Clinical Chemistry 60:12
1486–1499 (2014)

Review

Laboratory Evaluation of Pheochromocytoma and Paraganglioma

Graeme Eisenhofer[1,2*] and Mirko Peitzsch[1]

BACKGROUND: Pheochromocytomas and paragangliomas (PPGLs) are potentially lethal yet usually surgically curable causes of endocrine hypertension; therefore, once clinical suspicion is aroused it is imperative that clinicians choose the most appropriate laboratory tests to identify the tumors.

CONTENT: Compelling evidence now indicates that initial screening for PPGLs should include measurements of plasma free metanephrines or urine fractionated metanephrines. LC-MS/MS offers numerous advantages over other analytical methods and is the method of choice when measurements include methoxytyramine, the *O*-methylated metabolite of dopamine. The plasma test offers advantages over the urine test, although it is rarely implemented correctly, rendering the urine test preferable for mainstream use. To ensure optimum diagnostic sensitivity for the plasma test, reference intervals must be established for blood samples collected after 30 min of supine rest and after an overnight fast when measurements include methoxytyramine. Similarly collected blood samples during screening, together with use of age-adjusted reference intervals, further minimize false-positive results. Extents and patterns of increases in plasma normetanephrine, metanephrine, and methoxytyramine can additionally help predict size and adrenal vs extraadrenal locations of tumors, as well as presence of metastases and underlying germline mutations of tumor susceptibility genes.

SUMMARY: Carried out correctly at specialist endocrine centers, collection of blood for measurements of plasma normetanephrine, metanephrine, and methoxytyramine not only provides high accuracy for diagnosis of PPGLs, but can also guide clinical decision-making about follow-up imaging strategies, genetic testing, and therapeutic options. At other centers, measurements of urine fractionated metanephrines will identify most PPGLs.

Pheochromocytomas and paragangliomas (PPGLs)[3] are neuroendocrine tumors arising from adrenal and extraadrenal chromaffin cells, respectively *(1)*. About half of adrenal tumors produce a mixture of norepinephrine and epinephrine and the other half nearly exclusively norepinephrine or in occasional cases norepinephrine and dopamine. In contrast, paragangliomas of the thorax, abdomen, and pelvis rarely produce significant amounts of epinephrine, with most producing solely norepinephrine, others a combination of norepinephrine and dopamine, and some exclusively dopamine; occasional cases have also been described that produce negligible amounts of any catecholamine.

It is the unregulated secretion of catecholamines by PPGLs that is largely responsible for hypertension and symptoms such as palpitations, headaches, and hyperhidrosis that classically characterize the tumors. In excessive amounts, released catecholamines can lead to serious cardiovascular manifestations, including hypertensive emergencies involving end-organ damage that can progress to multisystem failure and death *(2, 3)*. Prompt diagnosis is therefore important; the first critical step is recognizing the possibility of the tumor *(2)*. Thereafter, choosing the most appropriate laboratory test and procedures for testing are crucial for reliable detection or exclusion of the tumor.

Traditionally biochemical testing for PPGLs relied largely on measurements of catecholamines in urine, often carried out in conjunction with measurements of catecholamine metabolites, such as vanillylmandelic acid (VMA) and the metanephrines *(4)*. Others championed measurements of catecholamines in plasma over urine *(5)*. More recently there has been a shift in

[1] Institute of Clinical Chemistry and Laboratory Medicine and [2] Department of Medicine III, University Hospital Carl Gustav Carus, Technische Universität Dresden, Germany.
* Address correspondence to this author at: Institute of Clinical Chemistry and Laboratory Medicine and the Department of Medicine, University Hospital Carl Gustav Carus, Technische Universität Dresden, Fetscherstraße 74, 01307 Dresden, Germany. Fax +49-351-458-6398; e-mail graeme.eisenhofer@uniklinikum-dresden.de.
Received July 23, 2014; accepted September 26, 2014.
Previously published online at DOI: 10.1373/clinchem.2014.224832

[3] Nonstandard abbreviations: PPGL, pheochromocytoma and paraganglioma; VMA, vanillylmandelic acid; COMT, catechol-*O*-methyltransferase; LC-ECD, liquid chromatography with electrochemical detection; SULT1A3, sulfotransferase family, cytosolic, 1A phenol-preferring member 3; MEN 2, multiple endocrine neoplasia type 2; NF1, neurofibromatosis type 1; VHL, von Hippel-Lindau; SDHB, succinate dehydrogenase subunit B.

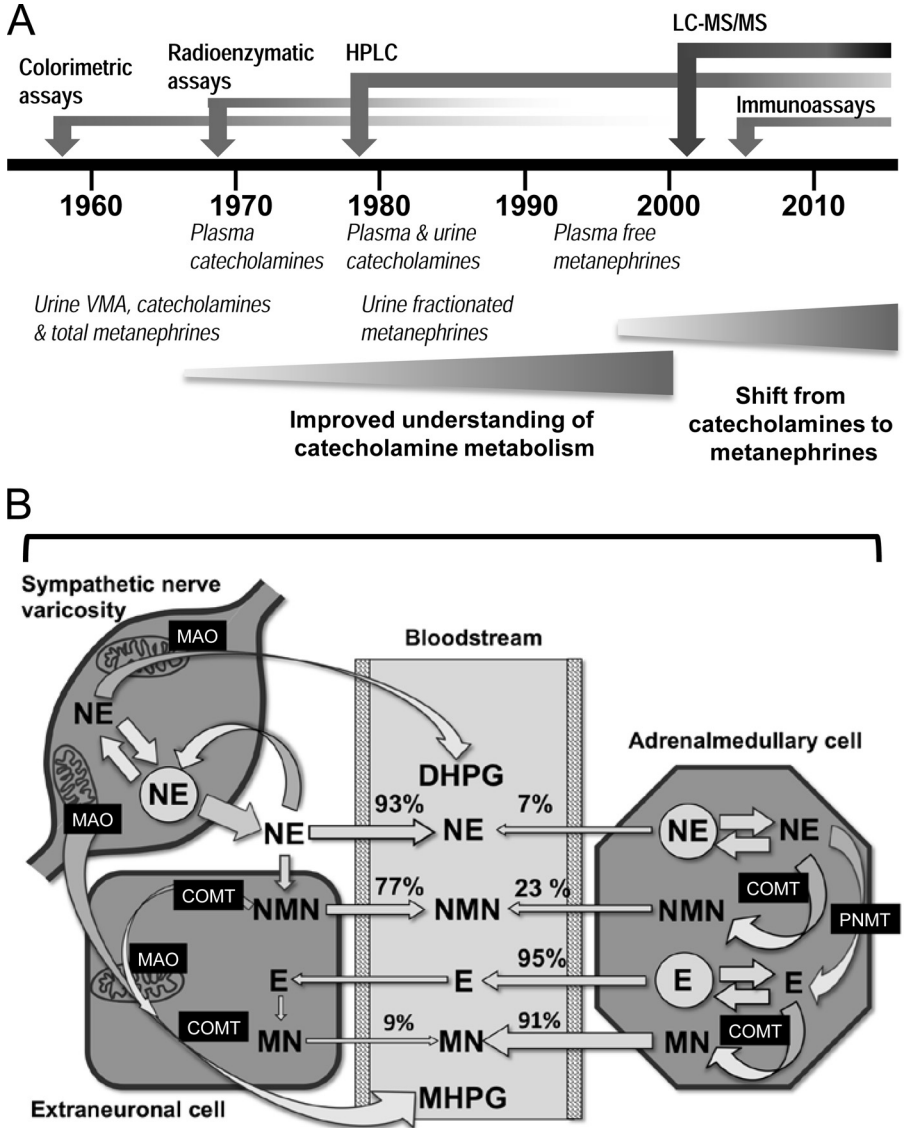

Fig. 1. Timeline illustrating developments in assay technology shifting emphasis from catecholamines to metanephrines for diagnosis of PPGLs (A) associated with improved understanding of catecholamine metabolism (B).

As illustrated in (B), most metabolism of norepinephrine (NE) occurs by deamination catalyzed by monoamine oxidase (MAO) within sympathetic nerves to form dihydroxyphenylglycol (DHPG), most of which is formed from NE leaking from storage vesicles into the sympathoneuronal cytoplasm. Similarly, in adrenalmedullary chromaffin cells and chromaffin tumor derivatives, metabolism occurs within cells from NE and epinephrine (E) leaking from storage vesicles into the cytoplasm, where the presence of COMT leads to formation of normetanephrine (NMN) and metanephrine (MN). COMT is not present in sympathetic nerves, but it is present in extraneuronal cells, where it metabolizes DHPG to 3-methoxy-4-hydroxyphenylglycol (MHPG), the main precursor of VMA. The COMT in extraneuronal tissues is also responsible for production of a small proportion of circulating MN (9%), derived from E metabolized after release from chromaffin cells, as well as a larger proportion of circulating NMN (77%) derived mainly from NE released by sympathetic nerves.

emphasis to measurements of urine and plasma metanephrines, including normetanephrine and metanephrine, the respective *O*-methylated metabolites of norepinephrine and epinephrine (Fig. 1). Some methods also allow additional measurements of methoxytyramine, the *O*-methylated metabolite of dopamine.

The shift in emphasis to metanephrines for diagnosis of PPGLs followed from advances in assay tech-

nology, allowing measurements of the low concentrations of free metanephrines in plasma *(6)*, as well as improved understanding of catecholamine metabolism that followed those technical advances *(7)*. It is now understood that the free metanephrines are produced within adrenal chromaffin or PPGL tumor cells after leakage of catecholamines from storage vesicles into the cytoplasm, where the presence of membrane-bound catechol-*O*-methyltransferase (COMT) leads to immediate metabolism. This process operates continuously and independently of exocytotic catecholamine release, providing an advantage for measurements of metanephrines during diagnosis of tumors that release catecholamines episodically or in low amounts (Fig. 1). Furthermore, VMA and other catecholamine metabolites such as 3-methoxy-4-hydroxyphenylglycol are formed primarily from the deaminated metabolite dihydroxyphenylglycol, which itself is formed mainly within sympathetic nerves *(7)*. Lack of COMT in sympathetic nerves, where most norepinephrine is initially metabolized, means that the metanephrines are relatively specific markers for catecholamines produced in chromaffin cells and their tumor derivatives.

Recommended Screening Tests

As outlined in the Endocrine Society Clinical Practice Guidelines on PPGLs *(8)*, biochemical testing for catecholamine-producing PPGLs is recommended to include measurements of plasma free or urine fractionated metanephrines. The diagnostic utility of measuring free metanephrines in plasma instead of urine was initially indicated in a series of studies from the NIH, culminating in a report with cumulative experience in over 800 patients tested for PPGLs *(9)*. That final study established that the superiority of plasma metanephrines over other tests for diagnosis of PPGLs remained significant even when compared to combinations of tests. It was thus concluded that combinations of tests offered no advantage over a single test of plasma metanephrines for diagnosis of PPGLs. Measurements of urine fractionated metanephrines represented the one test for which diagnostic sensitivity approached that for plasma metanephrines (97% vs 99%). Nevertheless, diagnostic specificity of urinary fractionated metanephrines was lower than for all other tests, including plasma free metanephrines (89% vs 69%).

As reviewed elsewhere *(8)*, numerous studies have confirmed the higher diagnostic sensitivity and advantages of plasma free metanephrines over other tests for detection of PPGLs. Several of these studies involved comparisons of plasma free with urine fractionated metanephrines *(9–12)*. Although all yielded consistently higher values for diagnostic sensitivity with the plasma than with the urine test, the differences were small and did not reach significance. Consistently higher specificity of the plasma than the urine test, a difference significant in 2 studies *(9, 11)*, is at odds with conclusions that plasma measurements of metanephrines offer inferior diagnostic specificity compared to urine measurements *(13)*. It thus remains a matter of debate whether one test is superior to the other or under what conditions one is preferable to the other *(14)*. Until resolved, measurements of both plasma free and urine fractionated metanephrines remain recommended as initial screening tests. As covered later, there are more relevant factors to consider that impact relative diagnostic accuracies and choice of plasma vs urine fractionated metanephrines for diagnosis of PPGLs.

Analytics

From bioassays, colorimetric, and fluorometric methods, to radioenzymatic assays, we have now progressed to liquid chromatographic–based methods employing electrochemical, fluorometric, or mass spectrometric detection for measurements of plasma or urine catecholamines and catecholamine metabolites (Fig. 1A). Liquid chromatography with electrochemical detection (LC-ECD) or coupled to tandem mass spectrometry (LC-MS/MS) has risen to the forefront of analytical techniques, with LC-MS/MS increasingly taking center stage *(15–19)*. Immunoassays have also become popular for measurements of plasma metanephrines *(10, 12)*. Together with chromatographic-based methods these assays allow "fractionated" measurements of normetanephrine and metanephrine, an important advance over earlier colorimetric or fluorometric measurements of "total metanephrines," representing the combined sum of normetanephrine and metanephrine. For LC-ECD and LC-MS/MS methods, additional measurements of methoxytyramine are also possible *(19)*.

FREE VS CONJUGATED METANEPHRINES

Metanephrines exist in plasma and urine in both free and much higher sulfate-conjugated forms, the latter produced by a specific sulfotransferase isoenzyme, sulfotransferase family, cytosolic, 1A phenol-preferring member 3 (SULT1A3) (Fig. 2A), present mainly in gastrointestinal tissues *(20)*. Measurements of urine metanephrines are usually carried out after an acid hydrolysis step that converts the higher concentrations of sulfate-conjugated metabolites to measureable free forms (Fig. 2B). Thus, such measurements reflect the combination of both free and conjugated metanephrines (i.e., deconjugated metabolites).

Importantly, efficiency of the acid hydrolysis conversion of sulfate conjugated to free metanephrines de-

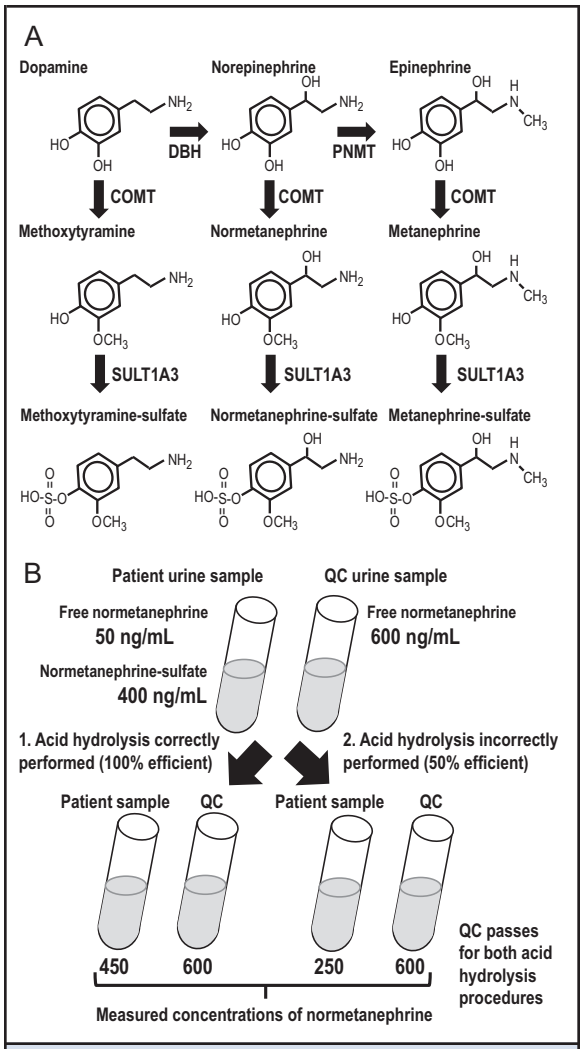

Fig. 2. Pathways of metabolism of catecholamines to free and sulfate-conjugated *O*-methylated metabolites (A) and pitfalls in measurements of urinary *O*-methylated metabolites after acid hydrolysis for measurements of free metabolites using methods that employ QC urine samples prepared with free metabolites (B).

DBH, dopamine β-hydroxylase; PNMT, phenylethanolamine *N*-methyltransferase.

pends on pH, temperature, and time of incubation *(21, 22)*. This would not be a problem if calibrators and QC samples recapitulated the proportions of free and conjugated metabolites in patient samples. However, commercially available calibrators and control samples used in assays of urine fractionated metanephrines are provided predominantly or entirely in the free form *(18, 21)*. Thus, unless deconjugation conditions are adequately controlled, concentrations of urine metanephrines in patient samples can suffer underestimation without parallel underestimation of controls (Fig. 2B). Consequently, assays can pass QC even without a deconjugation procedure, a shortcoming of current quality assurance procedures best addressed by use of urine controls containing natural forms of both conjugated and free metabolites *(23)*.

With improved analytical sensitivity of modern LC-ECD and LC-MS/MS instruments, it has become possible to measure urine free metanephrines *(16, 24)*, which also provide improved diagnosis of PPGLs compared to catecholamines and VMA *(25)*. Such measurements not only avoid the quality assurance problems associated with measurements of deconjugated metanephrines, but also offer theoretical advantages related to measurements of free metabolites formed within chromaffin cells and their tumor derivatives *(18, 23)*.

As yet, however, it is not established whether urine free metanephrines are superior to commonly used urine deconjugated metanephrines for diagnosis of PPGLs. On the other hand, comparisons of plasma free with deconjugated metanephrines show that the former measurements offer improved diagnostic efficacy over the latter *(26)*. This was ascribed to the large amounts of sulfate-conjugated normetanephrine produced in gastro-intestinal tissues acting to dilute the diagnostic signal of the deconjugated normetanephrine compared to the free metabolites produced directly within tumors.

LC-MS/MS VS LC-ECD AND IMMUNOASSAYS

Among analytical methods, LC-MS/MS has several advantages over LC-ECD, with accumulating evidence indicating that both offer superior accuracy and precision compared to immunoassays. Findings from quality assurance programs and head-to-head comparisons indicate that LC-MS/MS and LC-ECD provide comparable analytical accuracy and precision *(19, 27)*. Relative freedom from analytical interferences, simpler sample preparation (in some methods involving automated on-line purification), as well as rapid and high sample throughput offer significant advantages of LC-MS/MS over HPLC-ECD. High sample throughput is particularly important for high-volume commercial laboratories, where the capital costs of instrumentation may be recouped within several months of operation. For smaller hospital-based laboratories, instrument costs and the need to develop in-house analytics can be a disincentive to establish LC-MS/MS, making immunoassays more advantageous.

As outlined in the Endocrine Society Guidelines on PPGLs *(8)*, immunoassays are not recommended as a first choice for measurements of plasma free metanephrines. This is because immunoassays suffer not

only from higher imprecision than LC-ECD and LC-MS/MS methods, but also from inaccuracy involving underestimation of plasma concentrations of metanephrine and normetanephrine *(27–29)*. As reported in an earlier study by Peaston et al. *(28)*, the negative bias of immunoassays can lead to repeatedly false-negative results, which by LC-MS/MS are consistently positive and appropriately indicate presence of disease.

Analyte Stability

Because metanephrines are considerably more stable than catecholamines, the need for stringent precautions to avoid their degradation can be relaxed, providing an analytical advantage for measurements of the metabolites over the parent catecholamines. Nevertheless, as observed by Willemsen et al. *(30)*, metanephrines in whole blood at room temperature show pronounced and variable time-dependent changes, presumably in part reflecting metabolism of catecholamines by catechol-*O*-methyltransferase in red blood cells. In the same study, metanephrines were stable in whole blood stored at 4 °C for up to 6 h. Therefore, blood samples should be chilled up until the time of centrifugation, similarly to procedures required for catecholamines. Thereafter, metanephrines are relatively stable for up to 3 days in separated plasma, provided samples are stored at 4 °C. For any longer storage before analysis, plasma samples should be kept at −20 °C or lower.

Because catecholamines are prone to autoxidation under alkaline conditions or exposure to light, acidification of urine and collection in light-proof containers with storage at 4 °C are all well-established precautions to minimize degradation before measurements. In contrast, acidification is unnecessary for measurements of metanephrines, including free metanephrines, which are stable in untreated urine for up to 3 days at room temperature *(18, 31)*. For measurements of free metanephrines, overacidification can in fact lead to deconjugation and spurious increases in measured concentrations of the free metabolites. Nevertheless, for urine samples collected for measurements of both metanephrines and catecholamines, use of stabilizers to reduce degradation remains prudent. When such measurements include free metanephrines, adjustments of pH can be performed after receipt of urine samples by the laboratory, with care not to overacidify when measurements involve free fractions. Refrigeration on receipt by the laboratory also remains prudent. Long-term storage, again as for plasma, should be at −20 °C or lower.

Reference Intervals

As outlined in published recommendations *(32)*, upper cutoffs of reference intervals for plasma and urine fractionated metanephrines should be established to ensure optimum diagnostic sensitivity, with specificity a secondary consideration. This recommendation recognizes the potential deadly consequences of a missed diagnosis. Appropriately determined cutoffs provide confidence that patients with PPGLs will not be missed and conversely that negative results can be used to reliably exclude disease.

Unfortunately, reference intervals for plasma free metanephrines are all too often inappropriately established. A prime example is illustrated by upper cutoffs for immunoassays described above, which commonly follow the information supplied with commercials kits without validation by the laboratories using the kits. Upper cutoffs for normetanephrine of 180 pg/mL (0.98 nmol/L) described for the immunoassay are already too high for any patient younger than 55 years, but combined with the 60% negative bias lead to a diagnostic sensitivity of <75%, meaning that over 25% of patients with PPGLs may remain undetected. As described by Pussard et al. *(12)*, with appropriate validations cutoffs can be established for immunoassays that are much lower than those described in commercial kit package inserts.

Use of inappropriately high upper cutoffs for tests of plasma free metanephrines is not, however, confined to commercial immunoassay kits. It is also a problem for many laboratories where upper cutoffs have been determined for reference populations in which samples were obtained from patients under conditions of sympathetic nervous system activation, involving the seated rather than the fully recumbent supine position (Fig. 3). Endocrine Society Guidelines therefore recommend that reference intervals for plasma free metanephrines should be established for blood sampling with patients in the supine position *(8)*. As further shown by Därr et al. *(33)*, upper cutoffs for plasma free normetanephrine determined from blood samples taken with patients in the seated position can be expected to result in a substantial drop in diagnostic sensitivity, with up to 15% of patients with PPGLs being missed. For plasma methoxytyramine, reference intervals must be established for blood samples taken after an overnight fast.

Although the primary consideration for reference intervals used in screening for PPGLs should be directed to optimization of diagnostic sensitivity, this does not imply that optimization for enhanced specificity need be neglected. Since plasma concentrations of normetanephrine increase with advancing age *(34)*, use of age-adjusted reference intervals has been proposed to minimize false-positive results *(35)*. As outlined in a subsequent study, such optimization, with a diagnostic specificity reaching

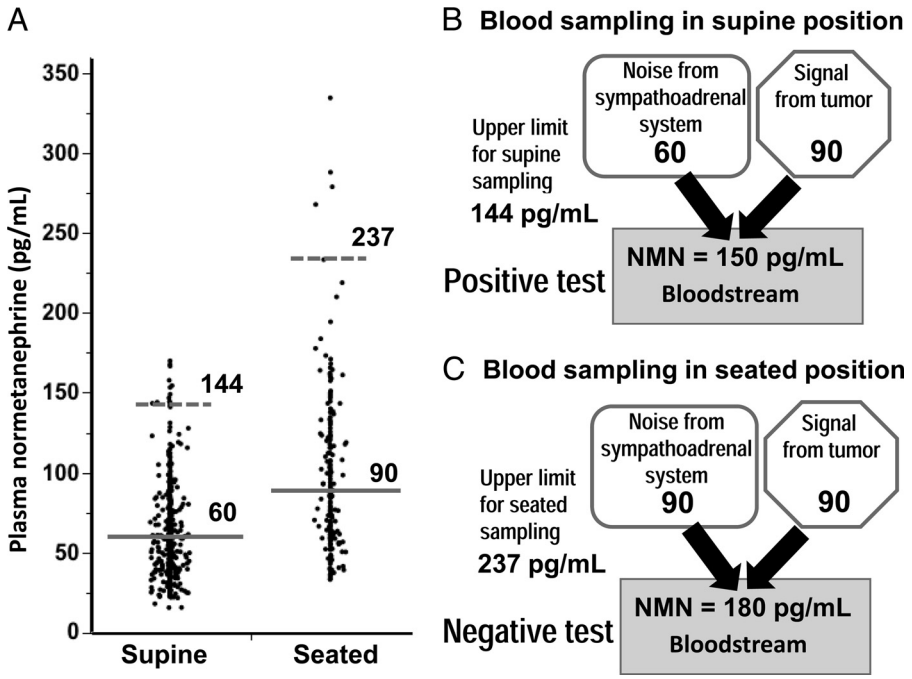

Fig. 3. Influence of blood sampling in the seated and supine positions on distributions of plasma concentrations of normetanephrine (A) and on outcomes of diagnostic testing with blood sampling in the supine (B) vs seated positions (C) in a hypothetical patient with a tumor that produces 50% more normetanephrine (NMN) than produced in the supine position from usual sympathoadrenal sources.

Data in (A) are derived from published results [Därr et al. *(33)*] of LC-MS/MS measurements of plasma normetanephrine from 433 patients sampled in the supine position (median age 51 years) and 195 patients sampled in the seated position (median age 52 years). Solid and dashed horizontal lines show the medians and upper cutoffs (latter determined from 97.5 percentiles) for supine (60 and 144 pg/mL) and seated (90 and 237 pg/mL) samplings. Calculations in (B) and (C) reflect the 50% higher median plasma concentrations and 65% higher upper cutoffs for samples obtained from a patient in the seated than in the supine position, as indicated in (A).

96%, can be achieved from an age adjustment for the upper cutoffs for plasma normetanephrine *(36)*. Such cutoffs increase from 99 pg/mL (0.54 nmol/L) for a 5-year-old patient to a maximum of 200 pg/mL (1.09 nmol/L) for patients 65 years and older and result in a substantial reduction in false-positive results with negligible loss in sensitivity (Fig. 4A).

Age-related increases in 24-h urine outputs of normetanephrine have also been described, with additional higher outputs in males than females for both normetanephrine and metanephrine *(37)*. This has led to the generation of sex- and age-specific reference intervals for urine outputs of normetanephrine and sex-specific reference intervals for both metabolites to provide optimized diagnostic sensitivity and specificity.

Use of age-appropriate reference intervals is particularly important for children, not only for plasma measurements of metanephrines *(38)*, but also for urine measurements for which it is useful to partition children across multiple age groups *(39–42)*. Due to difficulties in obtaining complete 24-h collections, urine samples from children are most commonly collected as spot urines with outputs of metanephrines normalized to creatinine. In contrast to 24-h samples, which show age-dependent increases in outputs of metanephrines *(39)*, when samples are normalized to creatinine the reverse is observed *(40–42)*. This appears to reflect dependence of creatinine on total muscle mass, which is also higher in males than females, so that sex differences in 24-h urine outputs can disappear or even be reversed when normalized to creatinine.

Preanalytics

Although appropriately determined reference intervals for plasma and urine fractionated metanephrines en-

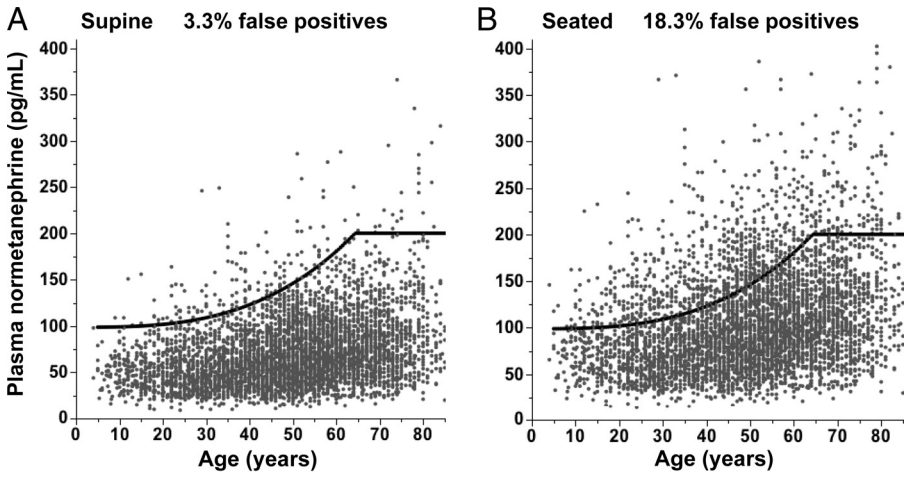

Fig. 4. Relationships of plasma normetanephrine concentrations with age for patients without PPGLs.

Relationships for supine samplings in 4994 patients, derived from previously published data [Därr et al. *(33)* and Eisenhofer et al. *(36)*], are shown in (A). Age-adjusted upper cutoffs (UC) of reference intervals, shown by solid lines, are determined by the equation ($UC = 3.79 \times 10^{-4}\ age^3 + 98.8$) for patients 5–65 years of age, and thereafter set to a maximum of 200 pg/mL (1.09 nmol/L). (B), The same data from (A), but with all results for plasma normetanephrine increased by 50% in accordance with expected increases associated with seated sampling *(33)*. Note that use of age-adjusted upper cutoffs optimized for supine sampling results in a 5.5-fold increase in false-positive results.

sures that almost all cases of PPGLs can be detected by positive results, this does not imply that all positive results indicate a tumor. The normally low pretest prevalence of PPGLs, combined with suboptimal diagnostic specificity, means that false-positive results far outnumber true-positive results. As reported by Yu and Wei *(43)*, false-positive results for plasma or urine fractionated metanephrines are common, with rates of about 20% during routine testing for PPGLs. For plasma metanephrines, normetanephrine was the analyte most usually showing a false-positive result, a conclusion also in agreement with an earlier study *(44)*. In both studies, false positives were usually of a borderline to mild nature. However, there are situations in which false-positive results can reach well into the high pathological range observed for patients with PPGLs.

SYMPATHOADRENAL ACTIVATION

The most common causes of false-positive results for measurements of plasma metanephrines are inappropriate blood sampling conditions associated with sympathoadrenal activation, particularly sampling performed with the patient in the seated rather than the supine position (Fig. 4B). Because more than 90% of plasma metanephrine and about 27% of circulating normetanephrine are produced from their catecholamine precursors within chromaffin cells and independently of exocytotic catecholamine release (Fig. 1B), these metabolites are less responsive to sympathoadrenal activation than their precursor catecholamines *(34)*. Nevertheless, a significant proportion of normetanephrine is produced from norepinephrine released by sympathetic nerves and is therefore responsive to changes in sympathetic nerve activity (Fig. 1B). Because of the rapid circulatory clearance of the metabolite, changes in its plasma concentrations follow relatively rapidly those of norepinephrine and include increases in plasma concentrations associated with assumption from supine of seated or standing positions *(45–47)*. Cold-associated increased sympathetic nerve activity, causing higher plasma concentrations of normetanephrine and lower diagnostic specificity in winter than in summer *(48)*, may further exaggerate increased plasma concentrations of normetanephrine in seated compared to supine positions of blood sampling.

As shown by Därr et al., blood sampling performed with the patient in the seated position without an overnight fast can be expected to return a close to 30% prevalence of false-positive results compared to a 5% prevalence for sampling performed on supine fasted patients *(33)*. The 30 min of supine rest required to minimize the prevalence of false-positive results can, however, be a problem to implement. Thus, as originally suggested by Lenders et al. *(45)*, and later reiterated by Därr et al. *(33)*, a practical solution is to per-

form screening with the patient in the seated position and follow up positive results with sampling performed with the patient in the supine position. Nevertheless, as indicated by an audit of patient follow-up after testing for PPGLs, such follow-up sampling is rarely practiced *(49)*; among the 76 patients with increased test results at screening there was follow-up in only 28% of cases.

Because use of plasma metanephrines with blood sampling performed with the patient seated offers significantly inferior diagnostic accuracy compared to sampling performed with the patient supine and has no diagnostic benefit over measurements of urine fractionated metanephrines, urine testing is preferable for centers where supine sampling cannot be offered *(8, 33)*. This proposal agrees with recommendations by others that measurements of urine metanephrines are best for low-risk populations in which overabundance of false-positive results can be a problem for the plasma test *(13)*. The problems remain, however, that 24-h collections of urine for fractionated metanephrines are not only inconvenient, but also suffer from false-positive results that are not as easily avoided as those for plasma measurements. First-morning spot urines offer a potential solution that is not only more convenient for patients, but also takes advantage of lowered sympathoadrenal activity associated with nighttime bed rest *(50, 51)*.

MEDICATIONS AND DIETARY INTERFERENCES

Troublesome interferences from medications or diet or measurements of plasma or urine metanephrines usually manifest as false-positive results in 2 main forms: (*a*) pharmacophysiological effects and (*b*) direct analytical interferences. The former are method independent and usually involve substances that influence the physiological disposition of catecholamines and their metabolites. In contrast, the latter are typically method dependent and important for clinical chemists to be aware of so as to modify methodology to minimize interference.

The most troublesome causes of method-independent false-positive results are from medications that block the neuronal uptake of catecholamines *(43, 44, 52, 53)*; these include tricyclic antidepressants and related drugs used to treat depression, insomnia, neuropathic pain, and other medical conditions. Phenoxybenzyamine, commonly used in preoperative preparation of patients with PPGLs, is another medication associated with increased prevalence of false-positive increases of plasma and urine normetanephrine due to blockade of sympathoinhibitory α_2-adrenoceptors *(44)*. Because these drugs increase only the likelihood of false-positive results, their withdrawal before testing is not necessary, but rather should be considered when initial testing reveals positive results. On the other hand, possible false-positive results from sympathomimetics and stimulants, such as caffeine, that can increase catecholamine release are best minimized by instructions to avoid such agents before testing.

Monoamine oxidase inhibitors, by blocking metabolism of metanephrines, can cause substantial increases in these metabolites *(54)*, but are infrequent causes of false-positive results since the drugs are rarely used. L-Dopa used in the treatment of Parkinson disease and restless leg syndrome is a more common cause of false-positive results, probably due to testing consequent to the blood pressure disturbances encountered in elderly patients receiving the drug *(55, 56)*. This immediate precursor of dopamine can increase plasma and urine concentrations of methoxytyramine with minimal influence on normetanephrine and metanephrine as measured by LC-MS/MS *(56)*. However, for LC-ECD methods, L-Dopa has been shown to increase all metabolites *(55)*, probably as a consequence of additional analytical interferences.

Dietary L-Dopa, dopamine, and other amines, common to many foods, have also been demonstrated to increase plasma levels of L-Dopa, dopamine, and particularly, sulfate-conjugated metabolites *(20)*. As shown by de Jong et al. *(46)*, such dietary compounds can lead to substantial increases of not only urine outputs of deconjugated normetanephrine and methoxytyramine, but also plasma free methoxytyramine. Thus, blood samples for measurements of the latter metabolite should be collected after an overnight fast. Procedures to avoid dietary influences on metabolites measured in urine are problematic and not commonly employed.

Antihypertensive medications, such as labetolol, commonly cited as causing analytical interferences with colorimetric measurement methods, have rarely been reported as causing problems with LC-ECD methods. Acetaminophen (paracetamol) *(6, 57)* and 5-aminosalicylic acid (mesalamine) and its prodrug sulfasalazine *(58, 59)* are more recently reported causes of analytical interferences with LC-ECD measurements of plasma and urine metanephrines. Unusual sources of interferences with internal standards used in LC-ECD assays of urine metanephrines have also been reported for methenamine *(60)* and a dietary component in curry leaves *(61)*, both leading to measurement underestimation. Although LC-MS/MS provides high analytical specificity, some interferences with measurements of *O*-methylated metabolites in plasma have been reported *(62, 63)*. Under some circumstances, patterns of ion pair fragmentation may be insufficient to avoid interference without chromato-

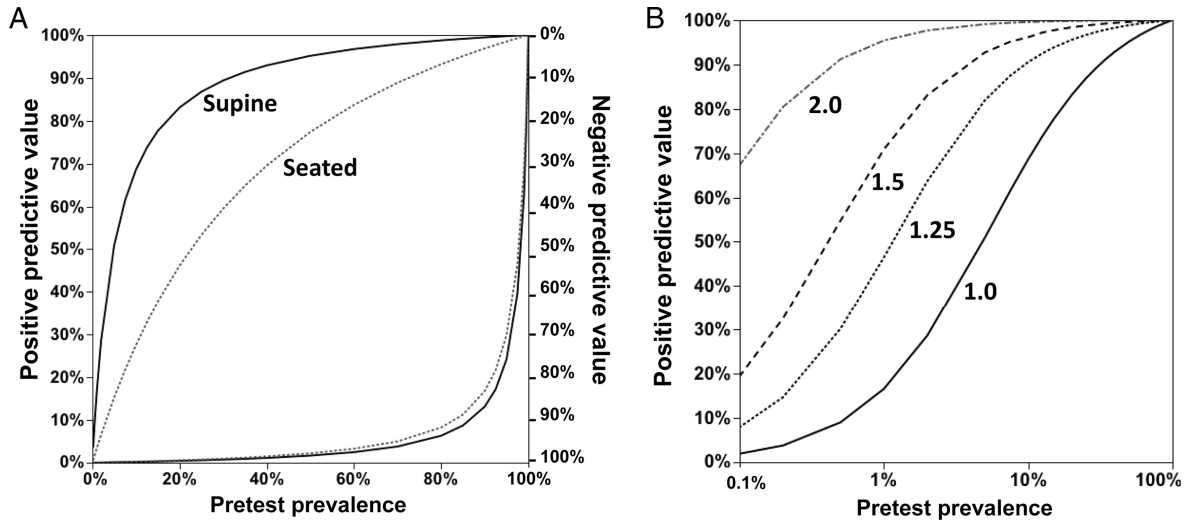

Fig. 5. Relationships of pretest prevalence with posttest probabilities of PPGLs according to measurements of plasma free metanephrines with sampling under supine and seated conditions (A) or under supine conditions using different upper cutoffs (B), all based on age-adjusted cutoffs for normetanephrine and cutoffs for metanephrine and methoxytyramine established previously [Eisenhofer et al. *(36)*].

(A), Positive and negative predictive values estimated according to a binary approach to test interpretation. (B), Positive predictive values estimated according to the continuous nature of test results. Relationships in (A) are derived from published data [Därr et al. *(33)*] for which diagnostic specificity was established at 95% under supine fasting sampling conditions and 71.3% under seated nonfasting sampling conditions (upper cutoffs optimized for supine fasting sampling). Relationships in (B), with a logarithmic scale for pretest prevalence, show increases in posttest probability of PPGLs according to any result for normetanephrine, metanephrine, or methoxytyramine increased by more than 100% (dashed dotted line), 50% (dashed line), and 25% (dotted lines) above established upper cutoffs (solid line). Note that at a 1% pretest prevalence of disease, increases in test results more than 2-fold above upper cutoffs indicate a 95% probability of a tumor compared to a 17% probability for a result only slightly increased above the upper cutoffs.

graphic resolution, which remains important even for many LC-MS/MS methods.

Laboratory Test Interpretation and Follow-up

EXCLUSION OF DISEASE

Provided that reference intervals have been appropriately established and measurement methods are accurate and precise, test results within reference intervals for plasma free metanephrines exclude almost all cases of PPGLs. Exceptions include microscopic recurrences or small tumors (<1 cm) found incidentally or during screening because of a hereditary predisposition to PPGLs or history of disease *(9)*. Tumors that produce only dopamine will also not be detected by measurements of metanephrines but can be detected by additional measurements of plasma methoxytyramine *(19, 64)*. Other exceptions include head and neck paragangliomas and rare phenotypically immature abdominal paragangliomas that do not synthesize catecholamines, the latter typically attaining a large size before detection *(65)*.

All of the above exceptions represent unusual presentations without the typical clinical signs and symptoms of catecholamine excess. In the more usual cases in which clinical suspicion is aroused by findings of hypertension associated with symptoms of catecholamine excess and in which the pretest prevalence of PPGLs is usually <2%, findings of test results within reference intervals for either plasma free or urine fractionated metanephrines carry high negative predictive value, reliably excluding disease so that no further immediate testing is warranted (Fig. 5A).

PRETEST VS POSTTEST PROBABILITY OF DISEASE

Pretest prevalence of PPGLs varies according to 4 main reasons for screening: (*a*) presence of signs and symptoms; (*b*) incidental findings of an adrenal or abdominal mass during imaging for an unrelated condition; (*c*) hereditary predisposition to PPGLs; and (*d*) history of

PPGL. Among unselected patients screened for PPGLs, prevalence of PPGLs ranges from 0.8% to 1.6% *(43, 66–68)*. Among patients with incidentalomas, prevalence of PPGLs is higher at 4%–9% *(69)*. Prevalence of PPGLs among patients with germline mutations of PPGL susceptibility genes runs even higher, reaching 40% in multiple endocrine neoplasia type 2 (MEN 2) *(70)*. Similarly, 16.5% of patients with a history of PPGLs develop recurrences *(71)*.

The above differences in pretest prevalence of PPGLs require consideration when interpreting positive results to assess posttest probability of a tumor (Fig. 5). For measurements of plasma free metanephrines, the conditions of blood sampling also impact posttest probability of disease, with substantially lower probability associated with seated than supine sampling (Fig. 5A). However, when sampling is carried out under supine fasting conditions more than 75% of all PPGLs can be easily recognized from the extent and nature of increased results *(44)*. Increases of both normetanephrine and metanephrine are rare as false-positive results but occur in at least half of all patients with pheochromocytoma. Similarly, solitary increases in either metabolite more than 2-fold above upper cutoffs are also rare as false positives. With such findings the posttest probability of PPGLs can be higher than 90% even at a pretest prevalence of only 1%, providing a high level of suspicion to justify imaging studies to locate the tumor (Fig. 5B).

The larger problem for interpreting positive results concerns those that are borderline in nature, comprising about 25% of all patients with PPGLs. Such patients with PPGLs, who present with borderline increased test results (true positives), are hidden among a much larger number of patients with similar elevations in test results but without tumors (false positives). For these patients the posttest probability is increased only marginally compared to the other patients with PPGLs in whom disease is clear from much larger increases. With this presentation, differences in disease prevalence substantially impact posttest probability of a tumor and must be considered in subsequent diagnostic decision-making (Fig. 5).

FOLLOW-UP TESTING

Follow-up testing is crucial to further exclude or confirm PPGLs in patients presenting with borderline-positive results but can also be important in some cases of markedly increased results. Physiological stress associated with extreme illness, as in intensive care settings, is an example that should be considered in interpreting marked increases of plasma or urine metanephrines. Inappropriate sampling conditions or medications, as discussed above, should also be considered as sources of borderline false-positive results. Confirmatory testing after exclusion of these and other sources of false-positive results is often useful for ruling out disease.

Once causes of false-positive results are ruled out, the clonidine suppression test with measurements of normetanephrine before and 3 h after the drug can be useful to distinguish true- from false-positive borderline elevations of plasma normetanephrine *(44)*. With appropriate interpretation, this method has a purported diagnostic specificity of 100% with a sensitivity of 97%, but as yet has not been validated in any prospective study.

In other situations of borderline positive test results and low probability of a tumor, a wait and retest approach can illuminate increased likelihood of an enlarging tumor. As outlined in the Endocrine Society Guidelines *(8)*, all positive test results should be followed up. However, the nature of this and whether to first follow-up with additional comprehensive or involved biochemical testing procedures, adopt a wait-and-retest approach, or proceed directly to imaging studies remains a matter of clinical judgment according to the extent of increases in test results in relation to changes in pretest to posttest probability of disease and other considerations impacting test interpretation.

TUMOR SIZE AND LOCATION

Tumor size is poorly correlated with plasma or urinary catecholamines but shows strong positive relationships with metanephrines *(72, 73)*, a consequence of their production within tumor cells by a process that is independent of variations in catecholamine release. Thus, summed plasma concentrations of metanephrines can be used to predict tumor diameter *(73)*. This information can be useful to relate to results of imaging studies, carried out either as a cause or consequence of clinical suspicion for a tumor. It also provides these metabolites with a potentially useful role as surrogate biomarkers of metastatic progression and response to therapy.

In addition to information about tumor size, patterns of increases in the different catecholamine *O*-methylated metabolites can be used to predict tumor location (Fig. 6). Chromaffin cell tumors that produce significant amounts of metanephrine alone, or in combination with normetanephrine, almost always have an adrenal location or reflect recurrence of a previous adrenal tumor *(73)*. Although solitary increases of normetanephrine cannot be used to predict tumor location, when these are accompanied by substantial increases in methoxytyramine the location is invariably extraadrenal. Similarly, tumors that produce exclusively methoxytyramine almost always have an extra-

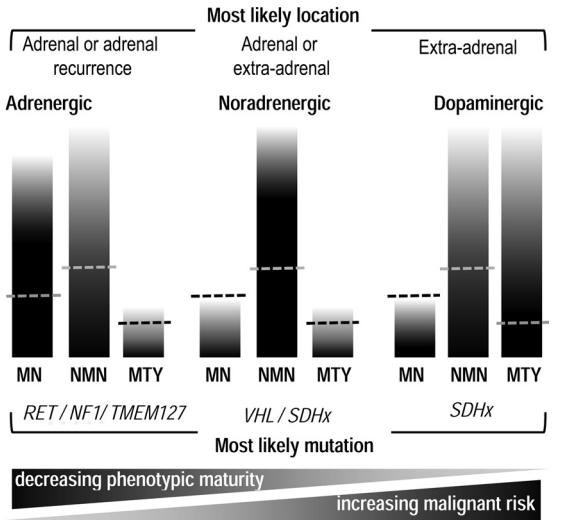

Fig. 6. Utility of PPGL catecholamine phenotypes, as reflected by patterns of increases in plasma normetanephrine (NMN), metanephrine (MN), and methoxytyramine (MTY), for predicting tumor location, underlying mutation, and malignant risk.

adrenal location *(64)*. Differences in biochemical phenotypes can therefore provide supplementary information to guide the focus of imaging studies and assist with interpretation of imaging results.

HEREDITARY CHROMAFFIN CELL TUMORS

It is now recognized that at least one-third of all PPGLs have a genetic basis due to mutations of over 14 tumor susceptibility genes identified to date *(8)*. MEN 2, neurofibromatosis type 1 (NF1), von Hippel-Lindau (VHL) syndrome, and familial paraganglioma syndromes due mutations of succinate dehydrogenase subunits B and D (SDHB and SDHD) represent the 5 main hereditary syndromes causing PPGLs among a diverse variety of other neoplasms. As shown by measurements of metanephrines in plasma and urine, these syndromes are characterized by distinct catecholamine biochemical profiles *(74, 75)*. Tumors due to mutations of neurofibromin 1 (*NF1*)[4] and ret proto-oncogene (*RET*) genes show increases in metanephrine, usually with additional increases in normetanephrine (Fig. 6). In contrast, tumors due to von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase (*VHL*) and succinate dehydrogenase complex, subunit B, iron sulfur (Ip) (*SDHB*) or succinate dehydrogenase complex, subunit D, integral membrane protein (*SDHD*) mutations lack increases in metanephrine and in patients with *VHL* mutations are characterized by solitary increases in normetanephrine; additional increases in methoxytyramine characterize 70% of tumors due to mutations of *SDHD* and *SDHB*.

The above observations have several implications for clinical management of patients with PPGLs. Because current guidelines indicate consideration of genetic testing in all patients with PPGLs, different patterns in biochemical test results can be used to determine which genes are the most appropriate to test *(8)*. For patients screened for PPGLs due to a mutation of a known tumor-susceptibility gene, emphasis during interpretation of results should be placed on the catecholamine metabolites expected to be increased according to the mutation; for example, testing should include measurements of both normetanephrine and methoxytyramine during screening of patients with *SDHB* and *SDHD* mutations.

METASTATIC DISEASE

Currently there are few established biomarkers for predicting or establishing metastatic disease in patients with PPGLs; the diagnosis remains reliant on evidence of metastases, usually obtained from imaging studies. Some studies have suggested that increases in urine dopamine could be useful for predicting metastatic disease *(76, 77)*. More recently, increases of plasma methoxytyramine have been shown to provide a much better predictor of the presence of metastatic PPGLs than urine dopamine, the latter formed mainly from renal uptake and decarboxylation of circulating L-Dopa *(78)*. Together with the presence of *SDHB* mutations as well as size and extraadrenal location of primary tumors, increases in plasma methoxytyramine provide useful information to assess the likelihood of metastatic disease (Fig. 6).

As subsequently illustrated in a small series of 64 patients with PPGLs, including 14 with metastatic disease, measurements of plasma methoxytyramine by LC-MS/MS provided a diagnostic sensitivity of 86% and specificity of 96% for identifying patients with malignancy *(19)*. Although not offering sufficient sensitivity to identify all patients with metastatic PPGLs, measurements of plasma methoxytyramine offer the most promising biomarker of malignant pheochromocytoma identified to date. These measurements should be considered for all patients at risk for metastatic PPGLs.

[4] Human genes: *NF1*, neurofibromin 1; *RET*, ret proto-oncogene; *VHL*, von Hippel-Lindau tumor suppressor, E3 ubiquitin protein ligase; *SDHB*, succinate dehydrogenase complex, subunit B, iron sulfur (Ip); *SDHD*, succinate dehydrogenase complex, subunit D, integral membrane protein.

Perspective

Advances in measurement methods and understanding of catecholamine metabolism now make biochemical diagnosis of PPGLs relative simple. Other related advances also mean it is now possible to glean considerably more information from laboratory evaluations than simply the presence or absence of a tumor. However, for use of plasma metanephrines, there remain considerable problems related to the transfer of the technology from research and development to the routine laboratory and clinical environment. Because of widespread use of inappropriate reference intervals, patients with PPGLs are at risk of being missed by a measurement method that is assumed to provide high diagnostic sensitivity. Use of inappropriate sampling conditions, leading to an overabundance of false-positive results and reduced motivation for patient follow-up, presents additional problems.

The above problems are best attacked with a 2-pronged benchside and bedside approach. At the benchside there is a need to provide the most accurate and precise test results possible, but with consideration of appropriately determined reference intervals and interpretative assistance. At the bedside there is a need for improved education and consideration of preanalytical precautions to reduce proportions of false-positive results. Physicians, however, prefer a test that is simple and without encumbrances. Measurements of plasma free metanephrines do not satisfy this preference unless they are obtained by use of procedures that severely compromise diagnostic performance. Although measurements of fractionated metanephrines in 24-h urine collections are inconvenient for patients, they are convenient for clinical staff and may be preferable for mainstream use. Measurements of urine free metanephrines, rather than urine deconjugated metanephrines, and use of spot first-morning urine samples, rather than 24-h collections, offer possible solutions to existing problems with urine collections. Such advances might also bring diagnostic test performance of the urine test to the same level as the plasma test.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: None declared.
Consultant or Advisory Role: None declared.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: M. Peitzsch, Deutsche Forschungsgesellschaft (EI855/1-1) and European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 259735 (project ENS@T-Cancer).
Expert Testimony: None declared.
Patents: None declared.

References

1. Lenders JW, Eisenhofer G, Mannelli M, Pacak K. Phaeochromocytoma. Lancet 2005;366:665–75.
2. Manger WM. The protean manifestations of pheochromocytoma. Horm Metab Res 2009;41:658–63.
3. Prejbisz A, Lenders JW, Eisenhofer G, Januszewicz A. Cardiovascular manifestations of phaeochromocytoma. J Hypertens 2011;29:2049–60.
4. Rosano TG, Swift TA, Hayes LW. Advances in catecholamine and metabolite measurements for diagnosis of pheochromocytoma. Clin Chem 1991;37:1854–67.
5. Bravo EL, Tarazi RC, Gifford RW, Stewart BH. Circulating and urinary catecholamines in pheochromocytoma. Diagnostic and pathophysiologic implications. NEJM 1979;301:682–6.
6. Lenders JW, Eisenhofer G, Armando I, Keiser HR, Goldstein DS, Kopin IJ. Determination of metanephrines in plasma by liquid chromatography with electrochemical detection. Clin Chem 1993;39:97–103.
7. Eisenhofer G, Huynh TT, Hiroi M, Pacak K. Understanding catecholamine metabolism as a guide to the biochemical diagnosis of pheochromocytoma. Rev Endocr Metab Disord 2001;2:297–311.
8. Lenders JWM, Duh QY, Eisenhofer G, Gimenez-Roqueplo AP, Grebe SK, Murad MH, et al. Pheochromocytoma and paraganglioma: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2014;99:1915–42.
9. Lenders JW, Pacak K, Walther MM, Linehan WM, Mannelli M, Friberg P, et al. Biochemical diagnosis of pheochromocytoma: which test is best? JAMA 2002;287:1427–34.
10. Unger N, Pitt C, Schmidt IL, Walz MK, Schmid KW, Philipp T, et al. Diagnostic value of various biochemical parameters for the diagnosis of pheochromocytoma in patients with adrenal mass. Eur J Endocrinol 2006;154:409–17.
11. Hickman PE, Leong M, Chang J, Wilson SR, McWhinney B. Plasma free metanephrines are superior to urine and plasma catecholamines and urine catecholamine metabolites for the investigation of phaeochromocytoma. Pathology 2009;41:173–7.
12. Pussard E, Chaouch A, Said T. Radioimmunoassay of free plasma metanephrines for the diagnosis of catecholamine-producing tumors. Clin Chem Lab Med 2014;52:437–44.
13. Kudva YC, Sawka AM, Young WF, Jr. Clinical review 164: The laboratory diagnosis of adrenal pheochromocytoma: the mayo clinic experience. J Clin Endocrinol Metab 2003;88:4533–9.
14. Eisenhofer G, Pacak K, Maher ER, Young WF, de Krijger RR. Pheochromocytoma. Clin Chem 2013;59:466–72.
15. Lagerstedt SA, O'Kane DJ, Singh RJ. Measurement of plasma free metanephrine and normetanephrine by liquid chromatography-tandem mass spectrometry for diagnosis of pheochromocytoma. Clin Chem 2004;50:603–11.
16. Whiting MJ. Simultaneous measurement of urinary metanephrines and catecholamines by liquid chromatography with tandem mass spectrometric detection. Ann Clin Biochem 2009;46:129–36.
17. Grebe SK, Singh RJ. LC-MS/MS in the clinical laboratory—where to from here? Clin Biochem Rev 2011;32:5–31.
18. Peitzsch M, Pelzel D, Glockner S, Prejbisz A, Fassnacht M, Beuschlein F, et al. Simultaneous liquid chromatography tandem mass spectrometric determination of urinary free metanephrines and catecholamines, with comparisons of free and deconjugated metabolites. Clin Chim Acta 2013;418:50–8.
19. Peitzsch M, Prejbisz A, Kroiss M, Beuschlein F, Arlt W, Januszewicz A, et al. Analysis of plasma

3-methoxytyramine, normetanephrine and metanephrine by ultraperformance liquid chromatography-tandem mass spectrometry: utility for diagnosis of dopamine-producing metastatic phaeochromocytoma. Ann Clin Biochem 2013;50:147–55.
20. Goldstein DS, Swoboda KJ, Miles JM, Coppack SW, Aneman A, Holmes C, et al. Sources and physiological significance of plasma dopamine sulfate. J Clin Endocrinol Metab 1999;84:2523–31.
21. Simonin J, Gerber-Lemaire S, Centeno C, Seghezzi C, Iglesias K, Abid K, Grouzmann E. Synthetic calibrators for the analysis of total metanephrines in urine: revisiting the conditions of hydrolysis. Clin Chim Acta 2012;413:998–1003.
22. Glauser M, Metrailler M, Gerber-Lemaire S, Centeno C, Seghezzi C, Dunand M, et al. Enzyme and acid deconjugation of plasma sulfated metanephrines. Clin Chim Acta 2014;430:125–8.
23. Davison AS. Urinary free metanephrines and suitability of available quality control material. Clin Chim Acta 2013;424:83–4.
24. Davidson DF. Phaeochromocytoma with normal urinary catecholamines: the potential value of urinary free metadrenalines. Ann Clin Biochem 2002;39:557–66.
25. Boyle JG, Davidson DF, Perry CG, Connell JM. Comparison of diagnostic accuracy of urinary free metanephrines, vanillyl mandelic acid, and catecholamines and plasma catecholamines for diagnosis of pheochromocytoma. J Clin Endocrinol Metab 2007;92:4602–8.
26. Pamporaki C, Därr R, Bursztyn M, Glockner S, Bornstein SR, Lenders JW, et al. Plasma-free vs deconjugated metanephrines for diagnosis of phaeochromocytoma. Clin Endocrinol 2013;79:476–83.
27. Pillai D, Callen S. Pilot quality assurance programme for plasma metanephrines. Ann Clin Biochem 2010;47:137–42.
28. Peaston RT, Graham KS, Chambers E, van der Molen JC, Ball S. Performance of plasma free metanephrines measured by liquid chromatography-tandem mass spectrometry in the diagnosis of pheochromocytoma. Clin Chim Acta 2010;411:546–52.
29. Mullins F, O'Shea P, Fitzgerald R, Tormey W. Enzyme-linked immunoassay for plasma-free metanephrines in the biochemical diagnosis of phaeochromocytoma in adults is not ideal. Clin Chem Lab Med 2011;50:105–10.
30. Willemsen JJ, Sweep CG, Lenders JW, Ross HA. Stability of plasma free metanephrines during collection and storage as assessed by an optimized HPLC method with electrochemical detection. Clin Chem 2003;49:1951–3.
31. Willemsen JJ, Ross HA, Lenders JW, Sweep FC. Stability of urinary fractionated metanephrines and catecholamines during collection, shipment, and storage of samples. Clin Chem 2007;53:268–72.
32. Pacak K, Eisenhofer G, Ahlman H, Bornstein SR, Gimenez-Roqueplo AP, Grossman AB, et al. Pheochromocytoma: recommendations for clinical practice from the first international symposium. Nat Clin Pract Endocrinol Metab 2007;3:92–102.
33. Därr R, Pamporaki C, Peitzsch M, Miehle K, Prejbisz A, Peczkowska M, et al. Biochemical diagnosis of phaeochromocytoma using plasma-free normetanephrine, metanephrine and methoxytyramine: importance of supine sampling under fasting conditions. Clin Endocrinol 2014;80:478–86.
34. Eisenhofer G, Friberg P, Pacak K, Goldstein DS, Murphy DL, Tsigos C, et al. Plasma metadrenalines: Do they provide useful information about sympatho-adrenal function and catecholamine metabolism? Clin Sci (Lond) 1995;88:533–42.
35. Sawka AM, Thabane L, Gafni A, Levine M, Young WF, Jr. Measurement of fractionated plasma metanephrines for exclusion of pheochromocytoma: can specificity be improved by adjustment for age? BMC Endocr Disord 2005;5:1.
36. Eisenhofer G, Lattke P, Herberg M, Siegert G, Qin N, Därr R, et al. Reference intervals for plasma free metanephrines with an age adjustment for normetanephrine for optimized laboratory testing of phaeochromocytoma. Ann Clin Biochem 2013;50:62–9.
37. Kairisto V, Koskinen P, Mattila K, Puikkonen J, Virtanen A, Kantola I, Irjala K. Reference intervals for 24-h urinary normetanephrine, metanephrine, and 3-methoxy-4-hydroxymandelic acid in hypertensive patients. Clin Chem 1992;38:416–20.
38. Weise M, Merke DP, Pacak K, Walther MM, Eisenhofer G. Utility of plasma free metanephrines for detecting childhood pheochromocytoma. J Clin Endocrinol Metab 2002;87:1955–60.
39. Fitzgibbon M, FitzGerald RJ, Tormey WP, O'Meara A, Kenny D. Reference values for urinary HMMA, HVA, noradrenaline, adrenaline, and dopamine excretion in children using random urine samples and HPLC with electrochemical detection. Ann Clin Biochem 1992;29:400–4.
40. Pussard E, Neveux M, Guigueno N. Reference intervals for urinary catecholamines and metabolites from birth to adulthood. Clin Biochem 2009;42:536–9.
41. Griffin A, O'Shea P, FitzGerald R, O'Connor G, Tormey W. Establishment of a paediatric age-related reference interval for the measurement of urinary total fractionated metanephrines. Ann Clin Biochem 2011;48:41–4.
42. Davidson DF, Hammond PJ, Murphy DL, Carachi R. Age-related medical decision limits for urinary free (unconjugated) metadrenalines, catecholamines and metabolites in random urine specimens from children. Ann Clin Biochem 2011;48:358–66.
43. Yu R, Wei M. False positive test results for pheochromocytoma from 2000 to 2008. Exp Clin Endocrinol Diabetes 2010;118:577–85.
44. Eisenhofer G, Goldstein DS, Walther MM, Friberg P, Lenders JW, Keiser HR, Pacak K. Biochemical diagnosis of pheochromocytoma: how to distinguish true- from false-positive test results. J Clin Endocrinol Metab 2003;88:2656–66.
45. Lenders JW, Willemsen JJ, Eisenhofer G, Ross HA, Pacak K, Timmers HJ, Sweep CG. Is supine rest necessary before blood sampling for plasma metanephrines? Clin Chem 2007;53:352–4.
46. de Jong WH, Eisenhofer G, Post WJ, Muskiet FA, de Vries EG, Kema IP. Dietary influences on plasma and urinary metanephrines: implications for diagnosis of catecholamine-producing tumors. J Clin Endocrinol Metab 2009;94:2841–9.
47. Deutschbein T, Unger N, Jaeger A, Broecker-Preuss M, Mann K, Petersenn S. Influence of various confounding variables and storage conditions on metanephrine and normetanephrine levels in plasma. Clin Endocrinol 2010;73:153–60.
48. Pamporaki C, Bursztyn M, Reimann M, Ziemssen T, Bornstein SR, Sweep FC, et al. Seasonal variation in plasma free normetanephrine concentrations: implications for biochemical diagnosis of pheochromocytoma. Eur J Endocrinol 2014;170:349–57.
49. Anas SS, Vasikaran SD. An audit of management of patients with borderline increased plasma-free metanephrines. Ann Clin Biochem 2010;47:554–8.
50. Peaston RT, Lennard TW, Lai LC. Overnight excretion of urinary catecholamines and metabolites in the detection of pheochromocytoma. J Clin Endocrinol Metab 1996;81:1378–84.
51. Peaston RT, Weinkove C. Measurement of catecholamines and their metabolites. Ann Clin Biochem 2004;41:17–38.
52. Harding JL, Yeh MW, Robinson BG, Delbridge LW, Sidhu SB. Potential pitfalls in the diagnosis of phaeochromocytoma. Med J Aust 2005;182:637–40.
53. Neary NM, King KS, Pacak K. Drugs and pheochromocytoma—don't be fooled by every elevated metanephrine. NEJM 2011;364:2268–70.
54. Lefebvre H, Noblet C, Moore N, Wolf LM. Pseudophaeochromocytoma after multiple drug interactions involving the selective monoamine oxidase inhibitor selegiline. Clin Endocrinol 1995;42:95–8.
55. Davidson DF, Grosset K, Grosset D. Parkinson's disease: The effect of L-dopa therapy on urinary free catecholamines and metabolites. Ann Clin Biochem 2007;44:364–8.
56. Eisenhofer G, Brown S, Peitzsch M, Pelzel D, Lattke P, Glockner S, et al. Levodopa therapy in Parkinson's disease: influence on liquid chromatographic tandem mass spectrometric-based measurements of plasma and urinary normetanephrine, metanephrine and methoxytyramine. Ann Clin Biochem 2014;51:38–46.
57. Davidson FD. Paracetamol-associated interference in an HPLC-ECD assay for urinary free metadrenalines and catecholamines. Ann Clin Biochem 2004;41:316–20.
58. Ito T, Imai T, Kikumori T, Shibata A, Horiba T, Kobayashi H, et al. Adrenal incidentaloma: review of 197 patients and report of a drug-related false-positive urinary normetanephrine result. Surg Today 2006;36:961–5.
59. Bouhanick B, Fauvel J, Pont F. Biochemical misdiagnosis of pheochromocytoma in patients treated with sulfasalazine. JAMA 2010;304:1898–901.
60. van Laarhoven HW, Willemsen JJ, Ross HA, Beex LV, Lenders JW, Sweep FC. Pitfall in HPLC assay for urinary metanephrines: An unusual type of interference caused by methenamine intake. Clin Chem 2004;50:1097–9.
61. Madhavaram H, Woollard GA. Interference from Indian diet on the internal standard in a commercial method for the measurement of urinary metanephrines by high-performance liquid chromatography with electrochemical detection. Ann Clin Biochem 2014;51:400–5.

62. Twentyman JM, Cradic KW, Singh RJ, Grebe SK. Ionic cross talk can lead to overestimation of 3-methoxytyramine during quantification of metanephrines by mass spectrometry. Clin Chem 2012;58:1156–8.
63. Dunand M, Donzelli M, Rickli A, Hysek CM, Liechti ME, Grouzmann E. Analytical interference of 4-hydroxy-3-methoxymethamphetamine with the measurement of plasma free normetanephrine by ultra-high pressure liquid chromatography-tandem mass spectrometry. Clin Biochem 2014;47:1121–3.
64. Eisenhofer G, Goldstein DS, Sullivan P, Csako G, Brouwers FM, Lai EW, et al. Biochemical and clinical manifestations of dopamine-producing paragangliomas: utility of plasma methoxytyramine. J Clin Endocrinol Metab 2005;90:2068–75.
65. Timmers HJ, Pacak K, Huynh TT, Abu-Asab M, Tsokos M, Merino MJ, et al. Biochemically silent abdominal paragangliomas in patients with mutations in the SDHB gene. J Clin Endocrinol Metab 2008;93:4826–32.
66. Hernandez FC, Sanchez M, Alvarez A, Diaz J, Pascual R, Perez M, et al. A five-year report on experience in the detection of pheochromocytoma. Clin Biochem 2000;33:649–55.
67. Vaclavik J, Stejskal D, Lacnak B, Lazarova M, Jedelsky L, Kadalova L, et al. Free plasma metanephrines as a screening test for pheochromocytoma in low-risk patients. J Hypertens 2007;25:1427–31.
68. Brain KL, Kay J, Shine B. Measurement of urinary metanephrines to screen for pheochromocytoma in an unselected hospital referral population. Clin Chem 2006;52:2060–4.
69. Terzolo M, Bovio S, Pia A, Reimondo G, Angeli A. Management of adrenal incidentaloma. Best Pract Res Clin Endocrinol Metab 2009;23:233–43.
70. Howe JR, Norton JA, Wells SA, Jr. Prevalence of pheochromocytoma and hyperparathyroidism in multiple endocrine neoplasia type 2a: Results of long-term follow-up. Surgery 1993;114:1070–7.
71. Amar L, Fassnacht M, Gimenez-Roqueplo AP, Januszewicz A, Prebjbisz A, Timmers H, Plouin PF. Long-term postoperative follow-up in patients with apparently benign pheochromocytoma and paraganglioma. Horm Metab Res 2012;44:385–9.
72. Stenstrom G, Waldenstrom J. Positive correlation between urinary excretion of catecholamine metabolites and tumour mass in pheochromocytoma. Results in patients with sustained and paroxysmal hypertension and multiple endocrine neoplasia. Acta Med Scand 1985;217:73–7.
73. Eisenhofer G, Lenders JW, Goldstein DS, Mannelli M, Csako G, Walther MM, et al. Pheochromocytoma catecholamine phenotypes and prediction of tumor size and location by use of plasma free metanephrines. Clin Chem 2005;51:735–44.
74. Eisenhofer G, Lenders JW, Timmers H, Mannelli M, Grebe SK, Hofbauer LC, et al. Measurements of plasma methoxytyramine, normetanephrine, and metanephrine as discriminators of different hereditary forms of pheochromocytoma. Clin Chem 2011;57:411–20.
75. Davidson DF, Bradshaw N, Perry CG, Lindsay R, Freel EM. Urinary free (unconjugated) metadrenalines in different hereditary forms of catecholamine-secreting phaeochromocytoma/paraganglioma. Ann Clin Biochem 2012;49:486–90.
76. John H, Ziegler WH, Hauri D, Jaeger P. Pheochromocytomas: can malignant potential be predicted? Urology 1999;53:679–83.
77. van der Harst E, de Herder WW, de Krijger RR, Bruining HA, Bonjer HJ, Lamberts SW, et al. The value of plasma markers for the clinical behaviour of phaeochromocytomas. Eur J Endocrinol 2002;147:85–94.
78. Eisenhofer G, Lenders JW, Siegert G, Bornstein SR, Friberg P, Milosevic D, et al. Plasma methoxytyramine: a novel biomarker of metastatic pheochromocytoma and paraganglioma in relation to established risk factors of tumour size, location and SDHB mutation status Eur J Cancer 2012;48:1739–49.