DIAGNOSIS OF ENDOCRINE DISEASE

Biochemical diagnosis of pheochromocytoma and paraganglioma

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Abstract

Adrenal pheochromocytomas and extra-adrenal sympathetic paragangliomas (PPGLs) are rare neuroendocrine tumours, characterised by production of the catecholamines: noradrenaline, adrenaline and dopamine. Tumoural secretion of catecholamines determines their clinical presentation which is highly variable among patients. Up to 10–15% of patients present entirely asymptomatic and in 5% of all adrenal incidentalomas a PPGL is found. Therefore, prompt diagnosis of PPGL remains a challenge for every clinician. Early consideration of the presence of a PPGL is of utmost importance, because missing the diagnosis can be devastating due to potential lethal cardiovascular complications of disease. First step in diagnosis is proper biochemical analysis to confirm or refute the presence of excess production of catecholamines or their metabolites. Biochemical testing is not only indicated in symptomatic patients but also in asymptomatic patients with adrenal incidentalomas or identified genetic predispositions. Measurements of metanephrines in plasma or urine offer the best diagnostic performance and are the tests of first choice. Paying attention to sampling conditions, patient preparation and use of interfering medications is important, as these factors can largely influence test results. When initial test results are inconclusive, additional tests can be performed, such as the clonidine suppression test. Test results can also be used for estimation of tumour size or prediction of tumour location and underlying genotype. Furthermore, tumoural production of 3-methoxytyramine is associated with presence of an underlying *SDHB* mutation and may be a biomarker of malignancy.

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Introduction

Pheochromocytomas and paragangliomas (PPGLs) are chromaffin cell tumours that arise from the adrenal medulla in 80–85% of patients and from extra-adrenal sympathetic tissue of abdomen, pelvis and chest (1) in

10–20% of patients. Head and neck paragangliomas represent the parasympathetic counterparts of PPGL. Sympathetic PPGLs usually produce significant amounts of catecholamines, whereas parasympathetic head and

Invited Author's profile

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neck paragangliomas are usually endocrinologically inactive (2). PPGLs are rare, occurring in ~2–5 patients per million per year, corresponding to a prevalence of 1.5–4 per 100 000 (3). In 0.1–0.6% of hypertensive patients, a PPGL tumour is found. However, still many patients remain undetected, therefore the prevalence of PPGL in autopsy studies is 0.05% (2). Most tumours are benign but ~10–15% are defined as malignant based on the development of metastases in nonchromaffin tissues such as lymph nodes, liver and bone (4).

A key feature of PPGLs is their genetic diversity. Today up to 35% can be attributed to a germline mutation and probably this percentage will rise in future when new susceptibility genes are discovered (5). Until now, germline mutations in ten genes have been identified to be responsible for genetic PPGL syndromes including von Hippel–Lindau (*VHL*), multiple endocrine neoplasia type 2 (*RET*), neurofibromatosis type 1 (*NF1*), succinate dehydrogenase subunits A, B, C and D (*SDHA/B/C/D*), succinate dehydrogenase complex assembly factor 2 (*SDHAF2*) and the more recently reported transmembrane protein 127 (*TMEM127*) and MYC associated factor X (*MAX*) (6, 7, 8). The presence of these germline mutations identifies patients who are at risk for syndromic presentation, multifocal PPGL (*SDHx*, *RET*, *TMEM127*), recurrent disease (all mutations) or malignancy (*SDHB* mutation) (9). Furthermore, the detection of somatic mutations in *NF1*, *VHL*, *RET*, *MAX*, *HIF2 α* and *SDHx* in at least 17% of sporadic tumours has brought the proportion of all patients with PPGL due to a genetic abnormality to ~50% (6, 10, 11, 12).

Most clinical features of PPGLs are secondary to tumoural secretion of the catecholamines (noradrenaline, adrenaline and dopamine). The clinical presentation depends on the amount, type and pattern of catecholamine secretion and is extremely variable among patients (13). However, many patients do report paroxysmal episodes of headache, sweating and palpitations. In addition, pallor, feelings of panic or anxiety, nausea, fever, flushing and constipation may occur. Hypertensive episodes are paroxysmal with either normal blood pressure between paroxysms or sustained hypertension. Paroxysms are mostly unpredictable but can be elicited by anaesthesia, micturition (in case of urinary bladder paraganglioma), tumour manipulation, tyramine-rich food and several drugs such as glucagon, metoclopramide and tricyclic antidepressants (14). The metabolic action of catecholamines may lead to weight loss and to disturbances in glucose metabolism, including diabetes mellitus or to lactate acidosis. However, symptoms are not always present, as up to 10–15% of cases are entirely

asymptomatic. In addition, PPGLs account for about 5% of all adrenal incidentalomas which usually also present without symptoms (2, 5). As signs and symptoms are quite similar to those observed in many other clinical conditions, a long diagnostic delay is not unusual. Accordingly, PPGL has been referred to as ‘the great mimic’ and proper diagnosis of PPGL remains a challenge for every clinician (15). If not timely recognised and treated, PPGL can have a devastating outcome due to myocardial infarction, severe hypertension, heart failure due to toxic cardiomyopathy, hypertensive encephalopathy, neurogenic pulmonary edema, stroke and/or arrhythmia caused by catecholamine excess (16, 17, 18). Recently, it has been reported that patients with PPGL do have a clearly higher rate of major cardiovascular events than patients with primary hypertension, probably due to prolonged exposure to the toxic effects of tumoural catecholamines (19). The serious and potentially lethal cardiovascular complications emphasise the importance of a rapid diagnosis.

Before any localisation procedure is initiated, the presence of these tumours first needs to be proven biochemically. The interpretation of biochemical test results for the diagnosis of PPGL can be challenging in daily clinical practice. Proper biochemical analysis is warranted to confirm or refute the unequivocal presence of excess production of catecholamines or their metabolites. This entails also avoiding unnecessary biochemical tests and imaging studies and inappropriate surgery in patients with a suspected PPGL. In this review, we will address several practical issues related to biochemical testing. This may assist clinicians to establish a proper and prompt diagnosis of PPGL.

Catecholamine synthesis and metabolisation

Before we address the approach of biochemical diagnosis, global understanding of catecholamine biosynthesis and metabolism is useful. Biosynthesis of catecholamines starts with conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase. DOPA is converted to dopamine which is translocated from the cytoplasm into catecholamine storage vesicles of chromaffin cells of the adrenal medulla, sympathetic nerves and paraganglia (Fig. 1). The presence of the enzyme dopamine- β hydroxylase within these vesicles is responsible for the conversion of dopamine into noradrenaline. In adrenal medullary chromaffin cells, noradrenaline is further converted to adrenaline by phenylethanolamine-N-methyltransferase (PNMT; Fig. 1).

Figure 1

Biosynthesis of catecholamines in (A) chromaffin cells of adrenal medulla and (B) sympathetic nerve cells. TH, tyrosine hydroxylase; L-AADC, aromatic L-amino-acid decarboxylase; DBH, dopamine- β -hydroxylase; PNMT, phenylethanolamine-*N*-methyltransferase.

As this enzyme is only present in these cells, adrenaline is nearly exclusively produced within the adrenal medulla (20).

One important mechanism to terminate the action of catecholamines is to breakdown to biologically inactive metabolites. Metabolism of catecholamines occurs through different pathways, resulting in numerous metabolites (Fig. 2) (21). Mostly, circulating noradrenaline is derived from noradrenergic neurons of the central and sympathetic nervous system. Deamination of neuronal noradrenaline to 3,4-dihydroxyphenylglycol (DHPG) occurs by monoamine oxidase (MAO) after neuronal reuptake or after leakage of the transmitter from storage vesicles into the neuronal cytosol. Noradrenaline is also partially metabolised in extra-neuronal tissues and adrenal chromaffin cells, where it is converted to normetanephrine by catechol-*O*-methyltransferase (COMT). Adrenaline is mainly metabolised within adrenal chromaffin cells by COMT, resulting in the *O*-methylated metabolite metanephrine. Metabolism of dopamine follows other pathways, resulting in production of the *O*-methylated metabolite methoxytyramine (20). Plasma free metanephrines are conjugated to sulphates by the gut wall enzymes.

Which patients need to be biochemically screened for PPGL?

All patients who present with signs or symptoms that suggest excessive catecholamine secretion should be screened by biochemical testing, independent of whether the patient has hypertension or not. Indications for biochemical screening are summarised in Table 1. Not all patients who present with new onset hypertension need to be tested but only those with additional clues for catecholamine excess. Initial testing is also required in patients with unexplainable variability of blood pressure or a paradoxical blood pressure response to anaesthesia, surgery or medications known to precipitate symptoms in patients with PPGL (see below). However, biochemical testing is not only indicated in symptomatic patients but also in asymptomatic patients with adrenal incidentalomas (22) or identified genetic predispositions to PPGL (23).

Which tests are available for the biochemical diagnosis of PPGL?

Traditional biochemical tests include measurements of urinary and plasma catecholamines, urinary and plasma free metanephrines (normetanephrine and metanephrine) and urinary VMA. Catecholamines and metabolites can be detected in either blood or urine and each medium has its own advantages and disadvantages (2). Urinary collection provides an integral measurement result over 24 h, but is potentially incomplete and is inconvenient for patients.

Figure 2

Metabolism of catecholamines. ADH, alcohol dehydrogenase; MAO, monoamine oxidase; COMT, catechol-*O*-methyltransferase; SULT1A3, sulphotransferase 1 A3.

Table 1 Indications for biochemical testing.

Symptomatic patients
Paroxysmal headaches, sweating, tachycardia, pallor, nausea, flushing and hypertension
Unexplainable variability of blood pressure
Paradoxal blood pressure response to anaesthesia, surgery or drugs
Orthostatic hypotension in an hypertensive patient
Non-symptomatic patients
Adrenal incidentaloma
Predisposition for hereditary PPGL ^a
New onset diabetes mellitus in a young lean hypertensive patient

^aPresence of syndromic features or proven pathogenic mutation in one of the known susceptibility genes in one of the family members, one or more family members with PPGL, recurrent or metastatic PPGL.

A blood sample can be taken any time, but for accurate results for measurement of plasma catecholamines or metanephrines, sampling conditions are critical (see below). Urinary metanephrines are usually measured after deconjugation (the result reflecting free and conjugated metanephrines), whereas plasma metanephrines are measured in the free form. Use of multiple biochemical tests during initial diagnostic workup in patients with a suspected PPGL tumour not only increases sensitivity but also lowers specificity. Therefore, for initial testing, a single test with a very high negative predictive value is preferred over of a combination of tests (24).

Analysis of catecholamines and metanephrines in plasma and urine can be done by several analytical techniques. Earlier procedures using radioenzymatic assays have generally been superseded by highly sensitive and specific HPLC methods using electrochemical detection (HPLC-ECD) (25, 26). Technical improvements have led to the development of a new technique: the liquid chromatography tandem quadrupole mass spectrometry (LC-MS/MS) (27). LC-MS/MS has an even higher specificity and can be used for smaller sample volumes. In addition, it is more cost-effective because it reduces specimen processing and analysis time compared with HPLC-ECD (28, 29, 30, 31, 32).

Which test is the best?

Measurements of metanephrines in plasma or urine are the tests of first choice because they have a higher diagnostic accuracy than catecholamines or other metabolites (24). Nowadays, it is well established that measurements of urinary and plasma catecholamines are insufficiently reliable because catecholamine secretion in PPGLs is often episodic or even negligible in

asymptomatic patients. This higher diagnostic accuracy of metanephrines can be attributed to continuous intratumoural production and secretion of metanephrines into the circulatory compartment (21, 33, 34). This secretion is independent of highly variable catecholamine release caused by the tumour or by sympathoadrenal excitation (35). Comparisons of different biochemical tests established that both plasma free metanephrines or urinary fractionated metanephrines offer a higher sensitivity for the diagnosis of PPGL than other traditional tests (24, 25, 33, 35, 36, 37, 38, 39, 40, 41). Plasma free metanephrines offers a sensitivity up to 96–99% (Table 2). So, in view of diagnostic sensitivity considerations, consensus statements recommended that initial biochemical testing should include measurements of plasma free metanephrines or measurements of urinary fractionated metanephrines (42, 43).

Plasma free metanephrines offers a specificity between 80 and 100% (Table 2) and therefore a relatively high rate of false-positive test results may be found (24). Although urinary VMA offers high specificity, this test should not be routinely used any more as an initial test because of its poor sensitivity.

Important differences exist in biochemical test performance between hereditary vs sporadic disease, of which tests show a consistently higher specificity and lower sensitivity in the hereditary group (24). Screening for

Table 2 Diagnostic performance of plasma free metanephrines. Adapted from Pacak K, Eisenhofer G, Ahlman H, Bornstein SR, Gimenez-Roqueplo AP, Grossman AB, Kimura N, Mannelli M, McNicol AM & Tischler AS. Pheochromocytoma: recommendations for clinical practice from the First International Symposium. October 2005. *Nature: Clinical Practice, Endocrinology and Medicine* 2007 3 92–102.

Centre	Study cohort (n)		Diagnostic performance	
	PPGL	No PPGL	Sensitivity (%)	Specificity (%)
Vienna, Austria (2000)	17	14	100	100
NIH, USA (2002)	214	644	99	91
Mayo Clinic, USA (2003)	56	445	96	85
Essen, Germany (2006)	24	126	96	80
Newcastle upon Tyne, UK (2006)	11	114	100	91
Prague, Czech Republic (2007)	25	1235	100	97
Queensland, Australia (2009)	22	71	100	98

disease in patients with a hereditary predisposition more often leads to detection of small tumours which release small amounts of catecholamines that may be insufficient to produce signs or symptoms. In contrast, sporadic disease is typically suspected by signs and symptoms of catecholamine excess, produced by larger and more easily detectable tumours.

Sources of false-positive test results

Several factors have to be taken into account for correct interpretation of biochemical test results. Elevated plasma levels of catecholamines or metanephrines are not specific for PPGL and do not always prove the presence of an underlying catecholamine-producing tumour but may also reflect increased sympathetic activity. Several pre-analytical factors may affect test results, such as exercise, posture, food, stress, hypoglycaemia and medications; these factors may alter production or disposition of catecholamines and their metabolites.

Conditions for blood sampling and urinary collection

Recommendations for testing conditions are listed in Table 3. A blood sample for measurement of metanephrines should ideally be taken after supine rest in a quiet room for at least 20–30 min, because samples obtained in sitting position without preceding rest provide a lower diagnostic accuracy (44). This is particularly important for the analysis of normetanephrine, the metabolite most sensitive to sympathoadrenal activation.

Dietary influence ► Numerous food products, including fruits (banana and pineapple), nuts and cereals, contain substantial quantities of biogenic amines that may produce false-positive test results. De Jong *et al.* (45) investigated the influence of a catecholamine-rich diet on catecholamine metabolites. Both plasma and urinary

metanephrines were not affected, whereas plasma and urinary free 3-methoxytyramine showed significant increases in their concentrations after ingestion of amine-rich foods. So, dietary restrictions are mainly necessary for the measurement of 3-methoxytyramine.

Drug interference ► Drugs can interfere analytically or pharmacodynamically with measurements of plasma and urinary catecholamines and metabolites, which may result in false-positive test results. Analytical interferences are usually method and analyte specific. Particularly, acetaminophen may interfere with HPLC-ECD assays of plasma free metanephrines and should be avoided (26, 46). Other interfering drugs that cause analytical interference are labetalol (47), buspirone (48), mesalamine (49) and sulphasalazine (50). LC-MS/MS methods are considered to be less susceptible for analytical interference than other biochemical assays (30, 51), although this needs confirmation in further studies.

Pharmacodynamic and pharmacokinetic interference involves the effects of drugs on secretion, metabolism and excretion of catecholamines or metabolites. Numerous drugs are known to increase catecholamine and metabolite concentrations, resulting in false-positive test results. Examples are sympathomimetic agents such as ephedrine, amphetamine, cocaine, caffeine and nicotine that increase the release of noradrenaline and adrenaline (52, 53). Drugs that inhibit reuptake of noradrenaline, such as serotonin–noradrenaline reuptake inhibitors (e.g. venlafaxine), ‘selective’ serotonin-reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs), may lead to increased concentrations of noradrenaline and normetanephrine and can give false-positive test results (52, 54, 55). Higher levels of catecholamines and metanephrines have also been observed in users of MAO inhibitors that block the conversion of noradrenaline and adrenaline to DHPG (56). Antihypertensive drugs

Table 3 Recommended conditions for biochemical testing.

Patient preparation	Avoid sympathomimetic agents (including ephedrine, amphetamine, nicotine) Avoid interfering medication (including labetalol, sotalol, acetaminophen, methyl dopa, antidepressants) Overnight fast, no caffeinated or decaffeinated beverages
Conditions for blood sampling of metanephrines	Supine condition, after 30 min rest ^a Collection in heparinised tubes on ice Storage of plasma in freezer at –20 °C if measured within 3 months
Conditions for urine sampling of metanephrines	Collection in a container without additives or eventually only sodium bisulphate Storage of urine container in a cold place Acidify urine in the laboratory to pH 4 before storing

^aIf a blood sample is taken in the sitting position and the test result is positive: repeat after 30 min supine rest.

including vasodilators (i.e. dihydropyridine calcium channel blockers) and selective α_1 -adrenoceptor blockers (i.e. doxazosin) can also give false-positive test results of catecholamines due to reflexive sympathetic activation (52). The non-selective α -adrenergic receptor antagonist phenoxybenzamine, which is commonly used in pre-surgical treatment of PPGL, can increase the levels of noradrenaline and normetanephrine. It blocks CNS and prejunctional sympathoinhibitory α_2 -adrenoceptors and thereby increases noradrenaline release possibly combined with reflexive sympathetic activation (52). In addition, L-DOPA, used for treating Parkinson's disease, was recently shown to cause false elevations of the dopamine metabolite 3-methoxytyramine and metanephrines (57).

Before sampling, patients should ideally discontinue all medications that might alter urinary and plasma concentrations of catecholamines or metanephrines. From a practical point of view, it might be better not to withdraw medication and repeat testing only when initial test results are elevated. It is often difficult to identify which medications interfere with a given test and in view of patient safety considerations, it is not always easy to discontinue all medications before sampling. Therefore, it is important to know that some medications are known to influence test results more than others. Phenoxybenzamine and TCAs are significant sources of false-positive elevations of plasma catecholamines and metanephrines, while selective α_1 -adrenergic receptor blockers (doxazosin), calcium channel blockers and most other antihypertensive medications are usually less problematic (52).

Sources of false-negative test results

Plasma levels of free metanephrines or urinary fractionated metanephrines within normal limits exclude a catecholamine-producing tumour with a high reliability. Exceptions include small tumours (<1 cm) in usually asymptomatic patients, dopamine-producing tumours, tumours in which noradrenaline and adrenaline are not synthesised or metabolised to normetanephrine and metanephrine and microscopic recurrence of disease (39). Tumours that predominantly or exclusively produce dopamine are rare and mainly found in extra-adrenal PPGL (58). Measurement of 3-methoxytyramine should be considered in patients with atypical presentation, in whom PPGL is strongly suspected despite normal plasma and urinary levels of metanephrines (59). Moreover, it has been shown that patients with *SDHx* mutations can present with biochemically silent tumours

without elevated levels of plasma and urinary levels of metanephrines (60). The biochemically silent phenotype in a small subset of tumours with *SDHB* mutations reflects a defect in catecholamine synthesis as a result of absence of tyrosine hydroxylase, the enzyme that catalyses the initial and rate-limiting step in catecholamine biosynthesis. Other potential mechanisms such as storage and secretion of catecholamines are still intact as secretory granules were observed in resected tumour tissue and tumours did show accumulation of the radiotracer MIBG, a catecholamine analogue that is taken up by and stored in secretory granules. Therefore, tumour screening in *SDHB* mutation carriers should not be limited to biochemical tests of catecholamine excess, but may include additional measurements including plasma chromogranin A, a nonspecific neuroendocrine secretory protein, and imaging studies (61, 62, 63, 64).

Reference intervals

The reliability of measurements of plasma free metanephrines for the diagnosis of PPGL is also dependent on use of appropriately established reference intervals. Such intervals may vary between laboratories but usually reflect differences in blood sampling conditions. Also the population that is used for establishing reference intervals is important. In case upper cut-offs for reference intervals are set too high, diagnostic sensitivity is compromised, whereas false-positive test results are a problem when upper cut-offs are set too low. Recently, Eisenhofer *et al.* (65) have shown that plasma free normetanephrine values are influenced by age and that age-adjusted cut-offs of reference intervals improve diagnostic test performance. Suggested upper cut-off levels of plasma normetanephrines for adult patients younger than 40 years are 0.62 nmol/l and for patients older than 60 years are 1.05 nmol/l. No effect of age was found for plasma metanephrine and 3-methoxytyramine with upper cut-off levels of 0.45 and 0.18 nmol/l respectively.

Interpretation of initial test results

The diagnostic work up after initial testing is shown in Fig. 3. PPGL can be excluded in symptomatic patients with normal test results of plasma free metanephrines, due to the high diagnostic sensitivity. An increase in plasma free normetanephrine above 2.2 nmol/l or plasma free metanephrine above 1.2 nmol/l, which is 3.5- to 4-fold above the upper limits of the adult reference intervals, makes the presence of PPGL extremely likely (~100% specificity).

Figure 3
Biochemical testing algorithm.

How to distinguish true-positive from false-positive test results?

Initial test results of plasma free metanephrines are not always immediately conclusive. When metanephrines are mildly elevated (plasma free normetanephrine: 0.61–2.20 nmol/l and/or plasma free metanephrine: 0.31–1.20 nmol/l), the question arises whether the test result is true or false positive. In these patients, additional biochemical tests are necessary to avoid unnecessary imaging studies. So what to do in these cases? First, measurements of plasma free metanephrines can be repeated after optimising sampling conditions and elimination of potential interfering drugs and substances which may be responsible for false-positive results (see before). Furthermore, one can perform additional measurements of urinary metanephrines. In some cases, simultaneous measurement of plasma catecholamines can help to establish the diagnosis. In patients with PPGL, larger increases in metanephrines than catecholamines are observed due to continuous production and release of metanephrines by tumour cells. Conversely, patients with false-positive results usually have larger increments in catecholamines as compared with plasma free metanephrines because of sympathoadrenal activation (52, 53). A ratio of normetanephrine to noradrenaline <0.52 or metanephrine to adrenaline <4.2 suggests sympathoadrenal activation (52).

Finally, if the previous tests are still inconclusive, a clonidine suppression test can be very useful (52). Clonidine suppresses release of neuronal noradrenaline (and thereby of normetanephrine) by stimulating brain and prejunctional neuronal α_2 -adrenergic receptors.

This test is only indicated in case of a mildly elevated plasma normetanephrine level. A fall in elevated plasma normetanephrine concentration to normal levels after administration of clonidine indicates that sympathetic activation is the cause of elevation, while a lack of decrease in plasma free normetanephrine (<40%) combined with a persisting high level of plasma free normetanephrine (>0.61 nmol/l) 3 h after administration of clonidine supports the presence of PPGL (sensitivity of 100% and specificity of 96% respectively) (52).

The glucagon stimulation test, previously used to unmask the presence of a PPGL, is obsolete due to the insufficient diagnostic sensitivity for reliable exclusion of PPGL. Furthermore, this test should not be used because of the substantial risk of hypertensive complications (66).

Catecholamine metabolites as biomarkers of tumour size, location and malignancy

Once biochemical diagnosis of PPGL is established, tumours can be divided into three groups according to their biochemical phenotype. Noradrenergic tumours secrete mainly noradrenaline while adrenergic tumours secrete mainly adrenaline in addition to some varying amounts of noradrenaline. The third group is formed by tumours in which dopamine is the predominantly secreted catecholamine. Catecholamine biochemical phenotypes can be more reliably determined from the measurements of plasma free metanephrines and 3-methoxytyramine. The secretion of metanephrines is relatively higher in noradrenergic tumours than adrenergic tumours and is positively correlated with tumour size (67). Therefore, measurements of plasma free metanephrines are useful for estimating tumour size. The biochemical phenotype can also be an indicator of tumour location, because adrenaline and metanephrine secretion is usually confined to adrenal tumours, whereas extra-adrenal tumours secrete predominantly or exclusively noradrenaline and normetanephrine. These differences in catecholamine synthesis can be attributed to the availability of cortisol derived from the adrenal cortex. Cortisol induces the expression of PNMT, the enzyme that converts noradrenaline to adrenaline (68).

Currently, there are no reliable pathological markers for metastatic disease in PPGL. An underlying *SDHB* mutation, a primary tumour size >5 cm and an extra-adrenal tumour location are predisposing for the development of metastatic disease. As metastatic disease occurs most commonly in extra-adrenal tumours, most patients with metastatic disease show a noradrenergic phenotype.

Metastatic disease in patients with exclusively adrenergic phenotype is extremely rare (4). Catecholamines and their metabolites could therefore be the potential biomarkers of malignant PPGL. Profound elevations of noradrenaline in malignant tumours most likely reflect increased tumour burden associated with metastatic spread (69). Dedifferentiated metastatic tissue potentially lacks mature enzymes of catecholamine synthesis, so that elevated levels of DOPA, dopamine and 3-methoxytyramine could be a marker for metastatic disease. Eisenhofer *et al.* (70) showed that plasma 3-methoxytyramine is a more sensitive biomarker of malignant disease than plasma or urinary dopamine. Tumoural production of 3-methoxytyramine is also associated with presence of an underlying *SDHB* mutation and extra-adrenal location of the primary tumour. Furthermore, malignant transformation of tumour cells does not necessarily result in the loss of biochemical phenotype.

Biochemical genotype–phenotype correlations

Among hereditary tumours, different patterns of catecholamine production and secretion are observed depending on the underlying mutation. The catecholamine biochemical phenotype can be used to guide cost-effective genotyping. Tumours with a *RET* or *NF1* mutation have a similar biochemical phenotype and show increased plasma concentrations of metanephrine, whereas *VHL* and *SDHx*-related tumours do not secrete adrenaline and show increased plasma concentrations of normetanephrine. *SDHB/D* tumours demonstrate additional or solitary increases in plasma 3-methoxytyramine, indicating dopamine production. Eisenhofer *et al.* (71) showed that patients with *NF1* and *MEN2* can be discriminated from those with *VHL*, *SDHB* and *SDHD* gene mutations in 100% of cases by the combined analysis of normetanephrine, metanephrine and 3-methoxytyramine. Within the subgroup of patients with *VHL*, *SDHB* and *SDHD* gene mutations, measurements of plasma 3-methoxytyramine discriminated patients with *SDHB* and *SDHD* mutations from those with *VHL* mutations in 78% of cases.

Summary

In conclusion, timely consideration and proper and prompt diagnosis of PPGLs is very important because missing the diagnosis can be devastating due to its potential lethal cardiovascular complications. Indiscriminate screening of all new hypertensive patients is,

however, not indicated unless the patients disclose any clues that indicate they may harbour a PPGL. The diagnosis has to be established biochemically in patients with PPGL symptoms, genetic predisposition or adrenal incidentalomas, before imaging studies are performed. Biochemical testing should include analysis of plasma free metanephrines or urinary fractionated metanephrines; these tests offer the highest diagnostic performance and are recommended as initial tests. Additional testing using the clonidine suppression test is indicated in case of mildly elevated test results of plasma normetanephrine that cannot be explained by faulty sampling conditions, patient preparation or by use of interfering medication. Blood samples should ideally be taken after fasting and after 30 min of supine rest with avoiding interfering drugs. Test results can be used to estimate tumour size and location and serve as a tool to guide genetic testing. Finally, plasma 3-methoxytyramine may serve as a biomarker of malignancy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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