Evolving Clinical Presentation and Assessment of Pheochromocytoma: A Review

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ABSTRACT

Pheochromocytoma (PHEO) is a neuroendocrine lesion in the adrenal medulla composed of chromaffin cells producing excess amount of catecholamines. These tumoral cells have the property to synthesize, metabolize, store, and secrete catecholamines and their metabolites. The clinical symptomatology is derived from the peripheral tissue effect of norepinephrine, epinephrine, and their by-products. Morbidity and mortality is increased due to the delay in the diagnosis and treatment. A high index of suspicion leads to testing for PHEO through biochemical, imaging, and genetic studies. Dilemma in its assessment comes about when the clinical picture is beset by too much catecholamine secretory periodicity, too little catecholamine secretion, in lesions less than 1 cm, in exclusively dopamine-secreting tumors, and in the unavailability of biochemical tests and imaging.

The aim of this review is to focus on the progress in the approach of early diagnosis of pheochromocytoma through improved clinical, biochemical, and imaging modalities. Emphasis is made on the early recognition of evolving clinical presentations, with the introduction of cardiovascular imaging, 2D echocardiogram, and cardiac MRI in the early diagnosis of patients with no risk factors and with equivocal biochemical and imaging results yet present with cardiovascular events. From the data reviewed and presented, several algorithms are proposed by the authors as an easy guide for clinicians in the diagnostic approach of pheochromocytoma.

Keywords: pheochromocytoma, catecholamines, metanephrines, methoxytyramine

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INTRODUCTION

Pheochromocytoma (PHEO) is a rare adrenomedullary tumor causing secondary hypertension, with an incidence of 0.1-0.6% (1-5). These tumors can synthesize, metabolize, store, and secrete catecholamines and their metabolites (6). A high index of clinical suspicion remains the pivotal point to initiate biochemical studies, particularly in those patients with certain patterns of spells, blood pressure elevations (paroxysmal or alternating with hypotension), drug-resistant hypertension, sudden palpitations with or without pallor, unexplained sweating particularly at night or in cold weather, unexplained hyperglycemia, and a hereditary predisposition for PHEO (7-13).

Although biochemical testing for PHEO is indicated for symptomatic patients as described above, it

is also indicated for patients with incidentally found adrenal lesions or identified genetic predispositions or syndromic presentations pointing towards a high likelihood to develop PHEO (e.g., in patients with multiple endocrine neoplasia type 2 [*MEN2*], von Hippel-Lindau syndrome [*VHL*], neurofibromatosis type 1 [*NF1*], mutations of the succinate dehydrogenase genes [*SDHB, SDHD*], and hypoxia-induced factor 2A [*HIF2A*]-related PHEO-polycythemia syndrome) (14-23). Only after PHEO is biochemically proven should imaging be performed. Current imaging modalities include anatomical (CT, MRI) and functional (molecular) imaging procedures using various radiopharmaceuticals, depending on the clinical situation. If a detailed clinical assessment together with well-thought and appropriate diagnostic approaches is not applied, consequences from improper or delayed diagnosis of PHEO almost always occur. This may lead to catastrophic consequences from sudden catecholamine release and their impact on cardiovascular and other systems, including lethal tachyarrhythmia, myocardial infarction, stroke, or death (24), and significant myocardial dysfunction persisting even after normalization of catecholamine levels postoperatively (25).

During the last few years, enormous progress has been made in the diagnosis of PHEO. These new discoveries include the inclusion of 3-methoxytyramine in the biochemical diagnosis (26-29), new reference values for seating and standing metanephrine levels, new reference values for children (11), metabolite profiling (metabolomics) and evaluation of relationships between metabotypes and genotypes (30), *in vivo* proton magnetic spectroscopy for the assessment of catecholamines and succinate, the use of new functional imaging modalities particularly somatostatin analogs radiolabeled with gallium-68 (^{68}Ga-DOTA-SSA) (31) in the localization of PHEO, and finally the advancement in the identification and characterization of new susceptible genes related to disruption of HIF degradation, such as prolyl hydroxylase (PHD) and HIF mutations (21,32), mutations in chromatin remodeling genes, e.g. *MERTK, MET*, and *H3F3A* (33), and disruption in DNA copy numbers (34). Also, new therapeutic approaches are on the horizon focusing on HIF-2β inhibitors, hypomethylating agents, and ^{177}Lu-DOTATATE for peptide receptor radionuclide therapy (PRRT) and precision medicine approach (35).

This review is undertaken to provide insight on the evolving clinical presentation of PHEO and to come up with diagnostic algorithms that will guide clinicians for early identification of the evolving clinical presentation and timely assessment of PHEO.

The Sympathoadrenal (Sa) Cell Lineage and the Adrenal Medulla

The SA cells are a sub-lineage of the neural crest giving rise to neuroendocrine chromaffin cells in the adrenal medulla and extra-adrenal neurons clinically called paraganglia (36). These SA derivatives have the common characteristics to synthesize, store, and release catecholamines. The migration of these cells is found along the sympathetic ganglia from the neck, mediastinum, and abdomen, down to the urinary system. The sympathetic neurons and chromaffin cells share the same progenitor in the neural crest. BMP-4 has shown to be the major induction factor for maturation of SA progenitor cells (Figure 1) (37). The differentiation of the SA cellular lineage to sympathetic neurons and chromaffin cells may be due to inherent environmental influence but it remains unclear. However, it is during this phase of NC cells migration in the presence of established transcription factors (TFs) including MASH-1, Phox2a, Phox2b, Hand2, Gata2/3 and Insm1 that they acquire catecholaminergic features and phenotypes (38-40). Other hypothetical suggested TFs are NOTCH-signaling, HAIRY1/2/3, and DELTA1, SERRATE, and NUMB, Inscuteable/dlg1 (40-41).

The temporal triggering events for specific chromaffin cells remain debatable due to lack of appropriate markers. The final target regions such as the adrenal medulla, paraganglia, and sympathetic ganglia have been demonstrated to be influenced by glucocorticoids (GCs) (42-44). The GC signaling has shown to not originate specifically from adrenal cortex and even present in GC-deficient mice. Several crosstalk pathways between GC/GR signaling, GR/MAPK pathways, IGF-1, FGF-2, and NGF receptor trkA have been described (45,46), which is vital for chromaffin survival. GC signaling is likewise crucial in the induction of adrenaline synthesizing enzyme phenyl ethanolamine-*N*-methytransferase (PNMT), so that GC-deficient mice only produce noradrenaline (46). In GC receptor knockout mice, chromaffin vesicles are intact and can still be identified by

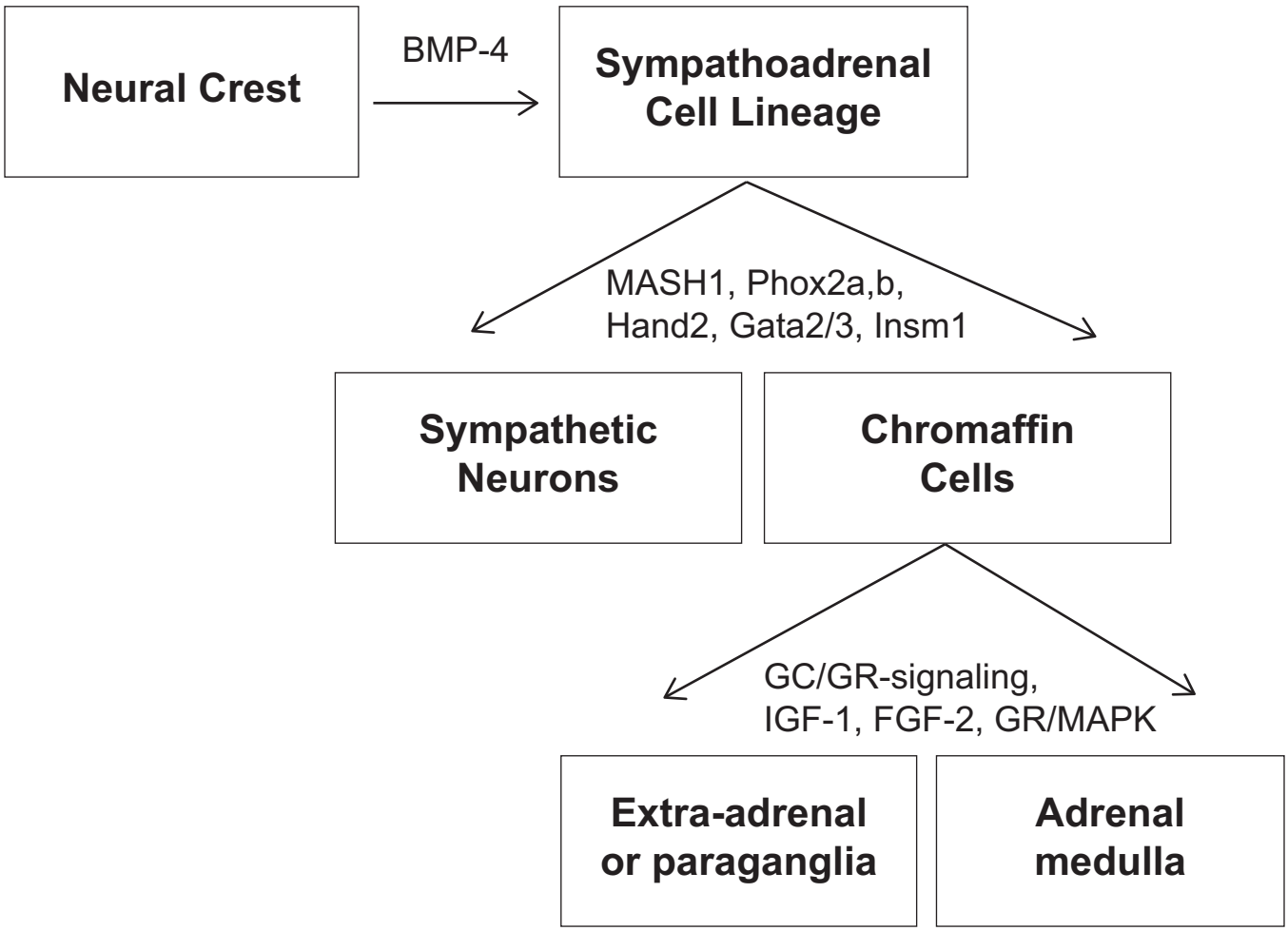

Figure 1 Sympathoadrenal cell lineage development to become adrenal and extra-adrenal chromaffin cells through different signaling pathways. BMP-4 has shown to be the major induction factor for maturation of SA progenitor cells. In the presence of transcription factors MASH-1, Phox2a, Phox2b, Hand2, Gata2/3 and Insm1 during the migration of chromaffin cells, they acquire catecholaminergic features and phenotype. Several crosstalk pathways between GC/GR signaling, GR/MAPK pathways, IGF-1, FGF-2, and NGF receptor trkA have been described which is vital for chromaffin survival. GC-signaling is likewise crucial in the induction of adrenaline synthesizing enzyme phenyl ethanolamine-N-methytransferase (PNMT).

molecular markers such as neuropeptides, transporters, and chromogranin B (47).

Catecholamine Synthesis and Metabolism: Physiologic Vs. Pathologic in Pheochromocytoma

The mother substance for catecholamine synthesis is amino acid L-tyrosine. Tyrosine is derived from the diet or synthesized from phenylalanine. Synthesis starts at the rate-limiting step of conversion of tyrosine 3,4-dihydroxyphenylalanine (DOPA) by the enzyme tyrosine hydroxylase (TH) (Figure 2A) (48). Conversion of DOPA to dopamine (DA) is catalyzed by aromatic L-amino acid decarboxylase (AADC). The DA formed in the cytoplasm by AADC is transported into vesicular storage granules. In dopaminergic neurons, DA is released without any further conversion to norepinephrine (NE), but in noradrenergic neurons and adrenal chromaffin cells DA is further converted to NE by dopamine β-hydroxylase (DBH) (16,49). The enzyme is present in vesicular storage granules, either bound to the vesicular membrane or present in the solute matrix core. In adrenomedullary cells, NE stored in vesicular storage granules can leak into the cytosol where enzyme phenylethanolamine N-methyltransferase (PNMT) is exclusively present and converts NE to epinephrine (E) (16,50). The formed E is translocated back into chromaffin granules (17).

Another major enzyme vital in the metabolism of catecholamines is catechol-O-methyltransferase (COMT) which is present both extramedullary and

intramedullary for conversion of DA to methoxytyramine (MTY), NE to normetanephrine (NMN), and E to metanephrine (MN) (51). Ninety percent of MN and 23% of NMN in the circulation come from this pathway (51,52). Sympathetic nerves contain the enzyme monoamine oxidase (MAO) which deaminates NE to 3-4-dihydroxyphenylglycol (DHPG). DHPG is methylated by COMT to 3-methoxy-4-hydroxyphenylglycol (MHPG), which is converted by aldehyde dehydrogenase (AD) in the liver to the metabolite vanillylmandelic acid (VMA), an end product of catecholamine metabolism in human urine (53,54). The O-methylated metabolites of NE, E, and DA are continuously released from PHEO and become major parameters to assess tumoral activity (27,55-59).

Apart from being excreted in the urine, the free metanephrines are also conjugated in the wall of the gastrointestinal tract by the enzyme sulfotransferase family, cytosolic, 1A, phenol-preferring, member 3 (SULT1A3) (60). These sulfate-conjugated forms of metanephrines prolong their plasma half-lives 30x higher than the free forms (61,62). Measurements of urine metanephrines utilize an acid hydrolysis step to convert the sulfate-conjugated metabolites to free forms. Thus, such measurements reflect the total metanephrines (Figure 2B) (6).

In PHEO, there are elevations of catecholamines and their metabolites specific for locations of lesions (adrenomedullary and extra-adrenal), abnormal tumor enzymatic activity (increased normetanephrine, metanephrine, and methoxytyramine) and altered pathway mechanisms (6).

Early Diagnosis of Pheochromocytoma

The two major factors for increased morbidity and mortality in PHEO are delay in the diagnosis (63) and late detection of metastasis (25). Recent developments addressed these concerns, such as improved biochemical analytical procedures (6), analysis and recognition of evolving clinical presentations (13,64-66), inclusion of methoxytyramine in the work-up of PHEO (17,67), improved imaging modalities (67,68), and correlation of biochemical and imaging profiles with phenotype and genotype of the patients (11).

Improved Sample Preparation, Specific Reference Values and Advanced Laboratory Methods

Catecholamines are measured in plasma and urine in several forms-as unconjugated norepinephrine, epinephrine, and dopamine, or by their *O*-methylated metabolites-normetanephrine, metanephrine, and methoxytyramine (6). Research institutions and commercial laboratories in most countries do not ordinarily measure these hormones and, therefore, have to be sent out through courier. It is imperative that preparation, collection, handling, storage, and packaging of these specimens must be done with utmost care, caution, and precision so as not to alter the true values of the hormones (69).

Prior to sample analysis, various important factors must be assessed in order to avoid or minimize false-positive and false-negative results, thus yielding better diagnostic accuracy. These factors are age, position of the patient during blood extraction, immediate dietary intake, and current medications. (Table 1) (6,11,70). Values of plasma catecholamines and metanephrines have shown to approximate tumoral activity if blood extraction is done in the supine position (69,70-74). Dietary restrictions for a tyramine-rich diet (cheese, nuts, cereal, beer, wine) are made mainly for the measurement of 3-methoxytyramine (MTY), a dopamine metabolite, and blood sample must be collected after an overnight fast (71).

Comorbidities have been reported to influence plasma and urine MN and NMN results, such as renal failure, stroke or intracerebral hemorrhage, decompensated congestive heart failure and obstructive sleep apnea (75). Stabilization of comorbidities is imperative to avoid false low (renal failure) or inadvertently high values (decompensated heart failure, stroke, obstructive sleep apnea). (Table 2). Plasma metanephrine has shown to be least affected by these conditions (75).

Diagnostic specificity and sensitivity of biochemical tests rely significantly on cut-offs of measured values of plasma catecholamines and their metabolites (76). Recently, Eisenhofer and his group established age-adjusted cut-offs of reference intervals for plasma normetanephrine and optimized cut-offs for metanephrine, minimizing false positive results, increasing diagnostic specificity to 96.0%, with minimal loss in diagnostic sensitivity of 93.6% (16,77). Plasma metanephrine, but not normetanephrine,

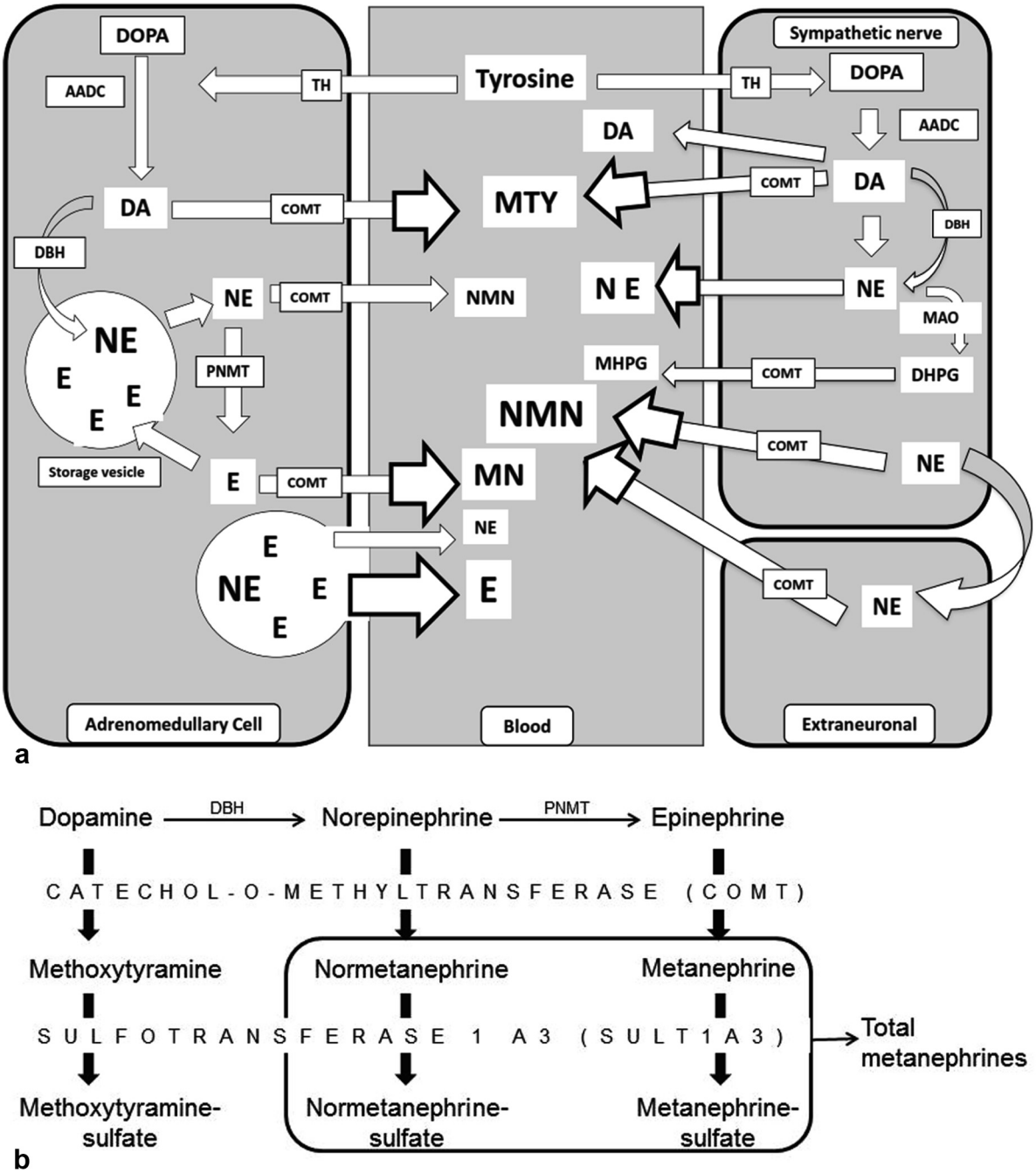

Figure 2 (A) Synthesis of catecholamines, namely, dopamine, epinephrine (E) and norepinephrine (NE) begins with the uptake of the amino acid L-tyrosine by adrenomedullary and sympathoneuronal cells. Tyrosine is derived from the diet or synthesized from phenylalanine and is converted to 3,4-dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase (TH), the rate-limiting step. L-DOPA is decarboxylysed to dopamine, which is actively transported to vesicles where intravesicular enzyme, dopamine-β-hydrolase (DPH) converts it to NE. NE leaks into the cytoplasm where phenylethanolamine N-methyltransferase (PNMT), an enzyme exclusive to adrenomedullary cells, converts it to E that is transported back into the vesicles. (B) The enzyme catechol-O-methyltransferase (COMT), which is present both extramedullary and intramedullary is responsible for conversion of DA to methoxytyramine (MT), NE to normetanephrine (NMN), and E to metanephrine (MN). Sympathetic nerves contain the enzyme monoamine oxidase (MAO) which deaminates NE to 3-4-dihydroxyphenylglycol (DHPG). DHPG is methylated by COMT to 3-methoxy-4-hydroxyphenylglycol (MHPG), which is converted by aldehyde dehydrogenase (AD) in the liver to the metabolite vanillylmandelic acid (VMA). These catechol O-methyl metabolites are produced in excess by tumorous chromaffin cells of pheochromocytoma in the adrenal, sympathetic neurons, and extraneuronal metastatic lesions. Metanephrines exist in plasma and urine in both free and sulfate-conjugated form. The sulfate-conjugated forms are catalyzed by a specific sulfotransferase enzyme, sulfotransferase family, cytosolic, 1A, phenol-preferring, member 3 (SULT1A3), which is found in gastrointestinal tissues. Measurements of urine metanephrines utilize an acid hydrolysis step to convert the sulfate-conjugated metabolites to free forms. Thus, such measurements reflect the total metanephrines.

Table 1 Sample preparation, collection, and storage for hormonal tests in pheochromocytoma

Hormonal test/s	Preparation & Collection	Storage
Norepinephrine & Epinephrine		
Plasma	Supine at least 20 mins with an indwelling cannula	Tubes with heparin or EDTA placed on ice and stored until -20°C, perform assay within 30 days
Urine		Containers with HCI light-proof containers. Storage at 4°C, long-term storage at -20°C or lower
Dopamine		
Plasma	Overnight fast. Avoid amine-rich foods* for 24 hrs. Supine at least 30 mins with an indwelling cannula	Tubes with heparin or EDTA placed on ice and stored until -20°C, perform assay within 30 days
Urine	Avoid amine-rich foods for 24 hrs	Containers with HCI light-proof containers. Storage at 4°C, long-term storage at -20°C or lower
Normetanephrine & Metanephrine		
Plasma	Supine at least 30 mins with an indwelling cannula	Tubes with heparin or EDTA placed on ice and stored until -20°C, perform assay within 30 days
Urine		Acidification of sample not needed. Light-proof containers. Storage at 4°C, long-term storage at -20°C or lower
Methoxytyramine		
Plasma	Overnight fast. Avoid amine-rich foods. Supine at least 30 mins with an indwelling cannula	Tubes with heparin or EDTA placed on ice and stored until -20°C, perform assay within 30 days
Urine	Avoid amine-rich foods	Acidification of sample not needed. Light-proof containers. Storage at 4°C, long-term storage at 20°C or lower

*Amine-rich foods: beer, wine, cheese, bananas, pineapple, nuts, cereals

Table 2 Precautionary measures in the sample preparation and biochemical interpretation of results in pheochromocytoma

Clinical Setting	Effect on Plasma and Urine NMN, MN or MTY	Precautionary Measures
Age	2x increase from childhood to 60 years old	Age-specific reference values
Position on blood extraction - seated vs. supine	Up to 30% increase in seated position in plasma NMN and MN	30 min rest before blood extraction
High amine-rich diet	Increase in urine NMN 2x increase in MTY	Avoid beer, wine, cheese, bananas, pineapple, nuts and cereals for 24 hours
Renal impairment	<4x increase in plasma NMN, <2x increase in MN	MN less affected. Correlate with other parameters
Essential hypertension	Up to 50% increase in plasma NMN and MN	Establish reference values
Decompensated congestive heart failure	2x to 4x increase in plasma NMN	Stabilize patient and repeat test. Plasma MN not affected
Stroke/Intracerebral hemorrhage	>2x increase in plasma NMN	Biochemical test one week after event
OSA	30% increase in urine NMN	Stabilize illness and repeat test. Plasma MN not affected

NMN; normetanephrine, MN; metanephrine, MTY; methoxytyramine, OSA; obstructive sleep apnea. Adapted from Dobri et al. 2014(75)

Table 3 Medians and reference intervals (2.5 and 97.5 percentiles) for plasma normetanephrine and metanephrine according to gender and six age groups

		Age (years)	Normetaneph-rine (nmol/L)	Metanephrine (nmol/L)				
	N	Median	Median	97.5 percentile	2.5 percentile	Median	97.5 percentile	2.5 percentile
All Subjects	1226	41.0	0.298	0.706	0.120	0.147	0.325	0.031
Women	679	40.2	0.293	0.710	0.125	0.132*	0.315	0.035
Men	547	41.0	0.302	0.704	0.120	0.170†	0.329	0.030
5-17y	116	13.2	0.248*	0.470	0.048	0.172†	0.333	0.045
18-29y	229	24.7	0.251*	0.588	0.118	0.137*	0.264	0.034
30-39y	232	34.5	0.273*†	0.618	0.126	0.138*	0.304	0.014
40-49y	283	45.0	0.300†	0.687	0.115	0.147*†	0.324	0.031
50-59y	241	53.0	0.362§	0.747	0.136	0.157†	0.375	0.046
>60y	125	65.4	0.355§	1.047	0.137	0.163†	0.358	0.051

• Presence of different symbols (*†§) indicates differences ($p<0.005$) in normetanephrine or metanephrine between men and women or among different age groups. Adapted from Eisenhofer et al. 20131(34)

Table 4 Diagnostic test performance of plasma metanephrines with different upper cut-offs and models adjusting for age

Model	Upper cut-offs (nmol/L)		Test performance	
	NMN	**MN**	**Sensitivity (%)**	**Specificity (%)**
Fixed – 97.5 percentiles	0.706	0.325	93.9*	88.3*
Age-dependent linear model	Variable	0.325	93.9*	91.2†
Age-dependent curvilin-ear model	Variable	0.325	93.7*	93.6§
Age-dependent curvilin-ear model	Variable	0.446	93.6*	96.0
Age-adjusted score model	NA	NA	79.5†	99.9#

• NMN, normetanephrine; MN, metanephrine; NA, not applicable (based on a score). Adapted from Eisenhofer et al. 2013(134).

was higher in men but reference interval did not differ. Upper cut-offs of reference intervals for normetanephrine increased from 0.47 nmol/L in children to 1.05 nmol in subjects older than 60 years (Table 3, Table 4) (16,77).

Equally important is the significant progress in the development of catecholamine assay methodology (6). Although immunoassays remain useful for measuring metanephrines (78,79) underestimation of plasma concentrations of metanephrines and normetanephrines have been reported (80). Recently, liquid chromatography with electrochemical detection (LC-ECD) or coupled to tandem mass spectrometry (LC-MS/MS) are currently becoming the preferred methods with favoring more of the latter (81-84). Aside from having superior accuracy and precision of catecholamine measurement with LC-ECD and LS-MS/MS (80-83), they allow fractionated measurements of normetanephrine and metanephrine versus colorimetric and fluorometric of total metanephrines (combined normetaphrine and metanephrine). Furthermore, there is an additional and important advantage of capability to measure 3-methoxytyramine (MTY) (80), a biomarker whose importance will be highlighted in a subsequent discussion.

Metabolomics, or global metabolite profiling, is a new technology of functional genomics used for

investigating metabolite changes associated with some gene mutations. LC-MS/MS, gas chromatography- mass spectrometry (GC-MS) (85), ultrahigh pressure liquid chromatography with tandem mass spectrometry (UPHPLC-MS/MS) (30), 1H nuclear magnetic resonance (NMR) spectroscopy (86) and recently, a new technique, the so-called ^{1}H high-resolution magic angle spinning (HRMAS) nuclear magnetic resonance (NMR) spectroscopy (87) have been employed with the advantages suited for a small sample of tissues with no chemical extraction and manipulation. The modality showed promising usefulness in the clinical assessment, specifically for SDHx-related tumors as a screening method and functional test for evaluating SDHx mutation of unknown pathogenicity (87).

Evolving Clinical Presentation of Pheochromocytoma

High index of suspicion remains the pivotal point to initiate biochemical and imaging studies in patients suspected to have PHEO. The clinical presentation is defined by the biochemical secretory characteristics of the lesion, NE, EPI, and their metabolites (15-19), dictated by enzymatic profile of the tumor (13). Basic knowledge on organ-specific roles of adrenoceptors is necessary in order to understand responses to catecholamines which are magnified in patients with PHEO due to excess secretion of the hormones and their metabolites (Table 5).

Based on ligand studies and their agonists and antagonists, adrenoceptors are classified into adrenergic (α1, α2, β1, β2, β3) and dopaminergic receptors (D1, D2) and their subtypes. Almost all tissues and organs of the body express these receptors (88). However, to date, there is no close relationship established between specific subtypes and signaling mechanisms. Each vascular structure may harbor mixtures of α1-adrenoceptor subtypes and may respond to the same stimuli at the same time (89-91). On the other hand, the maximal β-mediated vasodilatation varies from one vascular bed to another and depends on the tone of the tissue (92,93).

Norepinephrine mainly signals α1, α2, and β1 receptors, while epinephrine mainly signals β1 and β2 receptors. Normally dopamine does not affect the adrenergic receptors, but with increased plasma concentrations, it can stimulate both α and β receptors (13).

In general, alpha-1 receptors, mostly found in smooth muscle, peripheral arteries and veins cause vasoconstriction upon stimulating and increasing systemic pressure. In PHEO, manifestations include hypertension, headache, and pallor. Stimulation of α2-adrenergic receptors located on smooth muscles will result in arterial vasodilation and coronary vasoconstriction; in PHEO typical manifestations may include diaphoresis and orthostatic hypotension. Stimulation

Table 5 Main actions of catecholamines on the various receptors and their common manifestations in pheochromocytoma

Target organ system	Receptor types	Sympathetic action	Common manifestations in pheochromocytoma
Skin and mucosa	α1, α2	Vasoconstriction, localized secretion of sweat glands	Pallor, diaphoresis
Peripheral vascular	α1, α2, β2	Vasoconstriction α1, α2 Vasodilation β2	Hypertension
Orthostatic hypotension			
Brain	α1, D1	Vasoconstriction	Headache
Heart	β1, β2, D1	Increase in heart rate, contractility, automaticity, conduction velocity	Palpitations, tachycardia, angina
Lungs	α1, β2	Pulmonary arteriole vasoconstriction, tracheal and bronchial muscle relaxation	Dyspnea
Gastrointestinal	α1, α2, β2	Decrease gastrointestinal motility and secretion, constricts sphincters, increases liver glycogenolysis and gluconeogenesis, increases pancreatic release of insulin and glucagon	Nausea, abdominal pain, constipation, hyperglycemia
Kidneys	β1	Increase renin secretion	Hypertension
Adipocytes	β1, β3	Increase lipolysis	Weight loss

Table 6 Dramatic clinical presentations, laboratory and imaging findings, and clinical outcome of patients with unsuspecting pheochromocytoma

Parameter	Clinical Features
Age (years)	20's to 40's
Signs and symptoms	Headache, agitations, diaphoresis, nausea, vomiting
	Acute coronary syndrome
	Severe congestive heart failure
	Arrhythmia
Laboratory	Elevated creatine kinase
	Normal to elevated troponin
Imaging	Normal angiogram
	Dyskinesia, hypokinesia, akinesia by 2D Echo
	Diffuse myocardial edema by cardiac MRI
	Postoperative persistence of myocardial fibrosis
Clinical outcome	Resolution of signs and symptoms after adrenalectomy
	Normalized LV function and ejection fraction
	Persistent systolic and diastolic impairment
	Death

of β1-adrenergic receptors has a positive chronotropic and inotropic effect in the heart and will also result in release of renin. In PHEO this can contribute to hypertension, palpitations, and tachycardia. Stimulation of β2-adrenergic receptors will induce vasodilation of muscular arteries, and some common effects in PHEO include constipation and nausea. β3-adrenergic receptors in adipocytes induces lipolysis and can cause weight loss in PHEO (13,17,94).

Pourian and his group (66) recently attempted to formulate the likelihood ratio (LR) of signs and symptoms to aid in PHEO diagnosis in the clinical setting. The most prevalent signs and symptoms were hypertension, headache, palpitation, and diaphoresis. But based on their calculated LR, the significant symptoms that could aid in diagnosis were diaphoresis, palpitations, and headache alone, with the exclusion of hypertension.

The Sweden National Cancer Registry reported a 4x higher risk for mortality in PHEO compared to the general population with deaths occurring from acute hypertensive crisis (63). Interestingly, Stolk et al. (65) have demonstrated that among PHEO versus patients with essential hypertension, there is clearly a higher rate of cardiovascular (CV) events in PHEO excluding differences in hypertension and other CV risk factors. Recently, literature has been showing case reports of young individuals in their 20's, unsuspected to harbor PHEO, presenting with dramatic CV events, with one succumbing to CV failure (95). Interestingly, CV anatomic and functional abnormalities reverse after adrenalectomy (Table 6) (95,96). This is similar to our case of a 20-year-old female diagnosed and operated for large malignant PHEO. Except for her hypertension of one year, she has no other risk factors for CVD. Her echocardiographic finding showed dyskinesia of the septum (Figure 3).

Early diagnosis of PHEO not only resolves the catecholamine-induced cardiomyopathy with timely treatment but also the arterial stiffness. It has been shown that those PHEO patients whose diagnosis and treatment happened within 4 years from onset of hypertension do not require antihypertensive medications postoperatively as opposed to those whose onset of hypertension is 10 years or later from diagnosis and treatment (97). Interestingly, early diagnosis treated with mere unloading of the circulation with excess catecholamine by removing the dominant catecholamine-secreting lesion in patients with bilateral PHEO results into complete resolution of symptomatology, significant lowering to normalization of blood pressure, decrease in the number of antihypertensive medications, and better quality of life (98,99).

It seems that the destructive effect of chronic hypercatecholaminemia happens insiduously if the diagnosis of PHEO is overlooked, and dramatic CVD events and death occurs when there is a sudden surge in the concentration of the hormones adding significant insult to a compromised cardiac function. This explosive cardiovascular picture of patients with PHEO may provide new insights in the paradigm shift in the clinical assessment of these patients. Research is warranted to demonstrate the value and cost-effectiveness of 2D echocardiogram, a test readily available, in the assessment and monitoring of patients with minimal or absent CVD risk factors and being suspected for PHEO but with vague clinical presentation and equivocal biochemical results. Cardiac MRI is worth doing in patients with severe clinical symptomatology of cardiac disease like dyspnea, orthopnea, and chest pain and with evidence of myocardial damage in the ECG and elevated cardiac enzymes (100).

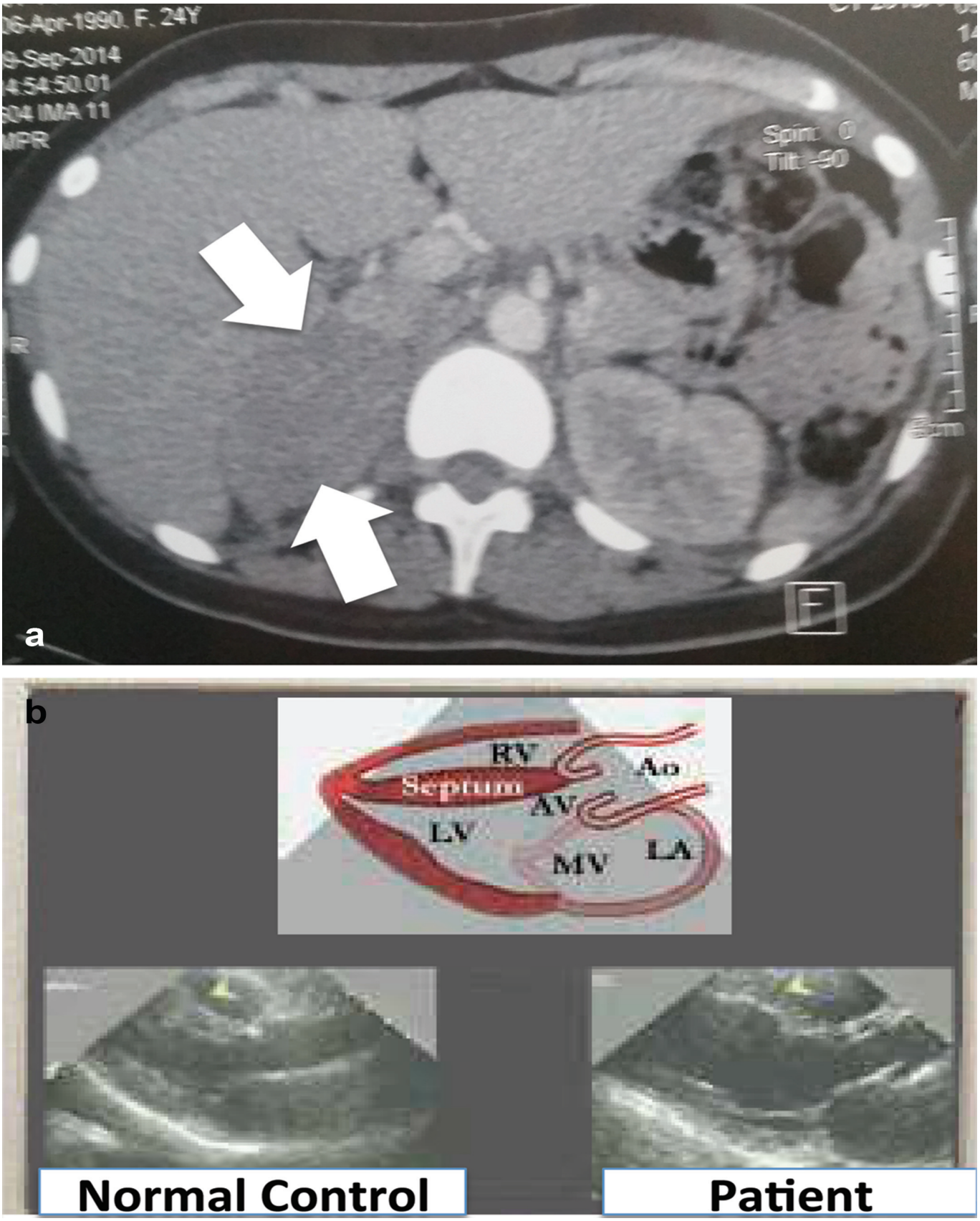

Figure 3 Computed tomography (CT) scan (A) of a 20-year-old female, non-obese, nonsmoker with 1-year history of hypertension and highest BP of 180/120. The adrenal mass measures 7.0 x 5.0 cm. Pathology confirmed PHEO with vascular invasion. Pre-operative 2D-echocardiographic study (B) showed dyskinesia of the septum.

Methoxytyramine: A Noble Biomarker for Early Diagnosis of Pheo and Early Detection of Metastasis

Until recently, the biggest challenge in the biochemical evaluation of PHEOs is those with minimal catecholamine secretion (101) or exclusively secreting dopamine (76), which often leads to delayed and missed diagnosis. These tumoral dopaminergic phenotypes are also observed to be mostly extra-adrenal, metastatic, and associated with hereditary lesions (17,26,77-102). The introduction of the measurement of plasma O-methylated dopamine metabolite and 3-methoxytyramine (3-MTY) made the evaluation of dopamine-producing PHEOs including their metastases possible and very useful, especially in those presenting with succinate dehydrogenase gene mutations (17,26,77).

The measurement of 3-MTY discriminated two distinct groupings; 1) *MEN*2 and *NF*1 and 2) *VHL* and *SDH*x (77). Patients with *VHL* and *SDH* mutations harbor immature tumors and lack PNMT necessary for epinephrine secretion; therefore, they do not synthesize metanephrine (MN) but only normetanephrine (NMN) and/or 3-MTY. The best biomarker for *SDH* tumors is 3-MTY since this is produced only by these tumors. Since *MEN*2 and *NF*1 tumors secrete both NE and E, measurement of NMN and MN best distinguishes these two from VHL and SDH tumors (17,26). With combined measurements of NMN, MN, and 3-MTY, patients with *NF*1 and *MEN*2 can be discriminated from those with *VHL*, *SDHB*, and *SDHD*, and 3-MTY can discriminate *SDHB* and *SDHD* from *VHL* in 78% of cases (17,26). Gupta and his colleagues recently demonstrated that 50% of malignant PHEO have increased levels of both NM and 3-MTY (35).

PHEOs of the dopaminergic phenotype generally are found to have reduced levels of the enzyme dopamine β-hydroxylase, which results in dopamine accumulation and a decreased production of norepinephrine (103,104). This finding may be due to proliferation of dedifferentiated progenitor cells giving rise to these tumors, as seen in patients with metastatic disease and mutations in SDHB and SDHB (105). Patients with these mutations show increases in the levels of dopamine and methoxytyramine in addition to elevations in the level of normetanephrine (77,106,107).

Biochemical secretory attributes of tumors using the 3-MTY biomarker has been shown also to assist in deciphering location and metastasis (26,77). In addition to being elevated in over two-thirds of patients with SDHB and SDHD mutations, 3-methoxytyramine is also a marker of multifocality and is extra-adrenal in location. Together with a diagnosis of SDHB mutation, a 5-fold higher levels of 3-MTY signifies malignant nature of the tumor with metastasis (26,77,108). Furthermore, although rare, cases of PHEOs in patients with NF1, VHL, MEN2A, and MEN2B secreting high levels of dopamine and/or 3-methoxytyramine, have also been reported (109-112).

Advanced Imaging Modalities

After catecholamine excess has been established biochemically, imaging studies for localization of primary tumor and determination of metastases should be done. The diagnosis of PHEO is usually challenging due to the variety of clinical presentations and anatomic and functional imaging results. It is important to use the most appropriate available imaging modality with good sensitivity without compromising specificity.

Anatomic imaging with the use of computerized tomography (CT) has been the preferred initial procedure for localization of PHEOs owing to its high sensitivity of 90% (11,113). However, its limitation has been observed in extra-adrenal, recurrent, and metastatic lesions (114,115). MRI, on the other hand is more advantageous in detecting extra-adrenal lesions, and is indicated in those with an allergy to contrast, pregnant or pediatric patients, and those whose contrast medium is a contraindication (10). Ultrasound sensitivity is poor but very useful in the detection of liver metastasis and lesions in the urinary bladder (116).

Table 7 reviews the sensitivity and specificity of imaging modalities in metastatic PHEO. The sensitivity of CT and MRI is variable. In a multicenter study involving patients with adrenal malignancy, the sensitivity of contrast-enhanced CT (CECT) was found to be at 59% only (117). In a study involving patients with biochemical catecholamine excess, the patient-based sensitivity of CT/MRI was 67% with lesion-based sensitivity of 44% (118). In contrast, a study involving 216 patients suspected of pheochromocytoma and paraganglioma, it has been found that CT/MRI is highly sensitive, as high as 95.7%

Table 7 Anatomic and functional imaging of pheochromocytoma.

	Author/Year	Sensitivity	Specificity
Anatomic Imaging			
CT	Cistaro A et al. (2015)	59%	100%
	Timmers HJ et al. (2009)	97% for nonmetastatic	38% for nonmetastatic
		100% for metastatic	-
MRI	Timmers HJ et al. (2009)	92% for nonmetastatic	58% nonmetastatic
		100% for metastatic	-
CT/MRI	Timmers HJ et al. (2012)	95.7% for nonmetastatic	90.2%
		74,4% for metastaatic	-
	Fiebrich HB et al. (2009)	67%	-
Functional Imaging			
MIBG	Bandopadhyaya GP et al. (2015)	68%	100%
	Timmers HJ et al. (2012)	75% for nonmetastatic	91.8%
		50 % for metastatic	-
	Fottner C et al. (2010)	53%	91%
	Fiebrich HB et al. (2009)	65%	-
	Timmers HJ et al. (2009)	78% for 123I-MIBG in nonmetastatic 76% for 131I-MIBG or 123I in nonmetastatic	92% for nonmetastatic 123I-MIBG, 131I-MIBG or 123I
		85% metastatic 123I-MIBG 65%131I-MIBG or 123I	
	Ilias I et al. (2008)	87.5% for nonmetastatic,	-
		88.9% for metastatic	
(18)F-DOPA PET/CT Scan	Bandopadhyaya GP et al. (2015)	82%	100%
	Cistaro A et al. (2015)	75%	100%
	Timmers HJ et al. (2012)	76.8% for nonmetastatic	90.2%
		82.5 % for metastatic	-
	Fottner C et al. (2010)	98%	100%
	Luster M et al. (2010)	100%	88%
	Flebrich HB et al. (2009)	90%	-
	Imani F et al. (2009)	84.6%	100%
	Timmers HJ et al. (2009)	78% nonmetastatic	77% nonmetastatic
		97% metastatic	-
	Ilias I et al. (2008)	87.5% for nonmetastatic	-
		91.4% for metastatic	
(68)Ga-DOTANOC PET/CT	Sharma P et al. (2014)	90.4%	85%
HED PET/CT	Yamamoto S et al. (2012)	91%	100%
	Trampal C et al. (2004)	92%	100%

^{123}I-metaiodobenzylguanidine (***MIBG***), positron emission tomography (**PET**), F-3,4-dihydroxyphenylalanine (**F-DOPA**), Hydroxyephedrine (**HED**)

for nonmetastatic tumors and 74.4% in metastatic tumors (119,120).

In contrast to anatomic imaging, functional imaging offers the advantage of higher specificity in detecting multifocal and metastatic tumors and can characterize tumoral metabolic activity (119-123). I- or (131) I-metaiodobenzylguanidine (MIBG) scintigraphy has the structure similar to NE so it can enter cells through NE transporters. 123I-MIBG is more sensitive and has better detection rate (68,116-124). On the other hand, single-photon emission computed chromatography (SPECT) has been used with CT/MRI

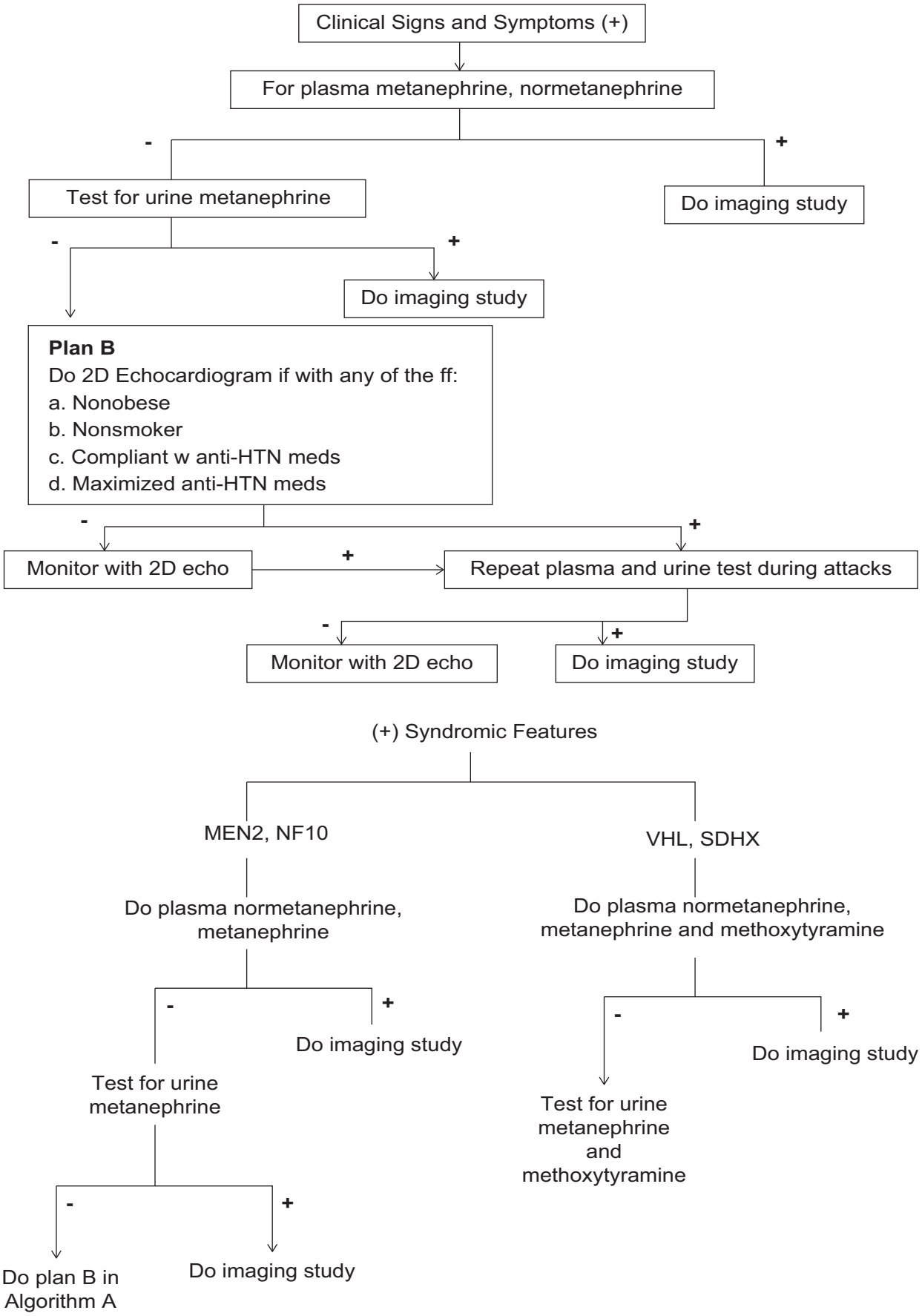

Figure 4 Algorithm for diagnosis of sporadic (A) and syndromic (B) pheochromocytoma. Algorithm C depicts the imaging modalities for both. Adapted from Martucci & Pacak (2014)* Do a cardiac MRI if the patient is with moderate to severe CV symptoms: dyspnea, orthopnea, chest pain with or without evidence of myocardial injury, such as abnormal ECG, elevated troponin or creatine kinase.

for colocalization. A recent report has shown the highest sensitivity with 123I-MIBG SPECT/MRI in the detection of adrenal PHEOs, especially in cases where PHEO is ruled out (125). With the advancement of imaging techniques, the limitation with MIBG becomes more apparent notably in missed metastasis yielding false-negative results in patients with succinate dehydrogenase subunit B (*SDHB*) mutations (68,126). (124) IMIBG is reserved for volume determination prior to 131IMIBG therapy for metastatic PHEO (127).

Positron emission tomography (PET) is being widely used and offers favorable attributes, such as less imaging time, low radiation exposure, and superior spatial resolution. 18F-fluorodeoxyglucose (FDG) PET is the preferred procedure for malignant tumors, especially SDHB-related PHEO since cancer cells readily take up glucose (120). However, its performance is not specific since it can detect other kinds of tumors (68).

18F-fluorodopamine (FDOPA) is a more specific tracer since its structure is similar to dopamine, a catecholamine precursor, and therefore enters the cell through NE transporter (116,124-128). This imaging modality has high sensitivity for metastatic tumors (129-131). Newer PET scanning tracers have been developed and showed promising results in detection of metastasis and characterization of metabolic activity of the tumor cells namely the DOTA peptides- DOTATATE, DOTATOC, and DOTANOC (68). Ga-DOTATOC PET/CT was found superior to FDOPA PET/CT in the diagnosis of metastatic tumors (132). Further ongoing research is being undertaken to observe these findings in a bigger cohort. For chromaffin tumors that express somatostatin receptors, $^{\circ 111}$In-DTPA octreotide or ^{111}In DTPA-pentetreotide have proven useful (68-133).

CONCLUSION AND INSIGHT

In this review, the diagnosis of PHEO is revisited to address the evolving clinical presentation that increases morbidity and mortality due to delay in diagnosis and treatment. The discussion centered on the progress in the approaches of early diagnosis of PHEO through complete history and physical examination, and improved analytical approaches and inclusion of an important metabolite, methoxytyramine, in the biochemical assessment. We have also emphasized the introduction of cardiovascular imaging 2D echocardiogram and cardiac MRI in the early assessment of patients with equivocal biochemical and imaging results. In this aspect, as have been shown in previous reports, the resultant cardiomyopathy from chronic catecholaminemia is reversible and catecholamine unloading in the circulation leads to significant clinical improvement. We have also pointed out the advances in imaging procedures, which led to better diagnostic accuracy in the early detection of metastasis and recurrence. Finally, we have elucidated how the clinical presentation, biochemical profile, and imaging characteristics of PHEO correlated well with specific gene mutations. With the aforementioned progress, we have come up with recommendations summarized in an algorithm shown in Figure 4.

DECLARATION OF INTERESTS

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

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