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Research Paper

Biochemical exploration tools for Pheochromocytoma: an overview

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Abstract
Pheochromocytomas are rare neuroendocrine tumors that can be challenging to diagnose due to their insidious presentation and ability to mimic other diseases. They belong to a heterogeneous group of chromaffin cell neoplasms with varying secretion profiles, locations, and potentials for malignancy depending on underlying genetic mutations. Biochemical diagnosis is based on detecting elevated levels of metanephrines, which are more sensitive and specific than catecholamines. Indeed, plasma and urine free metanephrines concentrations serve as a reliable indicator of tumor production, as they are minimally affected by factors such as renal insufficiency. Liquid chromatography coupled with tandem mass spectrometry is currently the preferred method for measuring metanephrine levels. However, their pre analytical phase can be challenging as a lot of interferences can occur . As for the treatment , it primarily involves surgical intervention with laparoscopic resection with or without partial adrenalectomy being the mainstay of management.

Keywords: *pheochromocytoma, metanephrines, catecholamines, liquid chromatography coupled with mass spectrometry.*

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I. Introduction

Pheochromocytomas are highly rare neuroendocrine tumors, affecting approximately 0.46 individuals per 100,000. They have the potential to be dangerous if not diagnosed and treated early (C). These tumors are characterized by their ability to synthesize catecholamines (norepinephrine, epinephrine, and dopamine) from chromaffin cells located in the medullary area of the adrenal glands (1). This secretion is usually paroxysmal, either spontaneous or provoked, and can cause clinical symptoms such as headaches, palpitations, sweating, and high blood pressure (2). While most pheochromocytomas are benign, some forms can be severe. The only criterion for distinguishing between these two forms is the presence of chromaffin tissue metastases in sites where it is normally absent (3). Given the lack of clinical specificity, diagnosis mainly relies on biological investigations and imaging (4). The aim of this work is to provide an overview of this relatively rare pathology and present the different techniques, mainly biochemical ones, used for its diagnosis.

Pathophysiology and clinical overview

Catecholamines are a class of neurotransmitters and neurohormones essential for the regulation of many physiological processes, including the regulation of heart rate, blood pressure, and respiratory rate (2). There are three types: dopamine, norepinephrine, and epinephrine (1). Their synthesis begins with tyrosine, a common precursor, which is converted into dopamine by the enzyme tyrosine hydroxylase, then transformed into norepinephrine and finally into epinephrine by N-methylation (5). Sympathetic neurons mainly synthesize norepinephrine, while the adrenal medulla, located above the kidney, produces both norepinephrine and epinephrine. These are rapidly degraded within the tissues where they are synthesized, which explains their low blood levels (6). Moreover, the metabolites of catecholamines, including 3-methoxytyramine, normetanephrine, and metanephrine, are converted into sulfated conjugates and eliminated in urine (7). It should be noted that most

circulating metanephrines and some normetanephrines originate from the adrenal medulla (8). Additionally, any disturbance in their synthesis has numerous repercussions on the body (high blood pressure, hyperglycemia…). The increase in the synthesis of catecholamines can be associated with diseases such as pheochromocytoma (9). This term is preferably reserved for chromaffin tumors of the adrenal medulla (Pacak et al, 2007c) (10). These tumors are rare and sporadic in 70% of cases, while in 30% of cases they are part of genetic predisposition syndromes (multiple endocrine neoplasia type 2A and 2B (RET gene), von Hippel-Lindau disease (VHL gene), hereditary paraganglioma syndromes (SDHAF2, SDHB, SDHC, SDHD genes), type 1 neurofibromatosis (NF1 gene)...) (11).

The clinical presentation of pheochromocytoma is highly variable, dependent on factors such as tumor location, size, type, and degree of catecholamine hypersecretion (10). Hypersecretion is responsible for a range of symptoms, most notably the development of arterial hypertension (AH) (1). AH is paroxysmal in approximately one-third of cases, presenting as crises characterized by a constellation of symptoms including headaches, palpitations, profuse sweating, pallor, abdominal or thoracic pain, and visual disturbances (12). These crises can lead to serious complications such as cerebral and meningeal hemorrhages, acute pulmonary edema, myocardial infarction, arrhythmias, and even life-threatening conditions (1). Although more than half of cases exhibit permanent, severe, systolodiastolic, resistant, and unstable hypertension, its absence does not exclude the presence of a pheochromocytoma (12). The tumor can be discovered during the exploration of adrenal incidentalomas (4-5% of cases), genetic screening investigations in first-degree relatives of affected individuals, or as part of the evaluation of syndromes such as multiple endocrine neoplasia, neurofibromatosis, or von Hippel-Lindau disease (6). Other clinical signs, while often nonspecific, may also contribute to the diagnosis. These include weight loss, a high frequency of the triad of headaches-palpitations-sweating, glucose intolerance or diabetes, and the occurrence of orthostatic hypotension (1).

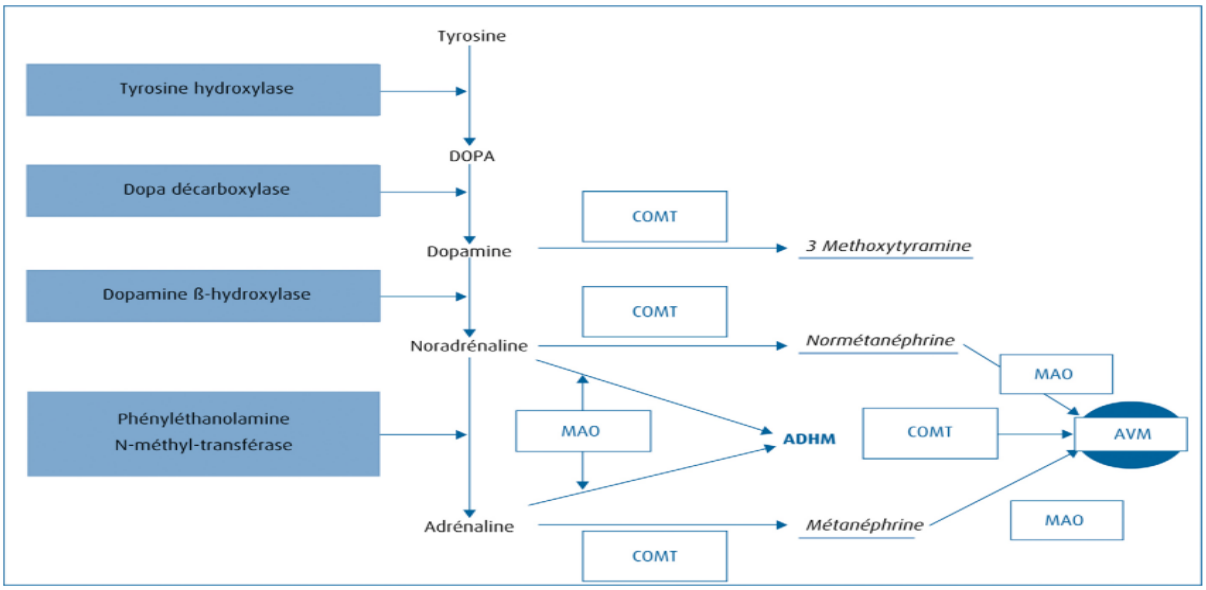

Figure 1: Synthesis and degradation of catecholamine (5)

Biochemical parameters explored in Pheochromocytoma

Early diagnosis of pheochromocytoma is critical due to the potential risks associated with catecholamine hypersecretion, tumoral syndrome, possible genetic origin, and potential malignancy (13). Diagnosis is based on the detection of an increase in catecholamine secretion. Recommended tests measure the degradation products of catecholamines, which are more stable and less influenced by environmental stress factors (14).

Metanephrines: While catecholamine secretion occurs intermittently, metanephrines and normetanephrines are continuously produced in pheochromocytomas and then released into the circulation (15). Although traditional biochemical tests used to measure urinary and plasma catecholamines or free urinary and plasma metanephrines (normetanephrine and metanephrine), current evidence suggests that the exclusive use of metanephrine assays is necessary for the diagnostic investigation of pheochromocytoma (6) (15) (16). In fact, plasma free metanephrines offer excellent sensitivity at 97% for pheochromocytoma diagnosis and diagnostic specificity between 80% and 100% (16) (17). Different analytical techniques are used for the measurement of plasma and urinary metanephrines. Currently, most laboratories favor high-performance liquid chromatography coupled with electrochemical detection (HPLC-ECD) methods (18). On the other hand, liquid chromatography with tandem

quadrupole mass spectrometry (LC MS/MS) is increasingly used due to its greater precision (19). In fact, Perry et al. reported that the measurement of fractionated 24-hour urinary metanephrines by mass spectrometry has excellent sensitivity and specificity in the diagnosis of pheochromocytomas with respective percentages of 97% and 91% (20). Although differences in the diagnostic sensitivity and specificity of fractionated urinary and plasma metanephrines have been observed using these different analytical methodologies (18), to date, no direct multicenter comparison using the reference method of tandem liquid chromatography and mass spectrometry for this analysis has supported these observations (21).

3-methoxytyramine (3MT): 3-MT, a dopamine metabolite, is a strong indicator of malignancy in the presence of a pheochromocytoma (22). It is measured by LC-MS/MS, with a prior solid phase extraction step, followed by ultra-high-performance liquid chromatography (UHPLC) in hydrophilic interaction mode (18).

Chromogranin A: Elevated levels of circulating chromogranin A have been associated with nearly all types of neuroendocrine neoplasms, including pheochromocytomas (20). Plasma chromogranin A, measured by immunological assay, can be used in addition to metanephrine assays in the biological diagnosis of patients with pheochromocytoma, especially in subjects suspected or diagnosed with non-functional pheochromocytoma (23).

Plasma/urinary catecholamines: They are poor indicators of the presence of a pheochromocytoma (24). Indeed, they are hormones widely secreted in situations of stress, leading to a significant number of "false positives" (17). Conversely, in patients with pheochromocytomas, they are extensively metabolized in the tumor, thus decreasing the amounts excreted and generating "false negatives". Thus, their measurement has little diagnostic value (22). However, measuring plasma catecholamines can be useful to exclude a pheochromocytoma if it is performed during a hypertensive crisis and shows low catecholamine levels (25). According to a study by Eisenhofer et al., this test has a good negative predictive value. Therefore, it is important to consider these limitations when interpreting the results of catecholamine measurements (22).

Pre analytical and interference factors

Studies such as the one carried out by Eisenhofer et al have shown that normal values of free normetanephrine in plasma vary with age (26). Thus, to improve test accuracy, it is recommended to use age-adjusted reference interval thresholds (27). Although food intake has only a minor effect on test results, it is preferable to take samples in a fasting state (13). However, it should be noted that certain foods such as fruits (bananas and pineapples), nuts, and cereals contain significant amounts of biogenic amines that can lead to falsely positive test results ; in particular, the measurement of free plasma and urinary 3-MT, which may show a significant increase in concentrations after ingestion of these foods (28). Therefore, dietary restrictions are mainly necessary for the measurement of 3-MT (13).

Before collecting metanephrine samples, it is recommended that the patient rest in a supine position for at least 30 minutes (29). This measure is essential for obtaining accurate diagnostic results, especially for normetanephrine, which is the most sensitive plasma metanephrine to sympathoadrenal activation (7). If collection is done without rest or in a seated position, diagnostic accuracy may be compromised (29). It is also important that patients stop all medications that may alter urinary or plasma concentrations of catecholamines or metanephrines before collection (30). Some medications may interfere with measurements of plasma and urinary catecholamines, leading to falsely positive test results. This interference can be analytical or pharmacodynamic, and may be caused by the effects of medications on the secretion, metabolism, and excretion of catecholamines or their metabolites (13). For example, ephedrine, amphetamine, cocaine, caffeine, and nicotine are sympathomimetic agents that increase the release of metanephrines (31) (32).

On the other hand, medications inhibiting the reuptake of norepinephrine, such as serotonin-norepinephrine reuptake inhibitors and tricyclic antidepressants, may induce an increase in concentrations of norepinephrine and normetanephrine, leading to falsely positive test results (31) (33). Moreover, some antihypertensive and vasodilator medications such as dihydropyridine calcium ,channel blockers and selective alpha1-adrenergic receptor inhibitors, may cause falsely positive results for catecholamines due to reflex sympathetic activation (31). Indeed, these medications can induce compensatory stimulation of the sympathetic nervous system to maintain blood pressure, which may increase the secretion of catecholamines and their metabolites (29). On the other hand, L-DOPA, commonly used in the treatment of Parkinson's disease, may cause false elevations of 3-MT and metanephrines (27). It is therefore important that patients inform their doctor of all medications they are taking before collection, to avoid any interference in test results (31). In addition, it is recommended that patients do not modify their usual medication regimen before collection , unless specifically requested by their doctor (34). In all cases, test results should be interpreted with caution, taking into account all factors that may affect the measurement of catecholamines and their metabolites (31).

In practice, it is recommended not to stop these medications before collection, unless specifically requested by the treating doctor. If initial test results are high, it may be necessary to repeat tests after a temporary cessation to confirm or refute the results (34). In all cases, it is important that the laboratory be informed of all medications taken by the patient before collection (32).

II. INTERPRETATION OF RESULTS:

In symptomatic patients, normal test results for plasma or urinary metanephrines exclude the presence of a pheochromocytoma with great accuracy due to the high sensitivity of these tests. However, any positive result does not guarantee the presence of a tumor (17). A retrospective study by Hoffman et al. showed a false positive rate of 19% to 21% for plasma free and fractionated urinary metanephrines (2). Drug interference can also lead to falsely positive results for metanephrines (27). Plasma methoxy derivatives concentrations are generally moderately increased in case of drug interference, whereas an increase greater than four times the normal values is considered pathognomonic for the diagnosis and found in 80% of pheochromocytomas. For intermediate concentrations (1 to 4 times normal values), it is recommended to repeat the measurements and to couple plasma methoxy derivatives determination with that of urinary derivatives, which is less sensitive but of equivalent or higher specificity. As for the measuring of catecholamines, even though they are part of the diagnosis of pheochromocytoma, in pheochromocytomas with paroxysmal secretion, their measurement alone may not be sufficient because the tumor can secrete normal levels of catecholamines between paroxysms (25)

III. MANAGEMENT OF PHEOCHROMOCYTOMA:

The management of pheochromocytoma follows a multidisciplinary approach and should be carried out in a specialized setting by an experienced team involving an endocrinologist, surgeon, and anesthetist (35). The management will depend on the type of pheochromocytoma, its location, size, and the presence or absence of metastases. The treatment is surgical resection of the tumor (36). Preoperative treatment, including alpha-blockers or calcium channel blockers, may be administered to the patient to control blood pressure and prevent hypertensive crisis during the procedure (36). The procedure is usually performed by median laparotomy, which allows initial access to the venous effluent of the tumor and good exploration of neighboring organs. Laparoscopic surgery is feasible, however, in cases of small pheochromocytomas. Regular postoperative monitoring is necessary to detect recurrence or the development of metastases (35).The five-year survival rate is 97% in initially benign cases and 23% in initially malignant cases (37).

IV. CONCLUSION:

Pheochromocytoma is a rare tumor with highly variable clinical expression and probably underestimated incidence. In recent years, significant progress has been made in biological assays, with metanephrine and normetanephrine being clearly the most efficient, which helped facilitating the diagnostic process. On the therapeutic level, progress remains to be made. Surgery is currently the only curative treatment.

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