Approach to the paediatric patient with suspected pheochromocytoma or paraganglioma versus neuroblastoma

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Abstract

Catecholamine producing tumours of childhood include neuroblastic tumours, phaeochromocytoma and paraganglioma (PPGL). PPGL and neuroblastic tumours can arise in similar anatomical locations and clinical presentations can overlap resulting in diagnostic challenges. Distinguishing between these tumour types is critical as management and long-term surveillance strategies differ depending on the diagnosis. Herein we describe two clinical cases and illustrate key considerations in the diagnostic work up of a neuroblastoma versus PPGL for patients presenting with adrenal, pelvic, and retroperitoneal masses in childhood.

Introduction

Catecholamine producing tumours arising in childhood include neuroblastoma, phaeochromocytoma and paraganglioma (PPGL). These tumours share a common cellular origin namely Schwann cell precursors that subsequently differentiate into neuroblasts from which neuroblastomas are derived and chromaffin cells from which PPGL are derived (1). Neuroblastomas are the most common extra cranial solid tumour in childhood, whilst PPGL in childhood are rare with an estimated annual incidence of 0.5-2/million children (2). Both neuroblastoma and PPGL exhibit significant clinical heterogeneity, have malignant potential, and pose a risk of significant morbidity and mortality (3–6). Neuroblastic tumours are neural crest-derived tumours and may occur in the adrenal glands or anywhere else where descendants of this lineage can be found in the body (e.g. neck, thorax, abdomen and pelvis). Neuroblastic tumours refer to a histological spectrum of tumours including neuroblastoma, ganglioneuroblastoma and ganglioneuroma, which can be separated by the tumoral content of neuroblastic cells and Schwannian stroma (1). Phaeochromocytomas arise in the adrenal medulla and paragangliomas refer to their extra adrenal counterpart which can arise from sympathetic or parasympathetic tissue anywhere from the bass of the skull to the pelvis. A recent study demonstrated that PPGL can be initially misdiagnosed as neuroblastoma in 30% of children (7). Neuroblastoma and PPGL can arise in similar locations including the adrenal, retroperitoneum, pelvis, neck, and mediastinum and clinical presentations can overlap. Neuroblastoma is the most likely diagnosis in young children (<2 years), but the median age at presentation for PPGL is 11-15 years (6, 8, 9). Children with PPGL may have symptoms related to catecholamine excess due to a more mature catecholaminergic biosynthetic and secretory pathway compared to neuroblastoma. Biochemical testing including plasma free or urine metanephrines is a helpful first step if there is clinical suspicion of PPGL, as a negative result will enable a safe diagnostic biopsy.

Case 1

A 12-year-old boy presented to his local hospital with a four-day history of acute headache and vomiting associated with abdominal pain. There was a longstanding two-year history of intermittent symptoms including severe headache, vomiting, fatigue, and sweating, exacerbated by exercise. Initial examination findings included an unwell, dehydrated child with poor capillary refill and a lower abdominal palpable mass. Blood pressure (BP) was 177/100 mmHg. Blood tests revealed a raised lactate dehydrogenase at 1.513 U/l (range 120-300) and serum creatinine on admission was elevated at 128 µmol/l (range 39-60), consistent with grade 2 acute kidney injury. Urinalysis revealed microscopic proteinuria and haematuria. Fluid resuscitation was performed resulting in clinical stabilisation and normalisation of creatinine to 58 µmol/l within 48 hours, but BP remained elevated. Abdominal ultrasound (USS) revealed normal kidneys, no hydronephrosis, and normal ureters, and confirmed the presence of a large, lower abdominal lobulated soft tissue mass. CT showed a 13cm x 10cm x 7cm diameter enhancing, highly vascular, heterogeneous mass with radiological appearances consistent with central necrosis, extending from the aortic bifurcation, where the common iliac arteries were splayed, into the suprapubic region (Figure 1). The patient was initially planned for emergency central line insertion, biopsy of the abdominal mass, bone marrow and cerebrospinal fluid examination for presumed high-grade lymphoma. However, on further enquiry and review, the longstanding nature of the symptoms were a concern, along with the elevated BP, responding poorly to nifedipine, and not explained by a presumed lymphoma diagnosis. In addition, the patient admitted that associated with the four-day acute episode leading to admission, he also had reduced, blurry vision. Ophthalmological examination revealed flame haemorrhages involving the central fovea, accounting for his reduced vision, caused by malignant hypertension. A CT head with contrast was normal. Further discussion of the CT imaging with radiologists at the principal treatment centre highlighted that the tumour arose from the aortic bifurcation, consistent with an origin from the organ of Zuckerkandl; therefore, the possibility of a paraganglioma (PGL) tumour was raised. Accordingly, the plan for emergency surgery was deconstructed to avoid life-threatening complications. Regarding hypertension, the 99th centile for BP for a patient of 12 years with height on the 25th centile was 131 mmHg systolic and 88 mmHg diastolic. In addition to calcium channel blockade with amlodipine, alpha-blockade with phenoxybenzamine resulted in immediate resolution of the patient's headache and symptoms. Echocardiogram was performed confirming left ventricular hypertrophy. Serum troponin I level was elevated at 125 ng/l (range 0-54), suggestive of a catecholamine cardiomyopathy. Measurements of plasma metanephrines and 3-methoxytyramine revealed a highly elevated normetanephrine of 18.849 pmol/l (range 0-1.180), elevated 3-methoxytyramine (3-MT) of 267 pmol/l (range 0-180) and normal metanephrine of 141 pmol/l (range 0-510), confirming paraganglioma. In view of the patient's young age, genetics referral and testing was undertaken, which identified a pathogenic germline *SDHB* variant. Ga-68-DOTATATE PET-CT showed three sites of uptake: the large pelvic tumour; a small 19mm x 9mm mass next to the uncinate process of the pancreas felt to be a metastatic lymph node or synchronous primary PGL; and a small left-sided thoracic paravertebral lesion, consistent with a further primary PGL (Figure 1). After surgical and anaesthetic multi-disciplinary discussion, the patient proceeded to abdominal surgery two months following initial presentation, allowing a period of BP and cardiac stabilisation before anaesthesia. An R0 removal of the large pelvic tumour was performed along with the second tumour next to the uncinate process of the pancreas. Histology confirmed two synchronous SDHB deficient paragangliomas with a ki67 of <5%. None of the sampled lymph nodes (0/2)

were positive for tumour. Following surgery, the patient's antihypertensive medication was successfully weaned and stopped. Post-operative plasma catecholamine metabolites were within the normal range. The patient underwent surveillance for the residual small left sided paravertebral PGL. On follow-up, this gradually increased in size, from 11 x 8 mm at initial presentation, to 15 x 11 mm six months later, to 21 x 18 mm one year later. A further Ga-68 DOTATATE PET-CT confirmed no other DOTATATE-avid disease. The patient underwent successful R0 resection of this lesion 13 months following initial presentation and now remains well in clinical follow-up. Lifelong surveillance will continue for further primary PGL lesions in view of the germline *SDHB* variant status.

Case 2

A 3-year-old boy presented to his local hospital with a one-month history of right sided leg pain, predominantly at night, difficulty in weight bearing, as well as intermittent fever, lethargy, and reduced appetite. He was a previously well child. On examination, relevant findings included fever and significant distress, particularly when moving his legs. The patient was able to weight bear but had an antalgic gait. Peripheral nervous system examination was unremarkable. BP was 120/50 mmHg (at the 99th centile for height). Initial blood tests revealed a mild normocytic anaemia of 92 g/L (range 107-139) with other cell lines preserved. C-reactive protein was elevated to 35 mg/L (range 0-9) and ESR to 97 mm/hr (range <10). The prolonged history of fever and additional systemic symptoms raised the concern for malignancy and an MRI of the patients' hips and spine was performed. This showed multiple bony lesions throughout both femurs, pelvic bones, and thoraco-lumbar spine, as well as several soft tissue lesions in the abdominal paraspinal region, extending through the left neural foramina of L1 and L2 into the spinal canal and compressing the thecal sac at L1 (Figure 2A). He subsequently underwent CT neck to pelvis for staging and to further delineate the abnormalities identified on MRI. This demonstrated a large volume of heterogeneous retroperitoneal soft tissue extending between T9 and L2 and just above the diaphragmatic hiatus, with numerous foci of high attenuation within the main volume of soft tissue in-keeping with calcification (Figure 2B). There were also numerous further foci of soft tissue in the left para-aortic region and left iliac chain with foci of calcification, likely representing infiltrated lymph nodes. Aspects of the main soft tissue volume encased both renal arteries. Additionally, a dumbbell shaped lesion bridging across and widening the left L1 neural foramina and partially effacing the thecal sac was seen and appeared distinct from the main body of retroperitoneal soft tissue (Figure 2C). The bony abnormalities seen on MRI were again seen on CT scan and suggestive of diffuse marrow infiltration. At this stage, the clinical and radiological features were most consistent with metastatic neuroblastoma. Urinary homovanillic (HVA) and vanillylmandelic acid (VMA) were markedly elevated, with an HVA creatinine ratio of 180.6 umol/mmol creatinine (range 0.0-12.3) and VMA creatinine ratio of 159.8 umol/mmol creatinine (range 0.0-8.6), supporting suspicion of metastatic neuroblastoma. Open biopsy of the paraspinal lesion was undertaken and a central line inserted, so that emergency treatment with Carboplatin and Etoposide could be started. The biopsy demonstrated Schwannian stroma and ganglion cells, consistent with maturation within a peripheral neuroblastic lesion, whilst bone marrow aspirates demonstrated widespread infiltration with small round blue cells consistent with neuroblastoma. Molecular tests confirmed the presence of segmental chromosomal abnormalities without MYCN amplification. An MIBG scan to complete staging showed widespread abnormal heterogeneous skeletal activity consistent with diffuse disease infiltration with neuroblastoma

(Figure 2D). The patient commenced treatment for high-risk neuroblastoma with multi-modal intensive treatment and has completed induction chemotherapy, myeloablative therapy with stem cell support and local radiotherapy to the primary tumour site. He currently has a small stable primary retroperitoneal tumour and no evidence of metastatic disease.

Clinical presentation

Outside of surveillance programmes, the diagnosis of PPGL should always be considered in a child presenting with hypertension (case 1) as primary hypertension is uncommon in childhood (1). However, as hypertension can be paroxysmal, the absence of hypertension in children does not exclude PPGL. In fact, most children first present with other signs and symptoms of catecholamine excess such as palpitations, hyperhidrosis, flushing, tremor, headaches, and nausea/vomiting during exercise (2–4). Some children may even present with reduced school performance or increased anxiety, making this diagnosis particularly challenging in this age group (5). Finally, children may also present with abdominal pain due to mass effect with larger PPGL. Clinical presentation of neuroblastoma is highly variable and depends on location of the tumour and stage. Children can present with consequences of compression from the mass on nearby organs, including bladder, bowel and spinal cord, pain from disseminated metastatic disease, or associated paraneoplastic phenomena including opsoclonus myoclonus syndrome and secretion of vasoactive intestinal peptide leading to intractable secretory diarrhoea. Some children, however, will present with a painless mass or a swollen or distended abdomen, sometimes found during bathing or clothing, especially in young age. Other common systemic signs or symptoms of neuroblastoma include fever, bone pain, lethargy, and weight loss. Blueberry muffin spots have also been described in infants with neuroblastoma, although rare and seen in other differential diagnoses. Hypertension can also be a feature at diagnosis but less commonly than in patients with PPGL. In patients with neuroblastoma, it is usually chronic and secondary to renal compression; however rarely, hypertensive crises can be induced during biopsy or surgery.

Biochemical testing

PPGL actively secrete catecholamines from storage vesicles by a process involving exocytosis. Catecholamine secretion may be low or episodic in some tumours and this process is independent of the continuous leakage of catecholamines in the cytoplasm and their metabolism to metanephrines through the actions of the catechol-O-methyltransferase (COMT) (6,7). In contrast, neuroblastomas display a relative lack of such catecholamine storage vesicles (8,9). Importantly, apart from COMT, neuroblastomas also express high amounts of monoamine oxidase (MAO), an enzyme that catalyses the continuous production of deaminated metabolites by catecholamines (10). Due to the lack of storage vesicles by neuroblastomas and the rather episodic secretion of catecholamines by PPGL, plasma or urinary catecholamines, have limited value for the biochemical diagnosis of these tumours. For PPGL, diagnosis is based on plasma free or urinary metanephrines, with the former showing higher diagnostic accuracy than the latter in patients at high risk of disease, such as children screened as part of surveillance programmes (11,12). Inclusion of methoxytyramine in the plasma panel is also important for the detection of dopamine producing tumours (12). For neuroblastomas biochemical testing has traditionally utilised measurements of urinary homovanillic (HVA) and vanillylmandelic acid (VMA), the respective end-products of dopamine and norepinephrine metabolism derived from combined actions of MAO and COMT.

Nevertheless, both HVA and VMA are normally produced in substantial amounts by other sources, which largely act to dilute any signal produced by neuroblastomas, thus restricting their diagnostic value (13,14). Despite the lack of large amounts of catecholamines produced by neuroblastomas, plasma free normetanephrine and methoxytyramine provide excellent biomarkers for their identification (15,16). One study has shown that plasma normetanephrine and methoxytyramine show a stronger diagnostic signal than VMA and HVA for the diagnosis of neuroblastoma (17). Despite their excellent diagnostic performance, normetanephrine and methoxytyramine cannot be used to distinguish neuroblastomas from PPGL, as both tumours show overlapping patterns of elevated metabolites. It should be noted that plasma 3-methoxytyramine is not widely available.

Addition of 3-O-methyldopa, the metabolite of Dopa, could add value in the differential diagnosis of the two neural-crest derived tumours. Approximately 75% of patients with neuroblastoma show significant increases in plasma concentrations of 3-O-methyldopa, whereas PPGL do not. This is particularly relevant for children with poorly differentiated neuroblastomas, where elevations of plasma Dopa and 3-O-methyldopa may be the only biochemical signal (17). However, differential diagnosis between PPGL and well differentiated neuroblastomas remains challenging. One direction towards improved diagnostics could be the provision of different deaminated metabolites in the panel of metanephrines, which is expected to be associated with significant increases for the MAO-expressing neuroblastomas. Nevertheless, further studies are needed to confirm this finding. Assessment of these metabolites should always be performed with mass spectrometric methods and age-specific reference intervals in children are important for the correct interpretation of test results, as catecholamine metabolites show variable difference related to age and sex (17-18). In summary, biochemical testing is crucial in informing the safety of proceeding with a biopsy but biochemistry, when interpreted in isolation, cannot reliably differentiate a PPGL from a neuroblastoma.

Molecular profiling

PPGL are frequently hereditary, and the predisposition genes can be subdivided into three main categories or ‘clusters’, classified by their impact on tumour signalling pathways (19,20). Cluster 1 (the ‘pseudohypoxic cluster’) includes *VHL*, *SDHx (SDHA, SDHB, SDHC, SDHD)*, *MDH2*, *FH*, and *EPAS1* genes and is characterised by transcriptional upregulation of genes implicated in angiogenesis, stabilisation of hypoxia-inducible factor (HIF) complex and hypermethylation resulting in dysregulated cellular proliferation. Cluster 2 genes include *RET*, *NF1*, *TMEM127*, *MAX* and *HRAS*, which activate kinase pathways (20). Cluster 3 includes somatic *CSDE1* mutations and *MAML3* fusion variants implicated in Wnt-pathway signal alteration (20)**.** The prevalence of hereditary PPGL is high in paediatric patients, estimated at 80% and genetic testing can inform tailored surveillance and management strategies (21). Cluster 1 gene variants are the most common gene variants in children with PPGL and pathogenic variants in the *SDHB* gene are particularly prevalent in children with PPGL, identified in 39% of children who underwent genetic testing in one study (21). *SDHx* variants are very rare in children with neuroblastoma and only a single case has been reported to date (22). Pathogenic variants in cluster 1 genes can influence the biochemical phenotype of PPGL and PPGL harbouring mutations in *SDHx* and other cluster 1 genes can have a non-secretory, predominately dopaminergic or predominately norepinephrine secretory phenotype (23). This biochemical phenotype associated with cluster 1 PPGL can further complicate the diagnosis

or differentiation of a PPGL from a neuroblastoma. Screening for variants in the *SDHx* genes can be rapidly performed using SDHB immunohistochemistry on biopsy or histological samples and can be a useful test to help confirm a diagnosis of PPGL versus neuroblastoma (24).

Most neuroblastoma cases are sporadic but germline variants in *ALK* and *PHOX2B* can predispose to familial neuroblastoma (25,26). There may also be an increased risk of neuroblastoma for patients with inherited pathogenic variants in *KIF1B*, *NF1* and patients with Costello, Noonan, Beckwith Weidemann (BWS) and Li Fraumeni syndromes (27). These can be diagnosed clinically or by whole genome sequencing (WGS), but methylation studies may be required to exclude BWS. Tumour molecular testing is widely employed for children with neuroblastoma as it can inform prognosis and stratify management and common molecular alterations include MYCN amplification, 1p deletion, 17q gain, 11q LOH and diploidy (28). There is some overlap in the molecular biology as 1p deletion, 17q gain and 11q LOH are reported in PPGL and germline variants in the myc-factor-associated X gene (*MAX*) have been reported in patients with PPGL and neuroblastoma (29-31). WGS sequencing of tumoural DNA is increasingly employed in paediatric oncology and the additional molecular information provided should aid in better differentiating PPGL from neuroblastoma. In the future, utilising technologies such as circulating tumour cells, circulating tumour transcripts or DNA may offer additional diagnostic potential for differentiating between neuroblastoma and PPGL.

Radiology

Imaging modalities employed in the diagnosis and staging of catecholamine producing tumours in childhood include ultrasound, MRI and CT. MRI and CT offer superior anatomical information compared to ultrasound. MRI has the advantage of sparing exposure to ionising radiation. The overlap in the primary anatomical location of PPGL and neuroblastomas poses a diagnostic challenge and is compounded by the overlapping radiological characteristics of both tumours on US, CT, and MRI.

Both PPGL and neuroblastoma have a heterogeneous and hypervascular appearance, looking hypointense on T1 weighted and hyperintense on T2 weighted MRI imaging and can demonstrate intense enhancement on contrast enhanced CT (32). Both tumours can invade local structures and encase neighbouring vessels, but this feature is more commonly noted in neuroblastoma compared to PPGL (33). Cystic change is more common in PPGL and intratumoural calcifications are more common in neuroblastoma, but neither are pathognomonic (See Table 1). Therefore, standard radiological modalities are useful in localising, staging, and defining the potential surgical approach but have limited specificity in differentiating between neuroblastoma and PPGL when interpreted in isolation.

For case 1, the tumour appeared to arise from the organ of Zuckerkandl and as such was predominantly pelvic in location. It had a characteristic hyperenhancement pattern peripherally on both CT and MRI but exhibited necrotic attenuation centrally. There was no calcification or discrete local lymphadenopathy. There was a small left paravertebral nodule at the level of T6. This was subtle on post-contrast CT, only seen in hindsight following functional imaging (Figure 1). Case 2 had a tumour which arose from the sympathetic chain and was extra-adrenal spanning the retroperitoneum from T9 to L2, extending just above the diaphragmatic hiatus and exhibiting an apparently discrete dumbbell component, which extended through

the left L1-2 neural foramina. The CT showed evidence of calcification in the primary mass and in adjacent retroperitoneal lymphadenopathy. There were widespread bone metastases at presentation with a florid periostitis at the level of both proximal femora (Figure 2).

Nuclear Imaging

Nuclear imaging has a role in staging, localisation of occult disease and selection of therapeutic radionuclide therapies in children with catecholamine producing tumours. There is limited data on the role of nuclear imaging for differentiating a neuroblastoma from PPGL. Commonly used functional imaging modalities include ^{123}I- metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy, 68Ga-DOTATATE PET CT, F-18-L-Fluoro-L-3, 4-dihydroxyphenylalanine (18F-FDOPA), and ^{18}F-flurodeoxyglucose (18F-FDG) PET CT. ^{123}I-MIBG scintigraphy has been used for decades for the diagnosis and staging of both neuroblastoma and PPGL and is mandatory before considering therapy with ^{131}I-MIBG radionuclide therapy. The target ligand for ^{123}I -MIBG scintigraphy is the norepinephrine transporter and is expressed by both tumour types reflecting their shared cellular origin. Similarly, ^{18}F-DOPA PET CT targets the L-amino acid transporter, a precursor in catecholamine synthesis and therefore expressed in both neuroblastoma and PPGL. Somatostatin receptor PET utilises somatostatin receptor agonists labelled with gallium such as 68Ga-DOTATATE PET and targets tumoral somatostatin receptor expression. Somatostatin receptor PET has demonstrated higher sensitivity for the diagnosis and staging of PPGL and neuroblastoma compared to ^{123}I-MIBG scintigraphy is the preferred nuclear imaging modality for PPGL (34-36). Finally, ^{18}F-FDG PET CT targets glucose uptake and utilisation by tumour cells and is sensitive for malignant tumours but cannot differentiate between specific malignancies. Once a diagnosis of PPGL or neuroblastoma has been established, nuclear imaging has diagnostic and theragnostic utility but has a limited role in distinguishing between PPGL and neuroblastoma.

Histopathology

Neuroblastoma is part of a spectrum of peripheral neuroblastic tumours, ranging from the least differentiated (neuroblastoma) to the most mature (ganglioneuroma). These tumours are classified based on the amount of Schwannian stroma (poor, rich, or predominant), the presence of neuropil, and the degree of differentiation of tumour cells (37,38).
Neuroblastoma can evolve over time, either spontaneously or after treatment, with the most primitive form characterized by undifferentiated small round blue cells, which must be differentiated from other small round blue cell tumours of childhood. Immunohistochemical markers are crucial for differentiating neuroblastoma from other similar tumours. Positive markers in neuroblastoma include Phox-2B, Synaptophysin, Chromogranin, and CD56, along with the retention of INI-1 expression. Negative markers, such as desmin, CD99, and WT-1, help exclude other diagnoses. Schwannian stroma can be confirmed using S100 immunostaining.

The most mature form of lesions on this spectrum, ganglioneuroma, consists mainly of Schwannian stroma with mature ganglion cells. Resected specimens after treatment may show a range of appearances with necrosis and calcification or alternatively maturation compared to the untreated original biopsy. It is important to consider sampling when reporting specimens at the more mature end of the spectrum. Nodular ganglioneuroblastoma contains both mature and immature components, making biopsy

interpretation challenging as the immature nodule may not have been sampled. Clinical risk stratification in neuroblastoma relies on molecular testing, with markers like NMYC amplification indicating high-risk disease. Segmental chromosome gains or losses also correlate with poorer outcomes. Based on tissue obtained before treatment, tumours are classified as favourable (FH) or unfavourable (UH) histology according to the International Neuroblastoma Pathology Classification, which considers factors like histopathological differentiation, the child's age, and the mitosis-karyorrhexis index (MKI) (39).

Paragangliomas exhibit significant inter- and intra-tumoral architectural and cytological variation. They most commonly consist of polygonal "chief cells" in a fibrous stroma, displaying a characteristic nested architecture known as "zellballen," bounded by sustentacular cells. Sustentacular cells can be difficult to discern on hematoxylin and eosin (H&E) staining but are positive for immunohistochemical markers like S100 and SOX-10. Some paragangliomas exhibit a trabecular or cord-like structure instead of the classic nests, making diagnosis more challenging.

Both neuroblastoma and paraganglioma cells share many immunohistochemical markers due to their common neural crest origin. Paragangliomas typically stain positively for synaptophysin, chromogranin, CD56, GATA-3, INSM1, and Phox-2B but should not express cytokeratins. The immunohistochemical profile can vary depending on the tumour's site. Immunostaining for SDHB is particularly useful for identifying SDH-deficient tumours caused by pathogenic variants in *SDHx* genes. The proportion of paragangliomas that exhibit malignant behaviour varies with site and genetic associations. Occasionally, composite tumours display features of both paraganglioma and ganglioneuroma/neuroblastoma (39). Comprehensive diagnostic frameworks for these tumours are provided in the WHO Blue Books, offering detailed guidance for pathologists (40).

Treatment and follow up.

Early and rapid diagnosis of a neuroblastoma versus a paraganglioma is crucial as management strategies differ for each tumour. Management of PPGL in children requires specialist multidisciplinary care in an experienced centre. Surgery is the only curative treatment option for PPGL, and preoperative optimization is crucial, with alpha-adrenoceptor blockade and/or calcium channel blockers initiated to stabilise haemodynamics and optimise surgical outcomes. In contrast, management of patients with neuroblastoma can vary widely from observation alone to intensive, multimodal therapy.

Children with PPGL have a high risk of multifocality, recurrence, and metastatic disease which complicates management, and 5-year mortality rate in paediatric metastatic PPGL remains high at 37% (2,21,41). Therapeutic options for metastatic disease are limited as is the evidence base in paediatric cohorts. Treatment choice should be individualised based on disease burden including tumour size and location, clinical symptoms, and progression rate. Additionally, broader considerations regarding the child's overall well-being, including their growth and development, should also be considered. Treatment options include a range of interventions such as repeat surgery, radionuclide therapy (e.g., 131I-MIBG or 177Lu-DOTATATE), and chemotherapy (cyclophosphamide, vincristine, and dacarbazine). Temozolomide has shown efficacy in retrospective series in adults and isolated case reports particularly in patients with *SDHx* mutations (42). Long-term surveillance is necessary due to

the risk of recurrence or development of new tumours or other syndromic manifestations in children with hereditary PPGL. Surveillance includes periodic clinical surveillance, measurement of plasma or urinary metanephrine levels and interval imaging studies. The interval between assessments should be tailored to the specific gene variant or clinical phenotype.

The treatment strategy for neuroblastoma depends on factors including age at diagnosis, tumour stage, histology and molecular profile, the combination of which allows patients to be assigned a risk group. Patients with low and intermediate risk disease have an excellent prognosis of >95% event free survival at 5 years (43). In contrast, the outlook for patients with high-risk disease has remained poor despite treatment with intensive induction chemotherapy, local treatment with surgery and radiotherapy, high-dose chemotherapy followed by autologous stem cell rescue and maintenance immunotherapy and differentiation therapy. Overall, long-term survival rates for these children are still approximately 50% (44). Up to 60% of children diagnosed with high-risk disease will relapse and long-term outlook for these patients is extremely poor with very few survivors. As with children with PPGL, patients who have been treated for neuroblastoma require long-term surveillance due to the risk of recurrence, which is highest in the first two years following treatment. Surveillance includes regular measurement of urinary VMA and HVA or plasma methoxytyramine and or plasma or urinary metanephrines if raised at diagnosis and imaging in the small group of patients with normal biochemistry at diagnosis. Surveillance for tumour recurrence typically continues until a child has reached 5 years from the end of treatment but is rarely needed lifelong compared to patients with PPGL. Following this period of surveillance, neuroblastoma survivors need life-long screening for late effects due to risk of developing chronic health conditions because of their treatment. This screening will vary depending on the treatment received; however, for high-risk neuroblastoma survivors, this will include monitoring cardiac and renal function and education regarding the risk of secondary malignancies, premature menopause, impaired fertility, hearing loss and neuro-psychological sequelae.

Summary

The differential diagnosis for an adrenal or extra-adrenal pelvic or retroperitoneal mass in childhood includes a neuroblastoma and PPGL. Determining the diagnosis is challenging and requires a careful clinical history and evaluation and a multipronged systematic diagnostic approach as outlined in this manuscript, as no one diagnostic strategy can specifically differentiate a PPGL from a neuroblastoma (Figure 3). Biochemical testing to exclude catecholamine excess is essential before diagnostic biopsy or surgery. In the future novel diagnostic strategies including plasma metabolomics and circulating DNA and RNA analysis may facilitate more rapid diagnosis of a neuroblastoma versus a PPGL.

Table 1: Summary of the cross-sectional imaging appearances of neuroblastoma and PPGL

Feature	Neuroblastoma	PPGL
Location	Often in adrenal glands, but can occur anywhere along sympathetic chain	Phaeochromocytoma arises in adrenal glands. Paraganglioma arise in sympathetic and parasympathetic ganglia and classically from the organ of Zuckerkandl
Attenuation (CT)/signal (MRI)	Variable due to haemorrhage and necrosis	Variable due to haemorrhage, necrosis and large tumours can demonstrate cystic degeneration
Calcifications	Frequently present (80-90%), seen as punctate or coarse densities on imaging (45)	Present in 15% of cases (46)
Contrast enhancement	May show hypervascularity, but not a defining characteristic	Demonstrate hypervascularity
Metastases	Approximately 50% of patients (47)Typical locations include bone, liver and lung (in that order) (45)	Up to 70% among *SDHB* mutation carriers. Typical locations include bones, lymph nodes, liver and lung (19,41)

Figure 1: Figure 1A-E demonstrates images for case 1. A demonstrates a Coronal view of a portal venous phase abdomen and pelvis CT performed at diagnosis showing large lower abdominal/upper pelvic mass with avid peripheral hyperenhancement and central necrosis. B demonstrates an axial slice from a post contrast MRI of the same tumour. Figure C shows a MIP image of Ga68-DOTATATE PET-CT performed at diagnosis showing a large avid lower abdominal mass and further avid focus in the left paravertebral region. D is a fused axial image of Ga68-DOTATATE PET-CT showing a small avid focal uptake anterior to IVC and posterior to uncinate process of the pancreas, not demonstrated on other cross-sectional imaging. Figure E demonstrates another fused axial slice of Ga68-DOTATATE PET-CT demonstrating an avid 9 mm left paravertebral nodule at the level of T6 vertebral body. Panel F shows a Haematoxylin and Eosin-stained section from Case 1 with the thoracic PPGL showing nests ("zellballen") with vascularised stroma and sustentacular cell nuclei.

Figure 2: Figure 2A-D demonstrates images for case 2. A demonstrates a sagittal T2 fat saturated slice from MRI performed at presentation showing a large retroperitoneal mass spanning between T9 and L2 and extending just above the diaphragmatic hiatus. Vertebral metastatic disease is also shown. B shows an axial slice from portal venous phase CT abdomen/pelvis performed at presentation showing retroperitoneal soft tissue lifting the aorta off the vertebral body. There is evidence of sparse speckled calcification. Figure C is another Axial slice from portal venous phase CT abdomen/pelvis performed at presentation showing a dumbbell-shaped mass extending through the left neural foramina. Figure D shows the Schwannian stroma and variably mature ganglion cells sampled from Case 2. Figure E shows whole body anterior and posterior planar images acquired 24 hours after administration of I123 MIBG demonstrated extensive skeletal involvement; the known retroperitoneal mass is not well delineated on planar images.

Figure 3: An outline of the suggested diagnostic considerations for differentiating PPGL from neuroblastoma in children.

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Data availability

Data available on request to the corresponding author

References

1. Mete O, Asa SL, Gill AJ, Kimura N, de Krijger RR, Tischler A. Overview of the 2022 WHO Classification of Paragangliomas and Pheochromocytomas. Endocr Pathol. 2022 Mar;33(1):90–114.
2. Lenders JWM, Eisenhofer G, Mannelli M, Pacak K. Phaeochromocytoma. Lancet. 366(9486):665–75.
3. Barontini M, Levin G, Sanso G. Characteristics of pheochromocytoma in a 4- to 20-year-old population. Ann N Y Acad Sci. 2006 Aug;1073:30–7.
4. Geroula A, Deutschbein T, Langton K, Masjkur J, Pamporaki C, Peitzsch M, et al. Pheochromocytoma and paraganglioma: clinical feature-based disease probability in relation to catecholamine biochemistry and reason for disease suspicion. Eur J Endocrinol. 2019 Oct;181(4):409–20.
5. King KS, Darmani NA, Hughes MS, Adams KT, Pacak K. Exercise-induced nausea and vomiting: another sign and symptom of pheochromocytoma and paraganglioma. Endocrine. 2010 Jun;37(3):403–7.
6. Batsis M, Dagalakis U, Stratakis CA, Prodanov T, Papadakis GZ, Adams K, et al. Attention Deficit Hyperactivity Disorder in Pediatric Patients with Pheochromocytoma and Paraganglioma. Horm Metab Res. 2016 Aug;48(8):509–13.
7. EISENHOFER G, KOPIN IJ, GOLDSTEIN DS. Leaky Catecholamine Stores: Undue Waste or a Stress Response Coping Mechanism? Ann N Y Acad Sci. 2004 Jun 16;1018(1):224–30.
8. Eisenhofer G, Keiser H, Friberg P, Mezey E, Huynh TT, Hiremagalur B, et al. Plasma metanephrines are markers of pheochromocytoma produced by catechol-O-methyltransferase within tumors. J Clin Endocrinol Metab. 1998 Jun;83(6):2175–85.
9. Verly IRN, van Kuilenburg ABP, Abeling NGGM, Goorden SMI, Fiocco M, Vaz FM, et al. Catecholamines profiles at diagnosis: Increased diagnostic sensitivity and correlation with biological and clinical features in neuroblastoma patients. Eur J Cancer. 2017 Feb;72:235–43.
10. Verly IRN, Leen R, Meinsma JR, Hooijer GKJ, Savci-Heijink CD, van Nes J, et al. Catecholamine excretion profiles identify clinical subgroups of neuroblastoma patients. Eur J Cancer. 2019 Apr;111:21–9.
11. Goldstein M, Anagnoste B, Goldstein MN. Tyramine-H ^{3} : Deaminated Metabolites in Neuroblastoma Tumors and in Continuous Cell Line of a Neuroblastoma. Science (1979). 1968 May 17;160(3829):767–8.
12. Lenders JWM, Duh QY, Eisenhofer G, Gimenez-Roqueplo AP, Grebe SKG, Murad MH, et al. Pheochromocytoma and Paraganglioma: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2014 Jun 1;99(6):1915–42.
13. Eisenhofer G, Prejbisz A, Peitzsch M, Pamporaki C, Masjkur J, Rogowski-Lehmann N, et al. Biochemical Diagnosis of Chromaffin Cell Tumors in Patients at High and Low Risk of Disease: Plasma versus Urinary Free or Deconjugated O-Methylated Catecholamine Metabolites. Clin Chem. 2018 Nov;64(11):1646–56.
14. Eisenhofer G, Aneman A, Hooper D, Holmes C, Goldstein DS, Friberg P. Production and metabolism of dopamine and norepinephrine in mesenteric

organs and liver of swine. American Journal of Physiology-Gastrointestinal and Liver Physiology. 1995 Apr 1;268(4):G641–9.
15. Eisenhofer G, Åneman A, Friberg P, Hooper D, Fåndriks L, Lonroth H, et al. Substantial Production of Dopamine in the Human Gastrointestinal Tract. J Clin Endocrinol Metab. 1997 Nov;82(11):3864–71.
16. Peitzsch M, Butch ER, Lovorn E, Mangelis A, Furman WL, Santana VM, et al. Biochemical testing for neuroblastoma using plasma free 3-O-methyldopa, 3-methoxytyramine, and normetanephrine. Pediatr Blood Cancer. 2020 Feb 14;67(2).
17. Barco S, Verly I, Corrias MV, Sorrentino S, Conte M, Tripodi G, et al. Plasma free metanephrines for diagnosis of neuroblastoma patients. Clin Biochem. 2019 Apr;66:57–62.
18. Peitzsch M, Butch ER, Lovorn E, Mangelis A, Furman WL, Santana VM, et al. Biochemical testing for neuroblastoma using plasma free 3-O-methyldopa, 3-methoxytyramine, and normetanephrine. Pediatr Blood Cancer. 2020 Feb 14;67(2).
19. Eisenhofer G, Peitzsch M, Bechmann N, Huebner A. Biochemical Diagnosis of Catecholamine-Producing Tumors of Childhood: Neuroblastoma, Pheochromocytoma and Paraganglioma. Front Endocrinol (Lausanne). 2022 Jul 26;13.,
20. Jochmanova I, Pacak K. Genomic Landscape of Pheochromocytoma and Paraganglioma. Trends Cancer. 2018 Jan;4(1):6–9.
21. Fishbein L, Leshchiner I, Walter V, Danilova L, Robertson AG, Johnson AR, et al. Comprehensive Molecular Characterization of Pheochromocytoma and Paraganglioma. Cancer Cell [Internet]. 2017;31(2):181–93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28162975
22. Pamporaki C, Hamplova B, Peitzsch M, Prejbisz A, Beuschlein F, Timmers HJLM, et al. Characteristics of Pediatric vs Adult Pheochromocytomas and Paragangliomas. J Clin Endocrinol Metab. 2017 Apr 1;102(4):1122–32.
23. Dubard Gault M, Mandelker D, DeLair D, Stewart CR, Kemel Y, Sheehan MR, et al. Germline SDHA mutations in children and adults with cancer. Cold Spring Harb Mol Case Stud. 2018 Aug;4(4).
24. Timmers HJLM, Kozupa A, Eisenhofer G, Raygada M, Adams KT, Solis D, et al. Clinical Presentations, Biochemical Phenotypes, and Genotype-Phenotype Correlations in Patients with Succinate Dehydrogenase Subunit B -Associated Pheochromocytomas and Paragangliomas. J Clin Endocrinol Metab [Internet]. 2007;92(3):779–86. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2006-2315
25. Papathomas TG, Oudijk L, Persu A, Gill AJ, van Nederveen F, Tischler AS, et al. SDHB/SDHA immunohistochemistry in pheochromocytomas and paragangliomas: a multicenter interobserver variation analysis using virtual microscopy: a Multinational Study of the European Network for the Study of Adrenal Tumors (ENS@T). Modern Pathology [Internet]. 2015 May;28(6):807–21. Available from: http://www.nature.com/articles/modpathol201541
26. Trochet D, Bourdeaut F, Janoueix-Lerosey I, Deville A, de Pontual L, Schleiermacher G, et al. Germline mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma. Am J Hum Genet. 2004 Apr;74(4):761–4.

27. Mossé YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, et al. Identification of ALK as a major familial neuroblastoma predisposition gene. Nature. 2008 Oct 16;455(7215):930–5.
28. Kamihara J, Bourdeaut F, Foulkes WD, Molenaar JJ, Mossé YP, Nakagawara A, et al. Retinoblastoma and Neuroblastoma Predisposition and Surveillance. Clinical Cancer Research. 2017 Jul 1;23(13):e98–106.
29. Aygun N. Biological and Genetic Features of Neuroblastoma and Their Clinical Importance. Curr Pediatr Rev. 2018;14(2):73–90.
30. Dannenberg H, Speel EJ, Zhao J, Saremaslani P, van Der Harst E, Roth J, et al. Losses of chromosomes 1p and 3q are early genetic events in the development of sporadic pheochromocytomas. Am J Pathol. 2000 Aug;157(2):353–9.
31. Duarte DB, Ferreira L, Santos AP, Costa C, Lima J, Santos C, et al. Case Report: Pheochromocytoma and Synchronous Neuroblastoma in a Family With Hereditary Pheochromocytoma Associated With a MAX Deleterious Variant. Front Endocrinol (Lausanne). 2021;12:609263.
32. Seabrook AJ, Harris JE, Velosa SB, Kim E, McInerney-Leo AM, Dwight T, et al. Multiple Endocrine Tumors Associated with Germline MAX Mutations: Multiple Endocrine Neoplasia Type 5? J Clin Endocrinol Metab. 2021 Mar 25;106(4):1163–82.
33. Kuhlen M, Pamporaki C, Kunstreich M, Wudy SA, Hartmann MF, Peitzsch M, et al. Adrenocortical Tumors and Pheochromocytoma/Paraganglioma Initially Mistaken as Neuroblastoma-Experiences From the GPOH-MET Registry. Front Endocrinol (Lausanne). 2022;13:918435.
34. Chen AM, Trout AT, Towbin AJ. A review of neuroblastoma image-defined risk factors on magnetic resonance imaging. Pediatr Radiol. 2018 Aug 4;48(9):1337–47.
35. AlSadi R, Maaz AUR, Bouhali O, Djekidel M. 68Ga-DOTATATE PET in Restaging and Response to Therapy in Neuroblastoma: A Case Series and a Mini Review. J Nucl Med Technol. 2023 Jun;51(2):140–6.
36. Kroiss A, Putzer D, Uprimny C, Decristoforo C, Gabriel M, Santner W, et al. Functional imaging in phaeochromocytoma and neuroblastoma with 68Ga-DOTA-Tyr 3-octreotide positron emission tomography and 123I-metaiodobenzylguanidine. Eur J Nucl Med Mol Imaging. 2011 May;38(5):865–73.
37. Taïeb D, Hicks RJ, Hindié E, Guillet BA, Avram A, Ghedini P, et al. European Association of Nuclear Medicine Practice Guideline/Society of Nuclear Medicine and Molecular Imaging Procedure Standard 2019 for radionuclide imaging of phaeochromocytoma and paraganglioma. Eur J Nucl Med Mol Imaging. 2019 Sep;46(10):2112–37.
38. Shimada H, Ambros IM, Dehner LP, Hata J, Joshi V V, Roald B, et al. The International Neuroblastoma Pathology Classification (the Shimada system). Cancer. 1999 Jul 15;86(2):364–72.
39. Jarzembowski JA SH et al. , editors, editor. Chapter 4: Peripheral Neuroblastic Tumours. In: WHO Classification of Tumours Editorial Board. Paediatric tumours. Lyon (France): International Agency for Research on Cancer; 2022. 5th ed. Vol. 7. 2022.

40. Mete O, Asa SL, Gill AJ, Kimura N, de Krijger RR, Tischler A. Overview of the 2022 WHO Classification of Paragangliomas and Pheochromocytomas. Endocr Pathol. 2022 Mar;33(1):90–114.
41. WHO Classification of tumour series Bluebooks Online (who.int).
42. Bausch B, Wellner U, Bausch D, Schiavi F, Barontini M, Sanso G, et al. Long-term prognosis of patients with pediatric pheochromocytoma. Endocr Relat Cancer. 2014 Feb;21(1):17–25.
43. Urquhart C, Fleming B, Harper I, Aloj L, Armstrong R, Hook L, et al. The use of temozolomide in paediatric metastatic phaeochromocytoma/paraganglioma: A case report and literature review. Front Endocrinol (Lausanne). 2022;13:1066208.
44. Irwin MS, Naranjo A, Zhang FF, Cohn SL, London WB, Gastier-Foster JM, et al. Revised Neuroblastoma Risk Classification System: A Report From the Children's Oncology Group. J Clin Oncol. 2021 Oct 10;39(29):3229–41.
45. Maris JM, Hogarty MD, Bagatell R, Cohn SL. Neuroblastoma. Lancet. 2007 Jun 23;369(9579):2106–20.
46. Xu Y, Wang J, Peng Y, Zeng J. CT characteristics of primary retroperitoneal neoplasms in children. Eur J Radiol. 2010 Sep;75(3):321–8.
47. Hayes WS, Davidson AJ, Grimley PM, Hartman DS. Extraadrenal retroperitoneal paraganglioma: clinical, pathologic, and CT findings. American Journal of Roentgenology. 1990 Dec;155(6):1247–50.
48. Liu S, Yin W, Lin Y, Huang S, Xue S, Sun G, et al. Metastasis pattern and prognosis in children with neuroblastoma. World J Surg Oncol. 2023 Apr 12;21(1):130.