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diagnostic term. Our cases also did not develop ischaemic complications. This suggests that the cases of ELP reported in the literature may represent only the more severe end of the spectrum. Long-term follow-up of more cases is necessary to determine the clinical behaviour of these findings.

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## Granulosa cell tumour of the adrenal

Sir,
Treatment resistant hypertension in the context of an adrenal mass is often assumed to be adrenocortical adenoma or phaeochromocytoma until proven otherwise. Histopathology may sometimes provide unexpected answers..

A 67-year-old female was referred by her general practitioner to a hypertension outpatient clinic for work-up of treatment resistant hypertension. Relevant background history included two cerebrovascular accidents (12 and 10 years ago), and a multinodular goitre (resected).

For the past 20 years she had refractory hypertension. On presentation, her blood pressure remained at 200/100 mmHg despite being on candesartan, perindopril, moxonidine and indapamide sustained release. The addition of spironolactone decreased her blood pressure to a systolic of 204 mmHg to 160 mmHg. She experienced headaches and visual symptoms whenever she did not take her antihypertensives. The patient was taking at least 3600 mg of potassium chloride tablets (Slow-K) a day in order to maintain her potassium within normal range. She had previously been intolerant to hydrochlorothiazide, verapamil, metoprolol, diltiazem and atenolol. She denied episodic sweating and palpitations, weight loss and polyuria. She did not complain of dysfunctional uterine bleeding, breast tenderness, vaginal secretions, hirsutism, acne and change in voice. There was no family history of breast or ovarian cancer. There was no smoking history. She was nulliparous and denied use of any oral contraceptives.

Her cardiovascular examination was significant for a hyperdynamic apex beat. Abdominal examination also revealed bilateral bruits. She did not have signs or symptoms of hyperthyroidism. There was neither radio-femoral delay nor a differential blood pressure between upper limbs. She was euvolaemic and did not have signs of heart failure. There was no peripheral oedema.

On investigation, her urine protein to creatinine ratio was 17 mg/mmol. No casts were present. Her creatinine was 70 μmol/L (GFR 82 mL/min/1.73m$^2$). Her sodium was 141 mmol/L, and bicarbonate 29 mmol/L. The patient's morning cortisol, thyroid function and inflammatory markers (erythrocyte sedimentation rate, white cell count and C reactive protein) were normal. A CT renal angiogram revealed a well-defined solid mass from the left adrenal gland. It measured $4.3 \times 3.2$ cm in size with minimal calcification. There was physiological tracer uptake on metaiodobenzylguanidine (MIBG) scan (Fig. 1). The right adrenal was normal. Calcification was noted in the renal arteries without haemodynamically significant stenosis.

Plasma metanephrine and normetanephrine levels, and urinary free noradrenaline, adrenaline, and dopamine were within

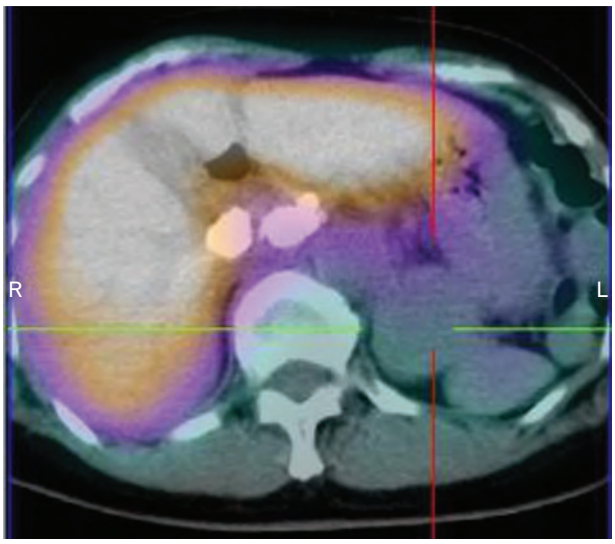


**Fig. 1** Well-defined, minimally calcified $4.3 \times 3.2$ cm solid mass from left adrenal. Physiological tracer uptake on MIBG scan.

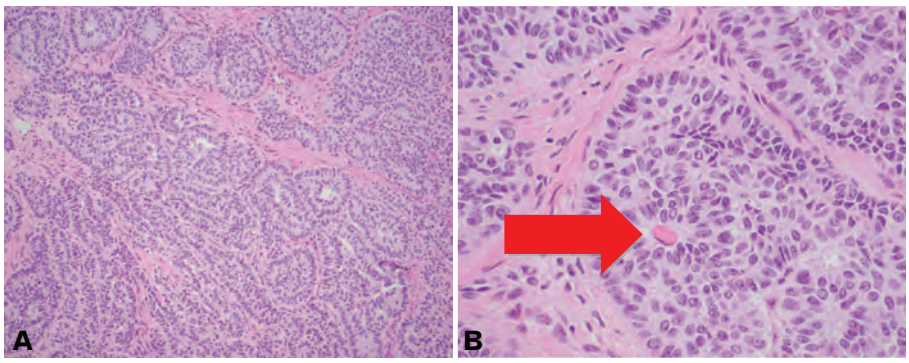


**Fig. 2** (A) The neoplastic cells are arranged in cords, trabeculae and nests (H&E). (B) High power view demonstrating a classical Call–Exner body (arrow) and occasional nuclear grooves in the neoplastic cells (H&E).

normal range. However antihypertensives may cause a false negative result. Episodic catecholamine release can also confound urine and plasma catecholamine levels. Therefore the decision was made to continue treating as a presumed phaeochromocytoma. Spironolactone was changed to phenoxybenzamine. This had a good effect, bringing the systolic blood pressure to 130 mmHg. After counselling of the advantages and disadvantages of further treatment, the patient elected for a left laparoscopic adrenalectomy. This proceeded without complications.

The resected adrenal tumour weighed 20 g and was 40 mm in maximum dimension. Histologically it demonstrated typical morphological features of granulosa cell tumour of adult type (Fig. 2A,B). Briefly, the tumour was composed of cords, trabeculae and nests of neoplastic cells (Fig. 2A). Many of the neoplastic cells demonstrated typical nuclear grooving and there were very occasional classical Call–Exner bodies (granulosa cells surrounding eosinophilic secretions, illustrated by arrow in Fig. 2B). By immunohistochemistry the neoplastic cells were negative for chromogranin (arguing very strongly against the possibility of phaeochromocytoma) but were positive for markers commonly seen in granulosa cell tumour including inhibin, calretinin and WT1. There was no vascular space invasion or perineural growth. Although the possibility of metastasis from an ovarian primary could not be excluded histologically, the favoured pathological diagnosis was a primary adrenal granulosa cell tumour of adult type. The patient subsequently underwent a dedicated pelvic ultrasound which failed to reveal any other ovarian or endometrial mass and further favoured the granulosa cell tumour as being of primary adrenal origin.

Interestingly, unilateral left adrenalectomy completely resolved the patient's hypertension. All antihypertensives were ceased over the following 6 months post-operatively. Urine protein to creatinine ratio normalised and her hypokalaemia resolved. Her renal function remained stable at a creatinine of 120 μmol/L (GFR 41 mL/min/1.73 $m^2$). She remained well on last review 28 months after initial presentation.

The ovaries and adrenal glands are embryologically related and rests of adrenal tissue are not uncommon incidental findings in normal ovaries and testis.[1] Ovarian tissue or ovarian type neoplasms in the adrenal are less common, but are well recognised and in fact two previous cases of primary granulosa cell tumour in the adrenal gland have been reported.[2,3]

This case highlights the link between hypertension, renin and granulosa cell tumour. This has only been published in the literature once before.[4] In that report electron microscopy was performed which suggested renin granules. Similar to our case, the patient had hypertension, high renin activity, hyperaldosteronism, hypokalaemia and a mass containing granulosa cells. Hypertension resolved after removal. Neither renin nor aldosterone levels were taken due to being on candesartan and perindopril. These antihypertensives confound the interpretation by blocking the renin-angiotensin axis. This increases the production of renin causing a falsely elevated result. It is recommended that antihypertensives be washed out for 2–3 weeks prior to testing. However, this was not feasible in our patient due to her refractory hypertension and risk of precipitating accelerated hypertension. Nonetheless, it is likely that our patient's pathology was renin mediated. This is implied by her features of hypertension and hypokalaemia.

Granulosa cell tumours are usually associated with hyperoestrogenism. Oestradiol levels were not performed, however our patient denied these symptoms. For most tumours, surgery alone is sufficient since most are early stage, as in our case. Disease free survival is approximately 90%.[5] Chemotherapy is required for women in later stages.

In summary, our patient demonstrated an interesting presentation of granulosa cell tumour. Whilst history, laboratory markers and radiological investigations were inconclusive, her hypertension improved on treatment aimed at hyperaldosteronism and phaeochromocytoma and, eventually resolved after unilateral adrenalectomy. The final diagnosis was yielded by histopathology. These results were altogether surprising.

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## Colonisation with *Pneumocystis jirovecii* in Australian infants

Sir,

*Pneumocystis jirovecii*, a fungus with worldwide distribution, causes a severe inflammatory pneumonia in immunocompromised adults and children. The association or causal link between *P. jirovecii* and sudden unexpected infant death is controversial.[1,2] Serological studies have indicated that exposure to *Pneumocystis* occurs at an early age.[3,4] Such colonisation in healthy children may lead to later reactivation and overt disease, although *de novo* infection also occurs.[5] The prevalence of colonisation in children, which appears to be higher than that in adults, is considered clinically relevant since colonised children are postulated to be a reservoir for *Pneumocystis*.[3] Studies in Chile, the US, Zambia and Europe have examined for the presence of *Pneumocystis* using direct detection methods (immunohistochemistry or polymerase chain reaction, PCR) in children and reported prevalence rates ranging between 29% and 100%.[1,3,6–9] However, there are no data on the prevalence of colonisation in Australian infants.

In this pilot study, we sought to determine if detection of *P. jirovecii* in young children without *Pneumocystis* pneumonia was feasible. We examined 50 non-duplicate nasopharyngeal aspirate (NPA) specimens that had been collected for routine virological testing, for the presence of *P. jirovecii* DNA. Nasopharyngeal aspirates from infants aged 2–8 months collected between December 2013 to March 2014 were retrieved from storage at −80°C. None of these patients had a clinical syndrome consistent with *P. jirovecii* pneumonia, nor were they HIV infected, but all had NPA specimens collected because they had respiratory symptoms. These specimens had been tested using the commercial Seeplex RV15 ACE Detection multiplex PCR assay according to manufacturer's instructions (Seegene, Korea). This assay detects influenza A, influenza B, parainfluenza 1, 2, 3 and 4, respiratory syncytial virus A and B, rhinovirus, enterovirus, adenovirus, coronavirus, metapneumovirus, and bocavirus. As part of the study design, we randomly selected 25 specimens from patients in whom no respiratory viruses were identified and 25 specimens from patients who were infected with one or more respiratory viruses.

Detection of *P. jirovecii* DNA by PCR was performed as previously described, with minor modifications.[10,11] DNA was extracted from 500 μL of nasopharyngeal aspirate sample. Briefly, the assay is an 'in-house' real-time TaqMan PCR assay that targets the single copy β-tubulin gene, performed on the LightCycler platform, a closed amplification system (Roche Diagnostics, Germany). In anticipation of lower levels of *Pneumocystis* burden in comparison to patients with overt *P. jirovecii* pneumonia, an additional 10 cycles of amplification were performed in order to increase the assay sensitivity. Therefore, the cycling parameters were 95°C for 10 min, followed by 50 cycles of 95°C for 5 s, 58°C for 20 s and 72°C for 20 s. Analysis of DNA extracts by multi-locus sequence typing (MLST) of four genetic loci: (1) internal transcribed spacer 1 and 2 (ITS1/2) regions of the nuclear rDNA gene cluster; (2) the *P. jirovecii*-specific β-tubulin; (3) mitochondrial large subunit (*mtLSU*); and (4) dihydropteroate synthase genes, was performed as previously described.[10]

*Pneumocystis jirovecii* DNA was detected in seven NPA specimens (14%). As expected, the burden of *P. jirovecii* was low with PCR cycle threshold (Ct) values ranging between 38 and 50. In contrast, when using this assay to diagnose *Pneumocystis* pneumonia and other disease, samples with a Ct value <37.3 cycles are typically classified as highly suggestive for *P. jirovecii* infection.[10]

The mean age of colonised infants was 190.7 days (range 62–469 days). Four of these patients were co-infected with respiratory viruses including rhinovirus ($n=2$), parainfluenza virus 3 ($n=2$) and adenovirus ($n=1$). In the other three patients, no respiratory viruses were detected. Attempts to perform MLST on the seven DNA extracts that yielded *P. jirovecii* DNA were unsuccessful for some of the loci despite multiple experiments. This was likely due to the low amounts of DNA template present in the extracts and was not further pursued. In contrast, the *mtLSU* gene target that is present in multiple copies in the *P. jirovecii* genome, was able to be amplified and sequenced in three of the DNA extracts.

In this study, we have demonstrated colonisation with *P. jirovecii* in Australian infants with a point prevalence of 14%, and as such, have opened up discussion on methodological issues that are relevant for future studies. Although reports from elsewhere have demonstrated a higher prevalence, there were a number of methodological differences in our study. Here, we used a single copy target for real-time PCR whereas other studies using PCR have employed multi-copy PCR amplification targets such as the *mtLSU* or the major surface glycoprotein (*MSG*) genes.[3,6,9] In addition, previous studies reporting a significantly higher prevalence examined autopsy lung specimens.[1,6] Because of the retrospective nature of the present pilot study, we were limited to using a small volume of specimen (500 μL) from the upper respiratory tract. Other studies using this specimen type have used between 200 and 3000 μL of sample for DNA extraction.[3,12] The relative sensitivities of *P. jirovecii* PCR using different types of respiratory tract specimens is not well defined in children, but in adults lower respiratory tract specimens are preferred.[13] However, in practice, when assessing for colonisation in infants, sampling the upper respiratory tract by the least invasive means is of high importance.

Previous studies have indicated that colonisation appears to be common in children during upper respiratory tract symptoms or infection.[3] Our pilot study was not designed to determine any associations between *P. jirovecii* colonisation and co-infection with respiratory viruses, but this is an important consideration for future studies. *Pneumocystis jirovecii* may provide an alternative aetiological diagnosis for upper respiratory tract symptoms,[3] and pathogens may co-exist and act synergistically in infection and disease. For instance, co-infection has been demonstrated to result in more severe disease caused by other