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Determination of catecholamines in plasma and urine

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For more than 20 years, measurement of catecholamines in plasma and urine in clinical chemistry laboratories has been the cornerstone of the diagnosis of neuroendocrine tumors deriving from the neural crest such as pheochromocytoma (PHEO) and neuroblastoma (NB), and is still used to assess sympathetic stress function in man and animals. Although assay of catecholamines in urine are still considered the biochemical standard for the diagnosis of NB, they have been progressively abandoned for excluding/confirming PHEOs to the advantage of metanephrines (MNs). Nevertheless, catecholamine determinations are still of interest to improve the biochemical diagnosis of PHEO in difficult cases that usually require a clonidine-suppression test, or to establish whether a patient with PHEO secretes high concentrations of catecholamines in addition to metanephrines.

The aim of this chapter is to provide an update about the catecholamine assays in plasma and urine and to show the most common pre-analytical and analytical pitfalls associated with their determination.

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Catecholamine biosynthesis

Catecholamines result from multi step biotransformation of tyrosine by a cascade of enzymes in neuronal structures of the central and peripheral nervous system. In circulation, the most abundant
catecholamines are norepinephrine (NE), epinephrine (E) and dopamine (DA). E and NE are produced by the adrenomedullary chromaffin cells whereas NE is also synthesized by sympathetic nerve endings. DA essentially results from the decarboxylation of 3,4-dihydroxyphenylalanine (L-DOPA) provided by the diet. Catecholamine synthesis is initiated by tyrosine hydroxylase (TH) generating L-DOPA from tyrosine. Conversion of tyrosine to L-DOPA by TH is the rate-limiting step in catecholamine biosynthesis. L-DOPA is then decarboxylated to dopamine via aromatic l-amino acid decarboxylase, which is then converted to NE via dopamine beta-hydroxylase; and eventually to E by the action of phenylethanolamine N-methyltransferase (PNMT) in adrenomedullary cells [1].

Catecholamine metabolism

The inactivation and metabolism of catecholamines is beyond the scope of this review but it is crucial to keep in mind that only a small fraction of catecholamines released from storage vesicles of sympathetic nerves will enter into the bloodstream because of their neuronal reuptake through specific transporters followed by inward active transport into storage pools [1]. These pools present a constant dynamic equilibrium controlled by vesicular monoamine transporters (VMAT) leading to a leakage into the cytoplasm of catecholamines that are also available for oxidative deamination by monoamine oxidase (MAO) into 3,4-dihydroxyphenylglycol (DHPG). Additionally, catecholamines may enter non-neuronal tissues to be methoxylated into metanephrines (NE to normetanephrine [NMN] and E to metanephrines [MN]) by catechol-O-methyl transferase (COMT). In contrast to sympathetic neurons, the adrenal medulla chromaffin cells express COMT and may also produce MNs. However, extraneuronal O-methylation of NE and E to MNs represents minor pathways of metabolism compared to intraneuronal deamination. Oxidation of MN and NMN by MAO or methoxylation of DHPG by COMT leads to the formation of methoxyhydroxyphenylglycol (MHPG) that is further metabolized in the liver into vanillylmandelic acid (VMA). These mechanisms explain in part the short half-life of plasma catecholamines. The dietary source of catecholamines undergoes a sulfotransferase isoenzyme SULT1A3 in the gastrointestinal tract before reaching the systemic circulation. Thus, free catecholamine concentrations that are measured in plasma and urine are not influenced by feeding. Consequently, 99% of dopamine and some 60–70% of circulating NE and E are sulfate-conjugated [1]. Unconjugated dopamine in urine is tenfold more abundant than NE, since it is derived from renal extraction and decarboxylation of circulating 3,4-dihydroxyphenylalanine (L-DOPA) provided by food, and may potentially be a misleading parameter for PHEO detection [2]. The metabolism of dopamine shows similar extraneuronal metabolism by COMT and MAO leading to the formation of 3-methoxytyramine (MT) and 3,4-dihydroxyphenylacetic acid (DOPAC). DOPAC undergoes O-methylation to produce homovanillic acid (HVA) as an end-product.

Measurement of catecholamines in pheochromocytoma workup

Analytical procedures and analytical pitfalls

The analysis of catecholamines has produced an extensive literature in terms of analytical methodology. In 1949, von Euler and Hamberg reported colorimetric assays that allowed differentiation of NE from E based on alumina oxide extraction [3]. Since these assays lack sensitivity they were replaced by fluorimetric assays that suffer from low specificity [4]. A major advance was done with radiometric-enzymatic assay for measuring simultaneously femtomole quantities of E, NE and DA, but this method is tedious [5]. High-performance liquid chromatography (HPLC) with fluorescence detection was a method of choice since catecholamines exhibited native fluorescence. Unfortunately, fluorescence detection suffers from major drawbacks since the emission wavelength for catecholamines is short and even after derivatization with chemical agents, fluorescence detection may be disturbed by endogenous co-eluting compounds [6]. Alpha-methylidopa and labetalol are classical sources of interferences observed when fluorescence detection is used [7,8]. In the early 80s, electrochemical detection (ECD) became popular since it allowed quantitating catecholamines in plasma from patients [9,10]. However, interference of labetalol causes spuriously raised E levels [11]. Paracetamol (acetaminophen) is also a cause of serious interferences with ECD methods requiring careful inspection of chromatograms to...
preclude misdiagnosis [12]. A methodological improvement has been achieved with coulometric arrays (HPLC–CoulArray) based on the generation of a specific voltammogram for each catecholamine [13]. The CoulArray method is also more sensitive than the amperometric assay since each analyte will exhibit a ratio profile in a series of potentials that should behave as the authentic substance present in a calibrator. Deviation from this ratio of responses is suggestive of a co-eluting interference. Nevertheless, HPLC–CoulArray requires well-trained technicians and frequent calibrations of the electrodes arrays. To maintain peak resolution, chromatographic runs are long (≈20 min) resulting in a late eluting peak of DA that is difficult to quantify.

Tandem mass spectrometry (MS/MS) methods interfaced with liquid chromatography (LC) have become popular in bioanalysis but their use for measuring catecholamines is demanding owing their high polarity and low ionizability. The first method published 10 years ago was based on a two-step liquid–liquid extraction of urines specific for compounds containing a catechol group [14]. The authors claimed that this method is free of interference caused by drugs.

More recently, simultaneous detection of free catecholamines and acid-hydrolyzed metanephrines in urine by tandem MS has been proposed. Quantification limits were ranging from 1.3 to 15.8 nmol/L depending on the measured catecholamine but well below concentrations found in normal urine [15–17].

The low circulating plasma concentrations of catecholamines make their determination by tandem MS a challenge since reported reference intervals for plasma catecholamines in man range between 0.05 and 5 nmol/L, which is far below the concentrations reported for urine. LC–MS/MS methods established to date have high limits of detection for NE, E and DA preventing their use for human plasma [18,19]. A method combining ultra performance liquid chromatography (UPLC) with a reductive ethylation technique has been proposed in order to reach subnanomolar limits of quantification of E and NE in 25 μL of plasma [20]. However, labeling by reductive ethylation of catecholamines requires the use of sodium cyanoborohydride, a highly toxic chemical producing hydrogen cyanide gas requiring specific equipment and a long extraction protocol [20]. We have recently set-up a new method combining an original solid phase extraction system with activated alumina combined with UPLC–MS/MS allowing quantification in plasma of concentrations as low as 0.05 nmol/L for E, 0.25 nmol/L for NE, and 0.15 nmol/L for DA [21]. In conclusion, we believe that UPLC–tandem mass spectrometry improves turnaround time and should exclude analytical pitfalls that were frequently found in the past.

Pre-analytical pitfalls of catecholamine assays

Pre-analytical pitfalls leading to false-positive (FP) test results. Drugs, dietary constituents as well as many physiological conditions (exercise, stress, posture) and diseases can influence the physiological processes that determine plasma and urinary levels of catecholamines and catecholamine metabolites. The impact of these confounding factors on catecholamine measurements is usually independent of analytical procedures and lead to false-positive (FP) test results [22].

Medication-associated FP results. Several medications are known to increase the levels of catecholamines and their metabolites by interfering with either their synthesis and storage, secretion or metabolism (Table 1). Tricyclic antidepressants (TCA) are a major source of pre-analytical FP results for catecholamine and metanephrine measurements [23,24]. Increase in plasma and urinary NE and NMN in patients on prolonged treatment with TCA is due to the inhibitory action of these drugs on NE re-uptake [25]. Prolonged TCA administration may also be associated with arterial hypertension which further complicates the differential diagnosis of PHEO [25,26].

Monoamine oxidase (MAO) inhibitors cause a substrate shift from MAO to COMT pathway leading to a substantial increase in plasma levels and urinary excretion of metanephrines [27]. Cyclobenzaprine, a centrally acting muscle relaxant used in the management of fibromyalgia is a tricyclic antidepressant-like agent that may increase plasma NE levels [28]. Phenoxybenzamine, a non-selective α-blocker used to preoperatively prepare patients with PHEO is another major source of FP results [24,29].

Drugs containing sympathomimetic agents significantly increase the levels of catecholamines and catecholamine metabolites. Several α-adrenoceptor agonists (e.g., ephedrine, pseudoephedrine, phenylephrine) are used extensively as nasal decongestants and as mydriatics [30]. Some of them are over the counter drugs and are therefore likely to be excessively used by patients. Levodopa is the
direct precursor of DA and therefore enhances catecholamines synthesis. Administration of levodopa and carbidopa was associated with a substantial increase in plasma and urinary dopamine levels which may be a major pitfall in patients treated for Parkinson’s disease and presenting with adrenal incidentaloma [31].

Selective α1-adrenoceptor blockers, calcium-channel antagonists, β-blockers, and vasodilators are minor sources of FP test results [24,32]. The antipsychotic agent clozapine was reported to be associated with slightly increased NE and NMN levels [33]. Self-administration of catecholamines or surreptitious catecholamine addition to urine is a very rare cause of FP catecholamine test results. Normal biochemical testing between attacks as well as discrepancies between dramatically increased catecholamine levels and inappropriately normal or slightly increased free metanephrines strongly suggests factitious PHEO [34].

Drug-abuse and intoxication-associated FP results. The use of cocaine as well as amphetamines such as ecstasy (3,4-methylenedioxymethamphetamine or MDMA) was reported to be associated with increased plasma catecholamine concentrations in the absence of PHEO [22,35]. As intoxication with either cocaine or amphetamines clinically mimics PHEO, differential diagnosis may be difficult if their use is undeclared [36].

Ethanol consumption can cause elevation of plasma catecholamine and metanephrine concentrations probably due to the known alcoholism-associated sympathetic overactivity [37]. Interpretation of high values for catecholamines and/or their metabolites in alcoholic patients presenting with hypertension may be a challenging issue.

Cigarette smokers may have increased plasma and urinary catecholamines and/or metanephrine levels as nicotine stimulates catecholamine release by activation of nicotinic acetylcholine receptors in the brain and the adrenal medulla [38].

Diet-associated FP results. Dietary constituents represent a common source of pre-analytical interference with assays of catecholamines and their metabolites. Caffeine can increase circulating catecholamines secondary to its stimulatory effects [22]. Catecholamine-rich foods (bananas, fruit juices, Table 1

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<td>Increase in plasma and urinary NE and NMN</td>
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<tr>
<td>α-blockers (non selective)</td>
<td>Increase in plasma and urinary NE and NMN</td>
<td>Significant [24,29]</td>
<td>Increase of NE release</td>
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<td>Phenoxycbenzamine</td>
<td>Increase in plasma catecholamines and metanephrines</td>
<td>Not significant [24]</td>
<td>Reflexive sympathetic stimulation</td>
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<tr>
<td>α-blockers (α1-selective) prazosin, doxazosin</td>
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<td>Not significant [24]</td>
<td>Reflexive sympathetic stimulation</td>
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<tr>
<td>Calcium-channel blockers (Nifedipine, Amlodipine)</td>
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<td>Not significant [24]</td>
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<td>β-adrenoceptor blockers (atenolol, metoprolol, propranolol, labetalol)</td>
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<td>Not significant [24]</td>
<td>Reflexive sympathetic stimulation</td>
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<td>Sympathomimetics (Ephedrine, pseudoephedrine, phenylephrine)</td>
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nuts, tomatoes, potatoes and beans) are major sources of diet-associated FP results [39,40]. Foodstuffs containing large amounts of tyramine such as hard cheeses and red wine can cause release of vesicular NE leading to FP test results [41]. However, food influences vary considerably depending on analytes and analytical procedures. Urinary excretion of deconjugated NE and DA is strongly affected by consumption of catecholamine-rich food, thereby increasing the likelihood of a FP test result when suspecting a PHEO. Therefore, urinary catecholamines are preferably measured in their free forms because the unconjugated fraction is the least affected by diet [40,42]. Indeed, subjects who consumed a catecholamine-rich nuts and fruits diet had up to 20-fold and 10-fold increases of urinary excretions of deconjugated DA and NE whereas urinary excretion of free DA and NE are limited (up to 1.5-fold increases). Outputs of urinary free and deconjugated E remained unaffected [40]. Dietary catecholamines have also substantial effects on urinary and plasma free MT but have no clinically relevant effects on levels of plasma and urinary free metanephrines [39].

**Medical conditions associated with FP results.** Catecholamine assays are most commonly requested in patients with clinical suspicion of PHEO. Severe and paroxysmal arterial hypertension with the classical triad of tachycardia, excessive sweating and headache is the typical presenting feature of PHEO. However, the clinical presentation of PHEO is highly variable and not specific with numerous features common to other hyperadrenergic conditions [43]. Some of these conditions such as hyperthyroidism and hypoglycaemia can be excluded readily. During migraine and cluster headaches attacks, patients may have paroxysmal symptoms suggestive of PHEO as well as increased plasma catecholamines levels [44]. However conversely to PHEO, rise in arterial blood pressure has not been reported with migraine attacks [45]. Subarachnoid hemorrhage is also a differential diagnosis of PHEO paroxysms and is associated with an increase in plasma catecholamines levels [46]. Nevertheless, intracranial hemorrhage may be an uncommon presenting feature of PHEO either as a complication of severe paroxysmal hypertension or the consequence of cerebral aneurysm rupture in the setting of Von Hipple Lindau syndrome [47]. Cardiovascular emergencies, such as congestive heart failure, acute coronary syndrome and acute myocardial infarction, may mimic clinical and laboratory features of PHEO [43,48]. However, the occurrence of unexplained myocardial ischemia or acute heart failure with angiographically normal coronary arteries along with the finding of high levels of catecholamines should prompt the physician to perform further investigations to reliably rule out PHEO because the latter condition may cause either vasoconstriction of the coronaries or a potentially reversible cardiac failure referred to as Takotsubo cardiomyopathy [49].

Panic attacks strongly mimic PHEO symptoms. Moreover, plasma and/or urinary NE levels may be increased in patients with panic disorders (PD) compared with normal individuals [50]. However, blood pressure rise is usually mild and anxiety is prominent in PD.

Severe and paroxysmal hypertension which has been referred to as primary pseudopheochromocytoma (pseudoPHEO) is the main differential diagnosis of PHEO and may be associated with higher baseline plasma concentrations of E and MN along with normal levels of NE [51,52]. Underlying pathophysiological mechanisms include both enhanced adrenal release of E in combination with β- and α1-adrenoceptor hypersensitivity [52]. Primary pseudoPHEO is a diagnosis of exclusion that should be considered once PHEO is carefully ruled out.

Obstructive sleep apnea (OSA) which is associated with increased sympathetic activity may result in drug-resistant or paroxysmal hypertension suggestive of PHEO as well as marked increase of catecholamine concentrations [53]. Distinctive features of OSA are isolated increase in NE and NMN levels with normal E levels as well as normalization or attenuation of catecholamines levels following OSA treatment [54].

Metabolic syndrome is characterized by marked sympathetic nerve hyperactivity [55]. Arterial hypertension as part of the metabolic syndrome may be considered as a differential diagnosis of PHEO in patients presenting with severe or resistant hypertension. Interpretation of catecholamines assays may be challenging in this setting as subjects with metabolic syndrome were shown to have higher 24-h urinary NE levels than healthy controls [56].

**How to overcome pre-analytical pitfalls leading to FP test results?.** FP test results usually lead to excessive health care expenditures because of subsequent expensive imaging and potentially inappropriate surgery to firmly confirm or exclude PHEO. It is therefore mandatory to identify sources of FP results.

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Several easy precautions can be taken before biochemical testing to attenuate drug, diet and stress interferences leading to FP results. A thorough drug-history is mandatory to provide a complete list of all ongoing medications and/or drugs and not to miss any self-medication. If the patient is taking substances known to increase catecholamines levels, progressive withdrawal of these substances and the use of alternative medications if necessary should be considered in collaboration with the treating physician. However, patient safety issues often make this difficult to apply. Interpretation of laboratory findings should therefore take into account the potential influence of ongoing treatments [24].

Patients should be instructed to avoid smoking, strenuous physical activity, caffeinated and alcoholic beverages, as well as catecholamine-rich foods for at least about 8–12 h before the testing. Blood samples should be collected via a previously inserted i.v. catheter with patients resting quietly supine for at least 15–20 min [23]. Because catecholamine and metanephrine excretion is overestimated when the period of urine collection is longer than 24 h, instructions for 24-h urine collection protocol should be clearly explained to the patient to ensure adequate collection. Given the circadian variation of catecholamine secretion with a maximum day-time secretion and a nocturnal decline [57], overnight urine samples circumvent diurnal variation and the stimulating effects of stress and exercise on increasing 24-h urinary catecholamine levels and may yield lower FP rates [58].

In patients with renal failure, plasma free MNs (which are relatively independent of renal function compared to sulfate-conjugated metabolites) are more adequate for diagnosis of PHEO than measurements of total plasma metanephrines (the sum of free plus conjugated MNs) [59].

Due consideration should be given to both the extent of the elevation in biochemical test results [60] and the degree of pre-test probability of the tumor [22]. A more than four-fold elevation in metanephrine concentrations above the reference range is associated with a close to 100% probability of a PHEO [60]. Conversely, in cases with moderate elevations in metanephrine concentrations, the potential confounding influence of medications or other causes of FP results should be carefully reconsidered. If there is difficulty in distinguishing FP from true-positive results, further follow-up biochemical testing based on MNs (combination of two of the following biomarkers: plasma free MNs, plasma total MNs, 24-h urinary fractionated MNs) is warranted before proceeding to localization studies [24,29,60–62]. If MNs remain elevated on adequate repeat testing, tumor likelihood remains possible. Clonidine-suppression test can be helpful to distinguish between FP and true-positive result [63]. Clonidine normally suppresses the release of NE from sympathetic nerve endings but does not affect the catecholamine secretion from a PHEO [64]. In patients with significantly increased baseline plasma NE concentrations, a fall to within the normal range or more than 50% after clonidine administration reliably excludes the diagnosis of PHEO [24]. In contrast, the clonidine test is unreliable in patients with only slightly increased plasma NE levels. In such patients, there may be normal suppression of plasma NE after clonidine despite the presence of a PHEO [22,24,65]. Additional measurements of plasma NMN before and after clonidine administration in patients with borderline elevated plasma NE levels enhance the diagnostic specificity of the clonidine test to 98% [24].

If clonidine-suppression test is unavailable or contraindicated, patterns of increases in MNs and catecholamines may provide additional information for distinguishing FP from true-positive tests. High plasma free NMN to NE or plasma free MN to E ratios may be considered as predictive factors of PHEO and should prompt the physician to perform tumor localization imaging [24].

Pre-analytical pitfalls leading to false-negative (FN) test results. False-negative (FN) results with catecholamine and catecholamine metabolite measurements are less frequent than FP results but can have dramatic and potentially lethal consequences related to a missed PHEO. FN results related to pre-analytical causes are primarily encountered with measurements of the parent catecholamines which suffer from problems of sampling timing because of intermittent secretion of catecholamine by tumors [66].

Incomplete collection of 24-h urinary specimens may result in falsely low urinary catecholamine and metanephrine results [60]. A plethora of articles have been published to assess the stability of catecholamines in urine and plasma and showed remarkable discrepancies. Catecholamines are prone to auto-oxidation at alkaline pH and they are therefore unstable if the urine has not been acidified to a pH below 4.0 [67]. MNs are stable in native urine [68].
Different procedures have been tested to study the stability of urinary catecholamines. Boomsma et al. found that the stability of catecholamines in EDTA/metabisulfite and untreated samples was maintained for up to 8 days at 20 °C and 35 days at 4 °C whereas acidified urines allowed to extend stability to 2 months at 20 °C [69]. Roberts et al. measured the catecholamine stability in commercially available urine samples after storage at various pH conditions, temperatures and time, from days up to 10 weeks and found that catecholamines were unchanged at pH 6.0 for at least 4 days and up to 10 weeks at pH 2.0 at either 4 °C or –18 °C [70]. Therefore, these studies indicated that pH is not an issue as long as the urine reaches the laboratory within 4 days.

This contrast with another study showing 50% loss of catecholamines during the first 24 h at room temperature in urine samples collected from 8 healthy individuals [68]. Immediate acidification, however, prevented degradation, while acidification after 24 h prevented further decay [68]. The stability of catecholamines by addition of disodium–ethylenediaminetetraacetic acid (Na₂–EDTA)/metabisulfite may be attributed to different antioxidative capacities of urine samples.

A similar stability study has been conducted with native urines from 9 individuals and demonstrated no significant degradation of catecholamines up to 3 days of storage at ambient temperature [17]. The authors postulate that catecholamine may have been protected from direct light exposure since samples were stored in a light-proof box. Indeed, direct light can affect the stability of catecholamines and this effect has not always been accounted for in past studies. In addition, acidification of the samples also resulted in a significant deconjugation of sulfated catecholamines leading to increase in dopamine concentrations, since the sulfate-conjugates of this amine occurs at relatively high concentrations compared to NE and E [17,71]. This may be of crucial importance for the diagnosis and monitoring of neuroblastomas.

FN test results may also be found in patients with silent PHEO which accounts for 7% of adrenal incidentaloma [72]. Exclusively or predominately dopamine-secreting tumors are very rare and are often overlooked because of their atypical clinical presentation (absence of hypertension and paroxysms) and because the biomarkers most commonly requested by physicians to screen for PHEOs are only E and NE and their O-methylated metabolites [73]. FN metanephrine test results may also be encountered in patients with genetic forms of PHEO or tumors that are too small (<1 cm) to produce sufficient amounts of catecholamines to be detected biochemically [74].

How to overcome pre-analytical pitfalls causing in FN results?. Measurements of the parent catecholamines should no longer be performed for screening and confirmatory diagnosis of PHEO because they offer the least diagnostic sensitivity which does not exceed 85% [60]. Diagnosis of PHEO should rely on the determination of either or both urinary and plasma fractionated MNs which have diagnostic sensitivities exceeding 97% [60,75]. Plasma free MNs have diagnostic sensitivity exceeding 96% with better specificity (85–100%) than plasma total MNs, but they are not widely available.

It is advisable for urinary catecholamine tests to acidify moderately the urine to pH 4.0 immediately after sample collection and protect the sample from light. Concurrent urine measurement of creatinine and correction of urinary metanephrines by urinary creatinine concentration circumvents FN pitfalls caused by incomplete collection of 24-h urinary specimens [76]. Due to the inconvenience of 24-h urine collections and the possibility of missing some samples, some investigators recommended overnight urine or spot collections as alternative urine collection times [58,77].

Plasma catecholamine stability has been extensively studied and an excellent review summarizes the important steps necessary to avoid catecholamine degradation [67]. As long as heparinized blood is centrifuged within 30 min after collection; the use of a refrigerated centrifuge is not mandatory. Once plasma is decanted from the blood cells, catecholamines are stable for 1 day at 20 °C, 2 days at 4 °C, 1 month at –20 °C (or 6 months with added glutathione), and up to 1 year at –70 °C [67,68].

Plasma DA and MT should be measured in patients presenting with paraganglioma and in patients harboring a large adrenal mass along with normal or marginally increased metanephrine levels in cases with atypical symptoms (e.g., arterial hypotension and vomiting) or extra-adrenal metastasis [78].

If initial catecholamine testing yields negative results despite high pre-test probability of PHEO particularly in carriers of mutations in one of the ten currently identified tumor susceptibility genes, comprehensive repeat routine screening is mandatory [78].
Post-analytical pitfall

Reference intervals for catecholamines in plasma and urine may vary to a large extent depending on the population investigated (e.g., normotensive, hypertensive, polymedicated and polymorbid patients) [67]. Therefore, it is mandatory to request that each laboratory establishes its own reference intervals including age and sex stratification for each population investigated.

Measurement of catecholamines in neuroblastoma workup

Neuroblastoma (NB) originates from the sympathetic nervous system. It is the third most frequent childhood cancer after leukemia and brain tumors and the most common cancer diagnosed in the first year of life [79]. The diagnosis and monitoring of the response to treatment of NB rely on an international consensus [80] based on elevated urinary NE and DA and their acid metabolites: VMA and HVA [81]. With VMA and HVA, NB can be diagnosed with a sensitivity ranging from 66 to 100% depending on disease stage [81]. Adding dopamine raises the sensitivity to 95% [82]. Of NBs, 5–10% are considered as non-secreting since none of these metabolites are detected. E is not a good marker for NB presumably because this tumor lacks PNMT expression. A potential pitfall is linked to the 24-h urine samples that are not always collected reliably, especially in neonates and pediatric patients. Therefore, it is more advisable to use random untimed urine specimens in young children and normalize them with urinary of creatinine concentration based on a standardized age-dependent population [81,83–87]. Since reference intervals may differ significantly between laboratories it is highly recommended that each laboratory establish its own reference limits in order to facilitate correct interpretation of the results.

Summary

The stability of catecholamine in urines is still controversial and needs further studies to warn the laboratory about potential pre-analytical pitfall linked to sample collection, shipment and storage conditions. With tandem mass spectrometry methodology, the accuracy and specificity of catecholamine quantification in urine and plasma are no longer an analytical issue. However, these bioamines are very sensitive to diet, medications and adrenosympathetic stimulation making their interpretation unreliable for the diagnosis and monitoring of pheochromocytomas whereas they remain the gold-standard for biochemical assessment of neuroblastomas when combined with VMA and HVA. Plasma and urinary metanephrines are now recognized as the most sensitive tests to exclude pheochromocytomas.

Practice points

- Plasma and urine catecholamine concentrations are increased during pathophysiological or pharmacological adrenosympathetic stimulation resulting in misdiagnosis of pheochromocytomas.
- The most frequent pre-analytical pitfalls are observed in patients who do not rest in supine position for at least 20 min before blood sampling as well as in patients who consume a catecholamine-rich diet or take medications that enhance catecholamine secretion.
- Metanephrines are the test of choice to exclude pheochromocytomas.
- Additional measurement of plasma normetanephrine during clonidine-suppression test enhances the specificity of the diagnosis of pheochromocytoma when plasma norepinephrine and/or normetanephrine levels are in the “grey zone”.
- Analytical pitfalls linked to measurement of catecholamines are prevented by using liquid chromatography tandem mass spectrometry methods.
- Urinary catecholamines (combined with VMA and HVA) are still considered as the gold-standard for the diagnosis of neuroblastoma whereas although accurate urine collection is uncertain problematic in neonates.
Research agenda

- The stability of catecholamines in urine is still controversial. Prospective studies are needed to determine optimal pH of urine to prevent deconjugation while maintaining catecholamine stability.
- Clinical studies are required to establish whether catecholamines in urine are better than metanephrines for the diagnosis and monitoring of neuroblastomas.

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