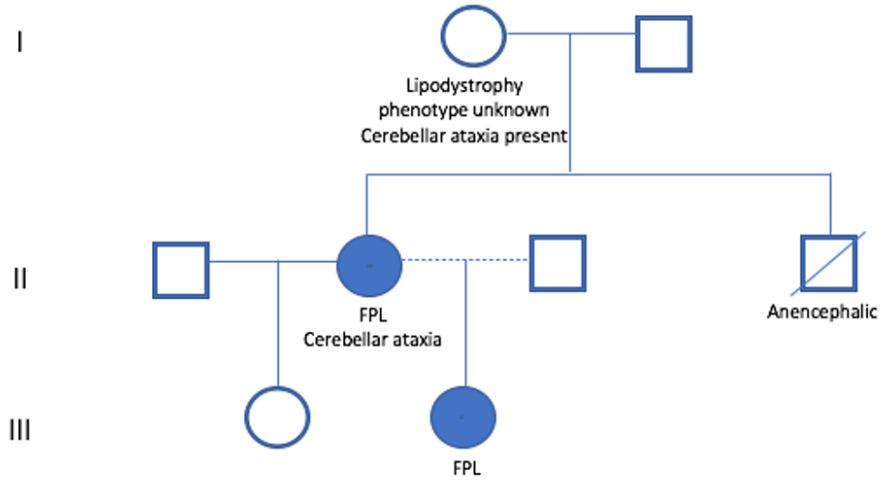
The *PTRF* (polymerase I and transcript release factor) gene encodes cavin-1. Cavin-1 is a highly abundant caveolae component and is suggested to play an essential role in caveolar formation. Caveolae are involved in several important cellular processes, including clathrin-independent endocytosis, regulation and transport of cellular cholesterol and signal transduction. Mice lacking *PTRF* do not have morphologically detectable caveolae and mimic lipodystrophy in humans with considerably reduced adipose tissue mass, high triglyceride levels, glucose intolerance and hyperinsulinaemia [2,3,4,5,6].

Homozygous variants in *PTRF* have recently been found to cause CGL4, an autosomal recessive form of congenital generalised lipodystrophy. CGL4 is characterised by generalised absence of adipose tissue and is associated with hypertriglyceridaemia and diabetes mellitus. Characteristic to this type of lipodystrophy is myokymia, muscle weakness and raised CK. Secondary features include cardiac arrhythmias, skeletal abnormalities, atlantoaxial instability, hepatomegaly, reduction of growth hormone levels, immunoglobulin A deficiency, and umbilical prominence. Since the characterisation of CGL4 in 2010, all affected individuals have been homozygous for truncating mutations in the *PTRF* gene [2,3,4,5, 6].

Case Report

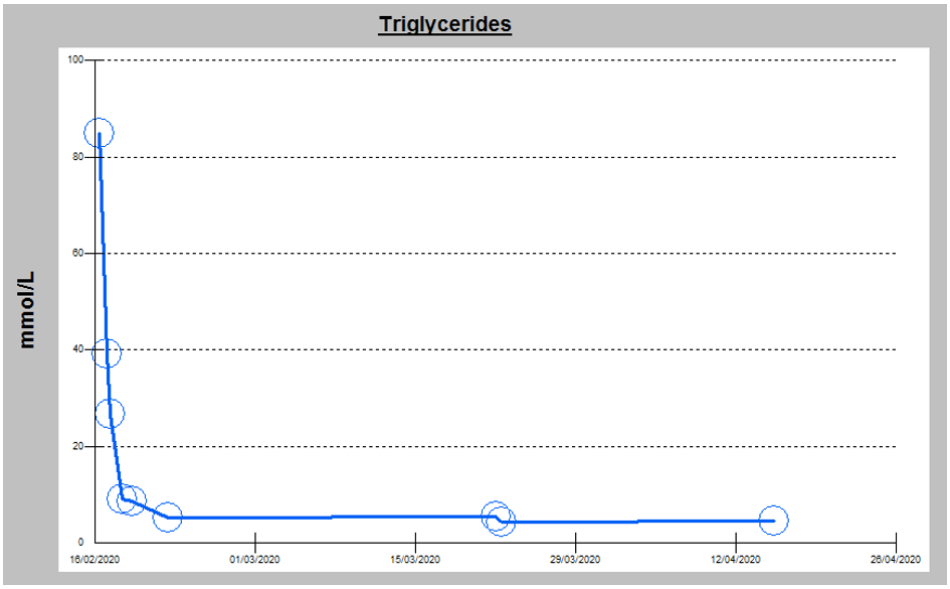
A 31-year-old woman was referred for assistance with weight reduction in the setting of metabolic syndrome. Her prior medical history included obesity, type 2 diabetes mellitus, polycystic ovarian syndrome, hepatic steatosis with bridging fibrosis and a recent hospitalisation for severe hypertriglyceridaemia-related pancreatitis requiring intensive care admission. Her obesity had been a life-long issue in the setting of chronic binge and purge behaviours, longstanding anxiety, depression and Tourette syndrome. She was a non-smoker and denied alcohol consumption. Current medications included Fenofibrate 145mg daily, Ezetimibe/ Rosuvastatin 10/20mg daily, Metformin 1000mg BD, Venlafaxine 225mg daily, Clonidine 100mg daily, Candesartan 4mg daily, Propranolol 20mg BD, Pregabalin 75mg BD, Melatonin 6mg nocte, Rabeprazole 20mg daily, Quetiapine 25mg nocte and Oxybutynin 5mg nocte.

Concerning her family history, her mother had suspected partial lipodystrophy given her Cushingoid habitus and background of hypercholesterolaemia, type 2 diabetes mellitus, obstructive sleep apnoea and severe hypertriglyceridaemia complicated by multiple episodes of pancreatitis. Her mother additionally had proximal myopathy, peripheral neuropathy and ataxia with cerebellar hypoplasia on CT scan Lipodystrophy testing found two novel missense mutations in the *PTRF* gene, the significance of which was unknown. There was a maternal family history of neuromuscular disorder, described as progressive ataxia, with a pattern of inheritance suggestive of a mitochondrial disorder (Figure 1). She was unaware of her father's medical history and she had one unaffected half-sister with a different father.



On physical examination, she had a lipodystrophic habitus with marked lipoatrophy of her arms and legs, appearing muscular with prominent visible surface veins. There was central adiposity and a moon-shaped face. Acanthosis nigricans was present on her neck and axillae. She did not have hirsutism or acne and there were no biochemical features of hyperandrogenism. She was 163 cm tall, with weight 92.1 kg, body mass index 34.7 kg/m^2 and waist circumference was 114.5 cm.

Her in-hospital laboratory results included: HbA1c 6.7% with a peak total cholesterol of 20.4 mmol/L (<5.5); triglycerides 84.8mmol/L (<2); and HDL-C, <0.08mmol/L (>1.2). She had a mild transaminitis with ALT 42 U/L (10-35), AST 41 U/L (10–35) and a raised GGT 102 U/L (5–35). She was treated with an insulin infusion in ICU, with excellent resolution of triglyceride levels.(Figure 2)



Owing to the presence of an elevated HbA1c, the patient was commenced on Empagliflozin and given lifestyle management advice including diet and exercise. In the setting of her hypertriglyceridaemia, she was commenced on fish oil. Genetic testing has been arranged to confirm her suspected diagnosis of FPL with assessment for *PTRF* gene mutation and family co-segregation studies.

Discussion

Lipodystrophic syndromes are a rare cause of type 2 diabetes with severe insulin resistance. This case demonstrates the need for awareness of this rare condition. Features suggestive of a diagnosis of partial lipodystrophy in a patient with metabolic syndrome include early onset diabetes, fatty liver disease, requirement of excessive doses of insulin, and persistently elevated triglycerides refractory to lifestyle and therapeutic interventions. Our case with suspected FPL demonstrated manifestations of both lipodystrophic habitus and metabolic derangements associated with metabolic syndrome, namely, diabetes mellitus, PCOS, NAFLD fibrosis and hypertriglyceridaemia, all of which are known to manifest in patients with FPL. These clinical manifestations are consistent with the lipid overflow hypothesis, in which excess triglycerides from reduced peripheral storage capacity accumulate in the liver, skeletal muscles and preserved visceral depots, subsequently leading to peripheral and hepatic insulin resistance and often, eventual diabetes mellitus [7,8,9].

For patients with a constellation of metabolic conditions and clinical features suggestive of lipodystrophy, consideration of genetic sequencing and molecular diagnosis may be useful [10]. A novel aspect of this case is the family history of a progressive neuromuscular disorder which led to the finding of two missense mutations in the *PTRF* gene in the mother. The first of these variants (c.923A>G) results in the substitution of a tyrosine to cysteine at position 308 in cavin-1. Of note, this tyrosine has been identified as a highly insulin-responsive phosphorylation site on the cavin-1 protein, suggesting that this may be a functional mutation, responsible for the phenotype observed in this mother-daughter pair. Although the clinical features of the kindred reported here are not consistent with CGL4, it is possible that they may have a milder spectrum of *PTRF*-related lipodystrophy.

Take Home Messages

- Familial partial lipodystrophy involves selective loss of subcutaneous fat in the arms and legs and abnormal accumulation of subcutaneous fat in other areas
- Lipodystrophy is commonly associated with a variety of metabolic complications including dyslipidaemia, hypertriglyceridaemia, hyperglycaemia, insulin resistance, diabetes mellitus, hepatic steatosis, increased central adiposity and increased cardiovascular risk.
- Many novel FPL genes remain to be discovered. We report a novel mutation in *PTRF* which may be pathogenic for FPL.

‘One of these things is not like the other’: Adrenal venous sampling in a patient with ACTH-independent Cushing’s syndrome and bilateral adrenal adenomas

Ryan Endall[1], Christopher Yates[1], Devaang Kevat[1], Jun Yang[2]

1. Department of Diabetes and Endocrinology, Western Health, Melbourne

2. Department of Endocrinology, Monash Health, Melbourne

A 40 year old woman was referred to our Endocrinology service following the incidental finding of bilateral adrenal adenomas on a CT scan performed to investigate back pain. Dedicated CT imaging of the adrenal glands showed a left adrenal nodule measuring 23x23x23mm, and a right adrenal nodule measuring 17x12x11mm; the nodules had pre-contrast Hounsfield unit values of 31 and 23, and absolute contrast washouts of 76.8% and 80%, respectively. The patient was from a Korean background with no significant medical history, took only over-the-counter vitamins, and did not consume alcohol or smoke. Systems review revealed two years of symptoms including generalised aches and pains, fatigue, weight gain (9kg over 12 months, up to 61kg), chronic constipation, thinning of the hair and skin, easy bruising, and oligomenorrhoea (three menstrual periods over 12 months). The patient had no history of fractures. On examination she had a body mass index of 20.32kg/m^2 (height 163cm, weight 54kg), blood pressure of 120/80, and no other clinical features of Cushing’s syndrome.

Morning plasma cortisol was normal at 498nmol/L (ref. range 145-619), with ACTH suppressed at 0.4pmol/L (ref. range 1.6-13.9). Hormonal evaluation demonstrated cortisol excess on two 24-hour urine cortisol assessments and low-dose (1mg) overnight dexamethasone suppression test (Table 1). Other hormonal evaluation, including aldosterone-renin ratio and plasma metanephrines, were normal. HbA1c was 5.2% (33.3mmol/mol). Bone mineral density assessment via dual-energy X-ray absorptiometry was unable to be performed due to COVID-19 restrictions; however CT imaging of the spine did not show any fractures or evidence of osteopaenia.

We made a diagnosis of ACTH-independent Cushing’s syndrome in the context of bilateral adrenal pathology. Autonomous cortisol secretion (ACS), whether associated with overt Cushing’s syndrome or otherwise, is seen in 5-30% of patients with incidentally-found adrenal lesions (1-3). The management of ACTH-independent Cushing’s syndrome is relatively straightforward where unilateral adrenal pathology is seen on imaging, with unilateral adrenalectomy being the usual recommendation (4). However, this decision is made more complicated in the setting of bilateral adrenal pathology, which carries with it a number of differential diagnoses, including bilateral macronodular adrenal hyperplasia, primary pigmented nodular adrenal disease, and bilateral adrenal adenomas (whereby one or both lesions may be autonomous). The distinction between functioning and non-functioning adrenal lesions cannot be made solely based on radiology findings, and although adrenal venous sampling (AVS) may aid in the lateralisation of cortisol excess, the current data are weak (1). Given our patient’s young age, symptom profile (including oligomenorrhoea and weight gain) and potential for future morbidity secondary to cortisol excess, we undertook a literature review for the use of AVS to guide further management. Although there is no currently accepted standard approach for the use of AVS for this indication, a number of alternative methods were identified in the literature, both for confirmation of catheter placement and for lateralisation of excess cortisol production.

Young *et al* (5) examined the use of AVS in 10 patients with bilateral adrenal lesions and ACS. An adrenal:peripheral adrenaline gradient was used to confirm catheter placement. The authors also used an adrenal:peripheral cortisol gradient cut-off of 6.5 to confirm the presence of a cortisol-secreting adenoma, with a high:low side adrenal vein cortisol gradient >2.3 sufficient to lateralise excess cortisol production to one side. All of the patients in this study had adrenalectomy guided by their AVS results; of the six patients who received unilateral adrenalectomy, none had recurrence of ACS (as shown by morning cortisol levels following overnight dexamethasone suppression test) at a mean follow-up of 36.1 months.

In a prospective study, Ueland *et al* (6) examined the use of AVS in 39 patients with adrenal lesions on CT imaging (both uni- and bilateral) and ACS. A metanephrine gradient was used to confirm catheter placement, and a high:low side adrenal vein cortisol gradient >2.3 was again used to confirm lateralisation. The results of AVS testing were also correlated with iodocholesterol scintigraphy in 18 patients, a method of imaging which has been utilised previously to demonstrate cortisol secretion from adrenal lesions. 11 of the 12 patients who underwent AVS-guided unilateral adrenalectomy in this study had resolution of excess cortisol production based on post-operative dexamethasone suppression testing during the 24-month follow-up period. In addition, iodocholesterol scintigraphy was concordant with AVS in 72% of cases.

The cortisol:aldosterone ratio has been used in an attempt to determine lateralisation in case reports by Wei *et al* (7) and Domino *et al* (8), although lateralisation was not demonstrated in these cases; it was also used successfully in a case series (9) which used a ratio of >2.0 to confirm lateralisation. In the setting of a normal aldosterone/renin profile, aldosterone may serve as a correction factor to account for dilutional differences between the adrenal veins.

Due to the lack of availability of the isotope required for iodocholesterol scintigraphy, we referred the patient to Monash Health for AVS. Results demonstrated bilateral ACS (adrenal:peripheral cortisol gradients >6.5 bilaterally). All high:low adrenal vein cortisol gradients were <2.0; the results were inconsistent, with the greatest lateralisation ratio (1.86, table 2) shown between the right and left common adrenal veins. When aldosterone was used as a correction factor for the lateralisation index, the maximal cortisol:aldosterone ratio was 6.5 (left:right common adrenal veins, table 2). Lateralisation results based on cortisol:metanephrine ratio were inconsistent. A decision was made to refer the patient for unilateral left adrenalectomy due to the larger size of the left-sided lesion and the greater cortisol:aldosterone ratio on the left.

In patients with ACS and bilateral adrenal lesions, using AVS to measure adrenal vein cortisol normalised to either aldosterone or metanephrines may help to lateralise the source of cortisol excess; however, a standard approach is lacking and the results may be inconsistent.

Key learning points:

- **Autonomous cortisol secretion is not an uncommon finding in patients with incidentally identified adrenal adenomas, with the optimal management made more challenging by the finding of bilateral adrenal pathology on imaging.**

- Adrenal venous sampling has been described as a method for lateralisation of excess cortisol production in this context, analogous to the procedure's use in evaluation of primary hyperaldosteronism.

- Challenges pertaining to the use of adrenal venous sampling for this indication include the lack of a standardised approach to biochemical confirmation of catheter placement, and the absence of an accepted correction factor to account for dilutional differences in cortisol between adrenal veins.

- Iodocholesterol scintigraphy is a potentially useful tool in this clinical scenario, however its use is precluded by limited

1. Fassnacht M, et al. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumours. European Journal of Endocrinology. 2016;175(2):G1-G34
2. Nieman LK, et al. The diagnosis of Cushing's syndrome: an endocrine society clinical practice guideline. Journal of Clinical Endocrinology and Metabolism. 2008;93(5):1526-1540
3. Terzolo M, et al. AME position statement on adrenal incidentaloma. European Journal of Endocrinology. 2011;164(6):851-870.
4. Nieman LK, et al. Treatment of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. Journal of Clinical Endocrinology and Metabolism. 2015;100(8):2807-2831.
5. Young WF, et al. The clinical conundrum of corticotropin-independent autonomous cortisol secretion in patients with bilateral adrenal masses. World Journal of Surgery. 2008;32(5):856-62.
6. Ueland GÅ, et al. Adrenal venous sampling for assessment of autonomous cortisol secretion. Journal of Clinical Endocrinology and Metabolism. 2018;103(12):4553-4560.
7. Wei J, et al. ACTH-independent Cushing's syndrome with bilateral cortisol-secreting adrenal adenomas: a case report and review of literatures. BMC Endocrine Disorders. 2018;18(22)
8. Domino JP, et al. Laparoscopic partial adrenalectomy for bilateral cortisol-secreting adenomas. Asian Journal of Surgery. 2007;30(2):154-7.
9. Papakokkinou E, et al. Adrenal venous sampling in patients with ACTH-independent hypercortisolism. Endocrine. 2019;66:338-348.

Conniption, Connundrum, Connfusion

Ruth Frampton[1], Anja Pluschke[1], Tim Greenaway[1]

1. The Canberra Hospital, Garran, ACT, Australia

Case report

We present the case of a 21 year old man referred for investigation of secondary causes of hypertension and found to have primary hyperaldosteronism with clear evidence of lateralisation on adrenal vein sampling, despite no radiological evidence of adrenal tumour or hyperplasia. He went on to have a laparoscopic adrenalectomy and remains normotensive six months post-operatively. Histopathology demonstrated micronodular adrenal disease.

Initial referral occurred after he presented to his General Practitioner with paroxysmal light-headedness, pre-syncopal symptoms and headache and was found to be persistently hypertensive. His medical history was significant for Systemic Lupus Erythematosus which had been diagnosed three years earlier after he presented with acute renal failure associated with joint pains and rash. His lupus was treated with hydroxychloroquine and methotrexate, and he had not had any flares since diagnosis. Renal function was normal. He had a mother with onset of hypertension before the age of 40 years. There was no other relevant family history.

Physical examination was unremarkable other than BP 147/88mmHg. Weight was 82.9kg Hypertension was confirmed by repeat testing, and home blood pressure monitoring showed systolic BP consistently 140-150mmHg.

Potassium level was normal at 4.3 mmol/L. A 24 hour Holter monitor had not shown any evidence of arrhythmia. Doppler Renal ultrasound was normal and in particular showed no evidence of renal artery stenosis, noting this did not exclude the diagnosis. Plasma metanephrines were normal.

The patient's aldosterone to renin ratio was found to be elevated at 124.0 (<70) with renin 9.6 mIU/L (4.4-46.1) and aldosterone 1190 pmol/L. He went on to a saline suppression test which showed failure to suppress with aldosterone 447 pmol/L (>170) following IV infusion of 2L normal saline over four hours during a seated procedure. He went on to have a triple phase CT abdomen which did not detect any adrenal abnormality.

Adrenal vein sampling was done without cosyntropin stimulation, which demonstrated lateralisation to the right. Both adrenal veins were successfully cannulated with a ratio of adrenal vein cortisol to peripheral cortisol greater than 2. The lateralisation index was > 4. The left adrenal had aldosterone to cortisol ratios of 1.1, 0.7 and 1.1, which were all less than the peripheral ratios of 1.6, 1.4 and 1.3 respectively.

Collection site	Time	Aldosterone (pmol/L)	Cortisol (nmol/L)	Aldosterone/Cortisol Ratio
L. adrenal 1	09:57	1360	1266	1.1
Peripheral	09:57	409	253	1.6
L. adrenal 2	10:00	1230	1756	0.7
Peripheral	10:00	371	261	1.4
L. adrenal 3	10:03	1760	1640	1.1
Peripheral	10:03	326	255	1.3
R. adrenal 1	10:25	6540	519	12.6
Peripheral	10:25	355	210	1.7
R. adrenal 2	10:30	22500	619	36.3
Peripheral	10:30	374	193	1.9
R. adrenal 3	10:37	4480	448	10.0
Peripheral	10:37	359	184	2.0
Low IVC	10:50	392	174	2.3
Peripheral	10:50	356	169	2.1

Figure 1: Adrenal Vein Sampling results

The patient then commenced antihypertensive treatment with olmesartan and was referred for surgical review. An elective laparoscopic right adrenalectomy was done. He had an uncomplicated admission and was discharged off all anti-hypertensive medication.

Macroscopically, the resected adrenal gland had generally normal size and appearance, measuring 60mm x 23mm x up to 5mm. Scattered subcapsular miniature nodules were visible.

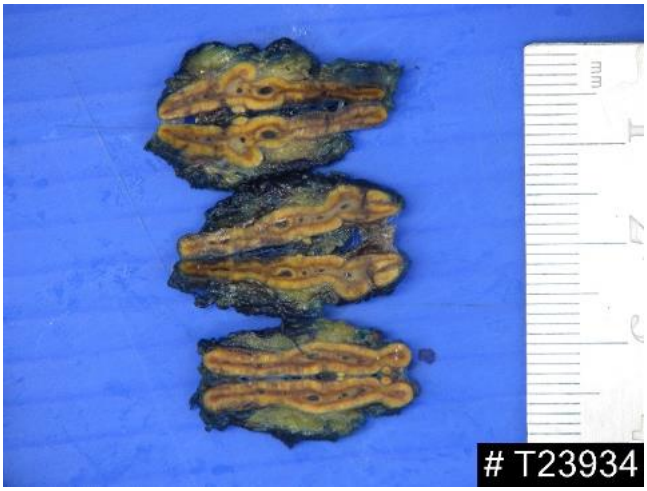


Figure 2: Macroscopic appearance of right adrenal gland

Microscopically there were small nodules of histologically unremarkable adrenocortical tissue in a subcapsular location and focally protruding into adjacent adipose tissue. In some areas, these nodules were entirely within adipose tissue and completely separate to the adjacent adrenal gland. All of the nodules had diameter less than 5mm. There was no necrosis and no mitoses seen. The histopathologic diagnosis was reported as micronodular hyperplasia.

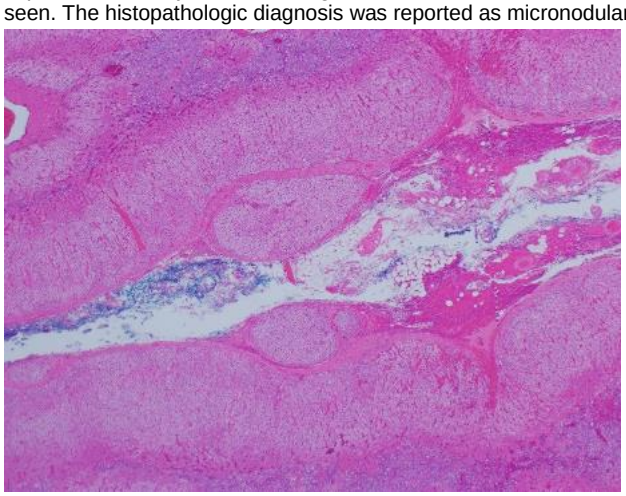


Figure 3: Microscopic appearance of right adrenal gland demonstrating micronodules

At clinic review four months post adrenalectomy the patient's blood pressure was 130/98mmHg. He reported improvement in his symptoms of pre-syncope and headache. His systolic blood pressure was below 130mmHg consistently on home monitoring.

Discussion

Primary aldosteronism describes a group of disorders in which production of aldosterone is inappropriately high for sodium status, fails to suppress after a sodium load, and is relatively autonomous of the other major regulators of secretion (angiotensin II and plasma potassium). It was initially described by Jerome Conn in 1955 and is a potentially curable cause of hypertension.

Though historically though to be a rare condition in which hypokalaemia was considered a *sine qua non* for diagnosis, recent cross-sectional and prospective studies report primary aldosteronism in greater than 5% of hypertension patients both in general and specialty settings, with prevalence of hypokalaemia estimated to be far from ubiquitous with estimates varying between 9 and 37%[2]. These data suggest that hyperaldosteronism may be underdiagnosed. However widespread screening with aldosterone to renin ratio is fraught given the multiple factors that can affect results, and the cost and expertise needed for confirmatory testing including adrenal vein sampling. Factors affecting aldosterone to renin ratio include posture, potassium status, dietary sodium intake, other hormones including ACTH, cortisol, and oestrogen, renal impairment, and multiple antihypertensive medications. Current Endocrine Society guidelines for case detection suggest screening with aldosterone to renin ratio in patients with sustained elevation of blood pressure >150/100 mmHg, or at lesser levels if multiple antihypertensive agents are required to achieve control. Screening is also recommended if hypertension associated with hypokalaemia, adrenal incidentaloma, sleep apnoea, or a positive family history (hypertension or stroke at age less than 40 years, or known primary hyperaldosteronism)[1].

Importantly, aldosterone excess has cardiovascular system effects at least partially independent of blood pressure effect. A 2018 meta-analysis of prospective and retrospective observational studies comparing patients with primary aldosteronism to those with essential hypertension found increased risk of stroke, coronary artery disease, atrial fibrillation, heart failure, diabetes, metabolic syndrome and left ventricular hypertrophy[3]. There was no difference demonstrated between patients with primary aldosteronism from unilateral aldosterone-producing adenoma or bilateral adrenal hyperplasia. Even after treatment

with mineralocorticoid antagonist, patients with primary aldosteronism have been found to have significantly higher incidence of cardiovascular events and death compared to age matched patients with essential hypertension and comparable cardiovascular risk profiles and blood pressure control[4]. Duration of hypertension is also a negative predictor of outcome after unilateral adrenalectomy, suggesting if surgical management is contemplated it is better to intervene earlier in the disease course.

The pathogenesis of primary aldosteronism remains incompletely understood. The most common cause of unilateral primary aldosteronism as demonstrated in our patient is an aldosterone producing adenoma. However in our case there was no evidence of adenoma on CT, and after resection the adrenals demonstrated generally normal appearance other than micronodules with a maximum dimension of 4mm. Proposed models for development of hyperaldosteronism include a neoplastic process where non-functional adenomas or hyperplasia progresses to become secretory, or a process whereby non-neoplastic secretory aldosterone producing cell clusters (APCCs), found to be commonly present in post mortem specimens, progress to a more severe neoplastic lesion[2].

It is unknown whether one, some or all of the micronodules present in our patient's right adrenal gland were aldosterone secreting, and whether similar micronodules are present in his left adrenal gland. Though at this stage our patient remains well and normotensive, it remains to be seen whether his remission from primary aldosteronism will be sustained or he will develop further contralateral secretory lesions with age. However this case demonstrates the utility of adrenal vein sampling in identifying unilateral disease that is not radiologically detectable, with likely significant benefits for his cardiometabolic risk into the future given his young age.

Discussion points

- Primary aldosteronism is likely underdiagnosed in all healthcare settings.
- Cardiovascular morbidity and mortality are higher than for age and sex matched controls with essential hypertension.
- Surgical management can be curative, even in the absence of an obvious adrenal lesion radiologically.

1. Funder JW, Carey RM, Mantero F, et al. The Management of Primary Aldosteronism: Case Detection, Diagnosis, and Treatment: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2016;101(5):1889-1916.
2. Vaidya A, Mulatero P, Baudrand R, Adler GK. The Expanding Spectrum of Primary Aldosteronism: Implications for Diagnosis, Pathogenesis, and Treatment.Endocr Rev. 2018;39(6):1057-1088.
3. Monticone S, D'Ascenzo F, Moretti C, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. Lancet Diabetes Endocrinol. 2018;6(1):41-50.
4. Hundemer GL, Curhan GC, Yozamp N, Wang M, Vaidya A. Cardiometabolic outcomes and mortality in medically treated primary aldosteronism: a retrospective cohort study. Lancet Diabetes Endocrinol. 2018;6(1):51-59.
5. Mulatero P, Monticone S, Bertello C, et al. Long-term cardio- and cerebrovascular events in patients with primary aldosteronism. J Clin Endocrinol Metab. 2013;98(12):4826-4833.

Hyogonadotrophic hypogondism and growth hormone deficiency in a man treated for Fanconi Anaemia

Gaurav Ghosh[1], Bu Yeap[1]

1. Fiona Stanley Hospital, Endocrinology, Perth, WA, Australia

Hypogonadotrophic hypogonadism and GH deficiency in a man treated for Fanconi Anaemia

Fanconi Anaemia (FA) is an inherited bone marrow failure syndrome characterised by pancytopaenia, predisposition to malignancy and physical abnormalities such as short stature, microcephaly, developmental delay, and café au lait skin lesions. Most patients with FA develop bone marrow failure requiring haematopoietic stem cell transplantation [1].

FA is caused by mutations in one of at least 17 different FA genes. In most cases, FA is inherited in an autosomal recessive manner via either homozygous or compound heterozygous mutations affecting an individual FA gene. There are two rare subtypes which are exceptions to this, FANCB gene mutation is X linked recessive and FANCR which is autosomal dominant [1]. FA is a rare condition with an estimated incidence of 1in 100 000-250 000 live births [2].

There are a wide range of endocrine disorders associated with FA and approximately 80 % of individuals with FA have at least one endocrine abnormality. Often these results from anatomical disruption of the hypothalamic-pituitary axis during development such as pituitary stalk interruption and septo-optic dysplasia but they can also be caused by hematopoietic stem cell transplantation associated therapies [3]. Endocrine abnormalities include short stature, growth hormone deficiency, abnormal glucose metabolism, dyslipidaemia, hypothyroidism and pubertal delay [3].

We discuss a case of a 20 year old gentleman with FA with hypogonadotrophic hypogonadism, growth hormone deficiency, type 2 diabetes, and subclinical primary hypothyroidism. Diagnosis of FA was confirmed at age 4 with bone marrow aspirate and chromosome fragility testing and he underwent stem cell transplantation at age 7.

He had delayed growth and short stature. Growth hormone deficiency was confirmed with serum IGF 1 testing, growth hormone sleep study and arginine stimulation test. He received growth hormone treatment between ages 11 and 18 with daily somatotropin injections. His final height was 164cm, slightly below mid parental height and approximately the third 3rd centile for height. He had delayed puberty and received 6 months of testosterone therapy which was discontinued due to evidence of endogenous puberty.

When assessed at age 19, weight was 68kg giving him a body mass index of 25.3. Examination found reduced testicular volume (5-6 ml bilaterally by orchidometer), sparse facial hair and Tanner stage II pubic hair. There was biochemical evidence of hypogonadotrophic hypogonadism with early morning testosterone by LCMS 4.3 nmol/L and LH 3.9 mU/L, FSH 7 mU/L, SHGB 16nmol/L. After discussion with treating clinician, the patient opted not to pursue fertility or sperm analysis at present and has recently commenced testosterone replacement with testosterone gel.

Growth hormone deficiency has been demonstrated biochemically on multiple occasions most recently with a glucagon stimulation test, demonstrating undetectable GH levels at baseline and all intervals after.

In terms of other pituitary hormones, he not had cortisol insufficiency previously and a recent short synacthen test was normal. He has had evidence of subclinical primary hypothyroidism with most recent TSH of 6.4 mU/L and T4 13 pmol/L. Prolactin levels have been normal, most recently 120mU/L.

He has a history of type 2 diabetes, diagnosed at age 19, controlled with metformin and sitagliptin. He has had other manifestations of FA including conductive deafness, cleft palate and bony deformities of the hands and skull.

A recent MRI scan of the pituitary demonstrated dysgenesis of the corpus callosum with pronounced thinning of the corpus callosum body, mild thinning of the splenium, normal bulk of the genu and absence of the septum pellucidum. The pituitary stalk was present but thin and displaced to the

right, contiguous with a small volume of adenohypophysis in the sella. Optic nerves, chiasm and tracts with present, with mild volume loss. Features are consistent with septo-optic dysplasia.

Despite having radiological features of loss of volume in the optic chiasm and optic nerves, visual acuity has been normal and he has had normal visual fields to confrontation .

He is followed up regularly in Endocrinology and haematology clinic. He is not working at present and is studying a computing course part time.

Discussion

Fanconi Anaemia is a rare genetic disorder which has multiple systemic manifestations beyond the haematological abnormalities. Most patients with FA will experience at least one endocrine complication and screening for and managing endocrine conditions across the lifespan is an important aspect of FA management.

The patient discussed has anatomical disruption of the hypothalamic pituitary axis with septo-optic dysplasia and has had multiple endocrinopathies related to FA; growth hormone deficiency, delayed puberty and hypogonadism, type 2 diabetes, and subclinical primary hypothyroidism [3].

Many different abnormalities on MRI brain are described in FA and these include abnormalities of the pituitary, corpus callosum, posterior fossa and optic chiasm.

The patient discussed had a small pituitary gland, hypoplasia of the corpus callosum and mild volume loss of the optic nerves and optic chiasm. A small volume pituitary gland is a common radiological finding in FA and structural abnormalities of the hypothalamo-pituitary axis account for some but not all of the endocrinopathies in FA [5]. Septooptic dysplasia is a disorder of early brain development characterised by optic nerve hypoplasia, abnormal development of the structures separating the left of and right halves of the brain such as the corpus callosum and septum pellucidum and pituitary hypoplasia. Septoptic dysplasia has estimated incidence of 1 in 10 000 overall. Abnormalities in different genes involved in embroyonic development of the brain such as HESX1, OTX2 and SOX2 are associated with septo-optic dysplasia, though the genetics of the condition are not fully understood [9]. Septo-optic dysplasia is reported as a CNS manifestation of FA, though its prevalence among FA patients is not well known [5].

The etiology of primary hypothyroidism which occurs in 60% of patients with FA is unknown [6].

Key points

Endocrine abnormalities are common in patients Fanconi Anaemia with around 80 % having one or more endocrine disorder

Anatomical disruption of the hypothalamic –pituitary axis during development is common and can result in result in insufficiency of pituitary hormones - most often growth hormone and gonadotrophin deficiency

Primary thyroid dysfunction, metabolic syndrome and disorders of bone mineral metabolism are other important manifestations of FA.

Regular assessment of growth, glucose and lipid metabolism, thyroid function, cortisol, puberty and gonadal function and bone density are important parts of FA management.

Hidden malignancies in large follicular thyroid neoplasms

Louise S Goodall[1], Sumathy Perampalam[1]

1. The Canberra Hospital, SYDNEY, NSW, Australia

Case 1

A 60-year-old male patient presented with right posterior chest wall pain. CT chest demonstrated a 112 x 63 mm right chest-wall lesion with destruction of the six, seventh and eight ribs and a pathological fracture of the T7 vertebral process. There were multiple bony metastasis on FDG-PET. Fine needle aspiration of the chest demonstrated likely primary thyroid cancer with oncocytic features**.** Thyroglobulin was >500 mcg/L.

He had undergone a right hemithyroidectomy in 2010 which was reported as 7cm "oncocytoma" with no evidence of malignancy. On retrospective interrogation of histopathology from 2010, it was thought there was a single focus of vascular invasion consistent with minimally invasive Hurthle cell carcinoma.

Thyroid ultrasound showed a suspicious hypoechoic nodule with one focus of calcification in the left thyroid bed measuring 1.9x1.6x1.6cm. He underwent completion left thyroidectomy. Histopathology revealed a 2.4 cm minimally invasive Hurthle cell carcinoma, focal capsular without vascular invasion. Thyroxine was commenced to supress TSH. He underwent radiation therapy to his posterior chest lesion and had radio-iodine therapy 200 mCi.

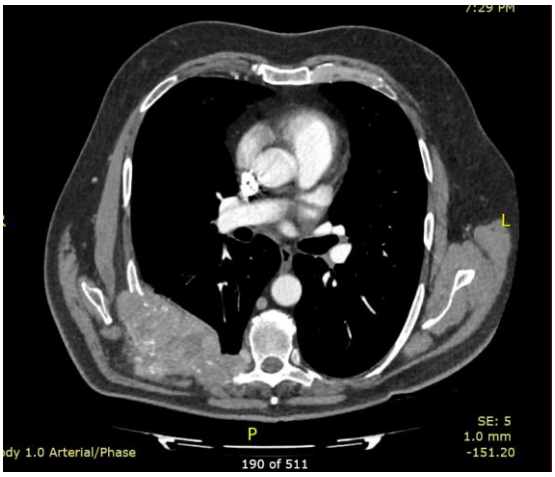


Figure 1 – CT demonstrating right seventh rib with large vascular soft tissue component measuring 12 cm in diameter

Case 2

A 68-year-old male was investigated for a chronic cough. A chest x-ray and CT chest confirmed multiple cannon-ball lesions with the largest measuring 14 x 8 x 7 cm in the right lobe. There were no other distant metastatic lesions identified on whole body CT and FDG PET scan. An ultrasound of the neck showed no radiological evidence of residual thyroid tissue and no mass lesions at the site of the thyroid bed or lymphadenopathy.

He had a background of total thyroidectomy for goitre in 2006. Histopathology was reported as right lobe 140 x 80 x 70 mm, completely occupied by a well-circumscribed light tan nodule with cystic and fibrotic areas consistent with benign follicular adenoma with no evidence of malignancy. Slides were not available for re-evaluation.

He underwent a biopsy of his lung lesion confirming metastatic neoplasm with features of thyroid origin with strongly positive PAX8, TTF-1 and thyroglobulin. His thyroglobulin was 1780 mcg/L (<30), consistent with metastatic thyroid carcinoma. After discussions with our cardio-thoracic surgical colleagues, resection of the largest the lesion was not recommended. He received RAI 200mCi and his lesions were all RAI avid.

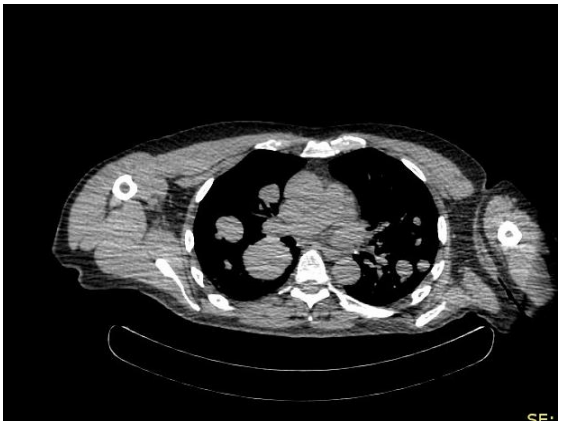


Figure 2 – Chest CT demonstrating innumerable bilateral lung metastases, largest in the inferior aspect of the right upper lobe measuring 66 x 73 mm

Discussion:

Follicular thyroid carcinoma is the second most prevalent form of thyroid cancer after papillary thyroid carcinoma, accounting for between 5-20% of all thyroid malignancies.[i] Despite locoregional lymphatic spread being rare (1-7%)[ii], haematogenous spread to lung and bone occurs more often (6-20%).[iii] Although metastatic disease may not be clinically evident for many years, such follicular neoplasms are usually large with evidence of capsule or vascular invasion or considerable cellular pleomorphism.[iv] Whilst minimally invasive follicular carcinomas have an excellent prognosis with cure rates of between 95-100%, widely invasive forms have a poorer prognosis with higher risk of recurrence and metastasis, and higher mortality.[v] The archaic diagnosis of 'benign metastasising goitre' arose due to the difficulty in recognising vascular invasion of follicular neoplasms after complete resection.

Ultrasound features are helpful in distinguishing papillary thyroid carcinoma (PTC), they are less useful in the diagnosis of follicular thyroid carcinoma. Cytology is also limited in its ability to distinguish between follicular thyroid carcinoma and benign thyroid adenomas as the same oncogenic drivers are found in follicular thyroid carcinomas and follicular adenomas. Utility of cytological biomarkers or mutations including B-galactose binding protein galectin-3, HMBE-1, PAX-PPARy translocations and RAS mutations is limited in the differentiation between follicular thyroid adenomas and carcinomas pre-operatively.[vi] Definitive classification of follicular neoplasms predominately requires post-operative histological diagnosis and examination of capsular and vascular invasion.[vii]

Despite advances in tumour markers, currently histological analysis of a very large number of tumour capsule samples is required to identify vascular invasiveness and malignant potential. However, minimally invasive carcinomas are often diagnosed with less than a millimetre of tissue differentiating them from adenomas and non-invasive thyroid neoplasm. Yamashima et al evaluated 14 encapsulated follicular neoplasms with extensive dissection concluded that although labour intensive, multiple sections produced by extensive dissection helped identify minute but definite evidence of angioinvasion.[vii]

There is debate internationally as to how much capsule should be examined to ensure a thorough examination, however complete microscopic examination of the tumour capsule is essentially impossible,[viii] and there is a high rate of intra-observer variation at histopathological diagnosis.[ix] A consensus for adequate sampling has not been established by the College of American Pathologists or the World Health Organization. [x] Current practice varies between submitting selected sections of the follicular capsule after gross examination for microscopic evaluation to the extreme approach of submitting the entire capsule for microscopic evaluation. With increasing use of molecular diagnostics, we may have a better appreciation of the underlying tumour biology as reflected by the histopathology.

In summary, large follicular lesions may harbour small focus of angioinvasion which may have not been sampled for microscopic evaluation. Until histopathological assessment improves, possibly combining immunohistochemistry and molecular diagnostics, yearly thyroglobulin assessment in the medium to long term may be prudent, regardless of if capsular or vascular invasion is identified.

Take home point:

- Long-term follow-up with thyroglobulin for late recurrence is warranted for large follicular lesions, regardless of if capsular or vascular invasion is identified.

[i] Hundahl SA, Cady MP, Cunningham MP, Mazzaferri E, McKee R, Rosai J, Shah JP, Fremgen AM, et al. Initial results from a prospective cohort study of 5583 cases of thyroid carcinoma treated in the United States during 1996. Cancer 2000; 89 (1): 202-217

[ii] Alfalah H, Crashaw I, Jany T, et al. Risk factors for lateral cervical lymph node involvement in follicular thyroid carcinoma. World J Surg 2008; 32 (12): 2623-2626

[iii] Parameswaran R, Hu JS, Min En N, et al. Patterns of metastasis in follicular thyroid carcinoma and the difference between early and delayed presentation. Ann R Coll Surg Engl 2017; 99 (2): 151-154

[iv] Mizukami Y, Nonmura A, Hayashi Y, Ohmura K, Michigishi T, Nogughi M, Nakamura S, Kshizaki T. Late bone metastasis from an encapsulated follicular carcinoma of the thyroid without capsular and vascular invasion. Pathology international 1996 (46): 457-461

[v] DeMay, RM. Follicular lesions of the thyroid. W(h)ither follicular carcinoma? Am J Clin Pathol 2000; 114: 681-683

[vi] Yamashina M. Follicular neoplasms of the thyroid. Total circumferential evaluation of the fibrous capsule. AmJ Surg pathol 1992; 16 (4): 392-400.

[vii] Staubitz JI, Musholt PB, Musholt TJ. The surgical dilemma of primary surgery for follicular thyroid neoplasms. Best Pract Res Clin endocrinol Metab. 2019; 33 (4): 101292.

[viii] Glomski K, Nose V, Faquin C, Sadow PM. Metastatic follicular thyroid carcinoma and the primary thyroid gross examination: Institutional review of cases from 1990 to 2015. Endorine Pathol 2017; 28: 177-185.

[ix] Hirokawa m, Carney JA, Goellner JR, DeLellis RA, Heffess CA, Katoh R, Mashahiko T, Kennichi K. Observer variation of encapsulated follicular lesions of the thyroid gland. Am J Surg Pathol 2002 (26): 1508-1514

[x] Seethala RR, Asa SL, Carty SE, et al. College of American Pathologists (CAP) protocol for examination of specimens from patients with carcinomas of the thyroid gland. Jan;2016 http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution%20Folders/WebContent/pdf/cp-thyroid-16protocol-3200.pdf

Was pain relief worth it?

Debra DM Gordon[1], Christopher C Gilfillan[2, 1], Rosemary R Wong[2]

1. Eastern Health, Department of Endocrinology, Box Hill Hospital, Melbourne, Victoria, Australia

2. Department of Endocrinology, Box Hill Hospital, Melbourne, Victoria, Australia

A 45 year old man, with 2 children, known to have depression and anxiety presented with a 2 year history of fatigue and lethargy and a recent history of loss of libido and a decreased need to shave. There was no associated visual disturbance or headaches and no complaints suggestive of a systemic aetiology for his symptoms, and no polydipsia/polyuria. Other than a history of previous heroin dependence some years before, there was no history of current illicit substance use. He had been taking various opioids and analgesia for some time for relief of back pain.

His clinical examination revealed bilateral gynaecomastia, sparse body hair, but normal testicular volume. Visual fields were intact. BMI 35kg/m2, BP 129/81 mmHg(lying), 115/75mmHg (standing).

Breast ultrasound confirmed gynaecomastia L>R.. Beta HCG was normal and oestradiol was low. Bioochemistry revealed profound hypogonadism, hypocortisolaemia, low serum TSH with low-normal fT4 suggestive of secondary hypothyroidism. Iron studies, angiotensin converting enzyme, copper and calcium were normal.

	Oct 2019 at diagnosis	August 2020 on recovery
Cortisol (172-497 nmol/L)	27	419
ACTH (1.6-13.9 pmol/L)	0.9	
TSH (0.5-5.5 mIU/L)	0.22	0.19
T4 (9-19 pmol/L)	11.1	14.6
PRL (90-400 units)	141	
TESTOSTERONE (9-27nmol/L)	<0.4	19.5
FSH (1.5-9.7 IU/L)	3.5	1.1
LH (1.8-9.2 IU/L)	7.0	0.8
IGF1 (12-34 nmol/L)	14	
GH mg/L	1.1	

An MRI of the pituitary and stalk was normal.

He required anterior pituitary hormone replacement with hydrocortisone, thyroxine and testosterone, which resulted in clinical improvement. As he gradually came off opioid medications over the next 10 months, his pituitary axis recovered fully. He was able to cease hydrocortisone, thyroxine and testosterone, and he remains well.

This case illustrates the need to consider rarer and unusual aetiologies of hypothalamic-pituitary axis (HPA) suppression that may be reversable. Many drugs affect the HPA, but usually do not affect all anterior hormones simultaneously.

The pulsatility and secretion of GnRH is under the control of many neuro-hormonal mechanisms, which include various neurotransmitters, endogenous opioids and steroid hormones. It has long been recognised that exogenous opioids exert various effects on this axis, either by inhibiting the release of GnRH, or by decreasing the responsiveness of FSH to negative feedback from sex steroids. The resultant effect is that LH is lower and sex steroids are suppressed, but FSH levels are only minimally affected. In addition to this effect, testosterone is under the control of LH, which too, is suppressed by exogenous opioids. This complex interaction of opioids causes overwhelming hypogonadism. (1)

The cortisol endocrinopathy associated with chronic opioid use, is more complex , as studies have indicated suppression of the cortisol axis with sustained oral morphine doses of 30-240mg /day, makes it likely that many people may be affected by chronic opioid use and under-recognized by clinicians. Still, not everyone with a low serum ACTH and cortisol level will need steroid replacement as stimulation studies have previously shown that the cortisol axis, although abnormal at baseline, may still be intact.(3)

Hyperprolactinaemia, not demonstrated in our case, is also a recognised consequence of opioid use despite the poorly understood mechanisms for this; however, this may have additional effects on hypogonadism and bone health. Opioids have a complex effect on the growth hormone axis in humans - the net effect is mild stimulation of GH release.(4)

Different opioids have shown different effects on thyroid hormone levels, the mechanism may be due to alterations in thyroid binding globulin, rather than direct effect on the HPA axis (5). Irrespective of this, clinically significant thyroid dysfunction is unusual.(5)

In summary, opioid suppression of the HPA can be a diagnostic challenge. This case illustrates opioid-induced hypopituitarism necessitating the need for anterior hormone replacement temporarily and alerts the clinician to the complex interplay of opioids on the HPA axis and the possible reversibility of the condition.

References:

1. Seyfried O. Hester J. Opioids and endocrine dysfunction. Br J Pain. 2012. Feb; 6 (1):17-24
2. Rhodin A. Stridsberg M. Gordh T. Opioid Endocrinopathy: a clinical problem in patients with chronic pain and long - term oral opioid treatment. Clin J Pain.2010.Jun;26(5)374-80
3. Palm S. Moenig H and Maier C. Effects of oral treatment with sustained release morphine tablets on hypothalamic-pituitary-adrenal axis. Methods Find ExpClin Pharmacol.1997 May;19(4):269-73
4. Delitala G. Thomasi PA.Palermo M. Ross RJM. Grossman A. Besser GM, Opioids stimulate GH release in man Independantly of GH-Releasing Hormone. JCEM.1989;69(2):356-358
5. Gozashi M. Mohammadzadeh E. Divsalar K. Shookoohi M. The effect of opium addiction on thyroid function tests.J Diabetes Metab Disord. 2014;13:5. doi:10.1186/2251-6581-13-5
6. Fountas A. Van Uum S. Karavitaki N. Opioid induced endocrinopathies. Lancet Diabetes Endocrinol. 2020 Jan;8(1)68-80

A clinically silent but aggressive, poorly differentiated Pit-1 lineage pituitary macroadenoma

Su Win Htike[1], Thomas Robertson[2], Thomas Dover[1]

1. Department of Endocrinology and Diabetes, Mater Hospital Brisbane, Woolloongabba, QLD, Australia

2. Department of Anatomical Pathology, Royal Brisbane and Women's Hospital, Brisbane, Queensland, Australia

Introduction

Silent pituitary adenomas are now being classified according to adenohypophyseal hormones and transcription factors in the latest 2017 WHO Classification of pituitary tumors[1-2]. Previously identified silent subtype 3 adenomas can be redefined as aggressive monomorphous plurihormonal adenomas of Pit-1 lineage, which are often non-functioning but may be associated with hyperthyroidism, acromegaly or galactorrhoea and amenorrhoea.

Case

A 19-year old man attended our Young Adult Endocrine clinic in February 2020 for a follow-up review of his pituitary macroadenoma. The patient was diagnosed with a non-functioning pituitary macroadenoma in August 2017 after presenting to Queensland Children's Hospital with worsening diplopia for 5 weeks. Radiologically, it was a homogeneous sellar mass (29x33x26mm) extending into the suprasellar region with bilateral cavernous sinus involvement (Figure1). Biochemically, he was found to have secondary adrenal and testosterone deficiency. However, his free T3 was marginally elevated with unsuppressed thyroid-stimulating hormone (TSH) and his thyroid ultrasound was unremarkable.

Initially, he was started on hydrocortisone and he underwent elective trans-sphenoidal 50% debulking surgery, which was uneventful. The histopathology report was consistent with atypical pituitary adenoma with proliferative activity (Ki-67 proliferation fraction 5% and up to three mitoses per 10 high power fields) and focal immunoreactivity for growth hormone. Despite that, his pre-surgery insulin-like growth factor 1(IGF-1) and growth hormone were within the range at 20 nmol/L (16-66 nmol/L) and 0.53 ug/L (0.05-3 ug/L) respectively. He was followed up regularly at Children's Hospital and replaced with hydrocortisone and intramuscular testosterone therapy. The rest of the anterior pituitary hormones were within the range.

In late 2018, he was referred to the Young Adult Endocrine team at Mater Hospital. Unfortunately, from mid-2018 to mid-2019, his repeat MRI revealed a gradual increase in the size of adenoma up to 22x25x28 mm extending to the right sphenoidal sinuses and positive mass effect on posterior pituitary and infundibulum. His free T3 was mildly elevated at the time of tumor regrowth. Subsequently, the patient had volumetric modulated arc therapy (VMAT) based salvage radiotherapy (50.4 Gray units in 28 fractions) in December 2019.

Throughout the reviews up to August 2020, the patient did not report any symptoms suggestive of uncontrolled endocrinopathy. The examination was similarly unremarkable. Moreover, he does not have any family history or clinical and biochemical evidence suggestive of multiple endocrine neoplasia (MEN). His last 2 MRI pituitary in March and June 2020 demonstrated an interval decrease in size reflecting a favorable response to radiotherapy.

Surprisingly, his free thyroid hormones were gradually rising with inappropriately normal TSH as depicted in Table 1 attached. His thyroid function tests were repeated in three different labs with different analytical platforms but they result in a similar pattern ruling out heterophile antibodies interferences. The rest of his recent anterior pituitary hormones can be reviewed in Table 2.

After review by the neuropathologist, immunohistochemistry for TSH was repeated on the 2017 biopsy material, which again demonstrated no discernible staining. However, the tumor showed strong diffuse staining for the transcription factor Pit-1 but was negative for GATA2 and estrogen receptor, confirming a poorly differentiated Pit-1 lineage pituitary adenoma. Although often silent, these adenomas can present with hormone excess, most commonly hyperthyroidism, and is probably the cause of the patient's central hyperthyroidism.

Thyrotropin-releasing hormone stimulation test resulted in blunted TSH response consistent with TSH producing pituitary macroadenoma (Table 3). This is further supported by evidence of elevated serum glycoprotein alpha-subunit at 2.12 IU/L (reference range 0.0-0.7).

The case was discussed in the monthly multidisciplinary pituitary meeting as clear guidelines are not widely available for this challenging and complex case. We would initiate the treatment with somatostatin analog subcutaneous injection to restore euthyroidism. The literature review also mentions that somatostatin analog treatment is a viable option as silent thyrotropinomas express somatostatin receptor (SSTR) [4]. In addition, he may likely require repeat surgery as the definitive treatment depending on his tumour growth and escape of thyroid function tests over time because there are a few cases reported with the resolution of hormonal excess post repeat surgery[5].

Learning points

- Immunoreactivity of various anterior pituitary hormones, pituitary lineage transcription factors, the ultrastructural features, and histological features can define pituitary adenomas. Moreover, they are helpful in the prediction of the disease course and response to adjunctive therapies.
- The standardized report for diagnosis of pituitary tumors, taking account of the invasion, the immunohistochemical (IHC) profile, and the proliferative markers have been recently proposed by the European Pituitary Pathology Group (EPPG)[3].

- 9% of silent pituitary adenomas are Pit-1 (GH/Prolactin/TSH) lineage positive[4].
- Pit-1 lineage adenomas possess aggressive characteristics with hormonal excess histologically and biochemically, though they can be clinically silent or borderline.
- They can be associated with multiple endocrine neoplasia type 1 syndrome in younger patients particularly if hyperprolactinemia is present[5].
- Mitotic count, Ki-67 proliferative index, and tumor suppressor gene p53 act as general histologic prognostic markers in pituitary neuroendocrine tumors[6].
- Multidisciplinary team effort is essential for a precise diagnosis, effective treatment with appropriate follow-up, and the best outcomes.
- Further studies and research are required in the areas of novel prognostic markers, emerging imaging, therapeutic guidelines, and drugs for managing challenging cases of complex and aggressive pituitary macroadenomas.

Table1: Thyroid hormone changes throughout the follow-up

Tests	Aug 2017	Aug 2017 (Post-op)	Dec 2017	Aug 2018	Sept 2019 (Relapse)	Feb 2020	March 2020	July 2020	Aug 2020
TSH (0.5-4.5 mU/L)	2.7	1.6	1.4	2.2	1.6	2.2	2	2.8	2.85
Free T4 (10-20 pmol/L)	18	14	13	14	17	**29**	**20**	**27**	**21**
Free T3 (2.8-6.8 pmol/L)	**9.9**				**8.2**	**10**	**9.5**	**12.5**	**12.8**

Table2: Recent pituitary hormones profile of the patient

Tests July 2020 At 11:00 am	ACTH (1.1-11.1 pmol/L)	Cortisol (160-520 nmol/L)	FSH (1-10 IU/L)	LH (1-10 IU/L)	Testosterone (10-33 nmol/L)	Prolactin (<300 mIU/L)	IGF-1 (20-45 nmol/L)
	1.2	250	3	2	8	98	12

Table3: Thyrotropin-releasing hormone (TRH) stimulation test

Tests	**Before TRH**	**20 mins after TRH**	**40 mins after TRH**	**60 mins after TRH**
TSH (0.5-4.5 mU/L)	2.53	2.34	2.32	2.32
Free T4 (10-20 pmol/L)	18			
Free T3 (2.8-6.8 pmol/L)	**10.4**			

Figure1: T2 Coronal view comparison of pituitary macroadenoma Pre (left) and post-surgery (right)

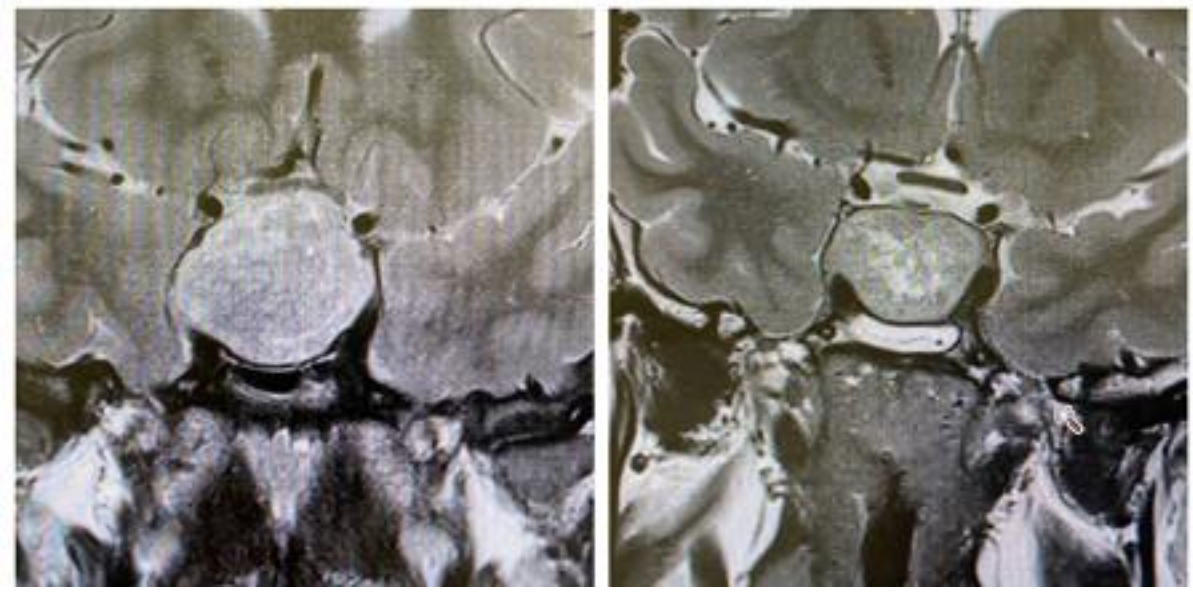


Figure 2: Pit 1 staining

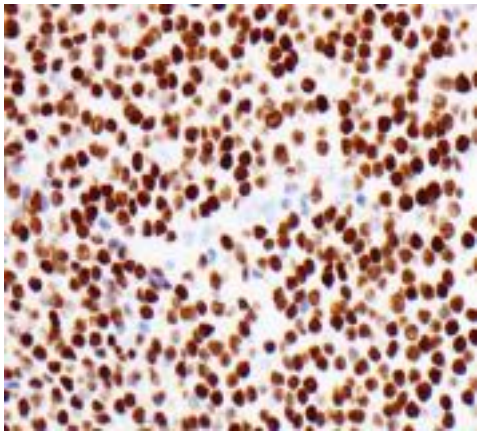


Figure3: HE staining with mitosis center

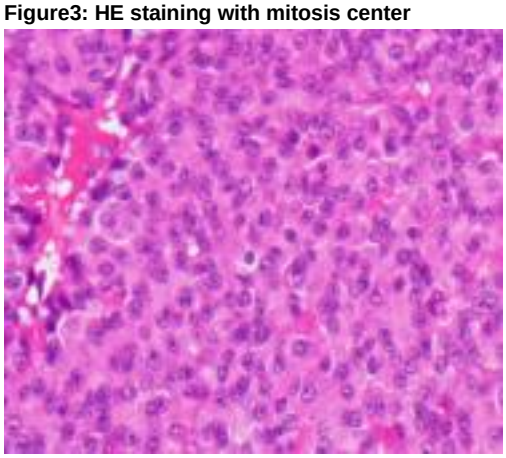


Figure 4: GH staining

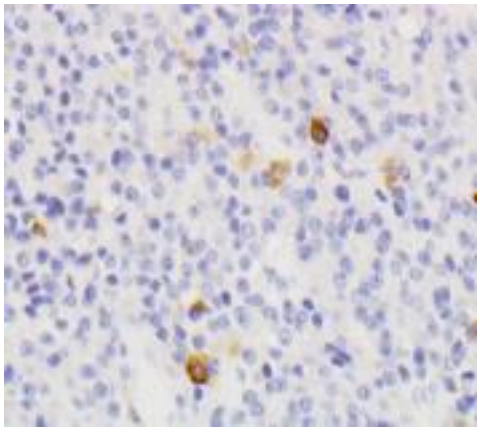
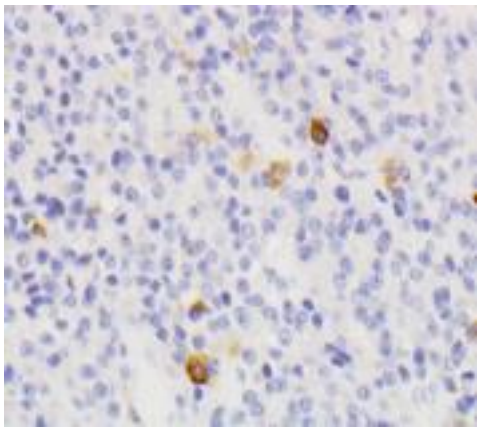


Figure 5: TSH Staining with mitosis center



1. Lloyd R, Osamura R, Kloppel G, Rosai J, eds. World Health Organization Classification of Tumours of Endocrine Organs, 4th ed. Volume 10. Lyon, France: IARC Publication; 2017.
2. Mete O, Lopes MB. Overview of the 2017 WHO classification of pituitary tumours. Endocr Pathol. 2017;28(3):228-243.
3. Trouillas J.; Jaffrain-Rea M.; Vasiljevic A.; Raverot G.; Roncaroli F.; Villa C.; How to classify Pituitary Neuroendocrine Tumours (PitNet)s in 2020, Cancers 2020, 12, 514; doi:10/3390/cancers12020514.
4. Drummond, J.; Roncaroli, F.; Grossman, A.B; Korbonits, M. Clinical and Pathological Aspects of Silent Pituitary Adenomas: A systemic review, J Clinic Endocrinol Metab, July 2019, 104(7)L2473-2489.
5. Mete et al, Silent subtype 3 pituitary adenomas are not always silent and represent poorly differentiated monomorphous plurihormonal Pit-1 lineage adenomas, Modern Pathology. 2016;29, 131-142.
6. Manojlovic-Gacic, E.; Endstrom, B.E.; Casar-Borota, O. Histopathological classification of non-functioning pituitary neuroendocrine tumors, Pituitary, 2018;21:119-129.

Genetic Hypoglycaemia: A Molecular Approach

Anojian Koneshamoorthy[1], Dilan Seniveratne-Epa[1], Stephen Farrell[1], Thomas Loudovaris[2], Helen Thomas[2], Richard MacIsaac[1], Nirupa Sachithanandan[1], Bala Krishnamurthy[1, 2]

1. St. Vincent's Hospital Melbourne, Fitzroy North, VIC, Australia

2. St. Vincent's Institute of Medical Research , Melbourne

Case Presentation:

A 22-year-old man was referred by a regional hospital for evaluation of asymptomatic hypoglycaemia (plasma glucose 2.4 mmol/L). His birthweight was 4.2 kilograms and his significant medical history included congenital hydrocephalus, congenital bilateral optic atrophy and obesity. There was no history of bariatric surgery. His mother had a partial pancreatectomy at age 6 for hypoglycaemic seizures, with development of diabetes in her fifth decade.

Initial investigations yielded random plasma glucose of 2.3 mmol/L (3.0 – 7.7 mmol/L), C-peptide of 0.9 pmol/L, insulin of 17.8 mU/L, proinsulin of 37.1 pmol/L, 3-hydroxybutyrate <0.01 mmol/L (0 – 0.61 mmol/L) and HbA1c of 3.6%. Sulphonylurea screen and insulin antibody was negative. Morning cortisol was 265 nmol/L with IGF-1 of 18 (12 – 42 nmol/L) (figure 1).

A 72 hour fast demonstrated endogenous hyperinsulinaemic hypoglycaemia. The fast ended at 10-hours with plasma glucose of 1.9 mmol/L, C-peptide of 3.51 pmol/mL, beta-hydroxybutyrate of 0.05 mmol/L and insulin of 7 mU/ml. Glucagon administration led to an increase in plasma glucose to 6.1 mmol/L (figure 2). Localisation studies including CT pancreas triple phase study, MRI pancreas, endoscopic ultrasound of pancreas, GLP-1 labelled PET scan and Dotatate PET scan did not reveal a pancreatic lesion.

A selective arterial calcium stimulation test localised areas of excess insulin production to the body and tail of the pancreas (figure 3). In consultation with the endocrine surgical unit at our hospital, the body and tail of pancreas (about 70% of his pancreas) was removed and spleen was preserved. An intraoperative ultrasound did not reveal any pancreatic lesions. The partial pancreatectomy did not correct hypoglycaemia. During the diagnostic evaluation and postoperatively, he was treated with verapamil and diazoxide, with minimal improvement in hypoglycaemia. Octreotide was commenced and blood glucose levels were maintained above 4 mmol/L.

Histopathology revealed diffuse nesidioblastosis. Islets were isolated for functional studies and in vitro stimulation of islets with glucose revealed a response of marked increase in intracellular calcium, a surrogate marker for insulin regulation (figure 4). Islets were also subjected to an unbiased gene expression analysis using 10x single cell sequencing and was compared with gene expression from islets isolated simultaneously from an organ donor. The patient's beta cells expressed significantly more insulin than the control subject. Despite the histopathological nesidioblastosis, there was no difference in the proportion of beta cells in the invitro islets and no difference in the expression of genes determining the cell cycle.

Genetic testing was performed in the patient and his mother. This revealed a Glucokinase (GCK) mutation (c.269A>C p.(Lys90Thr)(figure 5) which was predicted to a pathological mutation by SIFT, Align-GVGD and PolyPhen-2. His sister, who had no known glycaemic issues, developed gestational diabetes and during her glucose monitoring, her fasting glucose levels were between 3.5 and 4.5 mmol/L. She delivered a baby of normal weight who developed neonatal hypoglycaemia, responsive to diazoxide. Both have subsequently been tested for this GCK mutation and are awaiting results (Figure 6).

Discussion:

Here we report an adult with noninsulinoma pancreatogenous hypoglycaemia syndrome (NIPHS) with nesidioblastosis due to GCK mutation. NIPHS is characterised by endogenous hyperinsulinaemic hypoglycaemia which is not attributed to an insulinoma.[1] Pancreatic histology from patients with NIPHS typically show beta cell hypertrophy, enlarged and hyperchromatic islet nuclei and increased islets budding from periductular epithelium, characteristic of nesidioblastosis, which were all apparent in our patient.[2]

Congenital hyperinsulinism (HI) is the most common cause of hypoglycaemia in children.[3,4] There are 11 genes associated with monogenic forms of HI (ABCC8, KCNJ11, GLUD1, GCK, HADH1, UCP2, MCT1, HNF4A, HNF1A, HK1, PGM1) along with syndromic conditions such as Beckwith-Wiedemann and Turner syndromes.[4] The molecular aetiology is not known in approximately 45% cases.[4]

GCK is the third most common gene associated with HI.[4] Dominant missense-activating mutations of GCK lower glucose threshold for insulin secretion, thus resulting in fasting hyperinsulinaemic hypoglycaemia.[5] Affected children tend to have macrosomia and present with severe hypoglycaemia at birth.[4]

GCK (also known as hexokinase IV) enables phosphorylation of glucose, the rate-limiting step of glycolysis in the liver and pancreas.[6] GCK shares extensive sequence identity with the three other human hexokinase isozymes. Despite these similarities, GCK is considered the primary glucose sensor as small fluctuations in its activity alter glucose-stimulated insulin secretion from pancreatic β-cells.[7] GCK's midpoint of glucose responsiveness ($K_{0.5}$) is ~30-fold lower than that of homologous isozymes (7 mmol/L for GCK vs. ~0.2 mmol/L for hexokinases I-III), which closely matches physiological, circulatory glucose concentrations.[7] Unlike the other hexokinases, GCK is not susceptible to feedback inhibition by physiological concentrations of its product glucose 6-phosphate.

GCK has been detected in the pancreas, liver, gut and the brain and is implicated in the regulation of carbohydrate metabolism. It acts as a glucose sensor in pancreatic beta cells and promotes the synthesis of glycogen and triglycerides in the liver.[8] The importance of precise control over GCK activity is emphasised by disease phenotypes resulting from mutations in the human GCK locus.[7] Maturity onset diabetes of the young type 2 and permanent neonatal diabetes mellitus are caused by heterozygous inactivating GCK mutations.[7] Conversely, activating GCK mutations produce persistent hyperinsulinemic hypoglycemia of infancy, with disease severity correlating with the level of enzyme activation.[7] Adults who are identified with activating GCK mutations are usually diagnosed as part of family screening following an identified neonate.[9]

GCK is regulated in the liver by liver-specific glucokinase regulatory protein (GKRP). Upon formation of the inhibitory complex with GKRP, GCK is sequestered into the hepatocyte nucleus after forming inhibitory complex with GKRP.[7] GKRP-mediated inhibition is modulated by several phosphorylated carbohydrates. The importance of GCK in glucose metabolism and disease has stimulated much clinical interest to develop activators of the enzyme. A variety of molecules that stimulate GCK have been identified, however a viable therapeutic agent has not yet developed.[7]

Despite carrying the same GCK mutation, the clinical presentation in the family we report on was vastly different among affected relatives. One possible explanation for this phenomenon is that there may be individual differences in the way GKRP mediates GCK inhibition in individuals. It is also interesting that increased insulin secretion should cause nesidioblastosis in a patient with a GCK mutation. A possibility could be that local high insulin concentration in islets could saturate insulin receptors and excess insulin could bind to IGF-1, inducing beta cell proliferation. More detailed analysis of our 10x sequencing results with more control subjects is required to explore further.

Diazoxide is considered the first line medication for alleviating hypoglycaemia in congenital hyperinsulinism.[4] However, in patients with GCK activating mutations, this is often not an effective therapy. Octreotide may be useful in individuals non-responsive to diazoxide.[4] In summary, this case highlights the variable phenotype of GCK mutations and despite its congenital nature, it may not become clinically apparent until adulthood.

Conclusion:

- Testing for genetic mutations which are linked to congenital hyperinsulinism should be considered in adults with NIPHS
- Individuals with the same GCK mutation may present with variable phenotypes
- In depth analysis using modern technology may reveal the pathogenesis of nesidioblastosis

Test	Value	Reference range
Plasma Glucose	2.3	3.0 – 7.7 mmol/L
C-Peptide	0.904	<0.7 pmol/L
Insulin	17.8	3- 25 mU/L
Pro-Insulin	37.1	<13 pmol/L
3-Betahydroxybutyrate	<0.01	0 – 0.61 mmol/L
HbA1c	3.6	4.0-6.0%
Sulphonylurea screen	Negative	
Insulin antibodies	Negative	
TSH	3.65	0.5-4.7 mIU/L
Free T4	13.9	11.5-22.7 pmol/L
Cortisol	265	nmol/L
IGF-1	18	12-42 nmol/L
HGH	<0.1	ug/L
Amino acids	Non diagnostic profile	
Lactate	1.5	0.5-2.2 mmol/L
Ammonia	55	16-53 umol/L

Figure 1. Initial Investigations

Time	Glucose (mmol/L)	Insulin (mU/L)	C-peptide (pmol/L)	Pro insulin (pmol/L)	B-hydroxy butyrate (mmol/L)	Comments
1200 (+ 0000 hrs)	3.8					Fasting from 1200
1800 (+ 0600 hrs)	2.5	24	1.26	>100	0.08	
2000 (+ 0800 hrs)	2.3	7	0.94	37.1	0.05	
2200 (+ 1000 hrs)	1.9	7	3.59	-	0.05	Given IV glucagon 1mg No symptoms
2210 (+ 1010 hrs)	6.1					
2220 (+ 1020 hrs)	5.2					

Figure 2. 72 Hour Fast

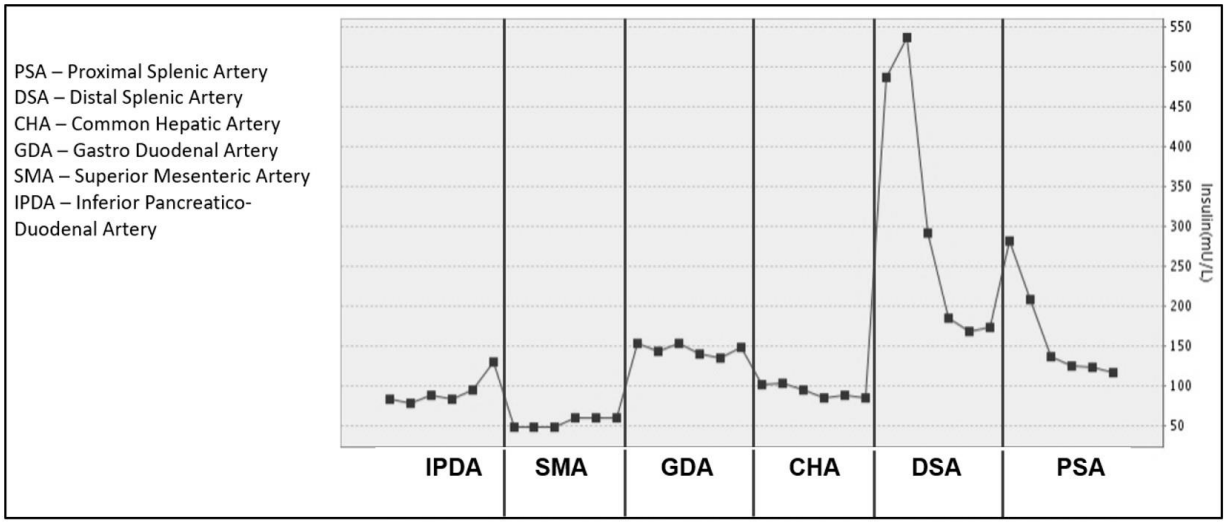


Figure 3. Insulin levels obtained during selective arterial calcium stimulation test

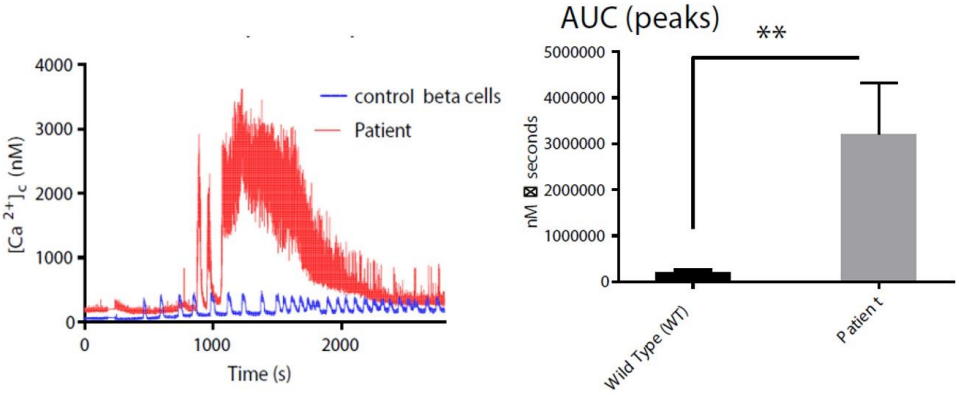


Figure 4 - Significant rise in intracellular calcium level (a surrogate marker of intracellular insulin) in response to the injection of 15 mM of glucose. AUC – Area under the curve

Variant details

Gene	Zygosity	HGVS description	Location: GRCh37 (hg19)	Classification
GCK	Heterozygous	NM_000162.5:c.269A>C p.(Lys90Thr)	Chr7:g.44191964T>G	Uncertain significance

Figure 5. Details of Novel GCK Missense Variant

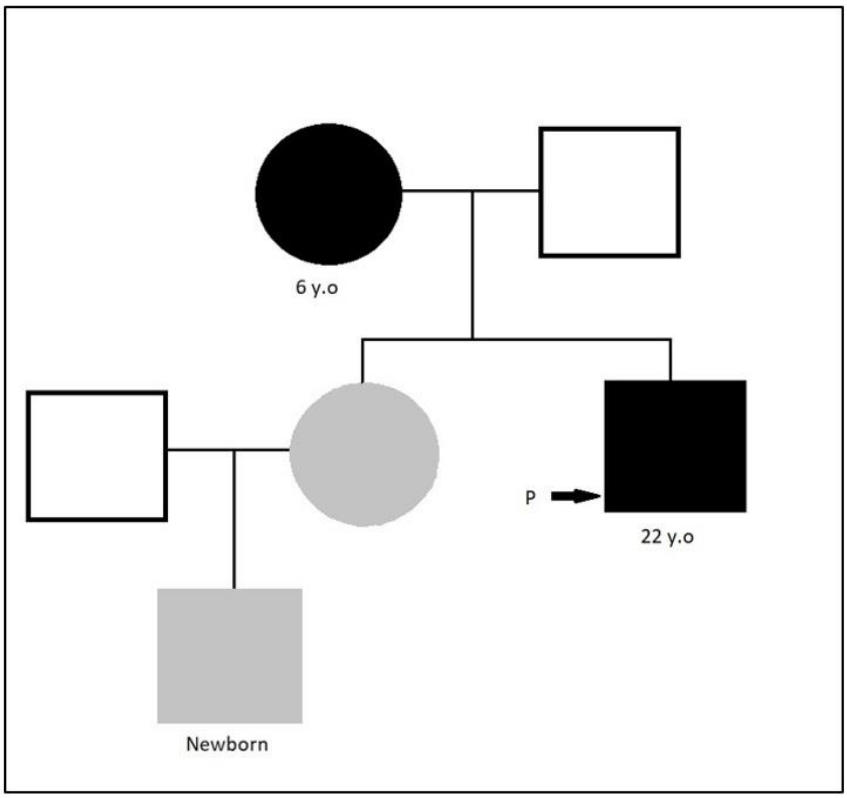


Figure 6. Pedigree of patient. Black represents individuals with confirmed GCK mutation and grey represents suspected individuals.

1. Anlauf M, Wieben D, Perren A, Sipos B, Komminoth P, Raffel A, Kruse ML, Fottner C, Knoefel WT, Mönig H, Heitz PU. Persistent hyperinsulinemic hypoglycemia in 15 adults with diffuse nesidioblastosis: diagnostic criteria, incidence, and characterization of β-cell changes. The American journal of surgical pathology. 2005 Apr 1;29(4):524-33.
2. Laidlaw GF. Nesidioblastoma, the islet tumor of the pancreas. The American journal of pathology. 1938 Mar;14(2):125.
3. Rumilla KM, Erickson LA, Service FJ, Vella A, Thompson GB, Grant CS, Lloyd RV. Hyperinsulinemic hypoglycemia with nesidioblastosis: histologic features and growth factor expression. Modern Pathology. 2009 Feb;22(2):239-45.
4. Stanley CA. Perspective on the genetics and diagnosis of congenital hyperinsulinism disorders. The Journal of Clinical Endocrinology & Metabolism. 2016 Mar 1;101(3):815-26.
5. Glaser B, Kesavan P, Heyman M, Davis E, Cuesta A, Buchs A, Stanley CA, Thornton PS, Permutt MA, Matschinsky FM, Herold KC. Familial hyperinsulinism caused by an activating glucokinase mutation. New England Journal of Medicine. 1998 Jan 22;338(4):226-30.
6. Irwin DM, Tan H. Evolution of glucose utilization: glucokinase and glucokinase regulator protein. Molecular phylogenetics and evolution. 2014 Jan 1;70:195-203.
7. Sternisha SM, Miller BG. Molecular and cellular regulation of human glucokinase. Archives of biochemistry and biophysics. 2019 Mar 15;663:199-213.
8. Morishita K, Kyo C, Yonemoto T, Kosugi R, Ogawa T, Inoue T. Asymptomatic congenital Hyperinsulinism due to a Glucokinase-activating mutation, treated as adrenal insufficiency for twelve years. Case reports in endocrinology. 2017 Jan 1;2017.
9. Challis BG, Harris J, Sleigh A, Isaac I, Orme SM, Seevaratnam N, Dhatariya K, Simpson HL, Semple RK. Familial adult onset hyperinsulinism due to an activating glucokinase mutation: implications for pharmacological glucokinase activation. Clinical endocrinology. 2014 Dec;81(6):855-61.

Hand Contracture And Xanthoma in Very Severe Hypertriglyceridemia and Diabetes Mellitus: a Case Report

Dananti Kusumawindani[1], Sony Wibisono[2], Deasy Ardiany[3]

1. Resident Of Internal Medicine, Airlangga University, Dr. Soetomo Teaching Hospital, Surabaya, East Java, Indonesia

2. Internal Of Medicine, RSUD Dr Soetomo, Surabaya, Jawa Timur, Indonesia

3. Endocrinology Division, Department of Internal Medicine, Airlangga University, Dr. Soetomo Teaching Hospital, Surabaya, East Java, Indonesia

Introduction

Xanthoma are deposits of lipid in tissue that are important clinical sign of systemic disease such as lipid metabolism disorder, particularly can be seen in severe hypertriglyceridemia (³1000 mg/dl) (1,2). Disorder of lipid metabolism or dyslipidemia divide into two categories, primary and secondary. Primary disorder occurs when alteration of genetic defects that directly affect lipoprotein. Secondary disorder occurs when other disorder alters lipoprotein metabolism indirectly. Often disorder of lipid metabolism results from combination of primary and secondary causes, as diabetes mellitus (DM) occurs in a person who has an inherited defect in lipoprotein metabolism (3,4).

Herein, a case presents a 30-year-old male patient came with multiple papules and nodules xanthoma in both hands and legs caused hand contracture, further investigation revealed that he had very severe hypertriglyceridemia and DM. Comprehensive management is needed to improve his problem and prevent other serious complication such as cardiovascular disease.

Case Presentation

30-year-old man has multiple lesions for a year. The lesions were progressively increasing size and number widespread over both hands and legs that caused hand contracture. There was no other complaint he had admitted. There was no past medical history. Alcohol consumption, smoking, hypertension, family history included diabetes mellitus, lipid disorder, and other metabolic disorder were denied. The physical examination revealed multiple yellow-brown papulonodular lesions in both hands and legs that some were coalesce one another. The lesions in his hand was known as xanthoma. Body mass index was 25.1 kg/m2, classified into overweight. A lipid profile examination revealed high triglyceride (TG) levels 7060 mg/dl, hypercholesterolemia 630 mg/dL, low HDL-C (high density lipoprotein cholesterol) 22 mg/dL, and high LDL-C (low density lipoprotein cholesterol) 110 mg/dL. Other laboratory abnormalities revealed high random blood glucose 370 mg/dL and high haemoglobin A1C (HbA1c) 11.2%. DNA analysis for any receptor gene mutation was not done due to its high cost. In conclusion, he had dyslipidaemia in domination of TG levels (very severe hypertriglyceridemia) and DM. The patient started on 145 mg fenofibrate as monotherapy. The patient also started 20 iu detemir insulin in the morning and 14 iu aspart insulin before meals. After two months consumed fenofibrate his TG levels 4380 mg/dL, cholesterol level 455 mg/dL, LDL-C 32.9 mg/dL, and HDL-C 23 mg/dL, fasting plasma glucose (FPG) 157 mg/dL, and 2-hour post prandial plasma glucose (2-h PPG) 239 mg/dL. As target TG level in this patient could not be achieved with monotherapy, combination with other lipid-lowering agent considered. He started to take 20 mg atorvastatin and added insulin dose gradually. After two month used combination of fenofibrate and statin, his TG level was 3275 mg/dL, cholesterol level 427 mg/dL, LDL-C 25.8 mg/dL, and HDL-C 23 mg/dL. Still combination of statin and fenofibrate had not been achieved the target, another lipid-lowering agent was added, 10 mg of ezetimibe. Lipid profile performed after he consumed combination of three drugs for a month, TG level 2984 mg/dl, cholesterol level 327 mg/dL, LDL-C 20 mg/dL, and HDL-C 24 mg/dL. However decreasing of cholesterol and triglyceride level was following by improvement of xanthomas.

Discussion

According to the World Health Organization (WHO), the prevalence of dyslipidemia in 2008 was 37% in men and 40% in woman that considered to cause mortality and morbidity (5).

Dyslipidemia is lipid metabolism disorder caused by either increased or decreased of plasma lipid. It is caused by increased of cholesterol, LDL-C or triglyceride, and decreased of HDL-C. Diagnosis of dyslipidemia based on laboratory of lipid plasma. For the measurement of triglyceride and LDL-C levels, patient must fast at least 12 hours before blood sampling (6). The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) classifies serum triglyceride levels into four categories: normal, <150 mg/dl; borderline high, 150–199 mg/dl; high, 200–499 mg/dl; and very high, ³500 mg/dl. NCEP ATP III also classifies serum cholesterol into three categories: desirable, <200 mg/dl; borderline high, 200-239 mg/dl; and high, ³240 mg/dl. HDL-c classifies into two categories: low, <40; and high > 60 (7). According to endocrine society clinical practice guideline, they modified the NCEP ATP III triglyceride classification to involve additional classification of severe hypertriglyceridemia, 1000-1999 mg/dl and very severe hypertriglyceridemia when TG levels reach ³2000 mg/dL (8).

Very high lipid plasma level can be caused by primary, secondary lipid metabolism disorder, or a combination of both (3,4,9). Despite familial aetiology of lipid metabolism disorder, either primary or secondary cause cerebrovascular disease as long term effect of uncontrolled lipid plasma level (10).

In our case, Patient came with multiple yellow-brown papulonodular lesions in both hands and legs that some were coalesce one another lead to hand contracture. Lipid plasma levels of this patient show he had very severe hypertriglyceridemia 7060 mg/dl, hypercholesterolemia 630 mg/dl, low HDL-C 22 mg/dL, and high LDL-C 110 mg/dL. He also had just diagnosed with DM according to his random blood glucose and HbA1C, 370 mg/dl and 11.2% respectively.

Recommendation based on ADA (American Diabetes Association) 2020, lipid management in diabetes include lifestyle therapy, optimize glycemic control for patients with elevated triglyceride levels and /or low HDL, statin, and other lipoprotein therapy. It is advised that diabetes patients aged 20-39 years with additional atherosclerotic cardiovascular disease risk factors to initiate statin therapy in addition to lifestyle therapy. It is reasonable to use high intensity statin therapy in patients with diabetes at higher risk. In very high risk patient, if LDL-C ³ 70 mg/dL on maximally tolerated statin dose, consider adding additional LDL-lowering therapy (such as ezetimibe or PCSK9 inhibitor). ADA also said that statin and fibrate combination therapy has not been shown to improve atherosclerotic disease outcomes and increase risk for transaminase levels, myositis, and rhabdomyolysis (9). Whereas according to ESC/EAS (European Society of Cardiology) 2019, if dominated by high TG levels, a fibrate often a combination of a statin and a fibrate may be needed (10).

In this patient initiated a daily dose of fenofibrate 145 mg, 20 iu detemir insulin in the morning and 14 iu aspart insulin before meals, after two months his TG level 4380 mg/dL, total cholesterol level 455 mg/dL, LDL-C 32.9 mg/dL, and HDL-C 23 mg/dL,

FPG 157 mg/dL, and 2-h PPG 239 mg/dL. As target TG level in this patient could not be achieved with monotherapy, combination with other lipid-lowering agent considered. He started to take 20 mg of atorvastatin and added insulin dose gradually, after two month used combination of fenofibrate and statin with close monitoring transaminase levels and sign of myositis and rhabdomyolysis, his TG level was 3275 mg/dL, cholesterol level 427 mg/dL, LDL-C 25.8 mg/dL, and HDL-C 23 mg/dL. Still combination of statin and fenofibrate had not been achieved the target, so another lipid-lowering agent was given, 10 mg of ezetimibe. Lipid profile performed after he consumed combination of three drugs for a month, TG level decreased into 2984 mg/dl, cholesterol level decreased into 327 mg/dL, LDL-C 20 mg/dL, and HDL-C 24 mg/dL. By decreasing lipid profile, this patient has an improvement of his xanthomas.

Summary

The importance of recognizing xanthomas that may has underlying disorder. This case provide very severe hypertriglyceridemia with xanthoma as first clinical presentation that result in hand contracture. In this case, very severe hypertriglyceridemia exacerbated by diabetes mellitus. Delaying treatment can lead to other serious complications.

1. James WD, Elston DM, Treat JR, Rosenbach MA, Neuhaus. 13th ed. Andrew's disease of the skin. New York: Elsevier; 2019. p. 530-534
2. Zak A, Zeman M, Slaby A, Vecka M. Xanthomas: clinical and pathophysiological relations. Biomedical Papers of the Medical Faculty of Palacky University in Olomouc. 2014 Jun 1;158(2).
3. Melmed S, Polonsky KS, Larsen PR, Kronenberg HM. William textbook of endocrinology. 13th ed. Philadelphia: Elsevier; 2016. p.1674-1684
4. Rader DJ, Hobbs HH. Disorder of lipoprotein metabolism. In: Longo DL, Kasper DL, Jameson JL, Fauci AS, Hauser SL, Loscalzo J, editors. Harrison's principle of internal medicine. 18th ed. New York: McGraw Hill; 2012. p. 3145-3161
5. World Health Organization (WHO). Global Health Observatory data. Available from: https://www.who.int/gho/ncd/risk_factors/cholesterol_text/en/. Accessed December 20, 2019.
6. Aman AM, Soewondo P, Soelistijo SA, Arsana PM, Wismandari, et al. Pedoman pengelolaan dislipidemia di Indonesia. Jakarta: PERKENI (perkumpulan Endokrinologi Indonesia);2019.
7. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults, 2001. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA: The Journal of the American Medical Association, 285(19), pp.2486-2497.
8. Berglund L, Brunzell JD, Goldberg AC, Goldberg IJ, Sacks F, Murad MH, Stalenhoef AF. Evaluation and treatment of hypertriglyceridemia: an Endocrine Society clinical practice guideline. The Journal of Clinical Endocrinology & Metabolism. 2012 Sep 1;97(9):2969-89.
9. Standards of Medical Care in Diabetes-2020. Diabetes Care. American Diabetes Association; 2020. Available from: https://care.diabetesjournals.org/content/43/Supplement_1
10. Mach F, Baigent C, Catapano AL, Koskinas KC, Casula M, Badimon L, Chapman MJ, De Backer GG, Delgado V, Ference BA, Graham IM. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. Atherosclerosis. 2019 Nov 1;290:140-205.

126

A case of clinical and biochemical manifestations of undiagnosed Hashimoto's thyroiditis

Sarah Lewis[1], Daniel Fineberg[1]

1. Alfred Health, South Yarra, VIC, Australia

We report the case of a 69 year old female who presented with mild cognitive impairment and difficulty mobilising in the setting of profound hypothyroidism secondary to Hashimoto's thyroiditis with associated elevated creatine kinase (CK), hyponatraemia, anaemia, renal impairment, hypercholesterolaemia and hypertryglyceridaemia. On initial investigations the patient had a thyroid stimulating hormone (TSH) of 49 mU/L, free T4 <5.4 pmol/L, thyroid peroxidase (TPO) antibody positive, CK 1628 units/L, sodium 120 mmol/L, haemoglobin 87 g/L, creatinine 109 mcmol/L, total cholesterol 8.1 mmol/L and tryglycerides 4.7 mmol/L. On examination the patient had no features of myxoedema coma but was found to have delayed relaxation of tendon reflexes, puffy facies with loss of outer one third of eyebrows, coarse hair, brittle nails and slowing of speech and movement with obvious cold intolerance. There was no muscle weakness on examination to suggest myositis although the patient complained of generalised aches and lethargy. The patient was initially treated with 100mcg oral thyroxine daily however this was increased and oral liothyroxine introduced following an inadequate improvement. Eleven days post admission the TSH was 6.26 mU/L and the free T4 was 12.4pmol/L following a total of 1500mcg oral thyroxine replacement and 60mcg oral liothyronine replacement. The hyponatraemia improved with a strict fluid restriction of 500 millilitres daily to sodium 133 mmol/L and the renal function improved to a creatinine of 70 mcmol/L. Atorvastatin was withheld due to the elevated CK which improved to 370 units/L and the anaemia remained stable throughout the admission. Although the patient refused formal cognitive assessments her functional abilities improved throughout the admission. This case highlights the clinical and biochemical features of severe hypothyroidism in the setting of undiagnosed Hashimoto's thyroiditis.

Suboptimal blood pressure control in medically treated bilateral primary aldosteronism

Jianbin Liu[1], Rosemary Wong[1], Christopher Gilfillan[1]

1. Endocrinology, Eastern Health , Box Hill, VIC, Australia

A 67 year-old woman was seen in 2014 for management of incidentally found asymptomatic hypokalaemia with a 10 year history of suboptimal blood pressure control despite four antihypertensive medications, Perindopril, Indapamide, Felodipine and Atenolol.

Primary Aldosteronism (PA) was diagnosed by classic clinical features of hypernatraemia, hypokalaemia, alkalosis and significantly elevated aldosterone/renin ratio of 1,132 (pmol/L)/(mIU/L). Serum metanephrines and urinary free cortisol levels were normal.

A dedicated adrenal CT scan showed one adenoma in the right adrenal gland and two in the left adrenal gland. Successful adrenal venous sampling did not show evidence of lateralization. Medical treatment with Spironolactone was commenced at 25 mg twice daily.

In the subsequent 6 years of treatment, the dose of Spironolactone was gradually up-titrated to 75mg twice daily, which elevated her serum renin from 0.5 mIU/L to 80-100 mIU/L (6-46 mIU/L). However, her blood pressure remained elevated, 140-150/80-85 mmHg despite increasing doses of Lercanidipine (from 10mg daily to 20mg daily) and Atenolol (from 25mg daily to 75mg daily). Along with this was her declining kidney function and borderline hyperkalaemia which ranged 4.8-5.4 mmol/L.

	2020	2019	2018	2017			2016	2015
	April			Aug	May	March		
Spironolactone	←			75mg BD	100mg BD	75mg BD	50mg BD	25 ->50mg BD
Lercanidipine	←			20mg daily	←			10mg daily
Atenolol	75mg daily	←						50mg daily
Blood pressure (mmHg)	150/85	150/80	140/80	146/89	145/85	132/83	138/80	130/75
Serum Sodium (mmol/L)	138 - 140	139 - 140	137 - 142		139	137	137	143 - 139
Serum Potassium (mmol/L)	4.8 - 5.3	4.8 - 5.4	4.9 - 5.2		5.3	5.1	4.7 – 5.0	3.5 – 4.7
eGFR (ml/min/1.73m2)	38 - 42	34 - 44	34 - 42		44	39	46 - 48	51 - 83
Serum Creatinine (mmol/L)	118 - 125	113 - 123	118 - 138		113	126	107 - 110	70 - 102
Renin (mIU/L)			110	83		34		

Nephrology review suggested that her renal function decline was likely caused by renovascular disease; glomerulonephritis screening tests were negative and she had small kidneys on ultrasound.

Bilateral disease comprises about 65% of PA [(1)], and highlights the importance of AVS in the workup of PA[(2)]. This case also illustrates the challenges of medical treatment of PA[(3)]. The goals are to fully block the effect of excessive aldosterone and to maintain optimal blood pressure [(2,3)]. However, the tendency to hyperkalaemia precluded the use of angiotensin converting enzyme inhibitor or angiotensin receptor blocker. Moreover, longstanding suboptimal blood pressure control has resulted in chronic kidney damage [(4)].

Questions:

- What is the target for mineralocorticoid receptor antagonist therapy?
- How should her blood pressure be managed now?
- What are the treatment options if a patient can not tolerate the needed dose of spironolactone due to its side effect or hyperkalaemia?

1. J Am Coll Cardiol. 2017;69(14):1811-1820
2. J Clin Endocrinol Metab, 2016, 101(5):1889-1916
3. Endocr Rev. 2018 Dec; 39(6): 1057–1088
4. Hypertension. 2018 September ; 72(3): 658–666

Craniotomy or Caution: COVID-19 or Keep Calm and Carry On

Jack Lockett[1, 2], Melissa Katz[1, 2], Emily Mackenzie[1, 3]

1. Department of Diabetes and Endocrinology, Princess Alexandra Hospital, Woolloongabba, QLD, Australia

2. Faculty of Medicine, University of Queensland, Brisbane, QLD, Australia

3. Nuclear Medicine, Department of Radiology, Princess Alexandra Hospital, Woolloongabba, QLD, Australia

Acromegaly occurs when chronic growth hormone (GH) excess leading to somatic tissue hypertrophy and an adverse metabolic profile.[1,2] The predominant cause (>95%) are pituitary somatotroph adenomas, representing 10-20% of pituitary adenomas.[1] International guidelines recommend transsphenoidal surgical resection (TSS) as first-line management.[1]

The novel coronavirus pandemic swept the world in 2020 with more than 24 million confirmed cases and 800,000 deaths.[3] Due to the high risk of infection and potential severity of subsequent illness, airbourne precautions are recommended for aerosol-generating procedures, such as the beginning of TSS. After reports of high rates of infection, and multiple deaths among ear, nose and throat surgeons overseas, in March 2020, the Australian Society of Otolaryngology, Head and Neck Surgery issued guidelines for addressing the COVID-19 pandemic.[4] This included undertaking only emergency and urgent upper airway operations for malignancy, threatened airway and bleeding. In response, all TSS were postponed at our centre, with craniotomy the approach chosen when requiring urgent pituitary surgery. Transcranial surgery (TCS) is currently reserved for large tumours with either dominant or eccentric extra-sellar extension and is believed to carry higher rates of post-operative complications.[5]

Here we present a case with newly diagnosed with acromegaly and visual field defects who avoided TCS through neoadjuvant medical therapy.

Case

A 45-year-old woman presented to her GP with 12-18 months of constant, pressure-sensation headache. On questioning, she had noted contemporaneous development of symptoms including soft tissue swelling, acral enlargement, skin oiliness, new skin tags, easy weight gain, jaw ache and onset of snoring. Oligomenorrhoea present for 18 months was associated with occasional vasomotor symptoms. During this time, the patient had also noticed dipoplia, ptosis of the left eye and frequently running into walls to suggest visual field deficits. Past medical history contained only a spleen-preserving distal pancreatectomy 3 years prior for solid pseudopapillary tumour, a distant smoking history, and previous hazardous, now infrequent alcohol use. Examination yielded typical features of acromegaly. Blood pressure 104/76mmHg, pulse rate 80bpm and regular, with no postural changes. There were no features of other endocrinopathy and cardio-respiratory examination was unremarkable. Visual acuity was normal, however there was a partial left ptosis, both bi- and monocular diplopia (worse in the left eye), no relative afferent papillary defect, normal ocular movements and no nystagmus. Visual perimetry results are included (figure 1) and are concerning for optic chiasm compression. Biochemistry is shown in table 1, in the setting of a markedly elevated insulin-like growth factor 1 (IGF-1), high random GH and clinical features consistent with acromegaly, a confirmatory oral glucose tolerance test was deemed unnecessary. Mild hyperprolactinaemia was present secondary to stalk effect from the 24x24x17mm macroadenoma with significant suprasellar extension, displacing and compressing the optic chiasm with extension into the cavernous sinus bilaterally (figure 2). The patient had been booked for emergent transcranial debulking to alleviate chiasmal compression. Her tumour would otherwise be amenable for TSS if not for the coronavirus embargo. After multi-disciplinary discussion and consent of the patient, she was commenced on intramuscular octreotide LAR 30mg monthly with 14 days of subcutaneous octreotide 100mcg TDS. We planned to repeat visual field testing after 2 weeks and proceed to surgery if no improvement. At 2 weeks, perimetry had improved (figure 1) and repeat MRI showed a 25% reduction in tumour volume (now 22x21x16mm) with improved effacement of the optic chiasm. Her symptoms had mostly resolved. After 4 months of treatment, visual fields had normalised, the tumour had not further involuted (figures 1 and 2), and IGF-1 remained elevated at 92nmol/L.

The diplopia was attributed by the neuro-ophthalmologist to unrelated corneal scarring with protective ptosis. She is now awaiting TSS which has since recommenced.

Discussion

The incidence of acromegaly is between 0.2-1.1 per 100,000 population.[2] The insidious onset of clinical features causes a median diagnostic delay of 4.5-5 years.[2] As a result, many patients present with macroadenomas with mass effect, the most concerning feature being visual field loss.[2] Additionally, it is associated with excess mortality double that of the general population, predominantly driven by cardiovascular disease.[1] The goals of treatment are a random GH level <1ug/L (shown to return mortality rates to that of the general population), age-adjusted normalisation of IGF-1, and management of comorbidities and complications of the disease.[1] TSS is recommended as first line management.[1] Pre-operative medical therapy is recommended in those patients with comorbidities which will heighten peri-operative risk, and are likely to improve with biochemical control of acromegaly.[1]

The first successful pituitary adenomectomy in 1905 was via a transfrontal approach.[5] Surgical techniques were refined in the following century, now TSS predominates. Less than 10% of resections occur transcranially (pterional).[5] It is thought that TCS is associated with higher rates of complications, however the data is limited to heterogeneous retrospective series of mixed adenoma types. Resolution of visual field defects is either better, no different, or less frequent in TCS compared to TSS.[5,6] Outcomes of anterior pituitary function may favour TCS for resolution of pre-operative deficits and are mixed with regards to new dysfunction.[5,6] Rates of permanent post-operative diabetes insipidus (DI) are consistently higher in TCS than TSS.[5-7] From the available data, the proportion of risk attribute to TCS (over TSS) cannot be separated from that secondary to the nature of tumours which require this approach. The degree of tumour deformation of the third ventricle/hypothalamus was a predictor of permanent DI, suggesting the latter may engender more of the risk.[7] Surgical remission of acromegaly is more than twice as likely in TSS than TCS, this is to be expected with lower rates of invasive adenomas in the TSS arms.[6] When considering cure in invasive lesions, TSS is still superior.[6]

Hypothalamic somatostatin inhibits GH secretion via binding to SST receptors (5 subtypes).[8] These G-protein couple receptors activate broad downstream signalling cascades reducing GH secretion, cell growth and inducing apoptosis.[8] SST2 (95%) and SST5 (85%) are the abundant subtypes on somatotroph adenomas.[8] First generation somatostatin analogues (SSA; octreotide,

lanreotide) preferentially bind SST2 and are effective at achieving biochemical control in 17-55% of patients in the adjuvant setting.[1,8] In a meta-analysis considering tumour shrinkage induced by primary SSA therapy, 36.6% of patients achieved significant reduction in tumour size (variable criterion) and the weighted mean reduction in size was 49.8% in those that responded, 19.4% for all patients.[9] A number of small studies using short-acting octreotide have shown tumour reduction occurs rapidly, between 18-30% within the first 2-4 weeks.[9,10] Further involution appears to plateau after 3 months.[9,10] This has been associated with improvement and even resolution of visual field defects in the first 2-4 weeks of treatment.[9]

As with previously described cases, our patient had an impressive reduction in tumour volume and improvement in visual field defects within two weeks of commencing treatment, her perimetry subsequently normalised with stability of tumour size at four months. This allowed her to avoid TCS and the potential increased risk of post-operative endocrine complications.

Take Home Messages:

- In acromegaly, pre-operative SSA therapy can rapidly induced tumour shrinkage and alleviate chiasm compression in 2-4 weeks. Most cases use subcutaneous octreotide ~300mcg/day.
- In pituitary adenomas, TCS may lead to higher rates of post-operative endocrine dysfunction and lower rates of cure (compared to TSS) but the evidence is conflicting, and the risk may be more attributable to tumour size/invasiveness.

Test	Result	Reference Range	Test	Result	Reference Range
Random GH	16.2 ug/L		**TSH**	0.8 mIU/L	0.3-3.5
IGF-1	104 nmol/L	10-32	**fT4**	12.2 pmol/L	9-19
PRL	672 mIU/L	<500	**fT3**	3.7 pmol/L	2.6-6
ACTH	41 ng/L	9-51	**FSH**	60 IU/L	
Cortisol	317 nmol/L	100-535	**LH**	20 IU/L	
Na	135 mmol/L	135-145	**E2**	70 pmol/L	
K	5.2 mmol/L	3.5-5.5	**FBC**	Normal	
Cr	50 umol/L	40-110	**LFT**	Normal	
eGFR	> 90 mL/min	>60	**HbA1c**	5.6%	
corrCa	2.41 mmol/L	2.3-2.6	**Vitamin D**	66 nmol/L	>50

Table 1. Biochemistry and pituitary hormonal panel at time of presentation, March 2020.

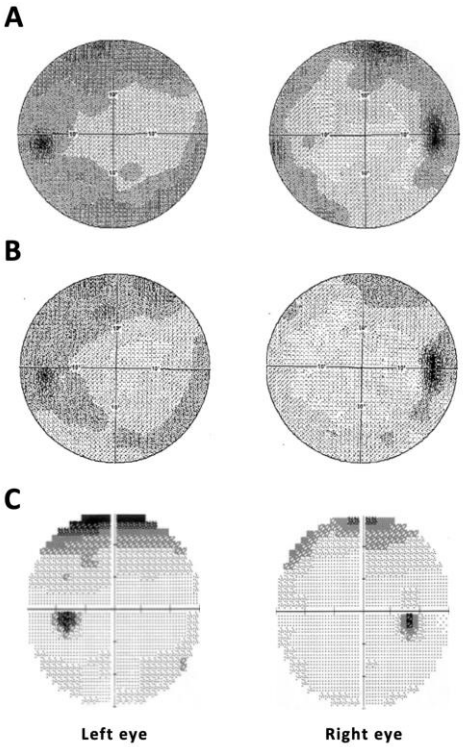


Figure 1. Evolution of visual perimetry over course of treatment. A) At time of presentation March 2020, left temporal hemianopia and right superior quandrantanopia; B) After two weeks of combined octreotide LAR and short-acting octreotide; C) After four months of continuing treatment with octreotide LAR, complete resolution of temporal field defects with residual superior loss due to ptosis.

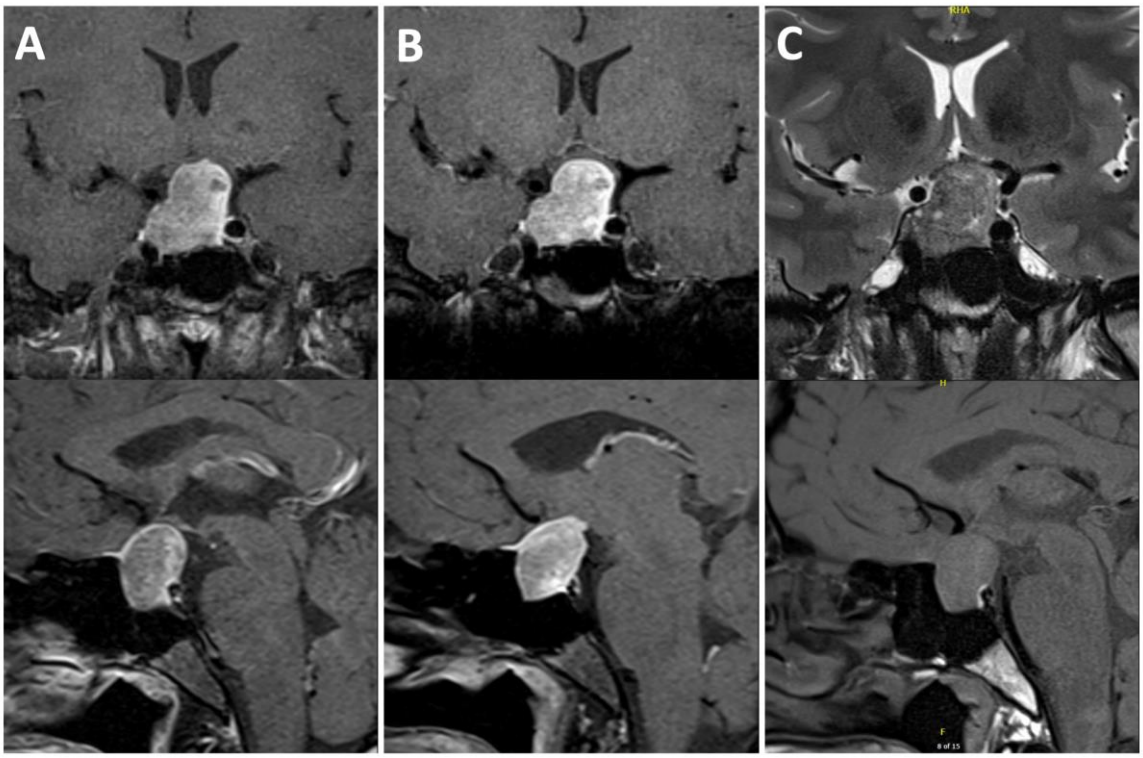


Figure 2. Reduction in size of tumour with treatment. A) At presentation, contrast-enhanced T1 images with fat saturation, 24x24x17mm (volume 9792mm^3); B) After 2 weeks of treatment, contrast-enhanced T1 images with fat saturation, 22x21x16mm (volume 7392mm^3), 25% reduction in tumour volume with reduced effacement of optic chiasm visible on coronal view; C) After 4 months of treatment, non-contrast T2 (coronal) and T1 (sagittal) images, no change in tumour dimensions compared to 2 weeks of treatment.

1. Katznelson L, Laws ER, Jr., Melmed S, et al. Acromegaly: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2014;99(11):3933-3951.
2. Lavrentaki A, Paluzzi A, Wass JA, Karavitaki N. Epidemiology of acromegaly: review of population studies. Pituitary. 2017;20(1):4-9.
3. Coronavirus disease (COVID-19) pandemic. World Health Organisation. https://www.who.int/emergencies/diseases/novel-coronavirus-2019. Published 2020. Updated 28th August 2020. Accessed 28th August, 2020.
4. ASOHNS Guidelines Addressing The COVID 19 Pandemic. Australian Society of Otolaryngology Head and Neck Surgery. http://www.asohns.org.au/about-us/news-and-announcements/latest-news?article=80. Published 2020. Updated 22nd March 2020. Accessed 20th August, 2020.
5. Buchfelder M, Kreutzer J. Transcranial surgery for pituitary adenomas. Pituitary. 2008;11(4):375-384.
6. Nomikos P, Buchfelder M, Fahlbusch R. The outcome of surgery in 668 patients with acromegaly using current criteria of biochemical 'cure'. Eur J Endocrinol. 2005;152(3):379-387.
7. Wang S, Li D, Ni M, et al. Clinical Predictors of Diabetes Insipidus After Transcranial Surgery for Pituitary Adenoma. World Neurosurg. 2017;101:1-10.
8. Gadelha MR, Wildemberg LE, Bronstein MD, Gatto F, Ferone D. Somatostatin receptor ligands in the treatment of acromegaly. Pituitary. 2017;20(1):100-108.
9. Melmed S, Sternberg R, Cook D, et al. A critical analysis of pituitary tumor shrinkage during primary medical therapy in acromegaly. J Clin Endocrinol Metab. 2005;90(7):4405-4410.
10. Caron PJ, Bevan JS, Petersenn S, et al. Tumor shrinkage with lanreotide Autogel 120 mg as primary therapy in acromegaly: results of a prospective multicenter clinical trial. J Clin Endocrinol Metab. 2014;99(4):1282-1290.

Agenesis of the dorsal pancreas: a rare incidental finding which is amongst the differential diagnoses for abdominal pain and diabetes mellitus.

Varun Manoharan[1, 3, 2], **Minoli V Abeysekera**[1, 4, 2], **Emily Hibbert**[1, 2]

1. Department of Endocrinology, Nepean Hospital, Penrith, NSW, Australia

2. Nepean Clinical School , Faculty of Medicine and Health, The University of Sydney, NSW

3. South Western Sydney Clinical School, University of New South Wales, Sydney, NSW

4. Griffith Medical School, Griffith University, Gold Coast, Queensland

Figure 1. Magnetic resonance cholangiopancreatography showing the head of pancreas (yellow) and the absence of the body and tail of the pancreas.
(A) Coronal section (B) axial section

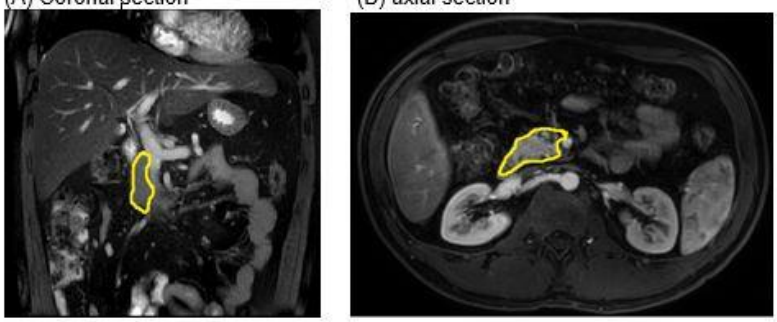


Table 1. Preliminary Investigations

	Results	References
Hb	160g/L	130-180 g/L
HbA1c	9.6%	4-6%
Fasting blood glucose	27.1mmol/L	3.5-7.7 mmol/L
C-peptide	1.0 ug/L	0.8-3.4 ug/L
Fasting insulin level	6 mU/L	<10 mU/L
Anti-insulin antibody	Not detected	-
Glutamic acid decarboxylase antibody	5 U/L	5 U/L
Islet cell antibody	Not detected	-
Zinc transporter 8 antibody	Not detected	-
TSH	8.1 mIU/L	0.5-4.0 mIU/L
Free thyroxine	15pmol/L	10-20 pmol/L
Thyroglobulin antibody	<20 IU/L	<40IU/L
Thyroid peroxidase antibody	>1300 IU/L	<60IU/L
Gliadin IgG antibody	3 U/mL	<20 U/mL
TTG IgA antibody	2 U/mL	<24 U/mL

Table 2. Most recent laboratory results

	Results	References
Hb	154 g/L	130-180 g/L
MCV	78 fL	82-95 fL
MCHC	346 g/L	300-350
Micronutrients		
Iron	24.4 umol/L	9-31 umol/L
Ferritin	71 ug/L	30-300 ug/L
Transferrin Saturation	37%	16-45 %
Folate	24nmol/L	>10 nmol/L
Vitamin B12	205 pmol/L	150pmol/L
Holocotranscobalamin	84 pmol/L	51pmol/L
Exocrine function		
Vitamin A	1.6 umol/L	1.4-4.0umol/L
25-OH Vitamin D	68 nmol/L	50 nmol/L
Vitamin E	31 umol/L	8-30 umol/L
Vitamin K	0.5 nmol/L	0.3-2.6 nmol/L
Amylase	43 U/L	<115U/L
Lipase	11 U/L	<60 U/L
Faecal elastase	317 ug/g	>100 ug/g
Endocrine Function		
Random glucose	11.4mmol/L	3.5-7.7 mmol/L
C peptide	0.68 ug/L	0.8-3.4 ug/L
HbA1c	8.6%	4-6%
Complication screen in type 1 diabetes mellitus		
TSH	1.32 mIU/L	0.4-3.5 mIU/L
Free thyroxine	12.8 pmol/L	9-19
Thyroglobulin antibody	20 IU/L	<60IU/L
Thyroid peroxidase antibody	289 IU/L	<35IU/L
TSH Receptor antibody	1.0 IU/L	
Gliadin IgG antibody	4 U/mL	<20 U/mL
TTG IgA antibody	1 U/mL	<4 U/mL
Total IgA antibody	1.2 g/L	0.8-4.4 g/L

INTRODUCTION

In parallel with improvement in imaging technologies, there has been an increase in the frequency of incidental diagnosis of agenesis of the dorsal pancreas. Yet this congenital malformation remains rare; its manifestations include diabetes mellitus, abdominal pain and pancreatitis[1]. We report a case of a 39 year old man with antibody-negative type 1 diabetes and abdominal pain, who was incidentally found to have dorsal agenesis of the pancreas on abdominal CT performed to investigate his chronic abdominal pain.

CASE SUMMARY

A 39 year old Caucasian man from non-consanguineous kinships presented to the Diabetes Service in 2020 for ongoing management of his type 1 diabetes mellitus. He reported longstanding colicky epigastric pain associated with intermittent diarrhoea without any blood or mucus in his stools. His abdominal pain was exacerbated by fatty meals. He reported significant loss of appetite but denied any extraintestinal symptoms suggestive of inflammatory bowel disease. His drug and alcohol history was unremarkable.

He was diagnosed with antibody negative type 1 diabetes mellitus three years prior, when he presented with polyuria, polydipsia and unintentional weight loss. Further investigations performed at diagnosis showed a relative insulin deficiency with a C-peptide level of 1.0 ug/L [0.8-3.4], fasting serum insulin level of 6 mu/l [<10] with a paired random plasma glucose level of 27.1 mmol/L and HbA1c of 9.6% (Table 1). Autoantibodies for Glutamic Acid Decarboxylase, Islet cell, Insulin and Zinc transporter 8 were not detected. However, he had other features of automimmunity. He was subclinically hypothyroid (Table 1) with elevated thyroid peroxidase antibody >1300 U/mL [<60]. His coeliac serology was negative and he had a vitamin B12 level of 205pmol/L [150pmol/L]. A diagnosis of Hashimoto's thyroiditis and failure to achieve good glycaemic control on oral hypoglycaemic agents led to a diagnosis of antibody negative type 1 diabetes mellitus. Notably, there was no history of diabetic ketoacidosis or evidence of diabetes complications.

His background history was significant for dyslipidaemia, gastroesophageal reflux disease and a family history of autoimmune thyroid disease. Regular medications included basal-bolus insulin therapy, thyroxine, atorvastatin, pantoprazole and panadeine forte for his colicky abdominal pain.

On examination, he weighed 79 kilograms and had a body mass index of 25.5kg/m2. Cardiorespiratory and gastrointestinal examinations were unremarkable.

Laboratory investigations in 2020 showed an HbA1c of 8.6%. He was replete for micronutrients and fat soluble vitamins, and was biochemically euthyroid. His full blood count, pancreatic enzymes and renal function were all within normal limits (Table 2). Repeat C-peptide level was low 0.68 ug/L [0.8-3.4] with a paired blood glucose level of 11.4 mmol/L. Agenesis of the dorsal pancreas was noted on CT imaging of the abdomen demonstrated agenesis of the dorsal pancreas. There were no focal pancreatic lesions or evidence of acute or chronic pancreatitis. This was confirmed on MRCP which also showed normal hepatobiliary anatomy (Figure 1). His faecal elastase was 317 microg/g [>100 microg/g].

His abdominal pain improved with cessation of panadeine forte and good glycaemic control was achieved with up titration of his insulin therapy.

DISCUSSION

ADP is a rare congenital abnormality that arises from dysgenesis of the dorsal pancreatic bud. The dorsal endodermal bud forms the upper part of the head, body and tail of the pancreas, whilst the ventral bud develops into the posterior part of the pancreatic head, uncinate process and main pancreatic duct of the pancreas[2]. Strict signalling pathways regulate the fusion of the ventral and dorsal buds and subsequent morphogenesis and differentiation of multipotent progenitor cells into acinar, ductal and endocrine cells. The acinar and ductal cells comprise 99% of the pancreatic mass and fulfil the exocrine functions, whilst the Islet cells of Langerhans comprise the remainder of pancreatic tissue. Associations between embryogenesis of the pancreas and genes such as HNF1B[3] and GATA6[4], and signalling pathways such as retinaldehyde dehydrogenase 2 (Raldh2)[5] have been reported in the literature. Yet this condition remains rare with less than one hundred and twenty cases reported in the literature[6-9].

Most patients with ADP remain asymptomatic; it is often identified incidentally on abdominal imaging. However, if symptomatic, abdominal pain is the commonest symptom at presentation[1]. Abdominal pain among patients with ADP has been postulated to be due to pancreatitis, duodenal obstruction and sphincter of Oddi dysfunction[6]. Our patient had normal amylase and lipase levels and there were no features suggestive of acute or chronic pancreatitis on abdominal imaging. We noted that he was on panadeine forte for abdominal pain, and cessation of panadeine forte and commencement of paracetamol for abdominal pain resulted in improvement in abdominal pain. Panadeine forte contains codeine, which has been shown to increase the basal pressure of the sphincter of Oddivia the non mu opioid receptor and results in spasm of the sphincter of Oddi.

Patients with ADP can present with symptoms of endocrine and exocrine deficiency. Approximately half of patients with ADP will develop diabetes mellitus, and up to half of them will require insulin therapy[7-9]. The age of onset of diabetes mellitus associated with ADP ranges between 28 to 39 years[7-9]. Although beta cell dysfunction and insulin deficiency have been implicated in the pathophysiology of diabetes mellitus among patients with ADP, there have only been three studies that reported a correlation between this congenital malformation and diabetic ketoacidosis[7,9]. Studies conducted on patients who have undergone surgical resection of the pancreas for other indications demonstrate that the pancreatic tail in humans is more densely populated with Islets of Langerhans than other parts of the pancreas. Animal studies have shown functional differences in these islets based on their location, whereby islets originating from the dorsal pancreatic bud have greater capacity to secrete and synthesise insulin than islets of ventral bud origins[10]. These studies have shown that removal of the pancreatic tail led to elevated fasting glucose and post-challenge hyperglycaemia, whilst removal of the pancreatic head caused an improvement in oral glucose tolerance. Very few cases of ADP reported in the literature have paired C-peptide levels measured. Our patient had a low-normal C-peptide level of 1.0 ug/L [0.8-3.4] and serum insulin level of 7 mu/l [<10] with a paired random blood glucose level 27.1 mmol/L around the time of diagnosis, highlighting how variable degrees of β-cell dysfunction contribute to dysglycaemia among patients with agenesis of the dorsal pancreas.

Steatorrhoea is a manifestation of exocrine deficiency and is commonly managed with supplementation of pancreatic enzymes. ADP has been associated with include polysplenia, ectopic spleen, bowel malrotation, horseshoe kidney, congenital heart disease and heterotaxy[7], none of which were noted on our patient's CT abdomen and MRCP.

There has been an increased frequency of incidental diagnosis of ADP[6,7]. Abdominal CT assists in the diagnosis of ADP, and MRCP helps characterise the anatomy of the biliary ductal system[6].

CONCLUSION

In summary, ADP is an extremely rare condition that is often asymptomatic but can also present with abdominal pain or with features of exocrine and endocrine deficiency. It is often diagnosed incidentally on abdominal imaging.

TAKE HOME MESSAGES

- Agenesis of dorsal pancreas is a rare clinical entity
- Abdominal pain is the commonest symptom at presentation, but it is often entirely asymptomatic

- It is usually diagnosed incidentally on imaging
- Patients can present with features of exocrine or endocrine deficiency
- Agenesis of dorsal pancreas is associated with other congenital abnormalities

1. Cienfuegos J, Rotellar F, Salguero J, et al. Agenesis of the dorsal pancreas: systematic review of a clinical challenge. Revista Espanola de Enfermedades Digestivas 2016;108:479–84.
2. Caetano LA, Santana LS, Costa-Riquetto AD, Lerario AM, Nery M, Nogueira GF, Ortega CD, Rocha MS, Jorge AAL, Teles MG. PDX1-MODY and dorsal pancreatic agenesis: new phenotype of a rare disease. Clinical Genetics 2018;93(2):382–6.
3. Schnedl WJ, Reisinger EC, Schreiber F, Pieber TR, Lipp RW, Krejs GJ. Complete and partial agenesis of the dorsal pancreas within one family. Gastrointestinal Endoscopy 1995;42(5):485-7.
4. Lango Allen H, Flanagan SE, Shaw-Smith C, et al., International Pancreatic Agenesis Consortium. GATA6 haploinsufficiency causes pancreatic agenesis in humans. Nature Genetics 2012;44:20–22.
5. Li H, Arber S, Jessell TM, Edlund H. Selective agenesis of the dorsal pancreas in mice lacking homeobox gene Hlxb9. Nat Genet. 1999;23(1):67–70.
6. Kumar R, Vyas K, Agrahari N, Kundu J, Jaiswal G. Complete agenesis of the dorsal pancreas: case report with imaging findings and review of the literature. Malawi Medical Journal. 2015;27(2):73–4.
7. Yang T, Yang X, Wang L, Mo J. Agenesis of the dorsal pancreas presenting with diabetic ketoacidosis – a case report and literature review. BMC Endocrine Disorders 2019; 19:120.
8. Sonkar SK, Kumar S, Singh NK. Agenesis of dorsal pancreas in a young adult: a rare cause of diabetes mellitus. BMJ Case Reports 2018.
9. Salman R, Barakat A, Berjawi G. Dorsal pancreatic agenesis in newly diagnosed type one diabetes mellitus: case report and review of literature. International Journal of Diabetes in Developing Countries 2019; 39: 228–231.
10. Xavier GDS. The Cells of the Islets of Langerhans. Journal of Clinical Medicine 2018; 7(3): 54.

Case report: Cushing's syndrome due to adrenocortical carcinoma during pregnancy

Jack Morris[1], Anthony O'Sullivan[2], Peter Campbell[3], Lily Xu[4]

1. Department of Endocrinology and Metabolism, Concord Repatriation General Hospital, Concord West, NSW, Australia

2. St George and Sutherland Clinical School , The University of New South Wales , Sydney, NSW, Australia

3. Department of Endocrine Surgery, St. George Hospital , Kogarah, NSW, Australia

4. St. George Hospital, Kogarah, NSW, Australia

Introduction

Adrenocortical carcinoma (ACC) is rare (incidence: one to two cases per million adults) and is more common in females, with a sex ratio of 4.2[1]. ACC diagnosed during pregnancy is exceedingly rare and studies are limited to case reports[2]. Cushing's syndrome (CS) is uncommon during pregnancy due to reduced fertility associated with hypercortisolism and elevated androgens[2]. Confirming the diagnosis of pathological hypercortisolism is also problematic due to changes in the hypothalamic-pituitary-adrenal (HPA) axis during pregnancy, which results in physiological hypercortisolism. Investigations commonly used in the non-pregnant state are not well validated during pregnancy[3]. Confirming the diagnosis of CS is imperative due to the adverse maternal and fetal outcomes experienced during pregnancy[4]. We present the case of a 31 year old pregnant woman who presented for management of recently diagnosed gestational diabetes mellitus (GDM) and was found to have features of CS on clinical examination. ACTH-independent CS and a 4cm adrenal tumour were subsequently diagnosed. The pregnancy was complicated by premature rupture of membranes and the patient delivered a female infant at 33 weeks gestation. Four weeks post-partum she underwent a left adrenalectomy, which subsequently identified an ACC on histopathology.

Case Report

A 31 year old caucasian woman (gravida 1, para 0) of 28 weeks gestation presented to the Endocrine Outpatient Department for the management of recently diagnosed GDM confirmed on 75g OGTT according to Australian criteria[5] (fasting glucose 5.3 mmol/L, 60 min glucose 14.0 mmol/L 120 min glucose 8.8 mmol/L). She had a past history of Hashimoto's hypothyroidism and was taking adequate thyroxine replacement therapy (TSH 0.3 mIU/L, reference range [RR] 0.31-2.75 mIU/L for 24-30 weeks gestation) and obesity (pre-pregnancy BMI 33.8 kg/m^2). In the initial consultation, she described developing coarse hair growth, facial and chest acne during the pregnancy. There was no history of polycystic ovarian syndrome, prior glucocorticoid use or adrenal disease. There was no family history of diabetes or adrenal disease. She had conceived naturally and her pregnancy was otherwise uncomplicated. An obstetric ultrasound at 22 weeks gestation demonstrated a singleton pregnancy with normal fetal morphology. On clinical examination, hirsuitism with male facial and chest hair distribution, acne and violaceous abdominal striae were confirmed (Figures 1a-c). The was no evidence of a myopathy. She was hypertensive (blood pressure 134/94 mmHg) and clinically euthyroid.

A 24-hour urinary free cortisol (UFC) was elevated (968 nmol/24h, RR <166 nmol/24h), as was testosterone (9.3 nmol/L, RR <2.0 nmol/L), and androstenedione (20.2 nmol/L, RR 0.9-7.5 nmol/L). Adrenocorticotropic hormone (ACTH) was suppressed (3.6ng/L, RR 7.2-63.3 ng/L) (table 1). Serum biochemistry, liver function, metanephrines, normetanephrines, aldosterone:renin ratio and DHEAS were within normal limits. Serially elevated 24 hour UFC and suppressed ACTH levels were used to confirm ACTH-independent hypercortisolism. A dexamethasone suppression test (DST) and midnight salivary cortisol levels were not performed due to lack of validation in pregnancy[2]. An abdominal ultrasound demonstrated a left adrenal lesion measuring 28x22x23mm. Her gestational diabetes was treated with metformin and subcutaneous basal glargine and bolus aspart insulin, which was subsequently well controlled. Endocrine surgery and obstetric medicine input was sought regarding further assessment and management of CS. As there was no evidence of foetal distress and the patient was otherwise stable, close and regular observation of the mother and foetus was decided upon until induction of labour after 34 weeks gestation. Postpartum, the patient would undergo a dedicated adrenal computed tomography (CT) for further characterisation of the lesion, before proceeding to surgery.

At 32 weeks gestation, the patient experienced premature prelabour rupture of membranes and was admitted to hospital. At 33 weeks gestation, she began to labour spontaneously and subsequently delivered a live female infant weighing 1879g via forceps-assisted vaginal delivery. The newborn was admitted to the special care nursery for observation and received a short course of dexamethasone to cover for possible adrenal insufficiency. There was no neonatal hypoglycaemia and the infant demonstrated normal appearing external genitalia, in contrast to previous case reports[5].

An adrenal CT scan performed post-partum confirmed the presence of a lesion measuring 3.2cm in maximal diameter. The lesion was homogenous (32 Hounsfield units). The absolute and relative washout ratios were 66% and 42% respectively, suggestive of an adenoma (Figure 2). Repeat 24h UFC performed post-partum remained elevated (693 nmol/24hr) and ACTH remained suppressed (1.9 ng/L). Four weeks post-partum, the patient underwent a laparoscopic left adrenalectomy, which was uncomplicated. Histopathology revealed a cortical lesion measuring 40x25x25mm with high-grade nuclear features with pleomorphism, presence of giant cells, bizarre nuclei, myxoid deposits and zones of tumour necrosis. Immunohistochemical staining was positive for inhibin, melanA. A Ki67 of 12% was identified in a few areas, but otherwise was very low (1-2%) for most of the tumour (Figure 3). There was no lymphovascular or capsular invasion. Overall the features were consistent with an adrenocortical carcinoma. The patient underwent fluorodeoxyglucose (FDG)-positron emission tomography (PET), which did not demonstrate evidence of residual or metastatic disease. The case was discussed at a regional Endocrine-Oncology Multi-disciplinary meeting and in consultation with the patient, a period of close surveillance was weighed against further surgery and chemo-radiotherapy. Due to the malignant nature of the lesion and strong preference to ensure microscopic clearance to protect against local or regional recurrence, the patient underwent further surgery to excise the residual left adrenal tissue.

Discussion

CS due to ACC during pregnancy is rare and associated with significant morbidity and mortality in both foetus and mother[2]. Comorbid hypertension, pre-eclampsia, weight gain, androgenisation and catabolic effects of hypercortisolism can occur. Diabetes mellitus is reported in 25% of pregnant women with CS and premature labour has been reported in 50% of cases[2-4]. Adrenal lesions are responsible for approximately 50% of CS during pregnancy, which is higher than CS in non-pregnant women (around 15%)[2]. Confirming pathological hypercortisolism during pregnancy is problematic due to physiological changes

that occur within the hypothalamic-pituitary axes[3]. Total cortisol levels are elevated twofold-threefold during pregnancy due to an increase in corticosteroid-binding globulin (CBG) and an increase in corticotropin secretion from both hypothalamic and placental sources[2]. The diurnal pattern of cortisol secretion is maintained in pregnancy, however suppression of cortisol by dexamethasone (DST) is reduced when measured against standard reference intervals derived from the non-pregnant population[2,4]. Whilst 24h UFC levels are elevated in second and third trimesters, measurement is considered the most reliable method of diagnosing pathological hypercortisolism during pregnancy, with concentrations greater than three times the upper limit of normal considered diagnostic[7-8]. An abdominal ultrasound was performed as the initial imaging modality due to its safety in pregnancy. The safety of magnetic resonance imaging (MRI) requires further evaluation, however it has been suggested as the preferred imaging modality in pregnancy[9]. There is no consensus on management of ACC during pregnancy and a paucity of data exists due to its rarity[9]. Compared with the non-pregnant population, patients diagnosed with ACC during pregnancy are reported to have a less favourable prognosis. One retrospective cohort study identified pregnant patients were more likely to be diagnosed at a later stage compared to non-pregnant women of childbearing age and had poorer overall survival[10]. Fortunately, our patients tumour was detected at an early stage and the patient remains disease free after surgical treatment alone. Despite delivery of a premature infant at 33 weeks gestation, foetal outcomes were also favourable.

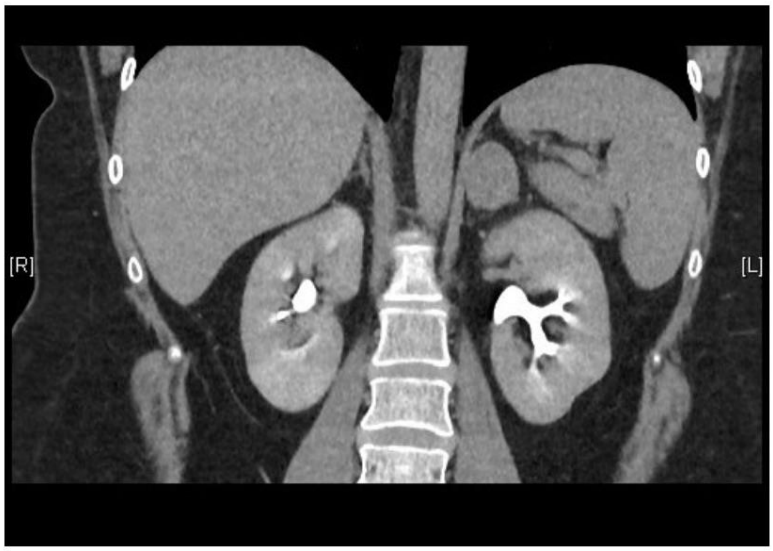


Figure 2: Adrenal CT demonstrating left adrenal lesion

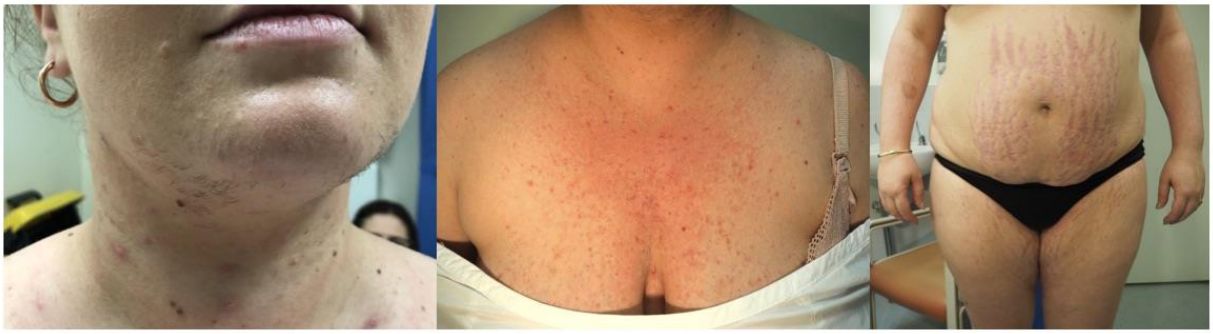


Figure 1a: male distribution facial hair, 1b: chest acne, 1c central adiposity and violaceous abdominal striae

Table 1: investigations for Cushing's syndrome

	Result	Reference range (RR)
24h urinary free cortisol (nmol/ 24h)	968	<166
ACTH ng/L	3.6	7.2-63.3
Testosterone nmol/L	9.3	<2.0
Androstenedione nmol/L	20.2	0.9-7.5
DHEAS umol/L	1.1	2.7-9.2
17-hydroxyprogesterone	2.3	<8.9

1. Luton J, Cergas S, Billaud L, Thomas G, Guilhaume B, Bertagna X, Laudat MH, Louvel A, Chapuis Y, Blondeau P, Bonnin A, Bricaire H. Clinical Features of adrenocortical carcinoma, prognostic factors and the effect of mitotane therapy. New England Journal of Medicine 1990: 322; 1195-1201
2. Abdelmannan D, Aron D. Special aspects of Cushing's syndrome: pregnancy. Contemporary Endocrinology: Cushing's syndrome: pathophysiology, diagnosis and treatment (book chapter). New York: Springer 2010 p. 259-71
3. Abdelmannan D, Aron D. Special aspects of Cushing's syndrome: pregnancy. Contemporary Endocrinology: Cushing's syndrome: pathophysiology, diagnosis and treatment (book chapter). New York: Springer 2010 p. 259-71
4. Lekarev O, New M. Adrenal disease in pregnancy Best Practice and Research Clinical Endocrinology and Metabolism 2011: 959-973
5. Nankervis A, McIntyre HD, Moses R, Ross GP, Callaway L, Porter C, Jeffries W, Boorman C, De Vries B, McElduff. A ADIPS Consensus Guidelines for the Testing and Diagnosis of Gestational Diabetes Mellitus in Australia Australasian Diabetes in Pregnancy Society, 2013
6. Morris L, Park S, Daskivich T, Churchill B, Rao C, Lei Z, Martinez D, Yeh M. Virilization of a female infant by a maternal adrenocortical carcinoma Endocrine Practice 2011: 17(2);e26-31
7. Sheeler L Cushing's syndrome and pregnancy. Endocrinology and Metabolism Clinics of North America 1994; 23:619-627
8. Pickard J, Jochen A, Sadur C, et al. Cushing's Syndrome in pregnancy Obstetrical and Gynaecological Survey 1990 45: 87-93
9. Eschler D, Kogekar N, Pessah-Pollack R. Management of adrenal tumours in pregnancy Endocrinology and Metabolism Clinics of North America 2015: 381-397
10. Abiven-Lepage G, Coste J, Tissier F, Groussin L, Billaud L, Dousset B, Goffinet F, Bertagna X, Bertherate J, Raffin-Sanson M. Adrenocortical carcinoma and pregnancy: clinical and biological features and prognosis European Journal of Endocrinology 2010 163; 793-800

Scratch beneath the surface to see why “mild” type 2 diabetes became a life-threatening diabetes emergency

Elisabeth Ng[1], Shoshana Sztal-Mazer[1, 2], Leon Bach[1, 2]

1. Department of Endocrinology & Diabetes, Alfred Health, Melbourne, Australia

2. Monash University, Melbourne, Victoria, Australia

Case

A 50-year-old female was brought in by ambulance after her 17-year-old son found her acutely confused and incoherent. Initial Glasgow Coma Scale score was 11 (E3V3M5). She was hypothermic (29.9°C), hypotensive (96/49), and hyperglycaemic (blood glucose level HI, ketones 4.8 mmol/L by glucometer). Past medical history comprised only type 2 diabetes mellitus (T2DM) treated with metformin 2 g and sitagliptin 100 mg. Of note, she was not prescribed an SGLT2 inhibitor. Her son perceived her to have good medication compliance and good general health; she was a non-smoker with rare alcohol consumption and worked full-time in an administrative role. There was a maternal family history of T2DM, but no autoimmune disease. Her son described a two-day history of nausea and vomiting preceding her acute presentation.

Initial biochemistry (table 1) demonstrated a marked metabolic acidosis (pH 6.59, bicarbonate 2 mmol/L), ketosis and a glucose level of 44.0 mmol/L, with renal impairment and raised inflammatory markers. Examination demonstrated hypovolaemia and cool peripheries, with an otherwise benign abdominal, respiratory and cardiovascular examination. A small skin wound was noted on the posterior thorax without overlying cellulitis or clinical evidence of abscess.

She was treated for severe diabetic ketoacidosis (DKA) in ICU with fluid rehydration, insulin infusion and potassium replacement. Management for presumed cold sepsis included empirical broad-spectrum intravenous antibiotics after a septic screen was sent including a COVID swab. She required vasopressor support as her blood pressure deteriorated further to 73/54 with a heart rate of 66 bpm. Oliguria developed in the context of acute kidney injury necessitating continuous renal replacement therapy. An agitated delirium developed requiring physical restraints and haloperidol sedation.

After commencing urgent resuscitation, attention was directed to the precipitant of severe DKA, particularly a source of sepsis. The posterior thorax skin lesion, initially deemed not amenable to incision and drainage, was reviewed by the surgeons 15 hours after presentation. The assessment was difficult due to patient agitation and wound location. Nevertheless, debridement was suggested once she improved metabolically. Venous pH normalised to 7.36 within 18 hours of admission, with a glucose of 16.1 mmol/L. Upon further surgical review, it was thought unlikely the skin lesion could have caused such a severe illness, thus a CT abdomen and pelvis was organised to exclude an intra-abdominal source of sepsis. This occurred 20 hours after admission and was unremarkable, as was chest imaging and respiratory viral swabs, including for COVID.

The skin wound was therefore explored under anaesthesia 26 hours after admission. Upon incision, ‘dirty dishwasher fluid’ and copious amounts of pus were expressed with clear evidence of necrotising fasciitis. A 15 x 8 cm incision was required for adequate access, with dissection to the trapezius muscle, and the wound was left open. Antibiotics were escalated from piperacillin-tazobactam to meropenem, clindamycin and vancomycin. Hyperbaric oxygen therapy was administered on day 0, 1 and 2 post-operatively. Cultures from the wound grew methicillin-sensitive staphylococcus aureus, and antibiotic therapy was rationalised.

She had a prolonged stay lasting 23 days for further washouts and debridements, VAC dressing management, antibiotic therapy, transition from nasogastric feeds to oral intake, and transition from intravenous to subcutaneous insulin (see Figure 1). Her back wound required a split skin graft from her right anterolateral thigh and subsequent wound care. She required intermittent haemodialysis on discharge for oliguric renal failure, likely due to acute tubular necrosis. HbA1c was 13.6% and fasting C peptide was 592 pmol/L (260-1730). Anti-GAD and IA2 antibodies were negative. After initial stabilisation, ongoing glycaemia was managed with once-daily mixed insulin, as per the patient's request.

Discussion

DKA is typically associated with type 1 diabetes but may also occur in patients with T2DM. Indeed, a recent study showed 125 cases of DKA in patients with T2DM who were not taking SGLT2 inhibitors in Victorian public hospitals over a 26-month period (1).

Identifying the precipitating event of DKA enables specific treatment and prevention of recurrence. In many cases the cause is clear from the onset, with the most common precipitants being infection and inadequacy of insulin therapy (under-dosing or omission) (2, 3). Where the cause is unclear, however, the importance of a thorough physical examination is amplified, especially when the patient has a diminished GCS which limits the availability of history. The examination may also be revealing when considering other precipitants such as pancreatitis, myocardial infarction, or stroke.

In this case, however, we see the value of combining the examination with an index of suspicion for necrotising fasciitis (NF) as a cause of what may appear to be occult sepsis. This rapidly progressive soft tissue infection involving fascia and subcutaneous tissue initially spares the cutaneous layer leading to a paucity of physical findings even when there is systemic and life-threatening toxicity (4). The most common symptoms when present are swelling, pain and erythema, while signs in advanced stages include bullae, skin discolouration and crepitus (5), but these only occur only in 14-33% of patients (6). It is well established that glucose toxicity in patients with diabetes mellitus is associated with increased susceptibility to infection (7, 8). In a systematic review of 1463 individuals with NF, diabetes was a comorbidity in 45% (range 15.2-71%) (5). Diabetes mellitus has been associated with atypical milder presentations of NF featuring fewer complaints of pain (9), further contributing to misdiagnosis. CT imaging may show gas in soft tissues, but the diagnosis is made by direct visualisation of the fascia intraoperatively. Delays in surgery can result in increased mortality (4), and limb NF in the presence of diabetes has been associated with higher amputation rates (10).

Our patient was noted to have an apparently benign skin lesion that was identified on detailed examination but thought unlikely to be the source of sepsis driving life-threatening DKA, so surgical debridement was pursued only once all other possible causes were excluded. The operative findings were especially alarming in this context, combined with the impression of apparently reasonably controlled T2DM although this proved not to be the case. In this individual who was not insulin-requiring but developed severe life-threatening DKA with no other obvious precipitant, an index of suspicion for NF may have expedited surgical exploration and debridement, which may have improved her outcome.

Our patient required multiple operative procedures for debridement, VAC management and grafting, and multiple sessions of hyperbaric oxygen therapy. For her severe DKA and associated multiorgan sequelae, she required intubation, a prolonged ICU admission, renal replacement therapy with transition to intermittent haemodialysis, and ongoing close outpatient monitoring.

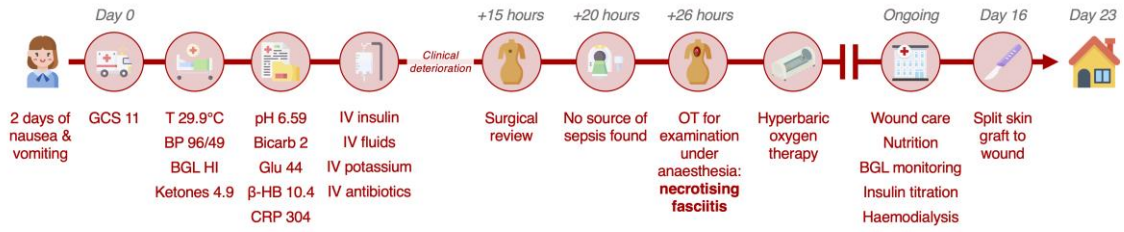
From this case we can be prompted to:

- Consider NF as a possible underlying precipitant for DKA with sepsis and no apparent source.
- View diabetes as a risk factor for NF which can mask the classic presenting features, hence delaying diagnosis and worsening outcomes.
- Examine a benign-appearing skin lesion in this context for crepitus, and if captured on CT imaging, assess for evidence of gas in the soft tissue.
- Have a low threshold to pursue surgical exploration for fascia visualisation of such skin lesions where no cause for sepsis is found in those unwell with DKA.
- Ensure that patients with T2DM:
    - Understand their condition implies an increased infection risk if poorly controlled.
    - Present early for acute management when unwell.

Table 1: Initial pathology results

Parameter	Value	Reference Range	Units
Arterial blood gas:			
pH	6.59	7.35 – 7.45	units
pCo2	27	35 – 45	mmHg
Bicarb calculated	2	22 – 32	mmol/L
Glucose	>41.6	3.50 – 7.7	mmol/L
Base excess	-35.8	-3.0 – 3.0	
Beta hydroxybutyrate	10.4	<0.5	mmol/L
Sodium	134	135 – 145	mmol/L
Potassium	3.3	3.5 – 5.2	mmol/L
Creatinine	171	45 – 90	mcmol/L
eGFR	30	>90	mL/min/1.73m^2
Glucose level	43.96	3.5 – 7.7	mmol/L
Lactate	1.9	0.6 – 2.2	mmol/L
Osmolality	351	275 – 300	mmol/kg
CRP	304	0 – 5	mg/L
Lipase	21	10 – 70	units/L
Haemoglobin	122	113 – 159	g/L
White cell count	35.05	3.90 – 12.70	10^9/L
Platelet count	584	150 – 396	10^9/L
Neutrophils	22.09	1.90 – 8.00	10^9/L

Figure 1: Timeline of events



1. Hamblin PS, Wong R, Ekinci EI, Fourlanos S, Shah S, Jones AR, et al. SGLT2 Inhibitors Increase the Risk of Diabetic Ketoacidosis Developing in the Community and During Hospital Admission. J Clin Endocrinol Metab. 2019;104(8):3077-87.
2. Hyperglycemic Crises in Patients With Diabetes Mellitus. Diabetes Care. 2002;25(suppl 1):s100.
3. Hjort U, Christensen JH. Diabetic ketoacidosis and compliance. The Lancet. 1998;351(9103):674-5.

4. Wong CH, Chang HC, Pasupathy S, Khin LW, Tan JL, Low CO. Necrotizing fasciitis: clinical presentation, microbiology, and determinants of mortality. J Bone Joint Surg Am. 2003;85(8):1454-60.
5. Goh T, Goh LG, Ang CH, Wong CH. Early diagnosis of necrotizing fasciitis. Br J Surg. 2014;101(1):e119-25.
6. Dworkin MS, Westercamp MD, Park L, McIntyre A. The epidemiology of necrotizing fasciitis including factors associated with death and amputation. Epidemiol Infect. 2009;137(11):1609-14.
7. Giaccari A, Sorice G, Muscogiuri G. Glucose toxicity: the leading actor in the pathogenesis and clinical history of type 2 diabetes - mechanisms and potentials for treatment. Nutr Metab Cardiovasc Dis. 2009;19(5):365-77.
8. Giri B, Dey S, Das T, Sarkar M, Banerjee J, Dash SK. Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: An update on glucose toxicity. Biomed Pharmacother. 2018;107:306-28.
9. Tan JH, Koh BTH, Hong CC, Lim SH, Liang S, Chan GWH, et al. A comparison of necrotising fasciitis in diabetics and non-diabetics. Bone Joint J. 2016;98-B(11):1563-8.
10. Cheng N-C, Tai H-C, Chang S-C, Chang C-H, Lai H-S. Necrotizing fasciitis in patients with diabetes mellitus: clinical characteristics and risk factors for mortality. BMC Infect Dis. 2015;15(1):417.

Tumour induced osteomalacia-the mystery illness beyond aches, pains and depression

Huajing Ni[1, 2], Roderick Clifton-Bligh[3, 4], Malgorzata Brzozowska [1, 2]

1. Endocrinology Department , The Sutherland Hospital , Sydney , NSW , Australia

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

3. Endocrinology Department , Royal North Shore Hospital , Sydney , NSW , Australia

4. Faculty of Medicine , University of Sydney , Sydney , NSW , Australia

Introduction

Tumour induced osteomalacia (TIO), previously known as oncogenic osteomalacia, is a rare paraneoplastic metabolic bone disorder characterised by renal phosphate wasting, hypophosphatemia, and osteomalacia (Carpenter, 2003). This complex paraneoplastic syndrome was first described in 1947 by McCance with less than 500 reported cases to date. Lack of awareness amongst clinicians often leads to substantial delays in the diagnosis of this rare condition with significant implications for affected individuals.

The pathogenesis of TIO is related to uncontrolled secretion of fibroblast growth factor 23 (FGF-23) by mesenchymal tumour tissues. FGF23 regulates vitamin D and phosphate metabolism as it inhibits phosphate reabsorption at the proximal tubule, promoting renal phosphate excretion and inhibiting calcitriol production (Perwad et al. 2007). Low serum phosphate is physiologically accompanied by suppressed FGF23; consequently, finding of elevated serum FGF23 in hypophosphatemia strongly suggests either TIO (Chong et al 2011) or a hereditary syndrome such as X-linked hypophosphatemic rickets (XLH), autosomal dominant (ADHR) and autosomal recessive (ARHR) hypophosphatemic rickets (Yamazaki et al. 2002).

We present a patient with TIO in whom the delayed diagnosis was accompanied by serious physical and psychosocial disabilities that have significantly improved after surgical resection of causative tumour.

Case Presentation

A 48-year-old man presented with an acute exacerbation of chronic lower back pain and severe bilateral leg weakness, present for at least 6 years. Pain severely restricted his mobility confining him to life in his apartment. He was of short stature with height of 155 cm, weight of 80kg, and BMI of 33. Neurological examination revealed predominantly proximal muscle weakness in both upper and lower limbs and associated muscle wasting. Whole spine MRI demonstrated mild disc disease without any obvious thoracolumbar spine fractures or enhancing lesions. Nerve conduction studies (2017) showed no evidence of generalised large fibre neuropathy or demyelinating features, and without clear neurogenic or myopathic features in the muscles sampled. Screens for vasculitis and multiple myeloma were negative.

Biochemistry found hypophosphatemia (0.21-0.76 mmol/L, reference range (RR) 0.75-1.50 mmol/L)) and despite intravenous and oral phosphate supplementations (up to 1g/day) his phosphate values did not revert to the normal reference range. Serum calcium level remained within normal range. He had consistently raised alkaline phosphatase (ALP) between 175-347 U/L (RR 30-110 U/L), elevated iPTH up to 27.4 pmol/L (RR 1.6-6.9 pmol/L) and low $1,25(OH)_2D$ of 42 nmol/L (60-200 nmol/L) which improved over time with calcitriol supplementation (up to 1.25 mcg per day). Estimated maximum tubular resorption of phosphorus corrected for glomerular filtration rate (TmP/GFR) revealed reduced fractional excretion of phosphate of 0.433 mmol/L (RR 0.9-1.35 mmol/L for males aged years 45-55), consistent with renal phosphate wasting. A whole-body bone scan revealed thoracolumbar scoliosis and lower limb arthritis. Bone mineral density (BMD) measured by Dual Energy X-ray Absorptiometry (DXA) was severely low with lumbar spine (LS) T score of -4.6, femoral neck (FN) T score of -2.9, and total hip (TH) T score of -4.1.

The patient continued to experience exacerbations of his back pain, not alleviated by CT guided steroid injections, as well pain radiating to his knees, shoulder, and chest. Pelvic CT, skeletal survey and repeat lumbar spine MRI showed no evidence of any lytic lesions or fractures. Over the next 18 months, the patient suffered from minimal trauma fractures involving his bilateral clavicles, multiple ribs, right humeral neck, and left humeral midshaft. After two years, a repeat DXA scan showed decrease in bone density with lumbar spine (LS) T score of -5.2 and total hip (TH) T score of -3.5 despite calcitriol (0.5 mcg twice daily), cholecalciferol (50 mcg daily) and a trial of denosumab. A review of his hormonal assays revealed hypogonadotrophic hypogonadism without evidence of an additional endocrinopathy. No pituitary pathology was found on MRI. The patient continued to find phosphate replacement challenging, due to ongoing diarrhoea, hence the progressive hypophosphatemia. Finally, his progressive physical disability led to financial crisis when he lost ownership of his home.

^{68}Ga-DOTATATE ([68Ga-DOTA0-Ty3] octreotate) PET CT scan showed a single focus of significant abnormal uptake in the left femoral head (Figure 1). At this time, serum intact FGF 23 was elevated at 205 pg/mL (DiaSorin Liaison, RR <50 pg/ml). MRI revealed bilateral non-acute femoral neck fractures and left sided avascular necrosis, treated subsequently with bilateral total hip replacement. Histological analysis confirmed the presence of a phosphaturic mesenchymal tumour in the left femoral head measuring 27 mm. Post-operative FGF 23 declined to 12.7 pg/mL post-operatively and remained within the normal reference at 43.1 pg/mL at 10 months after the surgery. His recovery was complicated by “Hungry Bone Syndrome” treated successfully with calcium and calcitriol replacement. He reported complete resolution of his chronic pain. He has become more independent with the activities of daily living without falls or fractures, at 10 months post-surgery. He no longer suffers from severe depression and he has actively participated in his rehabilitation program. Mark’s progress was assessed by the 36-Item Short Form Health Survey (SF-36). Postoperative improvement in Mark’s health status (quality of life (QoL) score of 80 out of 100)) was reflected by his highly perceived satisfaction with change in his overall health (QoL score of 100). In particular, Mark reported a major improvement in his social functioning (QoL score of 50) with lesser change (QoL scores of 30) for both physical functioning and emotional well-being.

Discussion and Literature Review

Classic features of osteomalacia of any cause include bone pain, musculoskeletal weakness, and recurrent pathological fractures (Econs at al.1994). Laboratory features of TIO include hypophosphatemia, phosphaturia, normal PTH and serum calcium, decreased $1,25(OH)_2D$, elevated ALP and raised FGF-23 (Jan de Beur, 2005). TIO tumours are divided into 4 subtypes with phosphaturic mesenchymal tumour being the most common (Econs et al. 1994). They are generally located in bone or soft tissues (Siegel et al. 2002).

Once diagnosed, TIO prognosis is generally good as most FGF23-secreting tumours are benign and rarely metastasise (Edmister et al. 2002). However, diagnosis of these tumours is frequently delayed by many years due to their occult nature,

small size, and atypical location (Jagtap et al. 2011). Tumour resection leads to prompt correction of all the clinical and biochemical manifestations of this syndrome (Chong et al. 2011; Ryan et al. 1984), and gradual re-mineralization of the osteoid matrix (Hautmann et al. 2015). Localisation of FGF23 secreting tumours is often challenging as they are often small in size and slow-growing, moreover, they are frequently located in atypical skeletal sites. Various imaging techniques are often used including functional imaging with octreotide scintigraphy, fluorodeoxyglucose (FDG)-positron emission tomography/computed tomography (PET/CT), ^{Ga}68 DOTATATE PET/CT, anatomical imaging with MRI and selective venous sampling for FGF-23 levels (Clifton-Bligh et al. 2013; Colt et al. 2005). In our case, ^{Ga}68 DOTATATE PET/CT detected the tumour lesion in our patient. The high sensitivity and specificity of ^{Ga}68 DOTATATE PET/CT favours its early use in suspected cases of TIO (Clifton-Bligh et al. 2013).

Medical treatment of TIO involves high doses of oral phosphate and calcitriol, as oral phosphate replacement alone is usually insufficient for correcting hypophosphatemia and often poorly tolerated. Prompt diagnosis and tumour excision can result in a dramatic improvement in the quality of life.

1. Carpenter T: Oncogenic osteomalacia--a complex dance of factors. N Engl J Med, 348(17), 1705-1708, 2003.
2. Chong W, Molinolo A, Chen C, Collins M: Tumor-induced osteomalacia. Endocr Relat Cancer, 18(3), R53-77, 2011.
3. Clifton-Bligh R, Hofman M, Duncan E, Sim Ie, Darnell D, Clarkson A, Wong T, Walsh J, Gill A,Ebeling P, Hicks R: Improving diagnosis of tumor-induced osteomalacia with Gallium-68 DOTATATE PET/CT. J Clin Endocrinol Metab, 98(2), 687-694, 2013.
4. Econs M, Drezner M: Tumor-induced osteomalacia--unveiling a new hormone. N Engl J Med, 330(23), 1679-1681. 1994.
5. Edmister K, Sundaram M: Oncogenic osteomalacia. Semin Musculoskelet Radiol, 6(3), 191-196. 2002.
6. El-Maouche D, Sadowski S, Papadakis G, Guthrie L, Cottle-Delisle C, Merkel R, Millo C, Chen C, Kebebew E, Collins M. T: (68)Ga-DOTATATE for Tumor Localization in Tumor-Induced Osteomalacia. J Clin Endocrinol Metab, 101(10), 3575-3581.2016.
7. Jan de Beur S: Tumor-induced osteomalacia. JAMA, 294(10), 1260-1267. 2005.
8. Ryan E, Reiss E: Oncogenous osteomalacia. Review of the world literature of 42 cases and report of two new cases. Am J Med, 77(3), 501-512. 1984.
9. Yamazaki Y, Okazaki R, Shibata M, Hasegawa Y, Satoh K, Tajima T, Takeuchi Y, Fujita T, Nakahara K, Yamashita T, Fukumoto S: Increased circulatory level of biologically active full-length FGF-23 in patients with hypophosphatemic rickets/osteomalacia. J Clin Endocrinol Metab, 87(11), 4957-4960.2002. doi:10.1210/jc.2002-021105
10. Perwad F, Zhang M, Tenenhouse H, Portale A: Fibroblast growth factor 23 impairs phosphorus and vitamin D metabolism in vivo and suppresses 25-hydroxyvitamin D-1alpha-hydroxylase expression in vitro. Am J Physiol Renal Physiol, 293(5), F1577-1583. 2007.
11. Hautmann A, Hautmann M, Kolbl O, Herr W, Fleck M: Tumor-Induced Osteomalacia: an Up-to-Date Review. Curr Rheumatol Rep, 17(6), 512. 2015.

Recurrent hyperparathyroidism following successful four gland parathyroidectomy and normalisation of pth in a renal transplant patient

Ryan Petrucci[1], Tamara Preda[1, 2], DP Johnston[3], Alar Enno[4]

1. Department of Endocrine, Head and Neck Surgery, Liverpool Hospital, Sydney, NSW, Australia

2. School of Medicine, Notre Dame University, Sydney, NSW, Australia

3. Clear View Medical Imaging, Sydney, NSW, Australia

4. Department of Anatomical Pathology, Liverpool Hospital, Sydney, NSW, Australia

Introduction

According to the Australian Bureau of Statistics 2011-12 Australian Health Survey, an estimated 10% of Australian adults over the age of 18 years had biomedical signs of chronic kidney disease (CKD) (1). The Kidney Disease: Improving Global Outcomes guidelines recommend that patients with stage 3 CKD undergo screening for secondary hyperparathyroidism (2).

Endocrine and biochemical anomalies associated with renal hyperparathyroidism relate to bone mineral/metabolic and cardiovascular disease. Secondary hyperparathyroidism (SHPT) in CKD patients is associated with increased fractures, hyperphosphataemia, anaemia, vascular and tissue calcification; these are important to treat before renal transplantation (3). There is also some evidence to suggest hypertension improves post parathyroidectomy in these patients and is a further benefit of parathyroidectomy prior to renal transplant (4).

Medical management for secondary hyperparathyroidism can be divided into pharmacological and surgical. Pharmacological agents include Vitamin D, Calcimimetics and phosphorus binders. Surgical options include total and subtotal parathyroidectomy (3). Internationally used guidelines such as *The National Kidney Foundation Kidney Disease Outcome Quality Initiative (KDOQI)* recommend parathyroidectomy for patients who are refractory to medical management (5).

Background

We present the case of a chronic renal failure patient who developed recurrent hyperparathyroidism despite previous 'total' parathyroidectomy and subsequent renal transplant.

Case Report

A 62 year old man was referred by his treating physicians to an endocrine surgeon with presumed tertiary hyperparathyroidism. He had a history of total parathyroidectomy and bilateral cervical thymectomy 17 years prior for secondary hyperparathyroidism with underlying chronic renal failure due to IgA nephropathy as a child.

Review of the histopathology report from this surgery confirmed removal of 4 hyperplastic parathyroid glands weighing between 0.285-0.815 grams.

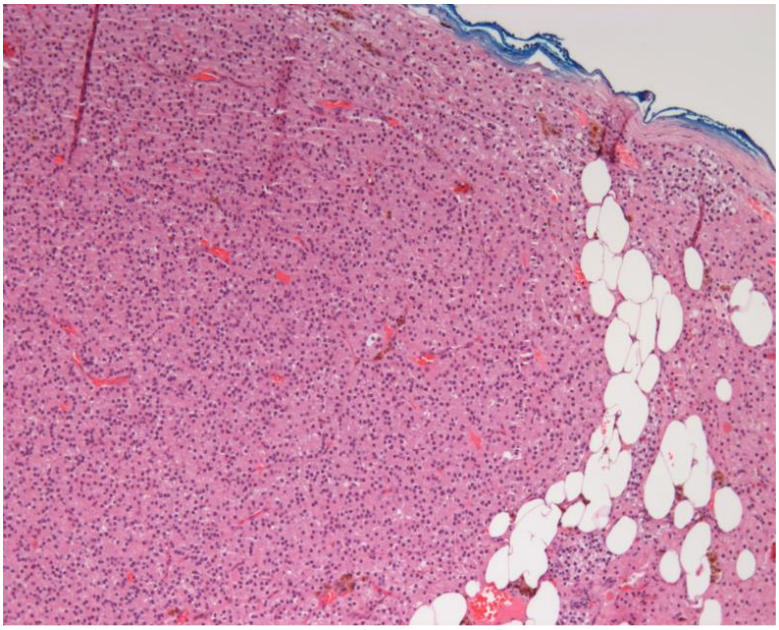


Figure 1- A low power (x10 magnification) image of the hyperplastic fat depleted parathyroid gland. There is adjacent normal parathyroid tissue visible.

The operation report noted four parathyroid glands in their expected locations in the neck. Comment was made that bilateral inferior parathyroids were found in the thyrothymic tract and were noted to be larger than their superior counterparts. The lower limit of dissection was the left brachiocephalic vein posterior to the manubrium (demarcated by surgical clips).

Following his original surgery PTH dropped from 93pmol/L to 4.6pmol/L with laboratory reference range 1.6 -6.9 pmol/L. This result was consistent with biochemical cure.

5 years after parathyroid surgery the patient underwent a renal transplant with excellent renal function for 8 years. His renal function then deteriorated culminating in a return to peritoneal dialysis (oliguric but not anuric). At the time of surgical review laboratory test results showed a PTH 120pmol/L (RR 1.6-6.9pmol/L), corrected serum calcium 2.60mmol/L (RR 2.15-2.55mmol/L) and eGFR 7 mL/min/1.73m^2. These results were indicative of tertiary hyperparathyroidism.

Imaging investigations were undertaken in order to localise the functioning parathyroid tissue responsible for elevation of PTH levels. A neck ultrasound did not detect any mass lesions. A SESTAMIBI-SPECT CT reported no focal uptake in or around the thyroid. A 12mm thoracic para-aortic nodule was seen in the left anterior/superior mediastinum adjacent to the aortic arch with moderate focal uptake (figure 2).

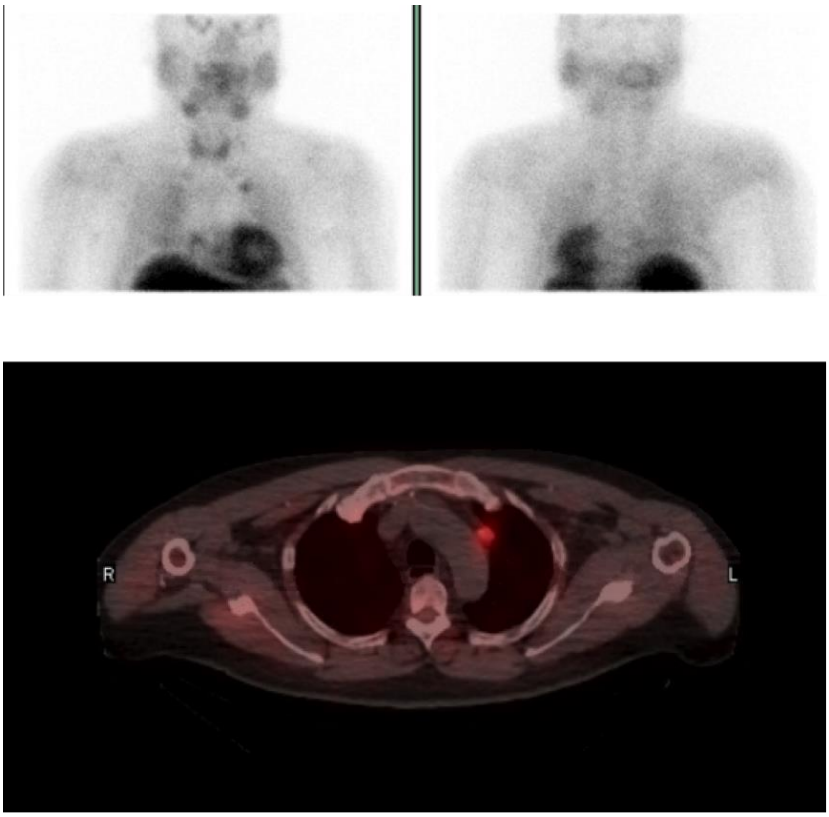
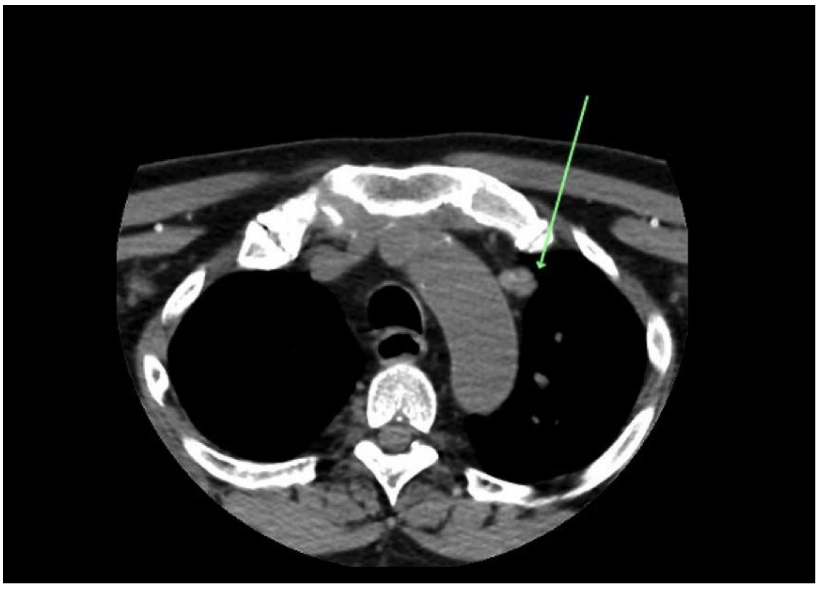


Figure 2- Sestamibi Parathyroid scan showing uptake in a mediastinal ectopic parathyroid gland situated adjacent to the arch of the aorta

The patient was referred for 4D Parathyroid CT. Non-contrast images were obtained given the patient was not anuric. A a mildly lobulated rounded lesion adjacent to the aortic arch in the superior mediastinum measuring 15 x 11mm with a single punctate focus of calcification was reported (figures 3 and 4).



Figures 3 – Axial CT image of mediastinal mass highlighted with the green arrow.

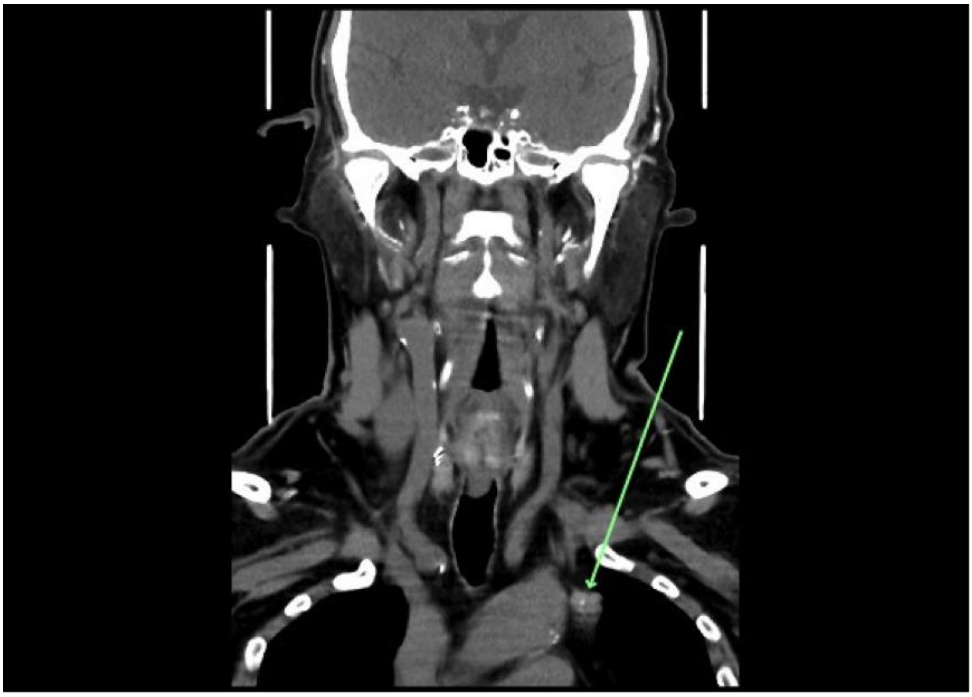


Figure 4- Coronal CT image of mediastinal mass highlighted with the green arrow

The patient was referred on to a cardiothoracic surgeon and a thoracoscopic excision of the lesion was undertaken in March 2020. The tumour was located in accordance with the imaging on the left side of the aortic arch and noted to be intimately associated with the phrenic nerve.

The surgical specimen was sent for histological assessment and reported a 1.7g fat depleted parathyroid gland with focal fibrosis and haemorrhage in keeping with an adenoma; no atypia.

Post-operative PTH dropped to 18.9pmol/L compared to 119pmol/L, which was taken 2 months prior to surgery. Post-operative corrected serum calcium normalised to 2.19 mmol/L. He was commenced on Calcium and 1,25 dihydroxycholecalciferol supplements to maintain normal corrected serum calcium levels and for treatment of bone disease.

He has been listed for a second renal transplant.

Discussion

Recurrent hyperparathyroidism although not common is of clinical significance due to its deleterious effects. Recurrence in patients who have undergone total parathyroidectomy has been attributed to incomplete cervical parathyroidectomy or supernumerary glands. A large-scale follow up study of 519 patients who underwent total parathyroidectomy showed a recurrence rate of 2.4% with 50% of these cases diagnosed with supernumerary mediastinal parathyroid glands (6). These patients required re-do surgery after imaging localisation.

Approximately 2-13% of the population have supernumerary (5 or 6) parathyroid glands (7,8). Ectopic parathyroids are defined as functional parathyroid glands located outside the normal anatomical position due to aberrant migration during foetal development. (9). They are found most commonly in the thymus, within the thyroid or in the mediastinum (6).

In CKD patients with secondary hyperparathyroidism it can take 6-12 months for PTH levels to normalise post transplant (10). Patients who continue to suffer from SHPT or recurrent disease need proper investigation and either pharmacological or surgical treatment. The current quoted prevalence of parathyroidectomy for this subgroup of patients is 1-5.6% (10).

In regards to this case it appears that the patient had an adequate PTH response to total parathyroidectomy initially. However, over time recurrent hyperparathyroidism manifested and was determined to be due to an ectopic supernumerary parathyroid gland. Whilst rare, this highlights the need for ongoing surveillance of renal hyperparathyroidism patients with consideration to the presence of supernumerary glands. Dual localisation with SESTAMIBI and CT is optimal when a non-neck location is likely.

Conclusion

- This case demonstrates that it is worthwhile to follow up patients who have been treated for secondary hyperparathyroidism, particularly once in receipt of renal transplant. Biochemical surveillance is simple and effective in detecting recurrence.
- Cross sectional thoracic imaging can and should be used to detect and localise supernumerary glands not apparent at the time of original surgery.

1. (1) Australian Institute of Health and Welfare 2020. Chronic kidney disease. Cat. no. CDK 16. Canberra: AIHW. [Cited 2020 Aug 25]. https://www.aihw.gov.au/reports/chronic-kidney-disease/chronic-kidney-disease
2. (2) Kidney Disease Improving Global Outcomes. Executive summary of the 2017 KDIGO Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD) Guideline Update: what's changed and why it matters. [Cited 2020 Aug 25]. https://kdigo.org/wp-content/uploads/2017/02/Executive-Summary-_2017-KDIGO-CKD-MBD-GL-Update_Corrected.pdf

3. (3) Lau WL, Obi Y, Kalantar-Zadeh K. Parathyroidectomy in the Management of Secondary Hyperparathyroidism. Clin J Am Soc Nephrol. 2018;13(6):952-961.
4. (4) Lorenz, Kerstin & Bartsch, Detlef & Sancho, Juan & Guigard, Sebastien & Triponez, Frederic. Surgical management of secondary hyperparathyroidism in chronic kidney disease—a consensus report of the European Society of Endocrine Surgeons. Langenbeck's Archives of Surgery. 2015;400:1344-5.
5. (5) Delos Santos R, Rossi A, Coyne D, Maw TT. Management of Post-transplant Hyperparathyroidism and Bone Disease. Drugs. 2019;79(5):501-513.
6. (6) Tominaga, Y., Numano, M., Tanaka, Y., Uchida, K. and Takagi, H. Surgical treatment of renal hyperparathyroidism. Semin. Surg. Oncol. 1997;13:87-96.
7. (7) Wang C. The Anatomic Basis of Parathyroid Surgery. Annals of Surgery. 1976;183:271-275.
8. (8) Pattou FN, Pellissier LC, Noël C, Wambergue F, Huglo DG, Proye CA. Supernumerary parathyroid glands: frequency and surgical significance in treatment of renal hyperparathyroidism. World J Surg. 2000;24(11):1330-1334.
9. (9) Noussios G, Anagnostis P, Natsis K. Ectopic parathyroid glands and their anatomical, clinical and surgical implications. Exp Clin Endocrinol Diabetes. 2012;120(10):604-610.
10. (10) Evenepoel P, Claes K, Kuypers DR, Debruyne F, Vanrenterghem Y. Parathyroidectomy after successful kidney transplantation: a single centre study. Nephrol Dial Transplant. 2007;22(6):1730–7.

A Painful Family

Ushank Ranasinghe[1], Shamasunder Acharya[1]

1. HNEHEALTH, Newcastle, NSW, Australia

Case Presentation

A 43-year-old white female (FH) was seen in the Hunter alliance diabetes clinic (integrated diabetes clinic). She was diagnosed with type 2 diabetes three years ago and was on metformin 1g twice daily and gliclazide 30mg once a day. The Hba1c was 7.1%. FH also had well-managed eczema and coeliac disease which was diagnosed about five years ago based on a history of recurrent episodes of diarrhoea. There was no biochemical or histological confirmation of coeliac disease.

FH has recurrent pancreatitis since the age of 10. The episodes could present as mild abdominal symptoms to severe episodes of pancreatitis needing hospital admission for resuscitation.

FM has many family members with pancreatic disease (**Figure 1**). Her mother and maternal grandmother died from pancreatic cancer in their 60s after experiencing decades of recurrent pancreatitis. FMs brother, maternal Aunt and their children have recurrent pancreatitis. FH has two daughters, age 20 and 10 who have recurrent pancreatitis, which started at the age of 2 and 5 respectively. Several family members' committed suicide for severe pain associated with pancreatitis.

FH has had several abdominal imaging over many years, and the most recent abdominal ultrasound in 2015 showed a pancreas with fatty infiltration with calcification. The pre-contrast CT scan showed significant pancreatic duct calcification consistent with sequala of recurrent episodes of chronic pancreatitis. The routine biochemistry was normal, and the coeliac screen was negative.

The strong family history suggested a familial cause of pancreatitis. We conducted genetic testing, which was positive for a pathogenic mutation in the PRSS1 gene. We have offered genetic testing for other family members. We started basal/bolus insulin to manage the diabetes and discontinued oral anti-diabetic medications. We also started pancreatic hormones 20000units (Creon) with each meal which significantly reduced the diarrhoea.FM remains concerned about the risk for herself and her children having pancreatic cancer which is known to have a poor prognosis. We are in discussion with our local gastroenterology service to decide on the best screening programme for FH and similar patients.

Discussion

Hereditary pancreatitis (HP) is a rare autosomal dominant disorder with variable penetrance causing recurrent pancreatitis. Epidemiological data shows that between five to ten percent of acute pancreatitis in children is from HP. The majority of families originate from the United States and Europe (Italy. Germany and Turkey). One family of Aboriginal descent was reported from New Zealand to carry the PRSS1 R122H variant[1].

The pathophysiology is based on unregulated activation of trypsinogen to trypsin leading to pancreatic inflammation. HP is generally caused by gain-of-function mutations in the cationic trypsinogen gene (PRSS1), although rare kindreds have been identified[2]**(table1**).

The clinical presentation varies from mild abdominal discomfort to severe acute pancreatitis, and cases vary substantially between individuals and families due to gene-gene and gene-environment interactions[4]. The majority of the patient would have had at least one episode of pancreatitis before the age of 20.

The complications occur later in life as a result of recurrent pancreatic insult. The exocrine dysfunction occurs typically early in the disease course, present as recurrent episodes of diarrhoea, malabsorption and malnutrition. The endocrine dysfunction follows a similar trajectory with some patients developing diabetes in their first decade of life with the risk increases with age. The most feared and severe complication which is pancreatic cancer mostly occurs after the 5th decade[6] (**figure 2**).

Early recognition of HP is vital, and management should comprise a multidisciplinary team involving a gastroenterologist, endocrinologist and general surgeon. The abdominal pain is managed with paracetamol, ibuprofen and opiates. There is evidence for using antioxidants which can reduce the oxidative stress in acinar cells which helps to reduce the pain[7]. For opiate naïve/dependent patients, pancreatectomy with islet cell auto transplantation can be considered[8]. The exocrine insufficiency is managed with pancreatic enzyme supplements with meals.

HP can lead to brittle diabetes following the destruction of both alpha and beta cells in the islets. Insulin secretion can be variable, and most patients will need insulin early in the course of diabetes[6].

Many studies from United States and Europe show that the occurrence of pancreatic cancer in HP was significantly higher than age- and sex-matched populations[3]. The risk goes up exponentially with older age[5]. The International Cancer of the Pancreas Screening (CAPS) Consortium summit made several recommendations on screening for patients with increased risk for familial pancreatic cancer. It highlighted the importance of early screening to detect margin negative pancreatic cancer and the screening of first-degree relatives. Initial screening should include endoscopic ultrasonography (EUS) and MRI/magnetic resonance cholangiopancreatography, not CT or abdominal ultrasound. Total pancreatectomy to prevent pancreatic cancer has been considered; however, long-term survival data is lacking[3, 4].

Learning points

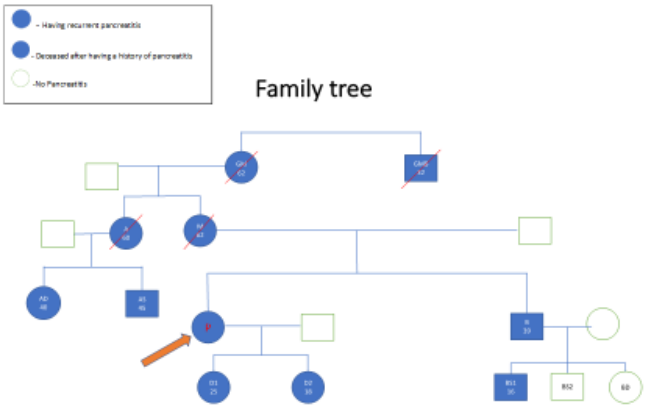
1 Hereditary pancreatitis should be considered in patients with diabetes who have a history of pancreatitis

2 Early initiation of insulin is warranted

3 Exocrine deficiency should be addressed with pancreatic enzyme supplementation

4 The patient needs to be managed by an MDT consist of an endocrinologist, gastroenterologist and a sergeant with a well-planned screening programme to detect pancreatic cancer.

Figure 1



P: FM - symptoms onset at the age of 10

D1: Daughter 1 -symptoms onset at the age of 2

D2-Daughter 2 - symptoms onset at the age of 10

B: Brother- symptoms onset at the age of 13

BS1: Brothers son 1-symptoms onset at the age of 12

BS2: Brothers son 2

BD: Brothers daughter

M: Mother-Died from pancreatic cancer at the age of 62

GM: Grandmother-Died from pancreatic cancer at the age of 62

GMB: Grandmothers brother -commit suicide for chronic abdominal pain

A: Aunt -Died from multiorgan failure following an attack of pancreatitis

AD: Aunts daughter Attempted suicide for abdominal pain

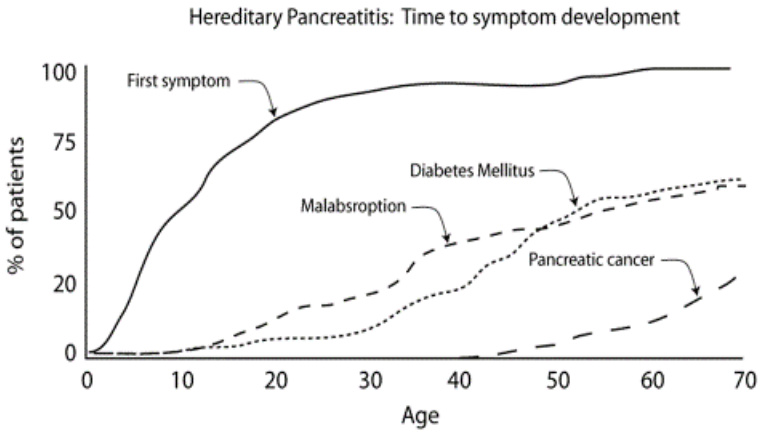
AS- Aunts son

Table 1

Genotype (variants)	Phenotype (syndromes)	Comment
PRSS1 GOF e.g. p.N29I, p. R122H, p.R122C	Autosomal dominant hereditary pancreatitis	Unregulated trypsin activity with premature activation Resistance to breakdown
PRSS1 LOF e.g. p.D100H, p.C139F, p.K92N,	Sporadic pancreatitis	Unfolded protein stress.
PRSS1 regulation e.g rs10273639, rs4726576 A	Protection from trypsin-associated pancreatitis	

Genotype-phenotype correlations of hereditary pancreatitis
GOF-gain of function; LOF-loss of function.

Figure 2



An unusual case of cardiomyopathy

Ushank Ranasinghe[1], Roger Smith[1]

1. HNEHEALTH, Newcastle, NSW, Australia

Case report

A 40-year-old man (MO) was admitted to the hospital with severe shortness of breath after experiencing several days of upper respiratory tract symptoms. He did not have other past medical history and was well and active. MO was a cigarette smoker and occasionally smoked marijuana which helped to manage his anxiety.

On arrival to the emergency department, MO was hypoxic with low oxygen saturations (60%), tachycardia (pulse 120 regular) with a blood pressure of 160/90mmHg.The clinical examination revealed respiratory distress, reduced air entry to lungs without hepato-splenomegaly or pitting oedema consistent with acute cardiac failure. The electrocardiogram showed sinus tachycardia with ST elevation in anterolateral leads (**image 1**). An emergency cardiac echocardiogram showed globally reduced left ventricular contractility with an ejection fraction of 10%. The initial troponin was 15000. The working diagnosis was a cardiogenic shock from myopericarditis.MO was admitted to the ICU for close observation and management.

A dobutamine infusion was administered to improve cardiac contractility. The patient was also treated with broad-spectrum antibiotics for a possible respiratory infection. A viral swab was positive for picornavirus, which raised the possibility of a viral myopericarditis. MO had a fast recovery and was soon transferred to a non-acute ward.

The repeat echocardiogram showed improved left ventricular contractility with an improved ejection fraction of 40%. MO was started on enalapril and bisoprolol for heart failure. His blood pressure remained elevated, and plasma catecholamines were requested as part of secondary screening which were elevated (noradrenalin 49.9nmol/L {ref 0.1 - 6.3}, adrenaline 24.9nmol/L {ref 0.1 - 6.3}, normetanephrine 11.748nmol/L {ref<0.670}, metanephrine 14.20nmol/L {ref< 0.447} (**chart 1 and table 1**). MO remained well, and the blood pressure normalised without further intervention. He was discharged following repeat collection of urine and plasma for catecholamines (results were pending) with an outpatient adrenal CT scan and endocrine follow-up.

MO remained asymptomatic with normal clinic blood pressure. The repeat catecholamines were elevated. The abdominal CT scan showed a left-sided adrenal mass (**image 2**).MO was readmitted for staging and preparation for surgery. A 68Ga-DOTATATE PET/CT showed a large dotatate avid left adrenal lesion consistent with pheochromocytoma. No extra-adrenal lesions were identified (**image 3**).

The case was discussed in the MDT meeting, and a decision was made for urgent surgery. The patient was commenced on phenoxybenzamine and high salt intake. The enalapril was discontinued. The patient underwent laparoscopic adrenalectomy eight weeks post initial presentation. The histology confirmed a 48 mm pheochromocytoma.

The patient is being followed up in the endocrinology outpatient clinic and is doing well. The post-surgery echocardiogram showed full recovery of cardiac function with an ejection fraction of 40%. The repeat catecholamines were normal, and the screening for genes associated with pheochromocytoma was (RET, SDHG, VHL) negative.

Discussion

Significant stress, such as burns, sepsis, surgery, or trauma, can raise plasma catecholamine levels. Following stress, the pituitary-adrenal axis and sympathetic division of the autonomic nervous system are activated, which promotes exocytosis of catecholamine-filled vesicles to plasma[5].

Catecholamines have multisystem actions. Their actions on the cardiovascular system regulate blood pressure by contracting the smooth muscle in the vasculature (via alpha-1 receptors) and enhance contractility of cardiac muscle (via beta-1 receptors)[1]. As a result, catecholamines are implicated in a multitude of diseases, including cardiogenic and non-cardiogenic shock. The levels of a physiological rise in plasma catecholamine during stress have not been quantified, and no studies are available comparing the difference between physiological and pathological release.

Most patients with a pheochromocytoma will present with clinical symptoms of diaphoresis, palpitations, and headaches, but our patient did not have any such symptoms[2]. The only relevant symptom was intermittent anxiety experienced for the past four months which settled with smoking marijuana. Also, the patient had a full recovery and remained asymptomatic following the initial admission and maintained a normal blood pressure which raised the question if it was, in fact, the stress from cardiac disease and assay interference of dobutamine infusion was responsible to the initial rise of the catecholamines. Studies show, heart failure with dilated cardiomyopathy can increase plasma catecholamines more than ten folds[7, 8]. However, measuring plasma catecholamines in patients with cardiogenic shock is challenging since exogenous catecholamines are frequently used in the treatment[9].

Catecholamine induced cardiomyopathy in the setting of pheochromocytoma is an unusual clinical entity. The overwhelming effect of catecholamines results in vasoconstriction of small arterioles and also cause a direct toxic effect on the myocardium. The majority of patients with pheochromocytoma have other associated symptoms of raised catecholamines[3].

The key to management of catecholamine-induced cardiomyopathy associated with pheochromocytoma is early intervention with surgical excision, which can result in complete reversibility of the myocardial dysfunction[6].There are case reports of the use of phosphodiesterase 3 inhibitors while waiting for surgery (ref). Such inhibitors enhance peripheral vasodilatation and cardiac contractility by increasing cyclic Adenosine Monophosphate (cAMP) activity and thereby increasing calcium influx into the cardiac sarcoplasmic reticulum[4].

This case highlights the highly variable presentation of pheochromocytoma and the importance of a broad understanding of possible presentations since it is imperative to diagnose and intervene early.

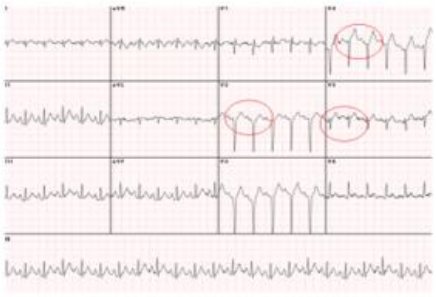
Take-home points

- Plasma catecholamines can rise following significant stress and can be affected by many medications.

- Pheochromocytoma can present as catecholamine's induced cardiac myopathy without any other symptoms in an otherwise well patient.
- Early intervention with surgery can rapidly and completely reverse catecholamine-induced cardiac disease.

Image 1

ECG- 31/08/2019

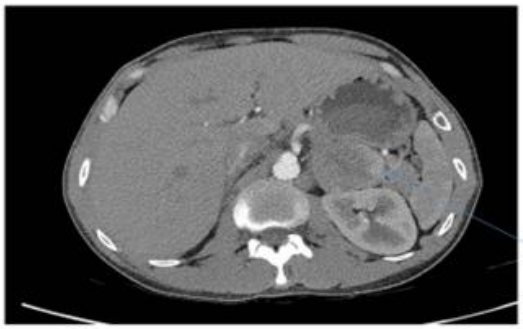


Mild ST segment changes

ECG- 31/08/2019

Image 2

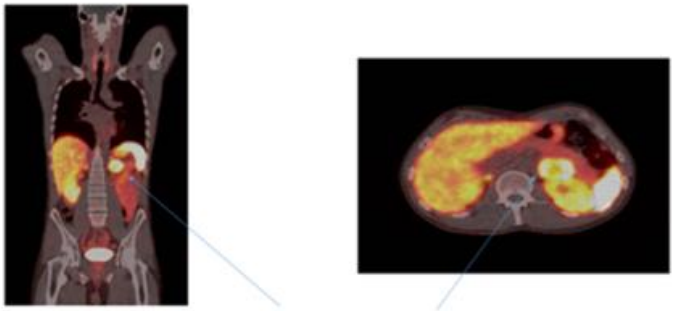
CT abdomen(contrast) 17/09/2019



5cm left adrenal mass

Image 3

68Ga-DOTATATE PET/CT



Dotatate avid left adrenal pheochromocytoma

Graph 1

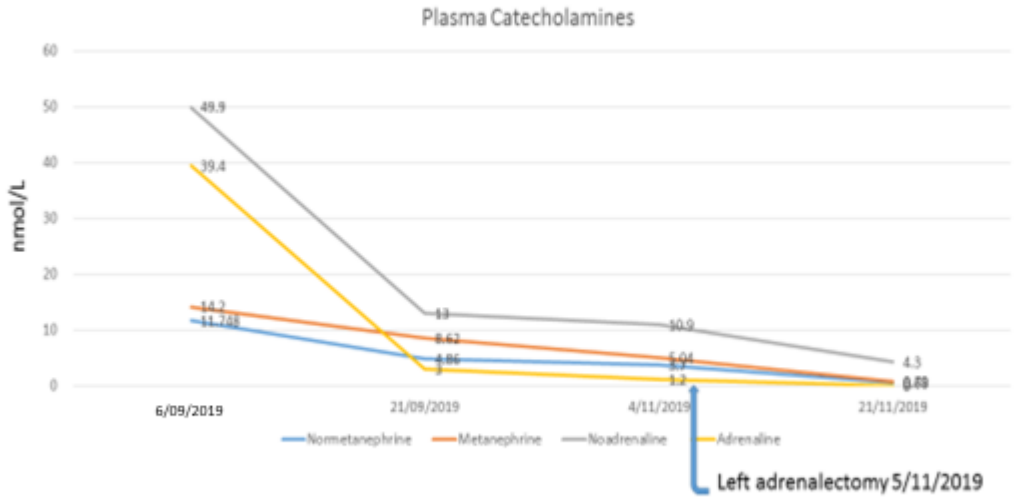


Table 1

Urine catecholamines

	Pre-surgery		Post-surgery	Ref. Range
	06/09/2019	17/09/2019 (different lab)	21/11/2019	
Dopamine/day	3.18		3.42	0.20-3.0umol/d
Noradrenaline/day	10.311		0.477	<0.600umol/d
Adrenaline/day	13.789		0.037	<0.100umol/d
Normetanephrine/day	17.9	15.7 (Ref <2.5umol/d)	1.1	<4.5umol/d
Metanephrine/day	55.7	58.8 (Ref<3.7umol/d)	0.6	<1.5umol/d
3-methoxytyramine		2.4		<1.3umol/d

Cushioning the impact of a 1st trimester hypertensive emergency

Ramessh Ranjan[1], Anthony Zimmermann[1]

1. Lyell McEwin Hospital, North Adelaide, SA, Australia

We present the case of Mrs OA, a 36-year-old woman, G3P1, who presented to hospital at six weeks gestation in hypertensive crisis. She described symptoms of exertional dyspnoea, intermittent chest pain and headaches of increasing severity over the preceding few weeks. At presentation, she had a blood pressure of 220/120 mmHg with a sinus tachycardia of 120 beats per minute. She gave a history of miscarriage earlier this year at 8 weeks gestation.

ECG revealed changes consistent with left ventricular hypertrophy with strain. Mrs OA was sequentially started on methyldopa 500mg QID, nifedipine XR 30mg daily and labetalol 100mg BD for the management of her hypertension. Echocardiogram revealed features of hypertensive cardiomyopathy, with left ventricular ejection fraction 35%.

Mrs OA had a similar presentation two years earlier, with hypertensive crisis requiring ICU admission. She was treated at that time with alpha and beta blockade after a CT pulmonary angiogram captured a right adrenal mass measuring 34 x 21 mm. Further investigation during that admission revealed normal plasma metanephrines and a 24-hour urine free cortisol of 1191 nmol/24 hour (0-150). Unfortunately, Mrs OA was lost to follow-up after failing to attend her clinic appointments. She stopped her anti-hypertensive medication shortly after.

History obtained during her current admission revealed weight gain of 50kg in the intervening 2 years. A repeat 24-hour urine free cortisol was similarly elevated at 1034 nmol/24 hour, while ACTH was suppressed at < 3 ng/L consistent with an ACTH independent cause for Cushing's syndrome. Her fasting plasma glucose was 5.4mmol/L. Imaging with an MRI of her adrenals again revealed a 34 x 23 mm right adrenal mass. Our patient was counselled regarding the high risk associated with continuing her pregnancy but expressed her wish to proceed with her pregnancy. At day 6 of her admission, in the setting of her significant hypertensive heart disease, metyrapone was started.

A diagnosis of Cushing's syndrome in pregnancy is rare, due to cortisol and androgen excess resulting in impaired fertility.[1] Cortisol inhibits GnRH secretion, and thus LH and FSH release. Oligomenorrhoea and amenorrhoea are reported in 75% of cases.[2] In addition, due to the overlap of the signs and symptoms of cortisol excess and pregnancy, such as weight gain, hypertension and hyperglycaemia - Cushing's syndrome is generally a delayed diagnosis in pregnancy.[3] A triad of hypertension, ecchymoses and muscle weakness has been suggested to suspect Cushing's syndrome in pregnancy.[1] As of 2018, there have been 200 case reports of Cushing's syndrome in pregnancy,[4] of which 50% have been of adrenal Cushing's. Despite Cushing's disease representing the majority of cases of Cushing's syndrome in non-pregnant women, it is less represented in pregnancy.[5]

Normal hormonal changes occurring during pregnancy may contribute to difficulty in making a diagnosis of Cushing's syndrome in pregnancy. A high index of clinical suspicion is required. Placental corticotropin-releasing hormone (CRH) and cortisol-binding globulin (CBG) are responsible for the physiological hypercortisolism in pregnancy (5). Placental CRH rises several hundred-fold during pregnancy. Placental CRH regulates placental ACTH and maternal ACTH release.[6] As a result, ACTH levels rise dramatically throughout pregnancy, with a surge at week 11, a further rise after 16-20 weeks and a final surge at labour.[6] The circadian rhythm of the HPA axis is preserved during normal pregnancy, although may be blunted.[6] In addition, placental oestrogen increases hepatic CBG production. This results in an increase in bound cortisol, which transiently reduces free cortisol levels, and thus stimulates pituitary ACTH secretion, also leading to in an increase in cortisol levels.[3] Therefore, total and free cortisol levels rise 2-3 fold throughout pregnancy, typically from the 11th week of gestation, and urine free cortisol levels increase from the second trimester.[3] 24-hour urine free cortisol is the recommended screening test for the diagnosis of Cushing's syndrome in pregnancy, with a reference range of 2-3 times the upper limit of normal from the second trimester.[37] ACTH levels are generally unhelpful, particularly from the second trimester.[5]

Cushing's syndrome is an important diagnosis to establish during pregnancy, due to its significant associated maternal and foetal morbidity and mortality.[8] Maternal morbidity occurs in 70% of cases with hypertension, diabetes mellitus and pre-eclampsia the most common complications. Maternal mortality is estimated at 2% compared with a background risk of < 0.01% in normal pregnancy.[8] The most common foetal complications are prematurity, intrauterine growth restriction and early spontaneous abortion, with foetal mortality estimated at 11%.[4]

The management of Cushing's syndrome in pregnancy is guided by disease severity and the time of gestation.[5] Surgical management is the first line option, with livebirth rates reported in up to 87% following surgical resolution.[6] Adrenalectomy has been found to be safe up to 32 weeks of gestation, however ideally surgery should be performed within the second trimester.[1] In some cases a conservative management approach may be possible. This depends on the degree of cortisol excess, and whether the associated comorbidities are able to be adequately controlled.[3] Cushing's syndrome diagnosed late in pregnancy may necessitate observation as the best management approach.[3]

Where surgery is contraindicated, and observation is not a feasible approach, medical management is available. Medical treatment is usually avoided in order to minimise the teratogenic risk and risk of induction of fetal adrenal insufficiency.[5] The agent most used in pregnancy is metyrapone, an inhibitor of 11 beta-hydroxylase leading to inhibition of cortisol production.[4] While it does cross the placenta, there is currently no evidence that it is teratogenic.[5] Metyrapone should be used with caution due to the total low number of reported cases, and the potential risk of hypertension due to deoxycorticosterone accumulation.[4] Another medical treatment option is ketoconazole which inhibits 11 alpha-hydroxylase and also leads to the inhibition of cortisol production.[4] It however has been associated with an increased rate of abortion in animal studies and crosses the placenta, hence carrying a possible teratogenic risk.[1]

Returning to our case, while control of Mrs OA's hypertension improved, and 24-hour urine cortisol reduced to 240 nmol/24 hour, pelvic ultrasound at week 8 gestation revealed a non-viable pregnancy. She underwent a dilatation and curettage with Mirena insertion and is currently awaiting a right adrenalectomy.

TAKE HOME MESSAGES

DETECTION

- Cushing's syndrome is uncommon in pregnancy due to cortisol and androgen excess impairing fertility.
- A diagnosis of Cushing's syndrome in pregnancy requires a high index of clinical suspicion as physiological hypercortisolism may make diagnosis difficult.
- Cushing's syndrome is associated with high maternal and fetal morbidity and mortality.

DIAGNOSIS

- The recommended screening test for Cushing's syndrome in pregnancy is a 24-hour urine free cortisol with a result greater than three times the upper normal limit from the 2nd trimester consistent with diagnosis.
- Distinguishing ACTH dependent and independent causes of Cushing's syndrome may be challenging during pregnancy as a consequence of placental CRH and ACTH.

MANAGEMENT

- Treatment of Cushing's syndrome is guided by disease severity and gestation.
- Surgical management is the preferred 1st line option in pregnancy.
- Metyrapone is the preferred agent for medical management during pregnancy.

1. 1. Brue T, et al. Management of Cushing's Syndrome during pregnancy: solved and unsolved questions. European Journal of Endocrinology. 2018; 178 R259 – R266.
2. 2. Alrt W, et al. Adrenal Corticosteroid Biosynthesis, Metabolism and Action. Endocrinol Metab Clin N Am. 2005; 34; 293-313.
3. 3. Karaca Z, et al. Pregnancy and pituitary disorders. Euro Journal of Endocrinology. 2010; 162; 453-475.
4. 4. Oksana Lekarev DO, et al. Adrenal disease in pregnancy. Best Practice and Research Clinical Endocrinology and Metabolism 25. 2011; 959-973.
5. 5. Ciftci Dogansen S, et al. Uncomplicated Pregnancy in a patient with Cushing's Disease. Acta Endocrinologica. 2017; vol. XIII, 215-219.
6. 6. Jolly K, et al. Surgery for Cushing's disease in pregnancy: our experience and a literature review. Ann R Coll Surg Engl. 2018; 101: e26-e31.
7. 7. Nieman L.K, et al. The Diagnosis of Cushing's Syndrome : An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2008; 93(5):1526-1540.
8. 8. Andreescu C.E, et al. Adrenal Cushing's syndrome during pregnancy. European Journal Endocrinology. 2017; 177, K13-K20.

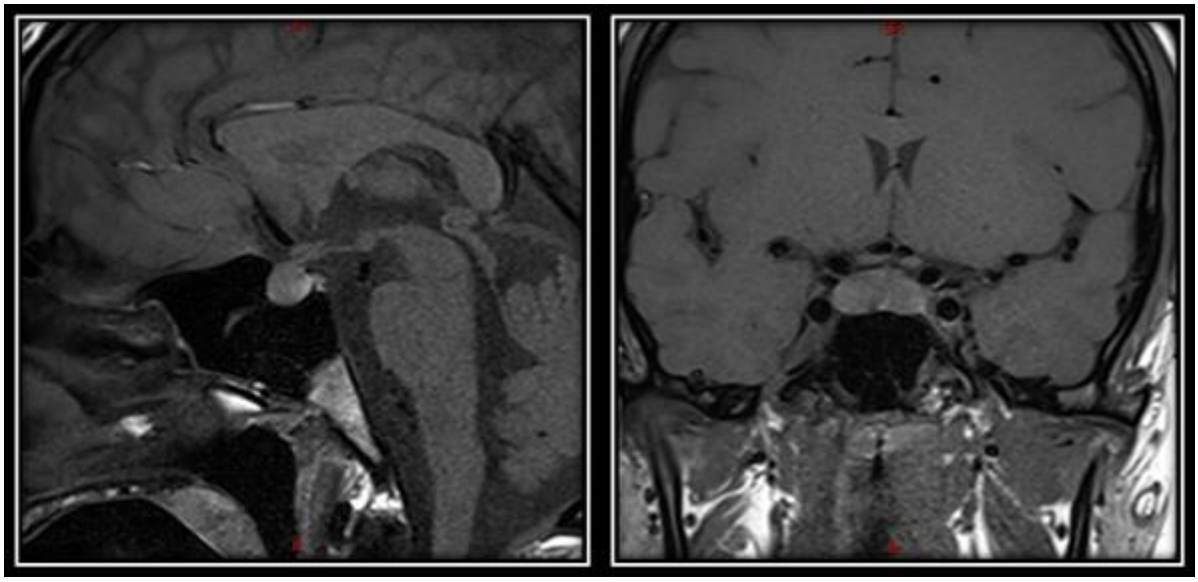
Gender affirming hormones - how much is too much of a good thing?

Lisa M Raven[1], Christopher A Muir[1]

1. Department of Endocrinology, St Vincent's Hospital, Darlinghurst, NSW, Australia

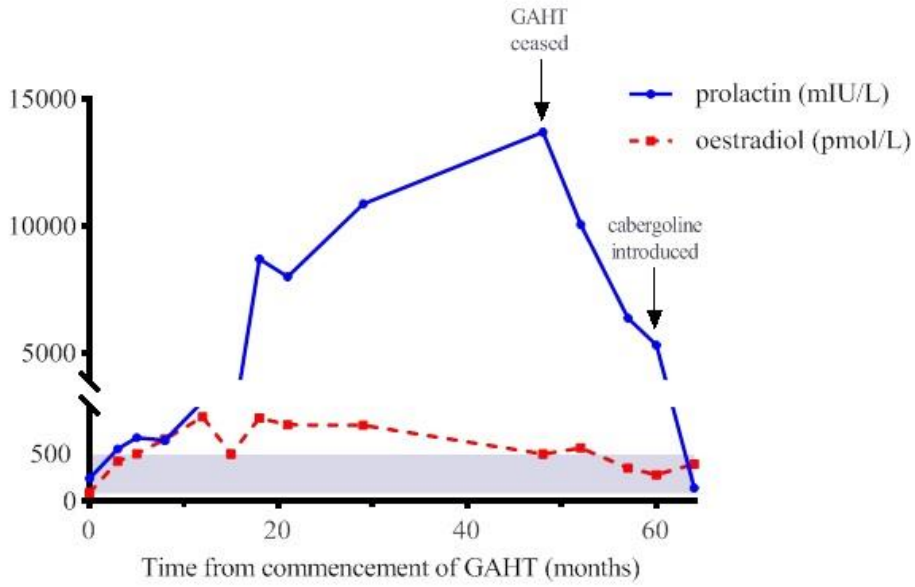
Case presentation:

A 23-year old transfeminine woman was referred for endocrine review of hyperprolactinaemia. Gender affirming hormone therapy (GAHT) had been commenced five-years prior and consisted of subdermal oestradiol 100 mg implants inserted 6-12 monthly, medroxyprogesterone acetate (MPA) 10 mg daily and spironolactone 100 mg daily. She was surgically naïve. Past medical history included depression, well controlled with fluvoxamine 100 mg daily and vitamin D insufficiency treated with cholecalciferol 1000 units daily. There was no significant family history. Physical examination identified gynecomastia (Tanner V) with expressible galactorrhea bilaterally. Resting blood pressure measured 117/80 mmHg and weight measured 97.6 kg, equating to a body mass index of 29.5 kg/m^2. There was no visual field deficit identified when assessed by confrontation. Hormonal analysis confirmed hyperprolactinaemia with serum prolactin measuring 13,696 mIU/L (normal range; NR 85-500). Magnetic resonance imaging (MRI) of the pituitary identified a right-sided 10.0 x 6.0 mm macroadenoma contained within the sella (Figure 1). A smaller, second adenoma measuring 5.0 x 3.0 mm was visualised within the left pituitary lobe, suggestive of a second adenoma. Cystic changes were present within both adenomas, raising the possibility of previous haemorrhage.



A review of prior results identified that serum prolactin levels had been elevated for several years prior to referral, with mild hyperprolactinaemia first documented three-months after commencement of GAHT. Mild low-level elevations in serum prolactin (<1000 mIU/L) persisted over the following 18-months, after which there was a progressive and exponential increase in serum prolactin to the current level of 13,696 mIU/L (NR 85-500), greater than 25-times the normal upper reference limit (Figure 2). Galactorrhoea had developed in conjunction with the marked increase in serum prolactin. There were no associated headaches or changes to vision or visual fields. Treatment with MPA and spironolactone was temporarily suspended and re-insertion of the next subdermal oestradiol implant was deferred. Prolactin levels decreased by approximately 50% following withdrawal of GAHT, but despite serum oestradiol levels of <300 pmol/L, prolactin remained grossly elevated at 5,307 mIU/L and galactorrhoea persisted.

Treatment with cabergoline was initiated at a dose of 0.5 mg weekly and MPA and spironolactone were recommenced. In place of a subdermal oestradiol implant, topical oestradiol was commenced via 50 mcg patch applied twice weekly. Following three months of treatment, serum prolactin concentrations improved to 140 mIU/L and galactorrhea had resolved.



Discussion:

Prolactin secretion is greater in women than in men. Lactotroph cells of the anterior pituitary are abundant in nuclear estrogen receptor alpha and estrogens are an important stimulator of pituitary lactotroph hyperplasia and prolactin release (1). In animal studies, administration of exogenous estrogens can induce formation of prolactin secreting pituitary tumours. Human data is limited, but also suggest exogenous oestradiol administration plays an important role in prolactin secretion (1).

Approximately one-in-five transgender women will develop hyperprolactinaemia following commencement of GAHT (2). Prolactin levels typically normalise with oestradiol dose reduction, although a subset will develop persistent hyperprolactinaemia with associated pituitary gland hyperplasia (2). In transwomen receiving feminising GAHT, prolactin levels increase in a dose dependent manner following commencement of oestradiol and decrease with dose reduction or oestradiol withdrawal (2, 3). Cyproterone acetate also increases serum prolactin through an unknown mechanism and hyperprolactinaemia can develop in transwomen treated with CPA independent of serum oestradiol concentration (4). Similar elevations in prolactin are not observed when oestradiol is used in combination with spironolactone or when CPA is withdrawn following gonadectomy (4-6). The long term consequences of hyperprolactinaemia in transwomen are unknown, but prolactinomas have been reported in association with GAHT suggesting a possible link between long term oestradiol use and risk of pituitary lactotroph adenomas (7).

Prolactinomas have been reported in multiple transgender women following long-term oestradiol treatment. Prolactinomas are benign functional tumours of the anterior pituitary lactotroph cell and oestrogens are primary regulators of lactotroph cell differentiation and proliferation (1). Transwomen are exposed to lifelong treatment with oestradiol, often at supraphysiologic doses and in conjunction with an anti-androgen. Anti-androgenic agents include cyproterone acetate and spironolactone. The incidence of prolactinoma in transwomen receiving GAHT is higher than in cis-gender women, men and transmasculine individuals, but despite the well-documented association, prolactinomas remain rare among transwomen. Current evidence suggests that risk of incident GAHT-associated prolactinoma is comparable to the increased risk in cis-gender women relative to cis-gender males (7). However, the true prevalence remains unknown and may be higher than reported, as transwomen do not develop typical signs of hyperprolactinaemia (menstrual irregularity) that typically lead to diagnosis in cis-gender women. The relative contribution of oestradiol and other factors to risk of prolactinoma is unknown (8). Whether higher doses and serum concentrations of oestradiol during GAHT proportionally increase risk remains controversial. Ethinyl oestradiol was historically used as the oestrogen component of GAHT and most reported GAHT-associated prolactinomas have occurred in association with ethinyl oestradiol (7). The relative risk of different oestradiol preparations is not known, although prolactinomas have been reported in association with conjugated oestradiol, oestradiol valerate and intramuscular oestradiol formulations (7). The majority of GAHT-associated prolactinomas have occurred when oestradiol was used in conjunction with CPA (7). Whether CPA exerts a direct effect via progestogenic suppression of hypothalamic dopamine release or acts indirectly via modulation of oestradiol or some alternate mechanism has yet to be determined. Interestingly, there is a clear association between CPA and meningioma, another benign brain tumor (9). However, prolactinoma has not been a prominent feature of CPA use outside of the transgender setting. Prior to this report, GAHT-associated prolactinoma had not been reported in association with alternative anti-androgens such as spironolactone. Spironolactone antagonises the androgen receptor, but unlike CPA does not act centrally to inhibit gonadotrophin releasing hormone secretion. The effect of spironolactone on pituitary lactotrophs is currently unknown, but appears less potent than CPA based on available observational data (4-6).

Predicting prolactin abnormalities is currently not possible and risk factors for development of GAHT-associated hyperprolactinaemia or prolactinoma are not known. Clinical practice guidelines currently recommend periodic monitoring of prolactin levels (10). However, no prolactin cut off has been established as pathogenic for transgender women receiving GAHT and the threshold for further investigation of hyperprolactinaemia remains arbitrary and subject to individual interpretation (10). Although marked hyperprolactinaemia and GAHT-associated prolactinoma are currently uncommon, they may become an increasingly important component of endocrine practice as the number of transwomen seeking GAHT continues to increase. Future research to further elucidate the mechanisms underlying the association between GAHT and hyperprolactinaemia will be important to identify women at risk of hyperprolactinaemia and GAHT-associated prolactinoma.

Take home messages:

- Transwomen receiving GAHT are at risk of hyperprolactinaemia, which can develop early in the course of GAHT
- Transwomen receiving GAHT may be at increased risk for prolactinoma
- Prolactinoma can occur in transwomen receiving spironolactone, and these women should receive similar monitoring to those prescribed CPA as an anti-androgenic agent
- Clinicians should maintain oestradiol at the lowest dose required to suppress testosterone and achieve adequate feminisation to reduce the risk of GAHT-associated hyperprolactinaemia and prolactinoma
- Screening of prolactin levels in transwomen receiving GAHT seems prudent to prevent morbidity related to hyperprolactinaemia and allow for early detection of prolactin secreting pituitary adenomas

1. Šošić-Jurjević B, Ajdžanović V, Miljić D, Trifunović S, Filipović B, Stanković S, et al. Pituitary hyperplasia, hormonal changes and prolactinoma development in males exposed to estrogens—an insight from translational studies. Int J Mol Sci. 2020;21(6):2024.
2. Asscheman H, Gooren L, Assies J, Smits J, De Slegte R. Prolactin levels and pituitary enlargement in hormone-treated male-to-female transsexuals. Clin Endocrinol. 1988;28(6):583-8.
3. Gooren L, Harmsen-Louman W, Van Kessel H. Follow-up of prolactin levels in long-term oestrogen-treated male-to-female transsexuals with regard to prolactinoma induction. Clin Endocrinol. 1985;22(2):201-7.
4. Defreyne J, Nota N, Pereira C, Schreiner T, Fisher AD, Den Heijer M, et al. Transient elevated serum prolactin in trans women is caused by cyproterone acetate treatment. LGBT health. 2017;4(5):328-36.
5. Bisson JR, Chan KJ, Safer JD. Prolactin levels do not rise among transgender women treated with estradiol and spironolactone. Endocr Pract. 2018;24(7):646-51.
6. Fung R, Hellstern-Layefsky M, Tastenhoye C, Lega I, Steele L. Differential effects of cyproterone acetate vs spironolactone on serum high-density lipoprotein and prolactin concentrations in the hormonal treatment of transgender women. J Sex Med. 2016;13(11):1765-72.
7. Nota NM, Wiepjes CM, De Blok CJ, Gooren LJ, Peerdeman SM, Kreukels BP, et al. The occurrence of benign brain tumours in transgender individuals during cross-sex hormone treatment. Brain. 2018;141(7):2047-54.
8. McFarlane T, Zajac JD, Cheung AS. Gender-affirming hormone therapy and the risk of sex hormone-dependent tumours in transgender individuals—A systematic review. Clin Endocrinol. 2018;89(6):700-11.
9. Gil M, Oliva B, Timoner J, Maciá MA, Bryant V, de Abajo FJ. Risk of meningioma among users of high doses of cyproterone acetate as compared with the general population: evidence from a population-based cohort study. Br J Clin Pharmacol. 2011;72(6):965-8.
10. Hembree WC, Cohen-Kettenis PT, Gooren L, Hannema SE, Meyer WJ, Murad MH, et al. Endocrine treatment of gender-dysphoric/gender-incongruent persons: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2017;102(11):3869-903.

Looking a little too tanned

Mayurapriya Raviskanthan[1], Jessie Teng[1]

1. Endocrinology Unit, Eastern Health, Melbourne

Case

Mr NJ, a 45 year old male, was referred to Endocrinology for investigation of hyponatremia during an admission with acute gastroenteritis complicated by acute kidney injury and hypotension.

Past history was significant for haemochromatosis, diagnosed age 19 after noticing skin bronzing. Genotypic analysis demonstrated compound heterozygosity for C282Y and H63D mutations. Despite previously requiring fortnightly venesections, his last venesection was 18 months prior and his most recent ferritin was 150microg/L (30-400microg/L). Liver ultrasound did not demonstrate cirrhotic features. His family history is significant for two brothers with compound heterozygous haemochromatosis, and one half-sister with hypothyroidism. Mr NJ lives at home with his wife and 3 biological children.

Investigations included a morning cortisol level of 58nmol/L performed on 2 separate occasions, with markedly elevated ACTH level of 941ng/L (7.2-63ng/L) consistent with primary adrenal insufficiency. Potassium levels were within normal limits. In retrospect, Mr N did note intermittent postural symptoms and lethargy at home. He denied weight loss, abdominal symptoms or any further changes to his skin colour.

Mr N was initially commenced on Hydrocortisone 20mg mane 10mg nocte, as well as fludrocortisone 100mcg daily with improvement in his Na levels, blood pressure and postural symptoms. CT abdomen and pelvis demonstrated shrunken adrenal glands. Thyroid function tests demonstrated mild primary hypothyroidism with a Thyroid Stimulating Hormone (TSH) of 9.64mIU/L and free T4 11.5pmol/L (12-22pmol/L), and he was commenced on thyroxine 50mcg daily.

Anti Thyroid Peroxidase (TPO) antibodies, anti Thyroglobulin (Tg) antibodies and Thyroid Stimulating Hormone Receptor antibodies (TSHrAbs) were all negative. Anti-adrenal cortical cell antibodies were also negative, raising the possibility of endocrine organ failure due to ferritin deposition from haemochromatosis. Further tests to exclude alternate causes of primary adrenal insufficiency, including 25-alpha hydroxylase antibodies and long chain fatty acids (to exclude a mild form of adrenoleukodystrophy), have been delayed due to the current COVID pandemic restrictions. Other relevant tests include normal LH of 4 IU/L (2-10 IU/L) and FSH of 4 IU/L (2-10 IU/L) with corresponding morning fasting total testosterone of 14.1nmol/L (9 - 35 nmol/L). Renin levels 2 months post commencement of fludrocortisone were 32mIU/L (10-50mIU/L). HbA1c was 5.2%.

Mr NJ's TSH and T4 have normalised with 50mcg daily thyroxine. However, despite changing his hydrocortisone to thrice daily dosing with a total daily dose of 32mg, he is still experiencing persistent lethargy and symptoms of hypocortisolemia. He is awaiting further review by the haematologists in regards to his haemochromatosis.

Review of the literature

Haemochromatosis is an autosomal recessive inherited condition, caused by defects in the HFE gene. Clinical manifestations commonly occur in patients with homozygous C282Y gene mutations, however heterozygotes with H63D and C282Y mutations may, in 10-15% of cases, develop clinical disease[1]. Clinical manifestations are generally the result of progressive iron overload, due to decreased expression of hepcidin, and resultant loss of inhibition of iron reabsorption from the gut and recycling of iron from red blood cells[2]. Haemachromatosis can manifest in cirrhosis, joint arthralgias and cardiomyopathy.

Endocrine dysfunction in haemochromatosis is a known phenomenon, and most commonly induces "bronze" diabetes, hypogonadotrophic hypogonadism, and osteoporosis[3]. Diabetes most commonly manifests in a Type 2 diabetes phenotype, although the pathophysiology is a combination of decreased insulin secretion due to damaged pancreatic islet cells, as well as increased insulin resistance due to hepatic dysfunction[3].

Endocrine disease is usually triggered by progressive years of iron loading, and is associated with cirrhosis. Incidence rates of endocrine disease (previously up to 80%) have dropped considerably, as disease is frequently ameliorated by venesection and lowering ferritin.

Adrenal insufficiency has been seen in 13 - 46% of patients with thalassemia, in the context of secondary haemochromatosis from transfusion dependence and subsequent iron deposition, manifesting in both primary and secondary adrenal insufficiency[3]. In hereditary haemochromatosis, isolated case reports of primary adrenal insufficiency have been seen, and iron deposition of the adrenal glands has been postulated as the cause[3]. Autopsy studies of patients with hereditary haemochromatosis have demonstrated iron deposition in the adrenal glands, although primarily in the zona glomerulosa rather than the fasciculata layer.

Iron deposition has also been seen in the thyroid gland of patients with hereditary haemochromatosis, although clinical hypothyroidism is rare[4]. A case series of 34 patients with hereditary haemochromatosis with cirrhosis secondary to iron accumulation, showed 8% (3) of patients had hypothyroidism, although all 3 patients subsequently tested positive to anti-TPO antibody testing[4] . Subsequent studies have demonstrated incidence rates of 1% of thyroid disease, primarily hypothyroidism, in patients with haemochromatosis, which is similar to the general population.

Quality of life is a common persistent concern in patients with treated adrenal insufficiency. Studies have demonstrated deficits in vitality, mental health, physical functioning and general health perception[5]. Thrice daily compared to twice daily hydrocortisone dosing has shown benefits in Health Related Quality of Life (HRQoL) scores, however pharmacological studies still demonstrate inconsistent target concentrations of glucocorticoid levels [6]. Daily prednisolone is an alternative glucocorticoid option, however its pharmacokinetic profile does not replicate the physiological diurnal variation of endogenous cortisol, and consequently may worsen both the lipid profile and bone health[7].

Plenadren, a modified release hydrocortisone, has become available in Europe, and has demonstrated pharmacokinetic similarities to normal endogenous production of cortisol. In spite of this, adequate quality of life improvement data is lacking, and trials have shown modest but not substantial improvements[7]. Chronocort, an alternate modified release hydrocortisone, which induces a physiological 'peak' in cortisol levels during the early hours of the morning, has shown some efficacy in the congenital adrenal hyperplasia population, and is currently in development for use in Addison's disease[8]. Subcutaneous hydrocortisone infusions have also been used on a case by case basis[8].

Differing routines can also complicate glucocorticoid regimens in patients with Addison's disease, and will require amendments to standard management. In fasting periods, such as during Ramadan, switching to once daily prednisolone to be taken at dawn, and ensuring intramuscular hydrocortisone is available are necessary considerations[9]. Similarly, patients who undertake shift work, particularly night shifts, may require an extra dose of hydrocortisone prior to commencing work.

In summary, although endocrine dysfunction is a common manifestation of hereditary haemochromatosis, the incidence is decreasing with early detection and initiation of venesection in patients. Primary hypothyroidism and primary adrenal insufficiency are exceedingly rare manifestations of disease. The solution to impaired quality of life in these patients has not been solved with currently available medications.

Take home points

- Haemochromatosis is a relatively common condition, and can manifest with endocrinological dysfunction, most commonly "bronze" diabetes, hypogonadotrophic hypogonadism and osteoporosis
- Treatment of endocrine manifestations are through amelioration of the iron burden with venesection
- Less common endocrinological manifestations of haemochromatosis can include primary adrenal and primary thyroid dysfunction
- Early screening for endocrine disease in patients with hereditary haemochromatosis can help to mitigate the disease burden and consequences.
- At diagnosis, patients should be screened for diabetes, hypogonadism and should be considered for a baseline DEXA scan, given this will likely be the point of greatest iron burden.
- Thereafter, patients should be tested if symptomatic, or more routinely if they develop other manifestations of iron overload such as cirrhosis.
- Quality of life is a persistent and unsolved issue in patients with adrenal insufficiency

1. Fleming, Robert E et al. "Pathophysiology of hereditary hemochromatosis." Seminars in liver disease vol. 25,4 (2005): 411-9. doi:10.1055/s-2005-923313
2. Vaulont S et al. "Of mice and men: The Iron Age". J Clin Invest. 2005;115(8):2079-2082. https://doi.org/10.1172/JCI25642.
3. Pelusi, C., Gasparini, D. I., Bianchi, N., & Pasquali, R. (2016). Endocrine dysfunction in hereditary hemochromatosis. Journal of Endocrinological Investigation.
4. Edwards, C. Q. (1983). Thyroid Disease in Hemochromatosis. Archives of Internal Medicine, 143(10), 1890. doi:10.1001/archinte.1983.00350100052015
5. Hahner, Loeffler et al. (2007) Impaired Subjective Health Status in 256 Patients with Adrenal Insufficiency on Standard Therapy Based on Cross-Sectional Analysis, JCEM 2007.
6. Howlett, T. A. (1997). An Assessment of Optimal Hydrocortisone Replacement Therapy. Clinical Endocrinology
7. Isidori AM, Venneri MA, Graziadio C, et al. Effect of once-daily, modified-release hydrocortisone versus standard glucocorticoid therapy on metabolism and innate immunity in patients with adrenal insufficiency (DREAM): a single-blind, randomised controlled trial. Lancet Diabetes Endocrinol. 2018;6(3):173-185. doi:10.1016/S2213-8587(17)30398-4
8. Isidori AM, Arnaldi G, Boscaro M, et al. Towards the tailoring of glucocorticoid replacement in adrenal insufficiency: the Italian Society of Endocrinology Expert Opinion. Journal of Endocrinological Investigation. 2020 May;43(5):683-696. DOI: 10.1007/s40618-019-01146-y.
9. Hussain, S, Hussain, S, Mohammed, R, Meeran, K, Ghouri, N. Fasting with adrenal insufficiency: Practical guidance for healthcare professionals managing patients on steroids during Ramadan. Clin Endocrinol (Oxf). 2020; 93: 87– 96. https://doi.org/10.1111/cen.14250

Pituitary abscess from a Rathke's cleft cyst: A case report

Alanna Tan[1], Jasmine Zhu[1], Felcity Stringer[2], Mathis Grossmann[1, 3], Jeffrey Zajac[1, 3]

1. Department of Endocrinology, Austin Health, Heidelberg, VIC, Australia

2. Barwon Health, Geelong

3. School of Medicine, The University of Melbourne, Melbourne, VIC, Australia

A 25-year old female presented to the Emergency Department with 2 days of rigors, nuchal rigidity and intermittent confusion on a background of 3 months of left sided headaches. Her past medical history included an ovarian cyst. She was not on any regular medications apart from paracetamol. A non-contrast Magnetic Resonance Imaging (MRI) of her brain performed four weeks prior demonstrated a 19 x 11 x 13mm pituitary cystic mass described as a pituitary adenoma.

On examination, she was febrile at 38.6°C, with nuchal rigidity. She was intermittently confused, scoring 3 out of 5 on serial 7s. She had no other neurological deficits. Her visual acuity was 6/5 bilaterally. Her visual fields were normal on confrontation testing and she had full range of eye movements. There was no papilloedema. A Gadolinium-enhanced MRI of her brain revealed a rim enhancing pituitary cystic lesion measuring 12.8 x 21 x 14mm that had increased in size and was impinging on the optic chiasm (*Figure 1*).

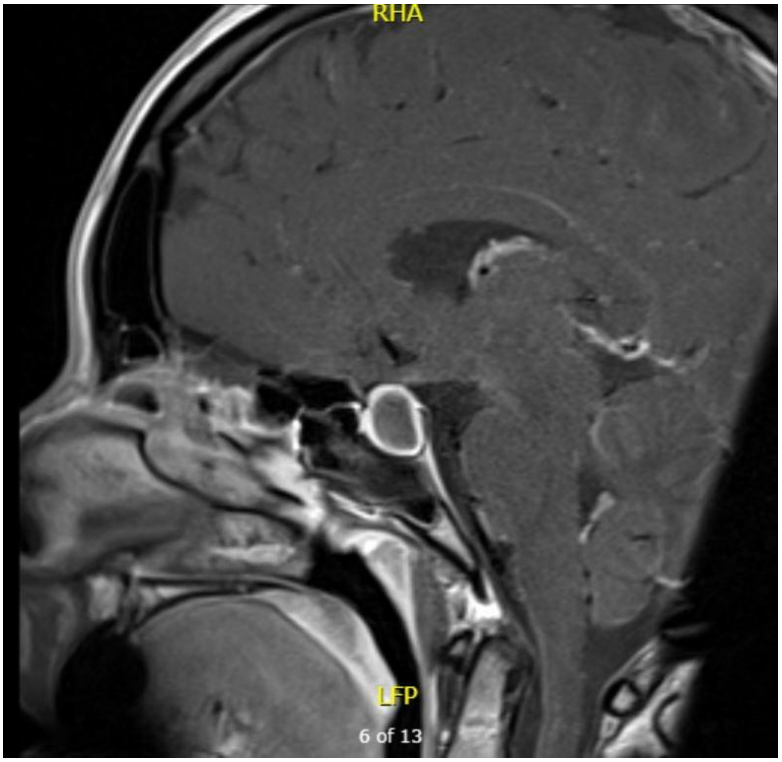


Figure 1: A Gadolinium enhanced MRI brain reveals a rim enhancing pituitary lesion measuring 12.8 x 21 x 14mm.

A lumbar puncture showed 1777x 10^6 leucocytes, 70% polymorphonuclear cells, 30% mononuclear cells, an elevated protein of 0.83 g/L (0.15-0.45) and a low glucose of 2.3 mmol/L (2.8-4.2). There were no organisms on gram stain. The cerebrospinal fluid was negative for Streptoccocus pneumoniae, Neisseria meningitidis and acid-fast bacilli. She had a peripheral leucocytosis with a white cell count of 18.x 10^9/L (4.0-12.0). Her HIV serology was non-reactive.

Given the ongoing fevers and meningism, there was a high index of suspicion for an infective process. She was commenced on intravenous benzylpenicillin, aciclovir, ceftriaxone and metronidazole. She received a single dose of dexamethasone and then was commenced on regular intravenous hydrocortisone pre-operatively. Her initial pituitary panel (after dexamethasone) revealed a low TSH and T4, appropriately suppressed ACTH and cortisol but no other abnormalities (Table 1).

Hormone	Baseline Level	Post-operative level	Reference range
TSH	0.83 mU/L	1.06 mIU/L**	0.38-5.30 mU/L
Free triiodothyronine (T3)	3.2 pmol/L	4.2 pmol/L**	3.8-6.0 pmol/L
Free thyroxine (T4)	7.8 pmol/L	13.9 pmol/L	8.0-16.5 pmol/L
FSH	6.0 IU/L	5 IU/L	
Oestradiol	171 pmol/L	232 pmol/L	
Prolactin	143 mIU/L	120 mIU/L	71-566 mIU/L
ACTH	< 1.5 ng/L*	9 ng/L**	7.2-63.3 ng/L
Cortisol	<11 nmol/L*	128 nmol/L**	185-624 nmol/L
Growth hormone (GH)	Not available	0.2 µg/L	<5.0 µg/L
Insulin-like growth factor 1(IGF-1)	Not available	20.8 nmol/L	11.0-33.7 nmol/L

Table 1: Baseline and post-operative pituitary panel

**After administration of dexamethasone*

*** After commencement of thyroxine and cortisone*

The patient had a transsphenoidal pituitary fossa exploration and frank pus was drained from the sella. Sixteen hours post-operatively, she developed polyuria and was treated with 1mg of intravenous desmopressin. Due to ongoing polyuria, she was converted to a regular intranasal dose of 10mcg twice daily. Her intravenous hydrocortisone was weaned to oral cortisone acetate and she was commenced on levothyroxine 75mcg.

Histological examination of the lesion revealed minimally inflamed pituitary gland tissue, fragmented proteinaceous debris with small numbers or neutrophils and focal benign epithelial lining suggestive of a Rathke's cleft cyst with superimposed inflammation. No pathogen was isolated from the pituitary abscess. She recovered rapidly and was discharged 5 days after her operation and continued intravenous ceftriaxone, oral metronidazole and oral doxycycline in the community for 4 weeks.

Eight weeks following her operation, a repeat pituitary panel did not demonstrate any abnormalities (Table 1).

Her thyroxine and cortisone were reduced to 50 mcg and 12.5mg BD respectively. She continued intranasal desmopressin. By this time, her menses had recommenced and headache had resolved.

In summary, a 25-year old immunocompetent female presented with crescendo headaches and was found to have an infected . No pathogen was found but she was managed empirically with antibiotics prior to surgery. At last follow up, she demonstrated partial recovery of her pituitary function.

Case discussion

We describe a case of a secondary pituitary abscess from an infected Rathke's cleft cyst in a patient with no identifiable risk factors who required thyroxine, cortisone and desmopressin post-operatively.

Pituitary abscesses are rare but serious intrasellar infections reported to comprise less than 1% of all cases of pituitary disease referrals in a tertiary specialist centre [1]. Most pituitary abscess occur in a previously healthy gland, while secondary pituitary abscess represent one-third of all pituitary abscesses[2]. The two common routes of abscess formation are haematogenous seeding, or direct extension from infected adjacent structures (eg meningitis, thrombophlebitis or sphenoid sinusitis). Common clinical manifestations include headache, visual disturbance, and features of pituitary insufficiency. The diagnosis of a pituitary abscess may be delayed due to the lack of systemic signs of inflammation (occurring only one-third of patients[3]), especially that radiologically, they are often mistaken for pituitary adenomas [4].

Rathke's cleft cyst (RCC) is an epithelial cell-lined cystic pituitary lesion derived from the remnant of Rathke's pouch which is usually asymptomatic. To date, there have been 21 cases of pituitary abscess from Rathke's cleft cyst published. Nine patients had endocrine dysfunction; 3 had multi-axis anterior failure, 3 had diabetes insipidus (DI) and 3 had hyperprolactinaemia at initial presentation. The latter 2 were caused by a stalk effect. In one patient, the was not specified. All but 1 patient presented with headache and/or visual disturbance; clinical signs of meningism were found in only 5 of 21 patients.

Most patients with RCC abscess presented after several months of symptoms. Rim-enhancement is a common feature on MRI. However, as the signal intensity of an abscess may be affected by its protein content, its appearance may be variable on MRI [5].

Once diagnosis is made, surgery should be performed promptly via a transsphenoidal approach as this provides a route for prolonged drainage and decompression of the suprasellar structures. Craniotomy is not routinely recommended due to the higher risk of exposure of infectious material to adjacent structures. Of the published 21 cases with an infected Rathke's cleft cyst, 8 had positive cultures from the abscess content: Acinetobacter, Staphylococcus epidermis, Staphylococcus aureus, Streptococcus pyogenes and Enterococcus faecium [6]. Whilst our patient did not have a positive culture, this may be due to the use of empirical antibiotics administered pre-operatively. Another differential that cannot be excluded is chemical meningitis.

Pituitary abscess formation within RCCs do not often cause DI. Furthermore, DI in RCCs have only been described in case reports describing atypical location of the cyst at the pituitary stalk, rapid growth, primary inflammation, stalk impairment or co-existence with destructive pituitary lesion[2]. A retrospective study of 700 patients who underwent endoscopic TSS for resection of a pituitary adenoma, RCC or craniopharyngioma found an overall rate of postoperative DI of 14.7%, but only 4.6% of patients had permanent DI (defined as the need for desmopressin more than 1-year post-operatively)[7]. Patients with RCC had a higher risk of developing DI compared to those with a pituitary adenoma (OR =2.2; 95% CI 1.2-3.9; P=0.009). In patients who underwent TSS for RCC, 26% developed post-operative DI while 10.3% developed permanent DI.

In conclusion, we have described a rare case of secondary pituitary abscess complicated by DI in a young non-immunocompromised and otherwise healthy patient.

Take home points:

- Rathke's cleft cyst abscesses are difficult to diagnose before surgery due to their indolent course of non-specific clinical symptoms and radiological findings.
- Symptoms may arise from mass effect (headache or visual disturbance) or pituitary hormone deficiency
- Whilst there are no specific features on MRI, a rim-enhancing cystic lesion should raise suspicion of the possibility of an abscess
- Surgery should occur promptly as source control of the infection alongside antibiotics

Adrenal nocardia in an immunocompetent patient

Shelley Vance[1], Elisabeth Ng[2], Natalie Harrison[1]

1. Department of Endocrinology and Diabetes, Barwon Health, Geelong, Victoria, Australia

2. Department of Endocrinology and Diabetes, Alfred Health, Melbourne, Victoria, Australia

Background

The investigation and management of patients with an adrenal mass is challenging. There are a wide range of differential diagnoses including, most commonly, a non-functioning adenoma, followed by a functioning adenoma, and more rarely, adrenocortical carcinoma, adrenal metastasis, and infection.[1,2]

Case Presentation

A 35-year-old male cropping and cattle farmer with no significant past medical history presented to a rural hospital with a three week history of dry cough, small volume haemoptysis and abdominal pain, compounded by a two week history of fevers, drenching night sweats and progressive dyspnoea. He had no significant travel history and no sick contacts. He was a non-smoker, with moderate alcohol intake and denied intravenous drug use.

Initial investigations demonstrated an elevated white cell count (WCC) with a neutrophilia and monocytosis, and a C-reactive protein (CRP) of 144mg/L (<3.0). Computed tomography (CT) imaging of the chest and abdomen identified a cavitating right upper lobe lung lesion and left adrenal lesion, reported to be concerning for adrenal carcinoma (figure 1). Community-acquired pneumonia was presumed, and he was admitted for treatment as such, initially with intravenous (IV) ceftriaxone and IV amoxicillin, followed by IV benzylpenicillin and oral doxycycline, then de-escalated to oral amoxicillin-clavulanate ongoing.

The adrenal lesion was investigated further with FDG-positron emission tomography (PET)/CT. This demonstrated intense heterogeneous avidity within the markedly enlarged left adrenal gland with features concerning for carcinoma and milder avidity within the cavitating lung lesion (figure 2). Attempted CT-guided lung biopsy was unsuccessful. He was discharged on day 9 with a plan for repeat outpatient CT-guided lung biopsy and follow up in the Surgical and Endocrinology Outpatient Clinics.

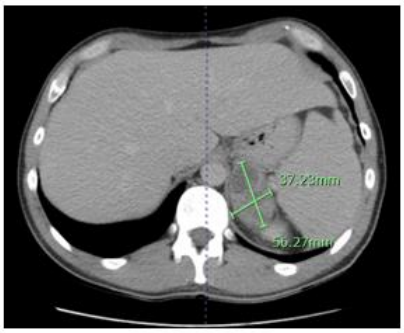


Figure 1: Computed tomography of the abdomen
Left renal adrenal lesion measuring 57 x 35 x 58 mm with no intra-lesional fat and an average attenuation of 22 Hounsfield units.

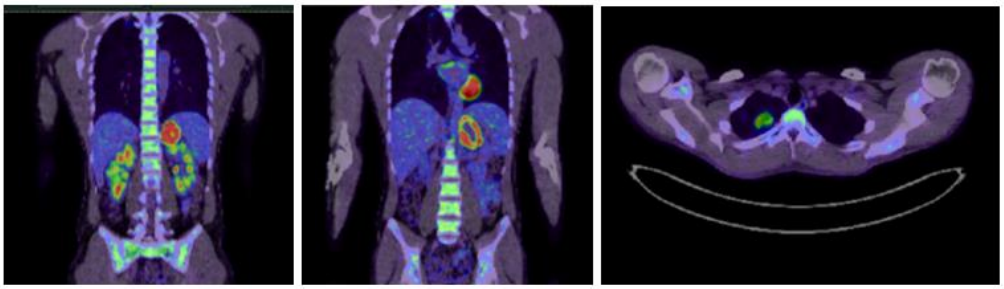


Figure 2: FDG-positron emission tomography / computed tomography
Intense heterogeneous FDG-avidity within the markedly enlarged left adrenal gland with areas of necrosis, an ill-defined periphery and a standardised uptake value (SUV) Max of 31.2 predominantly in the periphery with central photopenia. Milder FDG-avidity of the cavitating right apical upper lobe lung lesion with a SUV Max of 5.7 with other mild-moderate FDG-avid patchy nodules scattered throughout both lung fields. There were no enhancing intracranial lesions detected.

Adrenal functional studies returned with an equivocal 1mg dexamethasone suppression test and mildly elevated late-night salivary cortisol, with the remainder appearing within normal limits (table 1).

Functional Studies	Result	Reference Range
1mg DST*	96 nmol/L	<50
Late night salivary cortisol	3.3 nmol/L	<3.2
Urinary free cortisol excretion	628 nmol/24h	200-1000
Plasma normetanephrine	302 pmol/L	<900
Plasma metanephrine	<70 pmol/L	<500
24h urinary adrenaline excretion	55 nmol/24h	5-80
24h urinary noradrenaline excretion	356 nmol/24h	40-780
24h urinary dopamine excretion	1986 nmol/24h	200-3500
Aldosterone to renin ratio	5	<70
DHEA sulphate	2.9 umol/L	2.2-12.4

*DST – dexamethasone suppression test

Table 1: adrenal functional studies

Upon discharge, he deteriorated with worsening abdominal pain and high-grade fevers up to 39.8 degrees Celsius with associated rigors. He was re-admitted within two days and found to have a rising WCC and CRP of 178mg/L (<3.0). He received a single dose of IV ceftriaxone in the Emergency Department and underwent repeat abdominal CT, demonstrating interval enlargement of the left adrenal lesion and development of a left renal vein thrombus (figure 3). Blood cultures were collected and later returned negative. He underwent transthoracic echocardiogram which was negative for valvular vegetations. During this period his analgesic agents were gradually escalated to provide adequate pain relief. He was stabilised and discharged home to await a planned open left adrenalectomy +/- left nephrectomy and splenectomy.

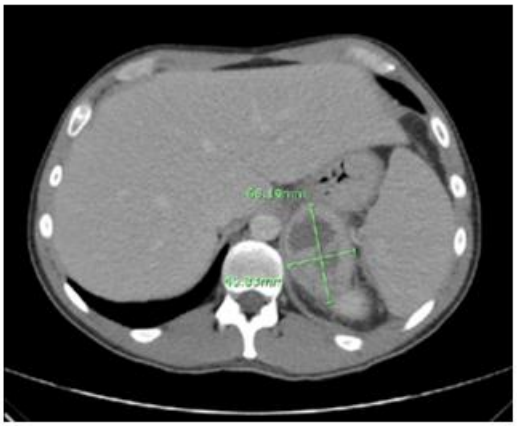


Figure 3: Computed tomography of the abdomen Left renal adrenal lesion measuring 68 x 47 x 80 mm, heterogeneous in nature with large cystic cavities internally, peri-lesional stranding and interval development of a left renal vein thrombus.

The following day he presented to a tertiary hospital with severe abdominal pain and ongoing fevers. On arrival his WCC was elevated and CRP 282mg/L (<5), and he was admitted under a surgical team for empirical IV piperacillin-tazobactam and monitoring. A repeat infective screen was performed including blood cultures, urine culture and a Covid-PCR swab. He reported progressively severe pain requiring stepwise escalation of multimodal analgesia including oxycodone, ketamine and clonidine.

Fevers continued despite IV piperacillin-tazobactam for 48 hours. He was reviewed by the Infectious Diseases and Respiratory teams with differential diagnoses including metastatic adrenal carcinoma, atypical pneumonia and tuberculosis.

On day 6 of this admission he underwent bronchoscopy and lavage, and an open left adrenalectomy. The latter procedure revealed a central abscess cavity containing thick yellow pus, adherent to the left renal vein with no invasion, without splenic pathology or free pus in the peritoneum. Blood cultures returned positive on the day of surgery, revealing gram positive rods, and raising the question of Nocardia. Antibiotic therapy was changed to IV meropenem and IV sulfamethoxazole-trimethoprim.

On day 2 post-operatively *Nocardia cyriacigeorgica* was confirmed in blood cultures with preliminary sensitivities to amoxicillin-clavulanate and amikacin. His treatment was refined to IV amoxicillin-clavulanate 1.2g three times daily, along with continued sulfamethoxazole-trimethoprim. Microbiology from bronchoscopy lavage and adrenal sample subsequently also identified *Nocardia cyriacigeorgica*. After final sensitivities returned his antibiotic therapy was tailored to IV ceftriaxone 2g daily for three weeks and oral sulfamethoxazole-trimethoprim 1600/320mg four times daily for six months. Subsequent blood cultures were negative.

He underwent immunodeficiency screening, which returned unremarkable. His main risk factor for this unusual infection was deemed to be his occupation. He was discharged on day 10 post-operatively with the hospital in the home service and has remained well.

Literature Review

Norcardia is a genus of aerobic gram positive acid-fast bacteria that live ubiquitously throughout the world, found in soil, fresh-water and marine-water.[3,4,5,6] Over eighty species have been identified and more than half have been shown to cause human infection with entry via lungs and skin.[3,5,6] Pulmonary nocardiosis is reported as the most common site of primary infection.[5] Nocardia is thought to disseminate into distant organs via the bloodstream, most commonly affecting the brain, kidney, joints, bone and eyes.[3] Adrenal nocardia has only been documented in case reports. Nocardia infection is more common in immunocompromised hosts, however, has been found in immunocompetent individuals.[3,4,5,6] The most common risk factors described are immunosuppression and malnutrition.[1,2]

Clinical presentations vary depending on the site of infection. Adrenal nocardia most commonly presents with fevers and abdominal pain on the side of the affected adrenal gland.[1] Diagnosis requires isolation and identification of the organism from a

clinical sample and is often delayed due to the specific culture medium required and slow growth which can take up to 3 weeks.[7]

Consensus recommendations for treatment of disseminated nocardiosis are based on observational studies. There is large diversity in antimicrobial susceptibility among species therefore combination treatment is often used whilst susceptibility testing is performed to guide directed treatment.[5] Australian guidelines recommend using trimethoprim-sulfamethoxazole alone for mild disease, adding ceftriaxone for moderate disease, and linezolid with amikacin, imipenem or meropenem for severe disease.[8] The rates of trimethoprim-sulfamethoxazole resistance appear low in Australia.[5] There is no consensus as to whether performing adjunctive adrenalectomy or laparoscopic drainage of the adrenal gland is beneficial.[1] Without appropriate treatment of disseminated nocardia, studies suggest a high mortality rate of 30-50 percent, which can be even higher in individuals with cerebral involvement.[1,4,5]

The work up and management of this patient was staggered across three admissions and two health services, after his presentation with this myriad of symptoms that raised concern for both a malignant and infective process. An adrenal lesion typically permits outpatient work up and management, but the diagnosis on this occasion warranted more intense inpatient work up. Surgery was deemed necessary given his clinical state, but consideration of this diagnosis in future patients may potentially avoid an invasive procedure.

Take Home Messages

- Nocardia infection should be considered as a differential diagnosis when evaluating patients with an adrenal mass, especially if presenting with systemic symptoms and signs of infection, in particular pulmonary infection.
- Nocardia can be difficult to diagnose due to the specific culture medium required and slow growth.
- Individual patient risk factors must be considered to prompt clinical suspicion for this rare but serious diagnosis.
- Consensus treatment involves trimethoprim-sulfamethoxazole plus an additional agent depending on severity, however antibiotic susceptibility should be performed in all patients due to the diversity in antimicrobial susceptibility of nocardia.
- All patients with nocardiosis should undergo cerebral imaging due to the heightened mortality associated with this, and immunodeficiency screening should be considered.

1. Jackson C, McCullar B, Joglekar K, et al. Disseminated Nocardia farcinica pneumonia with left adrenal gland abscess. Cureus 2017; 9(4): e1160.
2. Fassnacht M, Arlt W, Bancos I, et al. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. Eur J Endocrinol 2016; 175: G1-34.
3. Tachezy M, Simon P, Ilchmann C, et al. Abscess of adrenal gland caused by disseminated subacute Nocardia farcinica pneumonia. A case report and mini-review of the literature. BMC Infect Dis 2009; 9:194.
4. Elsayed S, Kealey A, Coffin C, et al. Nocardia cyriacigeorgica septicaemia. J Clin Microbiol 2006; 44(1): 280-282.
5. McGuinness S, Whiting S, Baird R, et al. Nocardiosis in the tropical northern territory of Australia, 1997-2014. Open Forum Infect Dis 2016; 3(4): ofw208.
6. Kibe S, Meigh R, Moon T, et al. Nocardia cyriacigeorgica in an immunocompetent patient. Ther Adv Respir 2015; 9(1): 28-30.
7. Saubolle M, Sussland D. Nocardiosis: review of clinical and laboratory experience. J Clin Microbiol 2003; 41(10): 4497-4501.
8. Nocardiosis [published 2019 April]. In: eTG complete [digital]. Melbourne: Therapeutic Guidelines Limited; 2020 March.

New Treatment Options Post Craniopharyngioma Resection: Glycogen Like Peptide 1 receptor agonist, Growth Hormone and Oxytocin.

Sneha Vidyasagar[1], Thomas Dover[1], Adam Morton[1]

1. Mater Hospital, Brisbane, QUEENSLAND, Australia

Case Description

A 27 year old male, presented at age 8 with failure to thrive. His Magnetic Resonance Imaging (MRI) revealed a multi-locular cystic mass, consistent with a craniopharyngioma which was resected with subsequent radiotherapy. His most recent MRI reveals no tumour recurrence. Other medical issues include narcolepsy and childhood epilepsy. He lives in supported accommodation with a disability pension but is independent with his activities of daily living and involved in volunteer work.

He developed pan hypopituitarism and diabetes insipidus with initial treatment consisting of hydrocortisone acetate 8mg orally in the morning and 4mg in the afternoon, thyroxine 150 mcg daily, testosterone undecanoate 1-gram IM three monthly and desmopressin 400mcg twice daily. Since the Pharmaceutical Benefits Scheme subsidised the growth hormone, he has commenced somatropin 0.2mg daily and is being titrated to a target Insulin like growth factor 1 (IGF1) level in mid-normal range. His Quality of Life Assessment of Growth Hormone Deficiency in Adults (QoL-AGHDA) score was 18/25 prior to commencing Growth Hormone and 9/25, one-year post Growth Hormone replacement, where a lower score indicates a better quality of life.

He developed hypothalamic hyperphagia resulting in morbid obesity (peak BMI 39.4 g/m2) and subsequent metabolic syndrome including Type 2 Diabetes and non-alcoholic fatty liver disease. He was treated with metformin extended release 1000mg daily, gliclazide modified release 120mg, insulin glargine U100 (70 units mane and 10 units nocte) and insulin aspart (28 units three times a day) prior to the initiation of a Glucagon Like Peptide 1 Receptor Agonist (GLP1RA) ; dulaglutide 1.5mg weekly

He subsequently lost 15kg over a 12-month period in addition to normalisation of his ALT and AST. A Sodium Glucose Transport Type 2 Inhibitor (SGLT2) was also initiated and he eventually was able to discontinue all insulin. He is now on metformin XR 2g, dulaglutide 1.5mg weekly and empagliflozin 12.5mg daily with a HbA1c of 7.0%.

He reported a low mood and an inability to 'feel emotions'. After assessment from psychology, depression was excluded and the association with hypothalamic damage and oxytocin deficiency was considered.

Discussion

100% of children have at least one pituitary axis affected, 80% developed metabolic syndrome and 6% suffered from depression post craniopharyngioma resection (1). This case report raises management of these complications, with a focus on growth hormone replacement, GLP1RA in hypothalamic obesity and oxytocin to improve mood.

Growth hormone (GH) deficiency is the most common endocrine disturbance that occurs secondary to craniopharyngioma (2). Replacement in GH-deficient children has been proven to have a positive impact on body composition and cardiovascular risk factors, including serum lipid profiles, insulin sensitivity and quality of life (3). A meta-analysis of 3487 children demonstrated that children treated with GH replacement had a lower recurrence rate of craniopharyngioma compared to those who were not (4). In assessing long term survival, psychological status and quality of survival with GH replacement, it was proven that early initiation of GH had better scores for emotional functioning and a lower physical fatigue than late substitution or patients who did not receive any GH substitution (5). Hence there is evidence to suggest early initiation of GH substitution after craniopharyngioma resection in young patients might have beneficial effects on quality of life and growth while reducing the rate of tumour recurrence.

The link between hypothalamic injury and obesity was first described by Dr. Fröhlich in 1993 (2) . Since then hypothalamic obesity has been associated with trauma, aneurysms, infiltrative diseases, tumours such as craniopharyngioma, genetic syndromes such as Prader-Willi syndrome, leptin deficiency, and melanocortin four receptor (MC4R) mutations. Risk factors for the development of hypothalamic obesity includes tumour location, radiation dose, extent of surgery, GH deficiency and tumour histology (6). Hypothalamic obesity complicates up to 52% of paediatric craniopharyngioma patients, however there are currently limited therapeutic options. These patients have an increased prevalence of Type 2 Diabetes Mellitus, Hypertension, Sleep Apnoea, Non Alcoholic Fatty Liver Disease and cardiovascular risk. Conventional treatments include a calorie-restricted diet, exercise therapy, pharmacologic treatment or bariatric surgery. Pharmacotherapy focuses on alterations in pathways, such as sympathomimetics, triiodothyronine, GLP1RA and somatostatin analogues. GLP-1 is secreted from L cells in the intestine and increases insulin secretion while inhibiting gastric emptying and subsequently food intake. It also acts on the GLP-1 receptor in the brain to suppress hunger. For this reason, GLP1RA are currently used as pharmacological therapies for Type 2 Diabetes Mellitus and obesity. A study by Zoicas et al observed nine patients with moderate to severe hypothalamic obesity treated with GLP1RA for up to 51 months. Eight of the nine patients experienced 13.1±5 kg weight loss (7). Weight loss was associated with improved insulin sensitivity, glycaemic control and lower triglycerides however cholesterol levels were not improved. GLP1RA work on receptors in the hindbrain, which are believed to be intact in patients with hypothalamic hyperphagia. A newer GLP1RA, Semaglutide, offers additional weight loss and glycaemic benefits over Dulaglutide, likely due to the smaller molecular size with a free, non-protein bound form able to cross the blood brain barrier and act on central nervous system receptors(8).

Oxytocin plays a role in the development of attachment, trust, behaviour and body composition (9). Following craniopharyngioma resection, patients report social-behavioural impairment, which may be a result of oxytocin deficiency (10). Gebert et al compared salivary oxytocin at baseline and following exercise in patients with and without craniopharyngioma and proved reduced oxytocin in craniopharyngioma patients with MRI proven hypothalamic damage (11). Oxytocin influences an individual's response to emotional situations, improving trust and social connnectivity in children with autism spectrum disorder, conduct disorders, attachment disorders, anxiety, and depression (10). Another case report described a 13-year-old boy with hypothalamic obesity post craniopharyngioma resection, who received 10 weeks of oxytocin. Following this he was found to lose 4.4kg, improved satiety, decreased preoccupation with food, implying a benefit of oxytocin replacement in this cohort of patients (12). However, peripherally administered oxytocin only has a 2-8 minute half-life and displays poor permeability across the blood-brain barrier, making it challenging to use as a therapeutic drug (13). Furthermore, it cannot be administered orally due to metabolism in the gut by chymotrypsin (14). Intranasal oxytocin is believed to be absorbed through the nasal mucosa and delivered to the central nervous system where it can exert its effect. While there is evidence that Oxytocin supplementation

can be beneficial in improving mood in patients with craniopharyngioma, current limitations include difficulties in measuring oxytocin levels to confirm oxytocin deficiency, determining the optimal mode of administration for oxytocin therapy and the safety profile for long-term use (13).

Take Home:

- Complications post craniopharyngioma resection and radiation can impact long-term health and well-being of patients.
- GH deficiency is the most common endocrine disturbance secondary to craniopharyngioma. GH replacement is subsidised in Australia and has a positive impact on body composition and cardiovascular risk factors, while reducing the rate of tumour recurrence
- Limited evidence is emerging for reducing appetite, improving satiety and glycaemic control in Hypothalamic obesity with GLP1RA, resulting in weight loss and improved metabolic parameters.
- Limited evidence to suggest social-behavioural impairment post craniopharyngioma can be improved with inhaled oxytocin. Current limitations include difficulties proving oxytocin deficiency, determining the optimal mode of administration for oxytocin therapy and the safety profile.

1. Jazbinsek S, Kolenc D, Bosnjak R, Faganel Kotnik B, Zadravec Zaletel L, Jenko Bizjan B, et al. Prevalence of Endocrine and Metabolic Comorbidities in a National Cohort of Patients with Craniopharyngioma. Horm Res Paediatr. 2020;93(1):46-57.
2. Alotaibi NM, Noormohamed N, Cote DJ, Alharthi S, Doucette J, Zaidi HA, et al. Physiologic Growth Hormone-Replacement Therapy and Craniopharyngioma Recurrence in Pediatric Patients: A Meta-Analysis. World Neurosurg. 2018;109:487-96 e1.
3. M Ahmid CGP, S F Ahmed, M G Shaikh. Growth hormone deficiency during young adulthood and the benefits of growth hormone replacement. Endocrine Connect. 2016;5(3):1-11.
4. Nawaf M. Alotaibi NN, David J. Cote, Salman Alharthi, Joanne Doucette, Hasan A. Zaidi, Rania A. Mekary, Timothy R. Smith. Physiologic Growth Hormone Replacement Therapy and Craniopharyngioma Recurrence in Pediatric Patients: A Meta-Analysis. World Neurosurgery. 2019;109:487-96.
5. Svenja Boekhoff AB, Anthe S Sterkenburg, Maria Eveslage,, Müller HL. Long-term effects of growth hormone replacement therapy in childhood-onset craniopharyngioma: results of the German Craniopharyngioma Registry (HIT-Endo). 2018;179(5):331-41.
6. Ja Hye Kim J-HC. Pathophysiology and clinical characteristics of hypothalamic obesity in children and adolescents. Annals of Pediatric Endocrinology & Metabolism. 2013;18(4):161-7.
7. Flavius Zoicas MD, Bernhard Mayr, Michael Buchfelder, Christof Schöfl. GLP-1 analogues as a new treatment option for hypothalamic obesity in adults: report of nine cases. European Journal of Endocrinology. 2013;168(5):699-706.
8. Pratley RE, Frias JP, Kumar H, Petrie J, Navarria A, Nielsen MA, et al. Efficacy of Semaglutide vs. Dulaglutide across Baseline HbA1c in SUSTAIN 7. Diabetes. 2018;67(Supplement 1).
9. Campbell A. Oxytocin and Human Social Behavior. Personality and Social Psychology Review. 2010;14(3):281-95.
10. Zada G KN, Pulido M, Amezcua L. Prevalence of neurobehavioral, social, and emotional dysfunction in patients treated for childhood craniopharyngioma: a systematic literature review. PLoS One. 2013;2013(8).
11. Gebert D AM, Stieg MR, Freitag MT, Lahne M, Fuss J, Schilbach K, Schopohl J, Stalla GK, Kopczak A. De-masking oxytocin-deficiency in craniopharyngioma and assessing its link with affective function. Psychneuroendocrinology. 2017;88:61-9.
12. Eugenie A Hsu JLM, Francisco A Perez, Christian L Roth. Oxytocin and Naltrexone Successfully Treat Hypothalamic Obesity in a Boy Post-Craniopharyngioma Resection The Journal of Clinical Endocrinology & Metabolism. 2018;103(2):370-5.
13. Raghav Bhargava KLD, Aled Rees. Oxytocin therapy in hypopituitarism: Challenges and opportunities. Clinical Endocrinology. 2018;90(2).
14. Shana E McCormack JEB, Elizabeth A Lawson. Metabolic Effects of Oxytocin. Endocrine Reviews. 2020;41(2):121-45.

A case of severe hypervitaminosis D with mild hypercalcaemia

Emma Whittle[1], Elzahn de Waal[2], Tony Huynh[2], Oliver Treacy[2], Adam Morton[1]

1. Endocrinology, Mater Hospital, Brisbane, Queensland, Australia

2. Chemical Pathology, Mater Pathology, Brisbane, Queensland, Australia

A 68 year-old woman presented to her general practitioner with fatigue and 5kg of weight loss. Her past medical history was significant for a hiatus hernia and hypercholesterolaemia. Her regular medications included rabeprazole and rosuvastatin. She intermittently took cholecalciferol 1000IU/day. Initial investigations revealed a haemoglobin of 87g/L (115-160), corrected calcium of 2.83mmol/L (2.1-2.6), and serum globulins of 61g/L (24-41). Her physical examination was unremarkable except for pallor. Further investigations in hospital showed a parathyroid hormone (PTH) of 4.9pmol/L (2.0-9.5), 25-hydroxy vitamin D (25OHD, Abbott Architect) of >400nmol/L (50-150nmol/L), phosphate of 1.44mmol/L (0.9-1.6), 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) of 152pmol/L (48-190), and an IgM kparaprotein in the gamma region of 44g/L (figure 1). A positron emission tomography/computer tomography scan showed extensive mildly fluorodeoxyglucose (FDG) avid lymphadenopathy above and below the diaphragm, with large FDG avid mass lesions in the pelvis, and widespread bone marrow infiltration. A bone marrow aspirate showed moderate to heavy marrow involvement with a mature lymphoproliferative disorder, consistent with a diagnosis of lymphoplasmacytic lymphoma/Waldenstrom's macroglobulinaemia (LPL/WM). Bone densitometry was normal.

The 25OHD level was felt to be inconsistent with clinical findings and the possibility of an aberrant result was considered.

Vitamin D results using different assays are summarised in table 1. The 25OHD result of >400 nmol/L was confirmed on repeat analysis using the Abbott Architect instrument. A subsequent 1-in-2 dilution of the sample returned a result of 84nmol/L – highly suggestive of the presence of an interferent. The presence of rheumatoid factor, a known potential interferent, was excluded with a measurement below the limit of quantitation of the assay. Treatment of the sample with antibody blocking reagent (Scantibodies) was not consistent with the presence of heterophile antibody interference. Analysis on a liquid chromatography tandem mass spectrometry (LC-MS/MS) vitamin D method returned a result of 82nmol/L. Measurement of the sample on a Siemens Centaur immunoassay platform produced a result of 75nmol/L, which was in agreement with the LC-MS/MS result. The sample analysed was collected prior to the patient starting chemotherapy and we concluded that the most likely interferent was the presence of the monoclonal IgM kappa paraprotein. Following chemotherapy, and in the setting of normal globulins, the patient's vitamin D result was 64nmol/L. Falsely elevated vitamin D levels due to assay interference have previously been reported with LPL/WM and myeloma (1).

DISCUSSION

The most striking feature of this patient's initial investigations was the 25OHD level of >400nmol/L. Possible causes of hypervitaminosis D include prolonged ingestion of large doses of cholecalciferol, deficiency or variants in CYP24A1, and an artefactual result due to assay interference.

Hypervitaminosis D due to excessive oral or intramuscular supplementation is rare. Development of hypercalcaemia due to excessive oral cholecalciferol requires prolonged ingestion of doses in the order of 40-50 000 IU daily for at least six months. A case series from India reported on 15 patients with hypercalcaemia secondary to intramuscular administration of cholecalciferol (2). The shortest period of time to develop hypercalcaemia was five weeks, during which time the patient received 3 000 000 IU of vitamin D. Hypervitaminosis D due to excessive exogenous intake is associated with hypercalcaemia, low PTH, and normal phosphate levels.

CYP24A1 deficiency is a rare cause of an elevated 25OHD and hypercalcaemia. The CYP24A1 gene encodes vitamin D 24 hydroxylase which metabolises both 25OHD and $1,25(OH)_2D$ to inactive metabolites 24,25-dihydroxyvitamin D ($24,25(OH)_2D$) and calcitroic acid (3). Expression of CYP24A1 is usually induced by both hypercalcaemia and $1,25(OH)_2D$, thereby preventing vitamin D-induced hypercalcaemia. CYP24A1 deficiency was first described in infants but can present at any age. CYP24A1 variants/deficiency are characterised biochemically by hypercalcaemia, hypercalciuria, undetectable PTH and parathyroid-hormone related peptide, low $24,25(OH)_2\ D_3$, and elevated $1,25(OH)_2\ D_3$. Clinical manifestations include nephrolithiasis and nephrocalcinosis. A definitive diagnosis can be made by genetic testing. Seven cases of hypercalcaemia with onset during pregnancy have been described in women with CYP24A1 variants/deficiency, in the setting of 25OHD 1-alpha hydroxylase expression by the placenta and enzyme upregulation in the maternal kidney(3).

Once pseudo-hypervitaminosis D from paraprotein interference was confirmed, the cause of the hypercalcaemia in this patient needed to be determined. Along with primary hyperparathyroidism (PHPT) and familial hypocalciuric hypercalcaemia (FHH), pseudohypercalcaemia (4)was also considered. Factitious or pseudohypercalcaemia may occur due to hyperalbuminaemia, thrombocytosis, or assay interference by paraproteins. Pseudohypercalcaemia leading to the incorrect diagnosis of PHPT has been reported with LPL/WM, myeloma, monoclonal gammopathy of uncertain significance, mixed cryoglobulinaemia with Sjogren's syndrome, and markedly elevated IgE levels. Pseudohypercalcaemia may be identified by the demonstration of normal ionised calcium levels. In the case presented, the patient's ionised calcium was mildly elevated at 1.33mmol/L (1.13-1.30), excluding pseudohypercalcaemia.

While PHPT is usually characterised by an elevated PTH level, 10 to 20% of patients will have an inappropriately "normal" PTH level, making it difficult to distinguish from FHH (5). The prevalence of FHH in the west of Scotland was estimated to be 1 in 78 000, compared with the estimated incidence of PHPT of 50 cases per 100 000 patient years (6,7). FHH is an inherited autosomal dominant condition with almost complete penetrance. The majority of mutations are linked to the gene encoding the calcium-sensing receptor on the long arm of chromosome 3(8). New mutations are relatively rare. Differentiating FHH from asymptomatic PHPT may be difficult because of considerable overlap in urine calcium excretion, serum calcium, serum phosphate, serum magnesium, and PTH between the two disorders. The best investigation to try to distinguish PHPT from FHH is the calcium:creatinine clearance ratio (CCCR). A CCCR of <0.01 is unusual for PHPT, but is seen in 80% of individuals with FHH (8). The CCCR may also be low with vitamin D deficiency and low dietary calcium intake. Pregnancy leads to an elevated CCCR in the setting of physiological hypercalciuria. In cases where results are repeatedly equivocal, testing of first-degree relatives or genetic testing may be useful.

On questioning previous pathology providers, multiple corrected serum calcium levels between 2011 and 2016 had been normal. A 24 hour urine collection demonstrated calcium excretion of 1.8mmol/day, with a CCCR of 0.005. While the patient's CCCR is low, the previous normal corrected serum calcium results make it most likely the patient has PHPT. In view of the patient's normal bone density, mild degree of hypercalcaemia, the absence of relatives for testing, and her diagnosis of

LPL/WM it was decided not to pursue gene testing to differentiate between the two disorders as it would not change management. On review of the literature, there does not appear to be an association between WM/LPL and PHPT.

In conclusion, this case highlights the potential for immunoassay interference when measuring 25OHD, and the potential for paraproteins to cause interference. The patient's history and presentation were not in keeping with vitamin D intoxication, which prompted consideration of other causes of an elevated 25OHD level. The presence of an elevated ionised calcium confirmed hypercalcaemia, as pseudohypercalcaemia should be considered in patients with WM. The most likely cause of hypercalcaemia in this patient is PHPT, a condition which is not typically associated with WM/LPL.

POINTS TO REMEMBER

- Hypervitaminosis D secondary to excessive supplementation is rare.
- It is important to consider assay interference when a result is unexpected clinically.
- Primary hyperparathyroidism can sometimes be difficult to distinguish from familial hypocalciuric hypercalcaemia.

1. Belaidi N, Georges A, Lacroix I, Croisonnier A, Ducros V, Souberbielle JC, Corcuff JB. Hypercalcemia and elevated concentration of vitamin D: a case report too easy to be true. Clin Chim Acta 2016;457:123-4.
2. Pandita KK, Razdan S, Kudyar RP, Beigh A, Kuchay S, Banday T. "Excess gooD can be dangerous". A case series of iatrogenic symptomatic hypercalcemia due to hypervitaminosis D. Clin Cases Miner Bone Metab 2012;9:118-20.
3. Arnold N, O'Toole V, Huynh T, Smith HC, Luxford C, Clifton-Bligh R, Eastman CJ. Intractable hypercalcaemia during pregnancy and the postpartum secondary to pathogenic variants in CYP24A1. Endocrinol Diabetes Metab Cae Rep 2019;2019.
4. Loh TP, Yang Z, Chong AT, Ng CH. Pseudohypercalcaemia in a patient with newly diagnosed Waldenstrom macroglobinaemia. Internal medicine journal 2013;43:950-1.
5. Silverberg SJ, Lewiecki EM, Mosekilde L, Peacock M, Rubin MR. Presentation of asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. J Clin Endocrinol Metab 2009;94:351-65.
6. Hinnie J, Bell E, McKillop E, Gallacher S. The prevalence of familial hypocalciuric hypercalcemia. Calcif Tissue Int 2001;68:216-8.
7. Yeh MW, Ituarte PH, Zhou HC, Nishimoto S, Liu IL, Harari A, Haigh PI, Adams AL. Incidence and prevalence of primary hyperparathyroidism in a racially mixed population. J Clin Endocrinol Metab 2013;98:1122-9.
8. Heath DA. Familial hypocalciuric hypercalcemia. Rev Endocr Metab Disord 2000;1:291-6.

Not a Bad Accident After All

Mimi Wong[1, 2], Usman Malabu[2, 3], Ipeson Korah[4], YongMong Tan[2, 3]

1. School of Medicine, University of Queensland, Brisbane, QLD, Australia

2. Department of Diabetes & Endocrinology, Townsville University Hospital, Townsville, Queensland, Australia

3. School of Dentistry & Medicine, James Cook University, Townsville, Queensland, Australia

4. Department of Radiology, Townsville University Hospital, Townsville, Queensland, Australia

Introduction:

This case describes a 60-year-old female with persistent Cushing's disease (CD) following transphenoidal surgery, who was treated with subcutaneous (SC) pasireotide for over 10 years, of which 6 years was inadvertently administered at a lower dose than conventionally recommended. Currently there is limited literature on long-term and low-dose pasireotide use in CD.

Case Presentation:

A 60-year old female was diagnosed with CD in June 2009. Ten years before that she had a coronary artery bypass graft for ischaemic heart disease, but no history of diabetes or hypertension. She had presented with a two-month history of fluid retention, increased perioral hair, easy bruising, weight gain, proximal muscle weakness, polydipsia and nocturia. On physical examination she was hypertensive with blood pressure (BP) of 150/100 mmHg and showed Cushingoid features of rounded facies, abdominal bruising and peripheral oedema. There was no visual field defect or cranial nerve palsies. Hypercortisolism from CD was confirmed by 24-hour urinary free cortisol (UFC) (4450 nmol/day; reference range 80 – 590 nmol/day), low and high dose dexamethasone suppression test and MRI revealing a right sided pituitary macroadenoma measuring 10.6 x 12.7 x 14.9 mm respectively of height, transverse and anterior-posterior dimensions with pituitary stalk deviation to the left and encompassment of the right internal carotid artery (figure 1A).

Transphenoidal debulking surgery was pursued in August 2009. As the pituitary macroadenoma was in close proximity to the internal carotid artery, complete resection was not possible. Pituitary MRI two months post-surgery showed a residual right-sided non-enhancing heterogeneous mass of 6.6 x 5.5 x 8.5 mm (figure 1B). Post-operatively she had ongoing Cushingoid features and biochemically still had hypercortisolism though it had improved (figure 2) (UFC 860 nmol/day).

In view of ongoing residual CD, in September 2009 she was commenced on SC pasireotide, inadvertently self-administered as 360mcg twice daily (BID). Within one month of pasireotide treatment, her UFC had normalised (240 nmol/day), and within a year she was clinically in remission. She became normotensive with BP of 100/80 mmHg and her Cushingoid features resolved.

She continued with SC pasireotide 360mcg BID from September 2009 to June 2015 when a medication audit discovered the mistaken lower dose and SC pasireotide dose was corrected to 600mcg BID. While on the lower pasireotide dose her diabetes was well controlled on metformin 2g daily and gliclazide modified release (MR) 30mg daily (figure 3). Her HbA1c was 6.8% after one year on low-dose pasireotide and 7.0% in May 2015, just prior to cessation of the low-dose pasireotide. Progress pituitary MRI over the 6 years on low-dose SC pasireotide revealed her residual tumour as measuring 8.2 x 6.6 x 12 mm (figure 1C), and did not show any residual tumour mass reduction despite her normal biochemistry (figure 2).

From June 2015 she started the higher dose SC pasireotide 600mcg BID. She has been on this higher dose for 5 years and remains in clinical and biochemical remission (figure 2). Despite the higher pasireotide dose and the addition of cabergoline 0.5mg twice a week from December 2014, there has not been any reduction in her residual pituitary mass on MRI (figure 1D). The higher dose pasireotide however has worsened her glycaemic control (figure 3) with HbA1c rising to 7.5% within 3 months. Despite increasing her gliclazide MR to 120mg and adding empagliflozin 10mg daily and sitagliptin 100mg daily to her metformin, her HbA1c progressively rose to 8.2% after 5 years of high-dose pasireotide. She has now commenced basal glargine insulin.

Discussion:

Cushing's disease (CD) is rare and the duration patients have hypercortisolism is predictive of morbidity and mortality, therefore achieving remission is important (1). First line treatment for CD is with transphenoidal surgery, with medical therapies suggested in those who are not surgical candidates or have persistent or recurrent disease (2). Corticotroph adenomas express somatostatin receptors (SSTR) and dopamine receptors, and both can be therapeutic targets (1, 2). Pasireotide is a somatostatin analogue, which binds to four of the five SSTR, with the highest affinity to SSTR-5, and can be used in the treatment of CD to improve Cushingoid features, pituitary volume and quality of life. The US Endocrine Society Clinical Guidelines recommends commencing SC pasireotide at 600mcg BID in CD. Pasireotide is generally well tolerated, though common adverse effects include symptoms related to administration, hyperglycaemia, gastrointestinal symptoms, cholelithiasis and liver derangement (2).

Low-dose, 360mcg BID, pasireotide was used to treat our patient for 6 years and she remained in clinical and biochemical remission throughout this period. This dosage appears to be the lowest dose of pasireotide in the literature used initially to treat CD. Feelders *et al* used 250mcg SC three times daily to treat patients with CD and achieved normalisation of UFC in 29% (3). Additionally there are case reports of low-dose SC pasireotide 150 - 300mcg BID being used in CD to maintain remission following initial treatment with 600 – 900mcg BID (4, 5). In these cases down titration was necessary due to development of adrenal insufficiency.

Amongst studies that compared the safety profile of patients managed with SC pasireotide 600mcg and 900mcg BID, there were increased rates of hyperglycaemia observed with higher doses (6). Pasireotide can bind to SSTR on pancreatic islet cells and K and L cells, reducing insulin, glucagon-like peptide-1 and glucose-independent insulinotropic polypeptide levels, and subsequently lead to hyperglycaemia (7). In our case, on low-dose pasireotide our patient's glycaemic control was well controlled over the 6 years. Following high-dose, 600mcg BID, pasireotide use her glycaemic control worsened over the 5 years with escalating anti-diabetes medications.

In our case both the low and high doses of pasireotide did not reduce residual pituitary tumour size. Tumour shrinkage appears to have a dose-dependent effect (6), though the mechanism of tumour shrinking following pasireotide use is unclear. There are

suggestions that pasireotide may have antiproliferative effects through angiogenesis inhibition and reduction of growth factors and trophic hormones (8).

Currently the literature regarding pasireotide and its long-term efficacy and tolerability is limited. In our case, our patient has been managed with pasireotide for the longest duration in Australia and in the literature. Our patient has sustained benefit for over 10 years and apart from hyperglycaemia, has not had any other adverse effect. An escape phenomenon was not observed, which can be associated with carbergoline (9). Petersenn *et al* reported data up to 5 years of pasireotide use with normalization of UFC in 11/16 patients, median tumour volume change of -3.5%, and safety profile at 5 years similar to that at 12 and 24 months (10). Following 5 years of pasireotide use though there is limited literature on its long-term use.

Learning Points:

- A lower dose of pasireotide may be effective in the initial treatment of CD than the recommended 600mcg BID dosage, though more studies are required to further explore this.
- Low-dose pasireotide use has the benefit of minimising adverse effects.
- In the long-term, pasireotide has a sustained clinical and biochemical effect in the treatment of CD and is well tolerated.

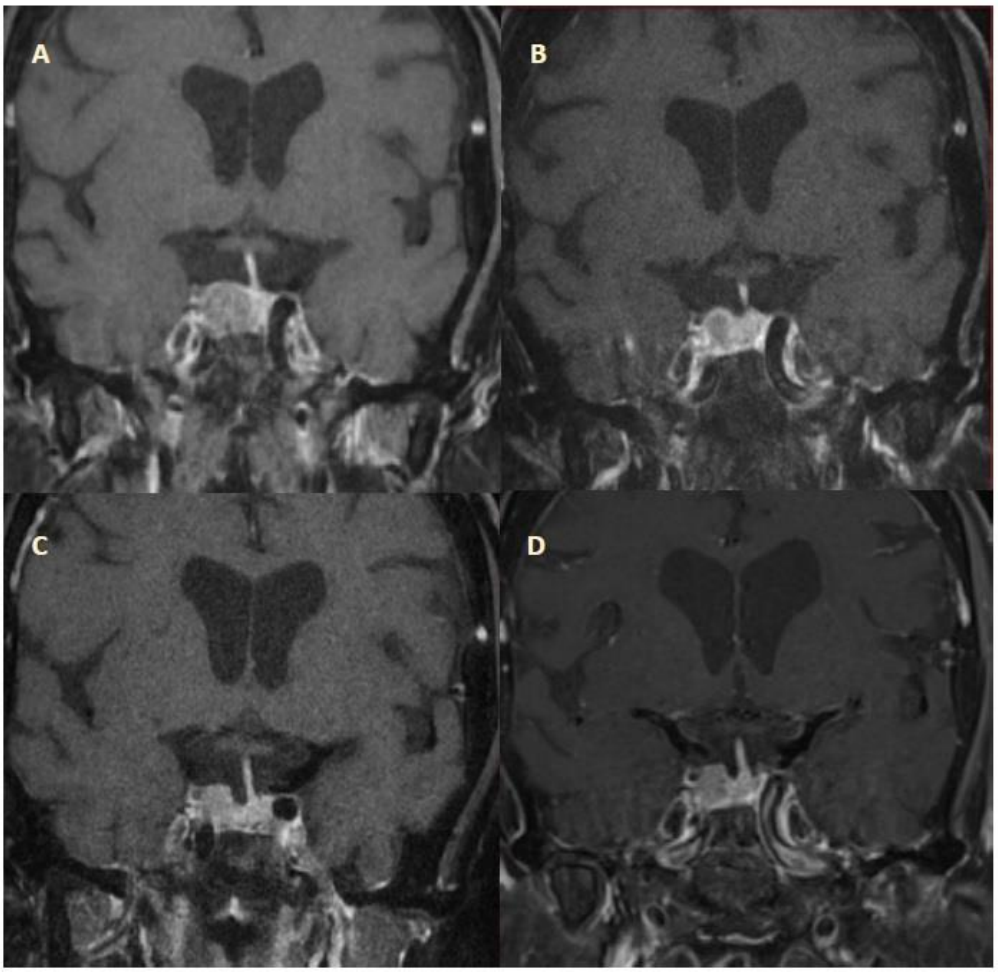


Figure 1: Coronal MRI pituitary view of pituitary macroadenoma over course of treatment. A – Pre-transphenoidal surgery. B – 2 months post-transphenoidal surgery. C – 6 years on subcutaneous pasireotide 360mcg twice daily. D – 5 years with the higher dose of subcutaenous pasireotide 600mcg twice daily (11 years of pasireotide treatment in total).

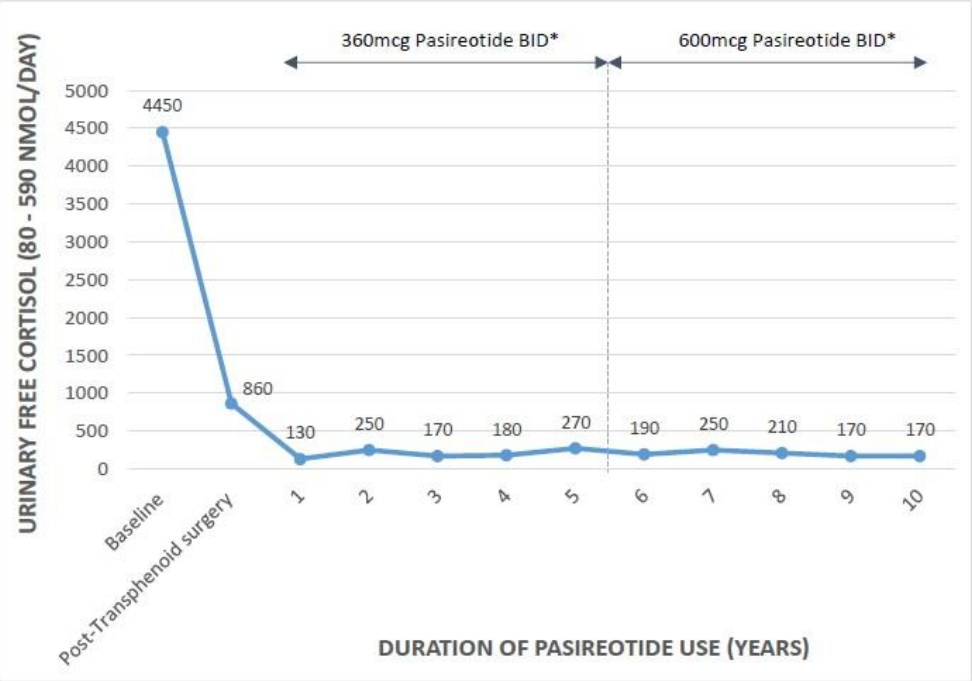


Figure 2: 24 hour urinary free cortisol measurements in relation to duration and dosage of pasireotide. BID* = twice daily

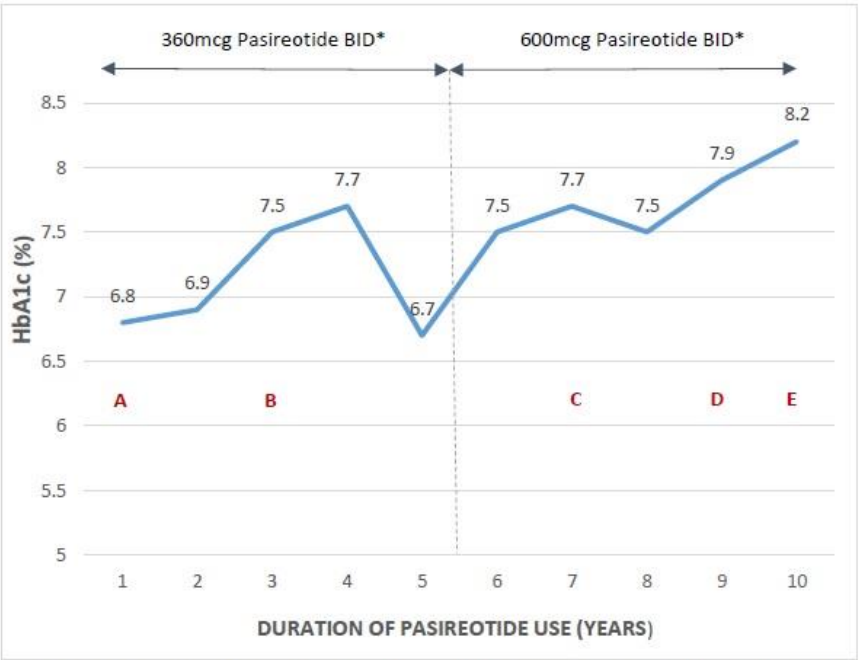


Figure 3: HbA1c (%) in relation to duration and dosage of pasireotide, and glycaemic management. BID* = twice daily. A, B, C, D and E shows escalating anti-diabetes medications with A (metformin), B (+ gliclazide modified release), C (+ empagliflozin), D (+ sitagliptin) and E (+glargine insulin).

1. Nishioka H, Yamada S. Cushing's Disease. J Clin Med. 2019;8(11):1951.
2. Nieman LK, Biller BMK, Findling JW, Murad MH, Newell-Price J, Savage MO, Tabarin A. Treatment of Cushing's Syndrome: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2015;100(8):2807-31.

3. Feelders RA, de Bruin C, Pereira AM, Romijn JA, Netea-Maier RT, Hermus AR, Zelissen PM, van Heerebeek R, de Jong FH, van der Lely AJ, de Herder WW, Hofland LJ, Lamberts SW. Pasireotide Alone or with Cabergoline and Ketoconazole in Cushing's Disease. N Engl J Med. 2010;362(19):1846-8.
4. Grossrubatscher E, Zampetti B, Dalino Ciaramella P, Doneda P, Loli P. Effectiveness of Low-Dose Pasireotide in a patient with Cushing's disease: Antiproliferative Effect and Predictivity of a Short Pasireotide Suppression Test. Clin Case Rep. 2015;3(8):718-22.
5. Trementino L, Cardinaletti M, Concettoni C, Marcelli G, Boscaro M, Arnaldi G. Up-to 5-year Efficacy of Pasireotide in a patient with Cushing's Disease and Pre-Existing Diabetes: Literature Review and Clinical Practice Considerations. Pituitary. 2015;18(3):359-65.
6. Colao A, Petersenn S, Newell-Price J, Findling JW, Gu F, Maldonado M, Schoenherr U, Mills D, Salgado LR, Biller BMK. A 12-Month Phase 3 Study of Pasireotide in Cushing's Disease. N Engl J Med. 2012;366(10):914-24.
7. Henry RR, Ciaraldi TP, Armstrong D, Burke P, Ligueros-Saylan M, Mudaliar S. Hyperglycemia Associated with Pasireotide: Results from a Mechanistic Study in Healthy Volunteers. J Clin Endocrinol Metab. 2013;98(8):3446-53.
8. Shimon I, Rot L, Inbar E. Pituitary-Directed Medical Therapy with Pasireotide for a Corticotroph Macroadenoma: Pituitary Volume Reduction and Literature Review. Pituitary. 2012;15(4):608-13.
9. Godbout A, Manavela M, Danilowicz K, Beauregard H, Bruno OD, Lacroix A. Cabergoline Monotherapy in the Long-Term Treatment of Cushing's Disease. Eur J Endocrinol. 2010;163(5):709-16.
10. Petersenn S, Salgado LR, Schopohl J, Portocarrero-Ortiz L, Arnaldi G, Lacroix A, Scaroni C, Ravichandran S, Kandra A, Biller BMK. Long-Term Treatment of Cushing's Disease with Pasireotide: 5-year Results from an Open-Label Extension Study of a Phase III Trial. Endocrine. 2017;57(1):156-65.

Epstein-Barr virus-associated smooth muscle tumour in the pituitary gland

Jasmine Zhu[1], Alanna Tan[1], Stephanie Lau[2], Mathis Grossman[1, 3], Jeffrey Zajac[1, 3]

1. Endocrine Centre of Excellence, Austin Health, Melbourne, Victoria, Australia

2. Anatomical Pathology, Austin Health, Melbourne, Victoria, Australia

3. School of Medicine, University of Melbourne, Melbourne, Victoria, Australia

Case:

A 27-year-old female presented with secondary amenorrhoea six months after the removal of her contraceptive progesterone implant for family planning. Her medical history was significant for congenital developmental abnormalities including a hypoplastic right kidney, which eventually required a nephrectomy. She also suffered from left-sided vesicoureteric reflux resulting in recurrent urosepsis and end-stage renal failure, leading to a renal transplant six years ago. This was complicated by Epstein-Barr virus (EBV)-positive diffuse large B-cell lymphoma and subsequent multifocal EBV-associated smooth muscle tumour (SMT) involving the cervical lymph nodes, lung, liver and bladder.

Several weeks earlier during an admission with urosepsis, the nephrology team had noted a TSH of 0.36 mU/L (0.38-5.30), fT4 6.0 pmol/L (8.00-16.50) and T3 2.5 pmol/L (3.80-6.90) and commenced her on thyroxine 75mcg. A subsequent pituitary panel at the time of endocrine review showed a prolactin level of 1132 mIU/L (71-566). She had a low morning cortisol of 59 nmol/L in the context of being on prednisolone therapy for immunosuppression of her renal transplant. FSH was 10.9 IU/L (1.7-21.5), LH 1.7 IU/L (1.0-96.0), oestradiol 51 pmol/L (45-1461), ACTH 1.7 ng/L (7.2-63.3), IGF-1 28.22 nmol/L (12.48-39.13), growth hormone 0.27 ug/L (< 3.60) and sodium 143 mmol/L (135-145).

On clinical examination, she had a new incomplete third nerve palsy with decreased pupillary light response. She had a mild superior temporal quadrantanopia in the left eye on formal visual field testing. This was in addition to a pre-existing right sixth nerve palsy since childhood. Imaging revealed a 35x 24x 22mm pituitary fossa lesion with bilateral cavernous sinus invasion, worse on the right, with left-sided optic chiasm compression (Figure 1).

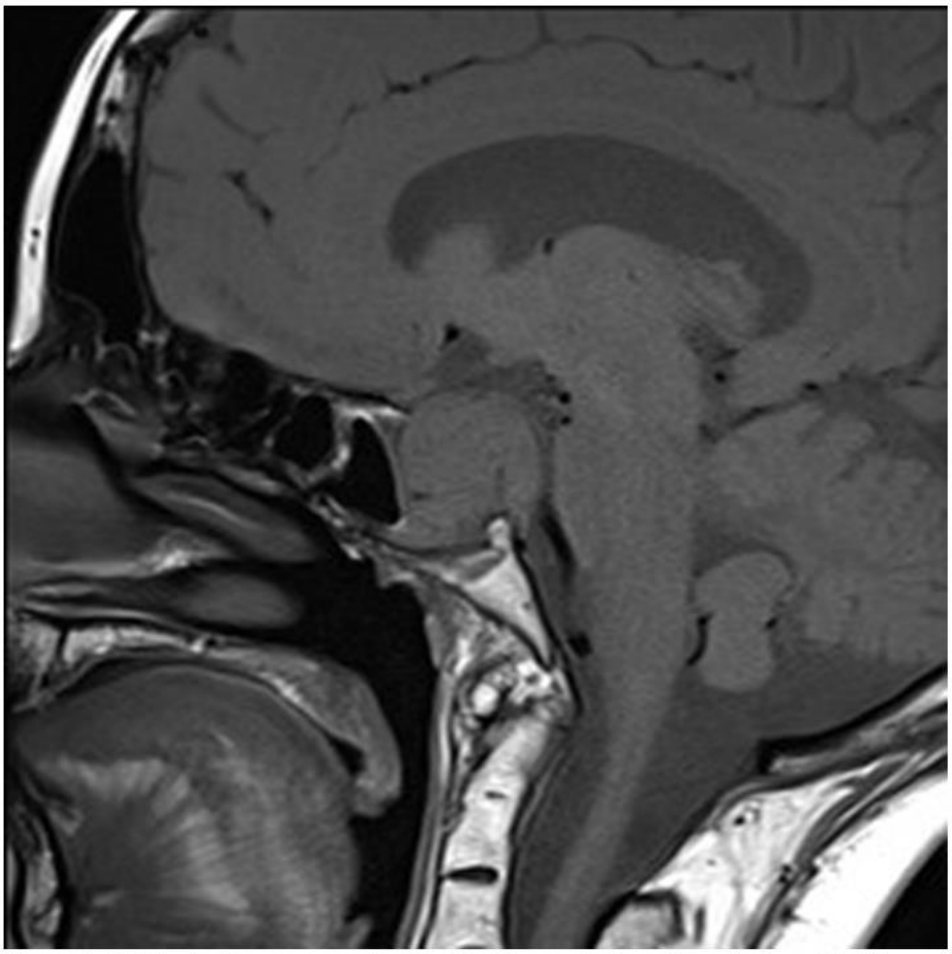


Figure 1. MRI brain demonstrated a 35x 24x 22mm pituitary fossa lesion with bilateral cavernous sinus invasion

Histologic examination of a trans-sphenoidal endoscopic biopsy of the suprasellar lesion showed a moderately cellular spindle cell neoplasm. Smooth muscle actin (SMA) immunohistochemistry and EBV-encoded RNA in situ hybridisation (EBER-ISH) were positive, consistent with EBV-SMT. She was admitted two weeks later for a planned two-stage tumour debulking.

Initially, she underwent a right-sided frontotemporal craniotomy and debulking of the cavernous tumour with peri-operative intravenous hydrocortisone. This was changed to oral dexamethasone 4mg QID post-operatively to reduce intracranial

oedema. Fifteen hours later, she became polyuric requiring a single dose of 1 mcg intravenous desmopressin. Histology confirmed EBV-SMT (Figure 2), associated with partially infarcted anterior pituitary tissue.

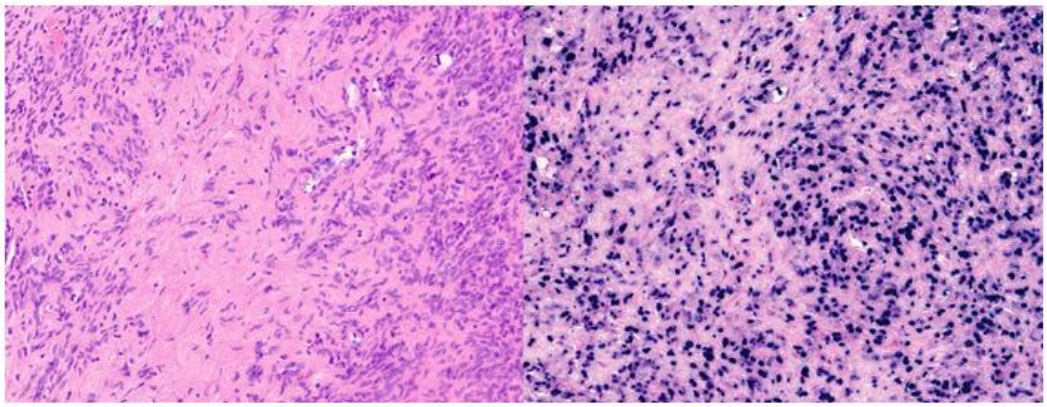


Figure 2. EBV-SMT, with variably cellular intersecting fascicles of bland spindled cells (H&E stain, left) and strong diffuse nuclear positivity for EBER-ISH (right).

Six days later, she underwent a stage 2 stereotactic trans-sphenoidal endoscopic resection of the residual tumour. Six hours later, she developed polyuria and required a further dose of 1mcg intravenous desmopressin. Her serum sodium levels remained within normal limits. Her post-operative course was otherwise uncomplicated and she continues to recover on the ward.

Discussion:

EBV-SMTs are rare neoplasms occurring almost exclusively in those who are immunosuppressed (1,2). A single-centre retrospective study of 5006 solid organ recipients over 31 years found only 3 cases of post-transplant EBV-SMT (3). Of those with solid organ transplants, EBV-SMTs occur most commonly in kidney transplant patients (4) with an incidence of 1.67% (5). They are considered a late onset complication of immunosuppression, occurring at a median of 9 years post kidney transplant (5). Irrespective of the organ transplanted, EBV-SMT can develop in either the graft or any other organ. Risk factors include EBV seronegativity followed by primary EBV infection and persistently high EBV viral loads (3).

The only reported pituitary EBV-SMTs are from a retrospective case series of 21 EBV-SMTs associated with HIV infection where 2 were in the pituitary (2). The clinical presentations of these cases were not characterised. A systematic review of 53 EBV-SMTs in the central nervous system found that the most common presenting symptom was a headache (1). However, the location within the central nervous system of these tumours was not specified and it is unknown whether any of these lesions occurred in the pituitary.

The pathogenesis of EBV-SMT is not fully understood. EBV infects smooth muscle cells using an unclear mechanism, with hypotheses including cellular fusion with infected lymphocytes, or via surface receptor CD21 (6). An analysis of renal transplant-associated EBV-SMTs demonstrated gene expression in keeping with a latency type III pattern, in which a range of potentially oncogenic nuclear antigens and latent membrane proteins (LMP) are expressed (7). LMP2A activates the mTOR/Akt pathway, and is thought to be a driver of oncogenesis. This is supported by the frequent response of these tumours to mTOR inhibitors (7). Everolimus was trialled in this patient on initial diagnosis of EBV-SMT with no success.

Intracranial tumours are hypothesised to derive from the myogenous vascular wall (8). EBV-SMT is frequently multifocal (1,5). Viral episomal analysis of multifocal lesions demonstrated independent viral clones, suggesting that they are the result of multiple infection events rather than metastasis (9). Histology characteristically demonstrates intersecting fascicles of spindled cells with blunt-ended nuclei, with positive immunohistochemistry for SMA and caldesmon (9) with diffuse EBER-ISH positivity. Cytologically malignant features including primitive areas, higher cellularity, necrosis, and mitotic activity can be observed; however, prognosis is independent of histology, relating primarily to patient immunity status.

The optimal management of EBV-SMT remains unclear. Surgical resection is the most common therapy (1,5,8). Other strategies have included involved field radiation therapy, stereotactic radiosurgery, systemic chemotherapy, mTOR inhibitors, modification of post-transplant immunosuppression therapy or a combination of these (1,4,5). However, there is no evidence supporting superiority of one modality over another. Previous studies have demonstrated comparable survival in those treated with resection versus reduced immunosuppression (4), sirolimus versus reduced immunosuppression (5), chemotherapy versus no chemotherapy (4) and complete versus partial resection (8).

Compared with those who develop EBV-SMT in the context of HIV, EBV-SMT arising in the setting of a solid organ transplant had poorer tumour outcomes, with only 20% of patients having no recurrence or stable disease on long term follow up (1). However, these tumours do not often cause mortality, and a retrospective study of 16 patients with EBV-SMT post kidney transplant found a survival rate of 75% over a mean follow up period of 5 years (5).

In a large case series of 29 tumours from 19 patients, mortality was recorded in only 3 patients, of whom only 1 died of tumour-related complications (9). This patient had widespread disease involving the spinal cord, gallbladder, lung, and liver. Another 5 patients from the same study with widespread disease were alive at the time of most recent follow-up. In another large series, death occurred in 17 of 25 patients, but was mainly the result of comorbid diseases (10).

Those with early-onset tumours and intracranial tumours have poorer survival (4,8). However, multiple tumours, tumour size or histological features of the tumour were not predictive of survival (8).

In conclusion, we have presented the rare case of an EBV-SMT occurring in the pituitary in a young woman with a renal transplant 6 years ago. This is the first report to our knowledge that characterises the clinical presentation of an EBV-SMT in the pituitary in a solid organ transplant recipient. EBV-SMTs are rare tumours and the optimal management of this condition remains unclear.

Learning points:

- EBV-SMTs are rare neoplasms occurring almost exclusively in immunosuppressed patients
- In such patients presenting with a pituitary mass, the differentials should be kept broad and EBV-SMT should be considered
- Due to the rarity of this condition, the optimal management remains unclear, but surgery is commonly performed

1. 1. Willeke VM, Anderson MP, Mahadevan A, Rojas R, Bhadelia R, Thomas AJ, Kasper EM. Epstein Barr virus associated smooth muscle tumors in the central nervous system: a case report and systematic review of the literature. Journal of neuro-oncology 2020; 147:247-260
2. 2. Pitjadi TM, Grayson W. Epstein-Barr Virus-Associated Smooth Muscle Tumour: A Case Series with a Significant Proportion of Tumours Showing Proclivity for Cutaneous Soft Tissues. Dermatopathology (Basel, Switzerland) 2019; 6:133-146
3. 3. Stubbins RJ, Alami Laroussi N, Peters AC, Urschel S, Dicke F, Lai RL, Zhu J, Mabilangan C, Preiksaitis JK. Epstein-Barr virus associated smooth muscle tumors in solid organ transplant recipients: Incidence over 31 years at a single institution and review of the literature. Transplant infectious disease : an official journal of the Transplantation Society 2019; 21:e13010
4. 4. Jonigk D, Laenger F, Maegel L, Izykowski N, Rische J, Tiede C, Klein C, Maecker-Kolhoff B, Kreipe H, Hussein K. Molecular and clinicopathological analysis of Epstein-Barr virus-associated posttransplant smooth muscle tumors. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2012; 12:1908-1917
5. 5. Tan CS, Loh HL, Foo MW, Choong LH, Wong KS, Kee TY. Epstein-Barr virus-associated smooth muscle tumors after kidney transplantation: treatment and outcomes in a single center. Clinical transplantation 2013; 27:E462-468
6. 6. Purgina B, Rao UNM, Miettinen M, Pantanowitz L. AIDS-Related EBV-Associated Smooth Muscle Tumors: A Review of 64 Published Cases. Patholog Res Int 2011; 2011:561548-561548
7. 7. Ong KW, Teo M, Lee V, Ong D, Lee A, Tan CS, Vathsala A, Toh HC. Expression of EBV Latent Antigens, Mammalian Target of Rapamycin, and Tumor Suppression Genes in EBV-Positive Smooth Muscle Tumors: Clinical and Therapeutic Implications. Clinical Cancer Research 2009; 15:5350-5358
8. 8. Hussein K, Rath B, Ludewig B, Kreipe H, Jonigk D. Clinico-pathological characteristics of different types of immunodeficiency-associated smooth muscle tumours. European journal of cancer (Oxford, England : 1990) 2014; 50:2417-2424
9. 9. Deyrup AT, Lee VK, Hill CE, Cheuk W, Toh HC, Kesavan S, Chan EW, Weiss SW. Epstein-Barr virus-associated smooth muscle tumors are distinctive mesenchymal tumors reflecting multiple infection events: a clinicopathologic and molecular analysis of 29 tumors from 19 patients. The American journal of surgical pathology 2006; 30:75-82
10. 10. Ramdial PK, Sing Y, Deonarain J, Vaubell JI, Naicker S, Sydney C, Hadley LG, Singh B, Kiratu E, Gundry B, Sewram V. Extra-uterine myoid tumours in patients with acquired immunodeficiency syndrome: a clinicopathological reappraisal. Histopathology 2011; 59:1122-1134

Refractory hypercalcaemia associated with disseminated *Cryptococcus neoformans* infection

Jasmine J Zhu[1], Will J Naughton[2], Kim Hay Be[3], Nic Ensor[3], Ada S Cheung[1, 4]

1. Endocrine Centre of Excellence, Austin Health, Melbourne, Victoria, Australia

2. Infectious Diseases, Austin Health, Melbourne, Victoria, Australia

3. Liver Transplant Unit, Austin Health, Melbourne, Victoria, Australia

4. School of Medicine, University of Melbourne, Melbourne, Victoria, Australia

Hypercalcaemia is a very common endocrine condition, yet severe hypercalcaemia as a result of fungal infection is rarely described (1-4).

A 55 year-old woman of Polynesian descent presented 6 weeks after her second liver transplant with tachycardia and rising inflammatory markers. She had a history of end stage liver disease due to non-alcoholic steatohepatitis cirrhosis, type 2 diabetes mellitus, obesity, hypertension and recurrent perianal abscesses. Her first liver transplant four months prior had failed as a result of acute rejection, hepatic bilomas, recurrent Enterococcus and Pseudomonas aeruginosa bacteraemia and cytomegalovirus viraemia.

There was no obvious cause of her tachycardia and rising inflammatory markers which were suspicious for infection. A computed-tomography pulmonary angiogram revealed bilateral air space opacities (Figure 1). Her Cryptococcus antigen titre was greater than 1 in 2560. Culture of bronchial washings demonstrated growth of *Cryptococcus neoformans*.

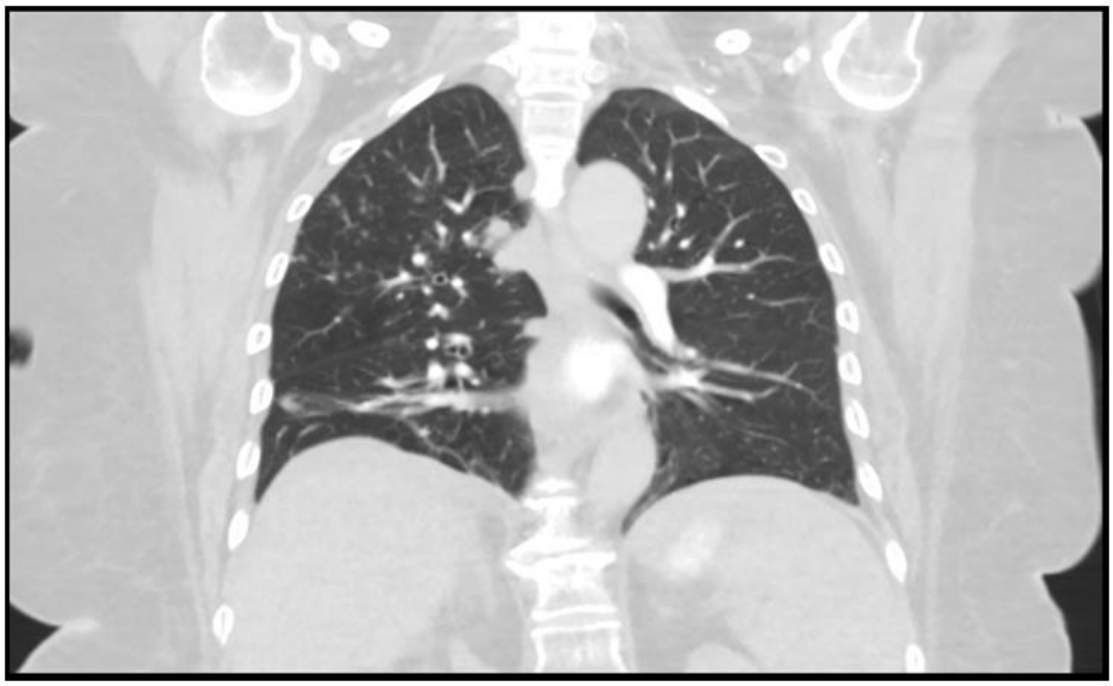


Figure 1. Bilateral scattered air space opacities demonstrated on a computer-tomography pulmonary angiogram.

The patient was commenced on four weeks of induction therapy with intravenous liposomal amphotericin and oral flucytosine. Despite antifungal treatment, she subsequently developed invasive fungal skin lesions over her abdomen and thighs (Figure 2) with biopsies demonstrating growth of *Cryptococcus neoformans*. She transitioned to consolidation therapy with oral fluconazole. Ten days later the patient developed further skin lesions, raising concerns about disease progression and she recommenced intravenous liposomal amphotericin and oral flucytosine. A fluorodeoxyglucose positron emission tomography (FDG-PET) scan demonstrated findings in keeping with disseminated cryptococcosis, with FDG-avid lesions in the lungs as well as substantial subcutaneous and muscle tissue avidity (Figure 3).

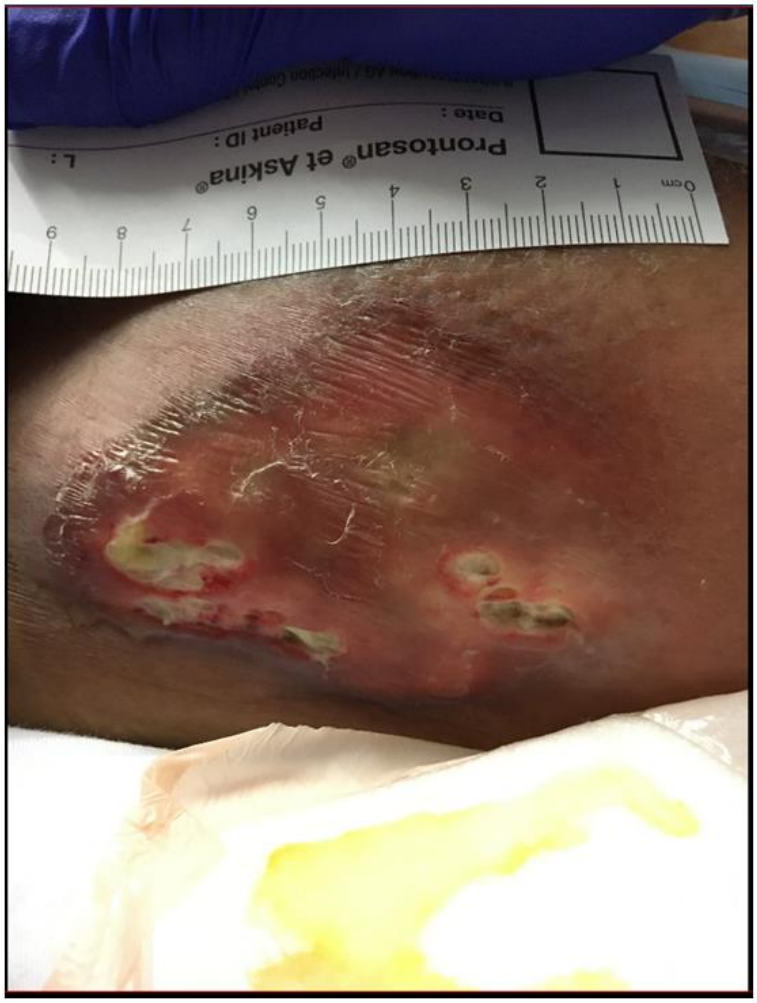


Figure 2. Cutaneous cryptococcosis with ulcerated pustular lesions.

Figure 3. FDG-PET demonstrating FDG-avid lesions in subcutaneous and muscle tissues of forearms, lower torso and lower limbs, and FDG-avid pulmonary nodules and right hilar lymphadenopathy.

Three weeks after her presentation, she was noted to have an elevated corrected calcium of 2.79 mmol/L. Her calcium concentrations were observed and continued to steadily rise. Further investigations when corrected calcium was 3.17 mmol/L

demonstrated that the ionised calcium was 1.69 mmol/L (1.12-1.30), the parathyroid hormone 1.1 pmol/L (0.7-4.1), 25-hydroxy-vitamin D (25-OH D) 59 nmol/L and 1,25-dihydroxy-vitamin D (1,25-OH D) 219 pmol/L (50-190). The parathyroid hormone was measured on the Diasorin Liaison (1-84) assay which was subsequently found to report falsely high values at the lower end of the reference range. Her albumin was 35 g/L and estimated glomerular filtration rate was 61 ml/min/1.73m^2.

The patient was surprisingly asymptomatic from the hypercalcaemia. She remained alert and oriented, and did not experience polyuria or polydipsia. Throughout her hospitalisation she remained ambulant, mobile and had consumed a regular diet without any nutritional or calcium supplementation. She was hypervolaemic due to large volumes of intravenous hydration to minimise amphotericin-induced nephrotoxicity. Consequently, she had signs of mild pulmonary oedema on chest radiography which improved with diuretic therapy.

As the cause of her hypercalcaemia was not immediately apparent, she was observed for several days before treatment with 30mg of intravenous pamidronate. Despite this, the corrected calcium continued to rise, peaking at 3.82 mmol/L. The patient received a 48 hour course of intravenous calcitonin 100mg six-hourly followed by 60mg of subcutaneous denosumab. The calcium slowly decreased, but remained above 3 mmol/L. Two weeks later, the patient had a liver biopsy demonstrating acute liver rejection and was treated with intravenous pulse methylprednisolone. The calcium levels sharply declined and normalised within several days (Figure 4). There were no granulomas on the skin or liver biopsies.

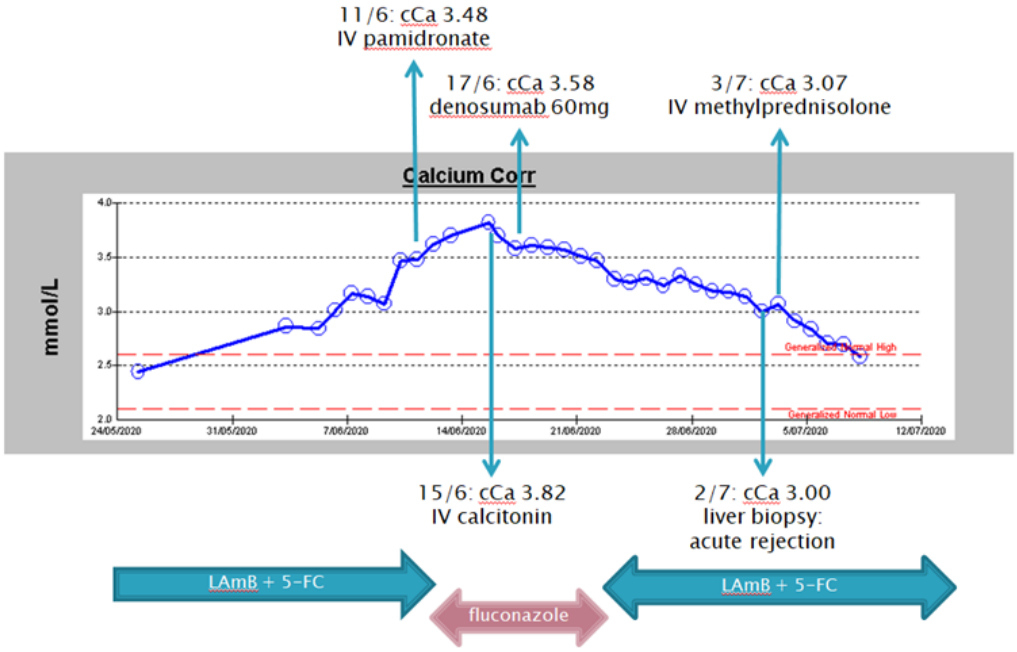


Figure 4. Trajectory of hypercalcaemia. The patient received initial treatment with intravenous liposomal amphotericin (LAmB) and oral flucytosine (5-FC). The calcium levels continued to rise following a pamidronate infusion, and then subsequently lowered after commencing a course of calcitonin followed by denosumab while on fluconazole. The hypercalcaemia resolved after methylprednisolone was administered for acute rejection of the liver graft.

Hypercalcaemia mediated by 1,25-OH D has been described as a rare complication of fungal infections. There are only two case reports in the literature of hypercalcaemia associated with *Cryptococcus neoformans* infection (1,2). In both of these cases, the patients were infected with HIV and had a low CD4 lymphocyte count. Whilst the case described by Spindel had disseminated multiorgan Cryptococcus infection (1), Ali et al describe isolated pulmonary *Cryptococcus neoformans* and *Coccidioides immitis* infection (2). Both cases had an elevated 1,25-OH D and a peak corrected calcium in the range of 3.30-3.60 mmol/L. Hypercalcaemia was managed with intravenous fluids, antifungal treatment and one of the cases required intravenous pamidronate and hydrocortisone. Resolution of the hypercalcaemia coincided with resolution of the Cryptococcus infection. Tuberculosis and lymphoma were excluded as alternative causes of hypercalcaemia.

Although the mechanism of hypercalcaemia in these infections is not clear, the fact that 1,25-OH D levels were elevated in all three cases suggests that the hypercalcaemia could be at least partly driven by the extra-renal production of 1-alpha-hydroxylase by macrophages in granulomas (1,3). Indeed, Cryptococcus is known to induce a granulomatous response (5), and granulomas were found in the skin and liver biopsies of the case reported by Spindel (1). A review has suggested that 1,25-OH D may play a role in regulating immune function (4) so this pathway may become activated through adaptive mechanisms. However, this theory has not been proven.

Management of 1,25-OH D-driven hypercalcaemia includes restriction of dietary calcium and sunlight exposure (4). Glucocorticoids inhibit 1-alpha-hydroxylase activity in macrophages as well as 1,25-OH D-mediated absorption of calcium from the gastrointestinal tract, and may also be given. However, the risks of exacerbating an underlying infection need to be carefully considered against the benefit of controlling the hypercalcaemia. In our case, methylprednisolone was administered as it was essential for the survival of the liver graft, and fortuitously resolved the hypercalcaemia.

In addition to its antifungal effects, fluconazole inhibits 25-hydroxylase and 1-alpha-hydroxylase, and its use in reducing 1,25-OH D levels in a patient with a CYP24A1 mutation has been described (6). In our case, the calcium levels dropped during fluconazole therapy, but this may have been confounded by concurrent calcitonin and denosumab therapy.

In summary, *Cryptococcus neoformans* is a rare cause of 1,25-OH D-mediated refractory hypercalcaemia which in our case required antifungal treatment, pamidronate, calcitonin, denosumab and high dose glucocorticoid treatment. Hypercalcaemia is a rare complication of disseminated fungal infection, which should be suspected in immunosuppressed individuals.

Take home messages

- In immunocompromised patients with unexplained hypercalcaemia, fungal infections should be considered in the differential diagnoses;
- Glucocorticoids may be considered to treat 1,25-OH D-driven hypercalcaemia, however, the benefits of lowering the calcium need to be balanced against the risk of exacerbating an underlying infection;
- Fluconazole might be an effective therapy for both treatment of the hypercalcaemia by lowering 1,25-OH D levels as well as of the fungal infection.

1. Spindel SJ, Hamill RJ, Georghiou PR, Lacke CE, Green LK, Mallette LE. Case report: vitamin D-mediated hypercalcemia in fungal infections. The American journal of the medical sciences 1995; 310:71-76
2. Ali MY, Gopal KV, Llerena LA, Taylor HC. Hypercalcemia associated with infection by Cryptococcus neoformans and Coccidioides immitis. The American journal of the medical sciences 1999; 318:419-423
3. Lionakis MS, Samonis G, Kontoyiannis DP. Endocrine and metabolic manifestations of invasive fungal infections and systemic antifungal treatment. Mayo Clinic proceedings 2008; 83:1046-1060
4. Sharma OP. Hypercalcemia in granulomatous disorders: a clinical review. Current opinion in pulmonary medicine 2000; 6:442-447
5. Shibuya K, Hirata A, Omuta J, Sugamata M, Katori S, Saito N, Murata N, Morita A, Takahashi K, Hasegawa C, Mitsuda A, Hatori T, Nonaka H. Granuloma and cryptococcosis. Journal of infection and chemotherapy : official journal of the Japan Society of Chemotherapy 2005; 11:115-122
6. Sayers J, Hynes AM, Srivastava S, Dowen F, Quinton R, Datta HK, Sayer JA. Successful treatment of hypercalcaemia associated with a CYP24A1 mutation with fluconazole. Clinical kidney journal 2015; 8:453-455

146

Keeping pace with the dynamic world in managing adult patients with type 2 diabetes

Jonathan Shaw[1]

1. Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia

About 2 in 3 Australian adults with type 2 diabetes (T2D) also have cardiovascular disease (CVD) and/or chronic kidney disease (CKD).[1,2] Indeed, CVD accounts for almost 1 in 3 deaths for people with T2D, and overall life expectancy is shortened by up to 8–9 years.[1] This burden of disease highlights the importance of selecting glucose-lowering therapies that minimise the risk of cardiovascular events. Additionally, recent updates to the ADA/EASD consensus statement emphasise the need to manage CV risks in patients with T2D.[3]

Join Professor Jonathan Shaw as he explains the recent guideline changes and the main findings of key cardiovascular outcomes trials in patients with T2D. The discussion will include key consideration for clinical practice when needing to minimise the risk of CV events in patients with T2D.

1. Shaw JE, Thomas M, Magliano D. The dark heart of type 2 diabetes. Baker Heart and Diabetes Institute, 2018. Available at https://www.baker.edu.au/-/media/documents/impact/Baker-Institute-The-dark-heart-of-type-2-diabetes.ashx?la=en. Accessed 1 October, 2020.
2. International Diabetes Federation. IDF Diabetes Atlas. 9th ed. 2019.
3. Buse JB et al. Diabetes Care 2020;43:487–93