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Clinical Practice Guidelines

Investigation for Adrenal diseases- An Overview

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Abstract

Adrenal diseases encompass a wide range of disorders involving hormone excess or deficiency, adrenal masses, and congenital anomalies. Accurate and timely diagnosis is crucial to guide effective management and avoid potentially life-threatening outcomes. This review provides a comprehensive overview of current investigative approaches for adrenal disorders, integrating recent advancements and established methodologies. It highlights the interpretation of hormonal assays for conditions such as primary adrenal insufficiency (Addison's disease), Cushing's syndrome, primary hyperaldosteronism (Conn's syndrome), Pheochromocytoma/paraganglioma (PCC/PGL) and congenital adrenal hyperplasia (CAH). The utility of dynamic endocrine tests, biochemical markers, and imaging modalities including CT, MRI, and nuclear imaging is discussed. Additionally, the role of genetic testing and emerging diagnostic algorithms is outlined. By summarizing practical approaches and updates in diagnostic strategies, this review aims to aid clinicians in the systematic evaluation of adrenal pathologies, ensuring accurate diagnosis and individualized patient care.

Key words: *Adrenal diseases, Addison's disease, Cushing's syndrome, Conn's syndrome, Pheochromocytoma*

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Introduction

Investigation of adrenal disorders has evolved significantly, integrating advanced biochemical assays, molecular imaging, genetics, and functional diagnostics.[1,2,3] This review summarizes the latest diagnostic advancements for Primary adrenal insufficiency (PAI) /Addison's disease, Cushing syndrome, primary hyperaldosteronism (Conn's syndrome), pheochromocytoma/paraganglioma (PCC/PGL) and congenital adrenal hyperplasia (CAH).

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A. Primary adrenal insufficiency (PAI)/Addison's disease:

Primary adrenal insufficiency (PAI) is a potentially life-threatening condition that requires a high index of clinical suspicion and prompt diagnostic evaluation. Early recognition is crucial to prevent adrenal crises, which occur in approximately 8% of PAI cases and are associated with high morbidity and mortality.[4,5] In a patient having suspected PAI, diagnosis typically begins with measurement of early morning serum cortisol (before 8:00 AM). A level <140 nmol/L (5 µg/dL) with a concurrently elevated plasma ACTH (more than twice the upper limit of normal) confirms PAI.[6] If the cortisol level is indeterminate, a short synacthen test (SST) using 250 µg of synthetic ACTH (cosyntropin) is performed, with cortisol levels measured at 30 and/or 60 minutes. A stimulated peak cortisol level 500 nmol/L (18 µg/dL) generally excludes adrenal insufficiency. Here is an algorithm for screening below[7]:

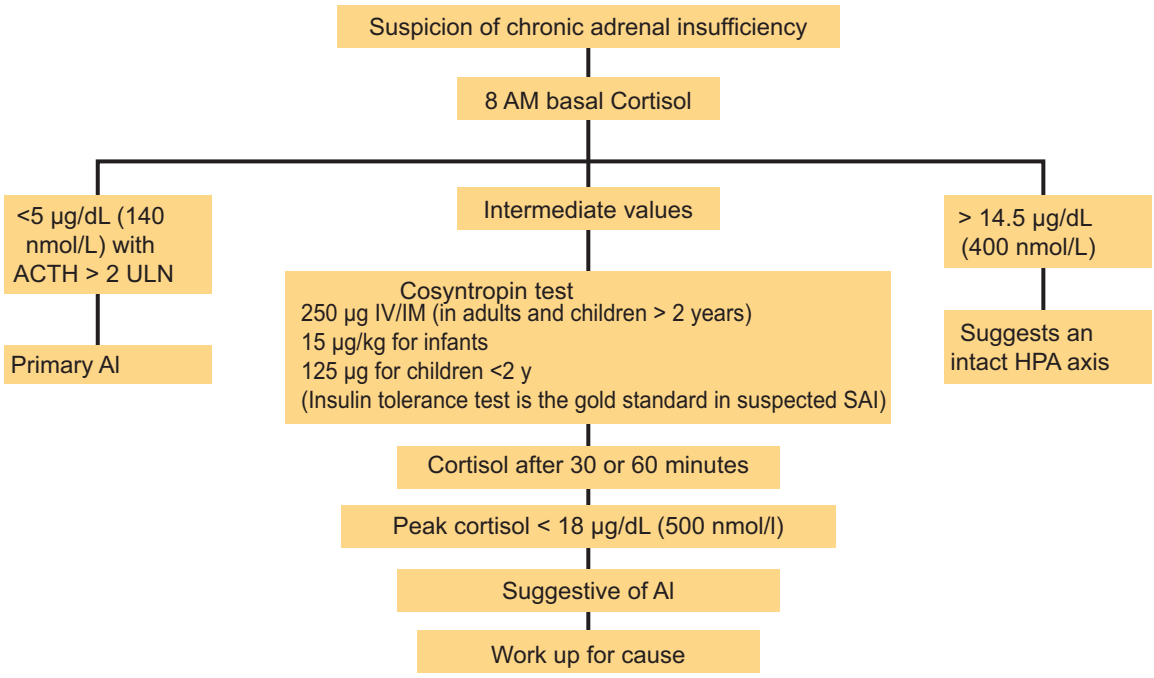

Figure 1: Biochemical screening evaluation for suspected adrenal insufficiency (AI). ACTH: adrenocorticotrophin; HPA axis: hypothalamic–pituitary–adrenal axis; SAI: secondary adrenal insufficiency; ULN: upper limit of normal. corticotrophin; HPA axis: hypothalamic –pituitary–adrenal axis; SAI: secondary adrenal insufficiency; ULN: upper limit of normal

Determining the Etiology

Etiological evaluation includes testing for 21-hydroxylase antibodies**,** which are positive in autoimmune adrenalitis, the most common cause in developed countries. In endemic regions, infections like tuberculosis and fungal diseases are prevalent causes. Imaging with CT scan helps identify structural lesions such as infiltrative disease, hemorrhage, metastasis, or infections.[8] In the absence of autoantibodies or evident structural lesions, genetic testing is warranted—particularly in patients under 20 years-to identify conditions like X-linked adrenoleukodystrophy.[8]

Other Relevant Investigations

Additional labs may show hyponatremia**,** hyperkalemia**,** hypoglycemia**,** and eosinophilia—typical features of aldosterone deficiency seen in PAI. Plasma renin measurement assists in assessing the degree of mineralocorticoid deficiency and guides fludrocortisone dosing.[9]

B. Cushing's syndrome

Cushing syndrome (CS) results from prolonged exposure to excess glucocorticoids, either from exogenous steroid use or an endogenous excess cortisol production. The most common cause of CS is iatrogenic, resulting from exogenous glucocorticoid use from any administration route, including topical or inhaled glucocorticoids. Endogenous CS is caused by ACTH-dependent or ACTH-independent source of excess cortisol production. The estimated incidence of Cushing's syndrome attributable to endogenous overproduction of cortisol ranges from 2 to 8 per million people annually.[10] ACTH-dependent CS accounts for 80% to 85% of cases and ACTH independent accounts for 15% to 20%.[11, 12]

If a patient is suspected of CS and exogenous glucocorticoid use is excluded, it is recommended to start by performing one of the first-line screening tests.[13] Recommended initial tests include:

1. overnight 1-mg DST;
2. 24-hour urinary free cortisol (UFC); and
3. late-night salivary cortisol test (LNSC).

At least 2 of the 3 tests should be positive to confirm Cushing's syndrome. A promising relatively novel test to detect chronic hypercortisolism is measurement of cortisol in scalp hair. Scalp hair analysis is a patient-friendly noninvasive method yielding cortisol values representing long-term cortisol exposure of the past months.[14]

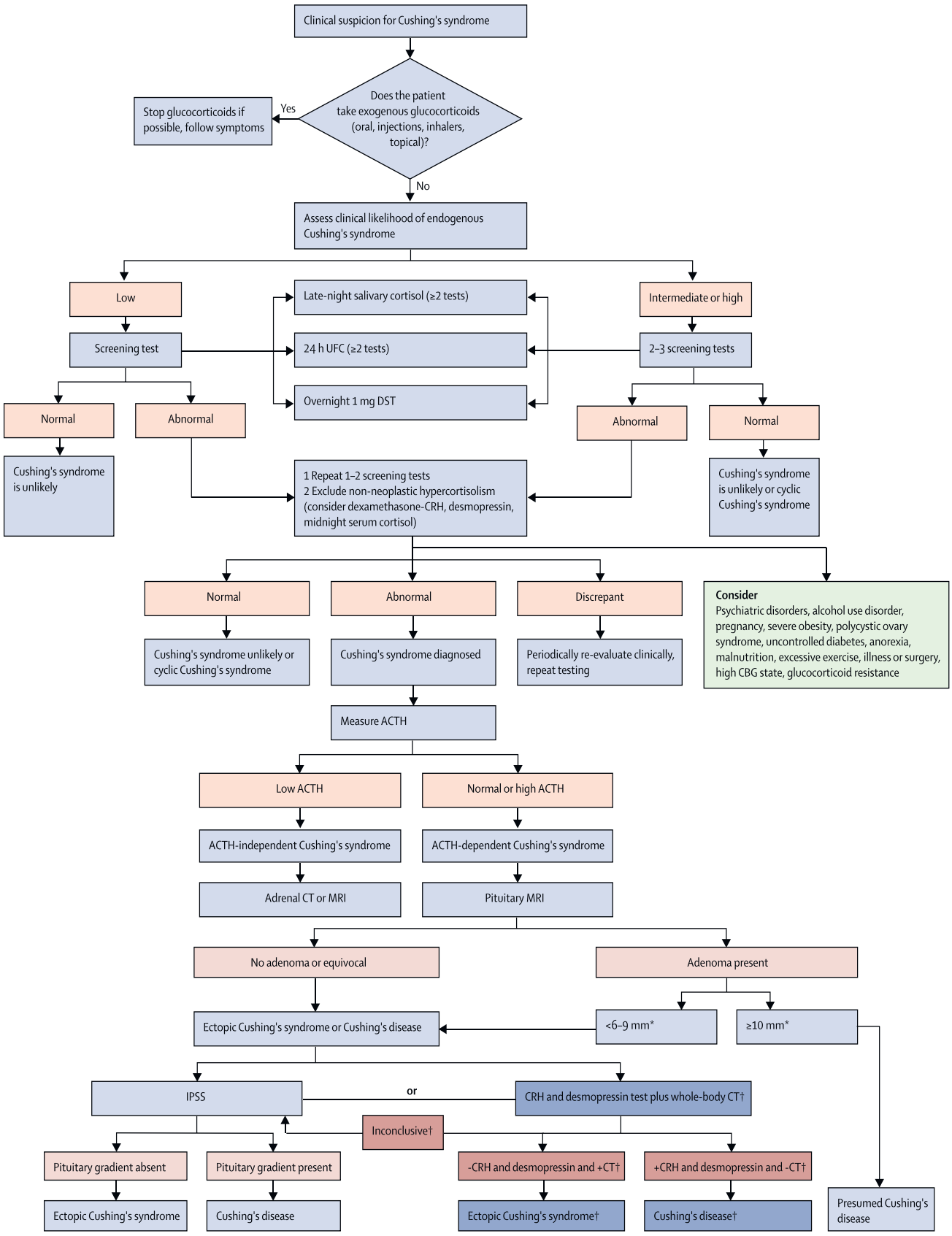

Figure 2: Algorithm of testing for suspected Cushing syndrome[15]

ACTH-dependent CS

Once CS has been established, plasma ACTH concentrations can help determine whether the cause is ACTH-dependent or ACTH-independent. In ACTH-dependent causes plasma ACTH levels will be in- appropriately normal or elevated (generally >20 pg/mL) and low (generally <10 pg/mL) in ACTH- independent causes of CS.[16,17] Thirty percent of the patients with CS have ACTH levels in the "gray zone" (5-20 pg/mL) and should have repeat testing and adrenal imaging should be considered to detect possible adrenal pathology.[18] ACTH-dependent CS comprises 80% to 85% of all CS cases.

Differentiation Between Cushing disease and Ectopic ACTH Secretion

Cushing disease, accounting for approximately 80% of ACTH-dependent CS cases, occurs when a pituitary adenoma secretes ACTH. Ectopic ACTH secretion (EAS) accounts for approximately 20% of ACTH- dependent CS. In EAS, most common sources of ACTH secretion are small cell lung carcinomas or pulmonary carcinoid tumors. Other causes include pancreatic neuroendocrine tumors, thymic neuro- endocrine tumors, gastrinomas, medullary thyroid cancer, and pheochromocytomas.

Pituitary MRI is essential for detecting pituitary adenomas. High-resolution 3T-MRI with 3-dimensional spoiled gradient-echo sequence, characterized by thinner sections and superior soft-tissue contrast can detect adenomas as small as 2 mm.[19] Nevertheless, pituitary MRI may be negative in up to 40-60% of Cushing disease cases. There can also be false-positive pituitary MRI findings (pituitary incidentaloma) in patients with EAS.[20] Bilateral inferior petrosal sinus sampling (BIPSS) is the gold standard to differentiate between Cushing disease and EAS. If a pituitary adenoma is >6 mm on MRI, the need for further testing with BIPSS is not necessary.[21] Dynamic testing with high-dose DST, CRH test, and desmopressin testing can also be used to help differentiate between Cushing disease and EAS.[22] 18 Fluorodeoxyglucose (FDG) PET/CT scans are useful for identifying rapidly proliferating neuroendocrine tumors.[23]

ACTH-independent CS

ACTH-independent CS is usually caused by an adrenal adenoma and less frequently by bilateral micro- or macronodular adrenal hyperplasia and adrenal carcinoma. Very rare causes include primary pigmented nodular adrenocortical disease, the Carney complex, and McCune-Albright syndrome. If after establishment of endogenous

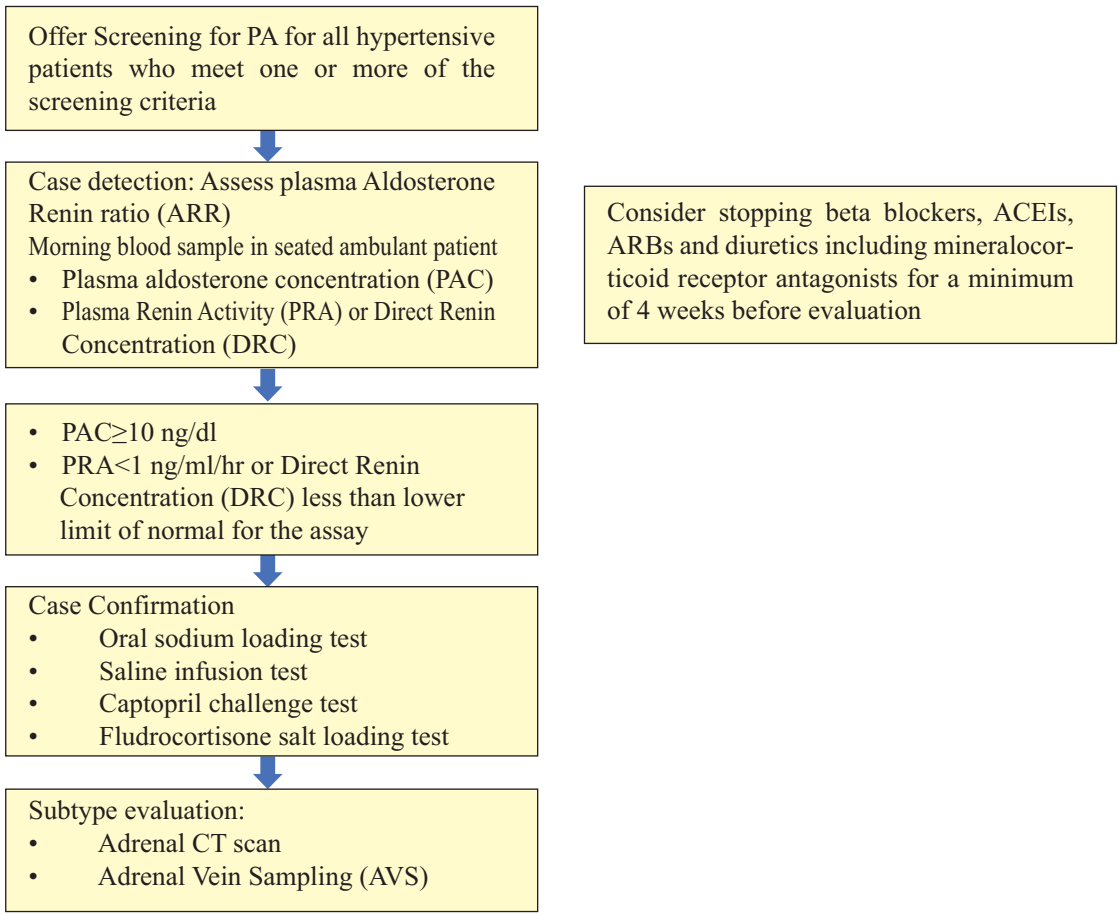

Figure 3: Schematic algorithm for diagnostic workup of primary hyperaldosteronism (PA) in patients with hypertension[24]

hypercortisolism, ACTH concentrations are suppressed (<10 pg/mL or <2.2 pmol/L), the next diagnostic step is imaging of the adrenal glands with CT or MRI.

Adrenal Imaging

Unilateral adrenal adenomas are usually small, with native fat equivalent density values less than 10 Hounsfield units (HU) and rapid contrast washout on contrast-enhanced CT scans. Adrenocortical carcinoma has an inhomogeneous tissue pattern with necrosis, calcifications, and more than 10 HU on unenhanced CT scans and with delayed contrast washout. In case of intermediate ACTH values, between 10 and 20 pg/mL (2.2 pmol/L and 4.4 pmol/L), the differentiation between an adrenal and a pituitary cause of CS can be difficult. In case of an adrenal cause, mild cortisol overproduction may be accompanied by incomplete ACTH suppression. Conversely, in case of more severe clinical and biochemical hypercortisolism an ACTH-dependent cause of CS is more likely.

C. Conn's Syndrome

Primary aldosteronism/Conn's Syndrome, characterized by aldosterone overproduction is a prevalent but underdiagnosed cause of secondary hypertension, contributing to increased cardiovascular and cerebrovascular events and end-organ damage. Unilateral adrenal disease (unilateral aldosterone producing adenoma [APA≥10 mm] or Aldosterone-producing micronodules [<10 mm]) and bilateral idiopathic hyperaldosteronism (IHA) are the most common subtypes; familial forms of primary aldosteronism due to germline variants in genes predisposing to this disorder and aldosterone producing carcinoma are rare causes.

Table1: Confirmatory tests used for PA diagnosis

Test	Procedure	Interpretation	Advantage	Disadvantage
Saline infusion test	Venous infusion of 2 L of saline solution in 4 hours (seated position preferred).	PAC not suppressed after saline infusion (>6.8 ng/dL (190 pmol/L) proposed as the most accurate threshold.	Relatively inexpensive and widely used Internationally.	To be performed in a medical facility with trained personnel. Hourly BP monitoring is recommended, and caution required in patients with severe kidney disease and/or heart failure.
Oral sodium loading test	Oral intake of 6 g/day sodium chloride for 3 days and potassium chloride to correct hypokalemia. 24-h urinary sodium (urinary sodium >200 mEq/24 h) U-Aldo sampling from day 3 at 08:00 am until day 4 at 08:00 h.	U-Aldo <10 µg/24 h (28 nmol/24 h) excludes PA. It has been proposed that U-Aldo >12 or 14 µg/24 h (33 or 39 nmol/24 h) could be used as cut off to confirms PA.	Relatively inexpensive and can be performed in outpatient setting. The sensitivity and specificity of the oral sodium loading test are 96% and 93%, respectively.	Its reliability depends on the accuracy of 24-h urine collection Contraindicated if severe hypertension, kidney failure, cardiac arrhythmia, severe hypokalemia.
Captopril challenge test	Oral intake of 25–50 mg captopril after 1 h in seated position. Blood sampling for ARR at baseline and after 2 hours.	After 2 hours PAC decrease <30% and suppressed renin confirms PA. Proposed threshold ARR > 30 ng/dL or PAC > 8 ng/dL after Challenge.	Relatively inexpensive and can be performed in outpatient setting.	Risk of angioedema and contraindicated in case of suspected renovascular hypertension. `

Biochemical Testing

The diagnostic cornerstone for pheochromocytoma remains plasma free metanephrines or 24-hour urinary fractionated metanephrines, both offering high sensitivity (96%) and specificity (85–99%).[49] In most center, the most practical strategy is to measure 24-hour urinary fractionated metanephrines and normetanephrines (Sensitivity and specificity 98%). Increase 24 urinary metanephrines >400 µg or normetanephrines >900 µg is the diagnostic cutoff. Supine plasma sampling is preferred, with elevated plasma normetanephrine (>2.5 pmol/mL) or metanephrine (>1.4 pmol/mL) highly indicative of PCC. To reduce false positives, pre-test avoidance of interfering medications and stressors is recommended.[26]

Imaging

Once biochemistry confirms the diagnosis**,** CT or MRI is the first-line anatomical imaging. CT offers ~88–95% sensitivity for lesions >1.3 cm, while MRI shows excellent contrast resolution, especially for extra- adrenal tumors.[27]

E. Congenital adrenal hyperplasia (CAH):

Congenital adrenal hyperplasia (CAH), most commonly due to 21-hydroxylase deficiency, is a group of autosomal recessive disorders characterized by impaired cortisol biosynthesis and variable degrees of aldosterone and androgen excess.[28,29] Diagnostic evaluation of CAH requires a tiered approach, integrating clinical, biochemical, and genetic assessments.[30]

The initial diagnostic cornerstone remains serum 17-hydroxyprogesterone (17-OHP) measurement. Elevated basal 17-OHP levels, especially >20 ng/ml in the neonatal period, are suggestive of classic CAH, while ACTH stimulation testing is essential for borderline or non-classic forms where stimulated cortisol level rise to 50-100 ng/ml. Tandem mass spectrometry has improved the specificity of steroid profiling by differentiating 17-OHP from cross-reacting steroids. Newborn screening programs, now implemented in many countries, utilize immunoassays to detect elevated 17-OHP in dried blood spots. However, false

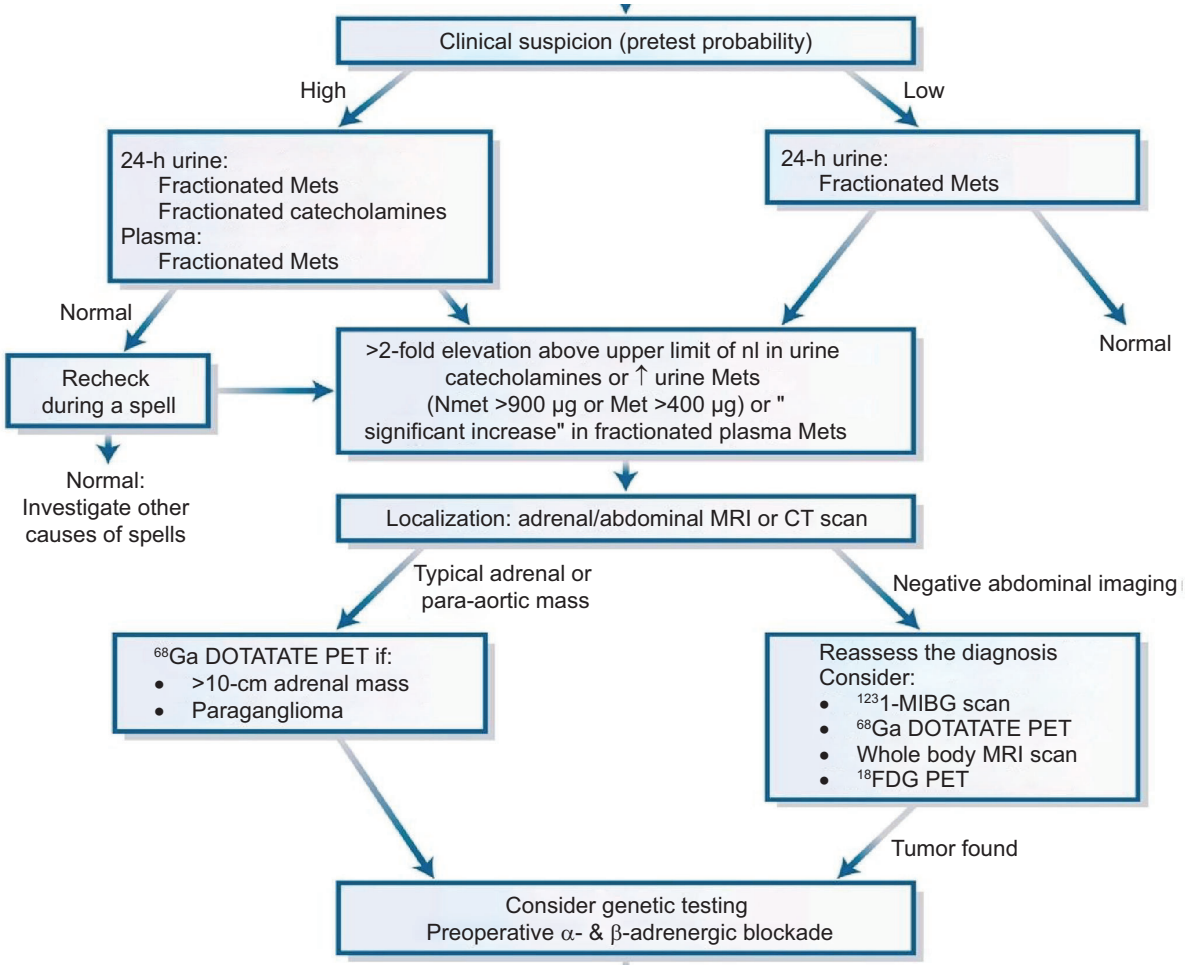

Fig.-4: Evaluation and treatment of catecholamine secreting tumours[24]

positives due to prematurity or stress necessitate second-tier testing using liquid chromatography–mass spectrometry (LC-MS/MS) to improve specificity.

Genetic testing has become increasingly important for confirmation and genotype-phenotype correlation. CYP21A2 gene analysis identifies the mutation spectrum and assists in predicting disease severity and guiding prenatal therapy. Additional markers such as androstenedione, testosterone, and plasma renin activity aid in assessing androgen and mineralocorticoid status.

Conclusion

Adrenal disorders encompass a diverse spectrum of endocrine conditions, each requiring a tailored diagnostic approach to ensure timely and accurate diagnosis. Advances in biochemical testing, functional imaging, and genetic profiling have significantly enhanced the diagnostic precision for disorders such as Addison's disease, Cushing syndrome, primary hyperaldosteronism (Conn's syndrome), pheochromocytoma, and congenital adrenal hyperplasia. Early recognition and appropriate use of both classical and emerging investigative tools are essential to optimize patient outcomes. As diagnostic modalities continue to evolve, integrating precision medicine and molecular diagnostics will play an increasingly central role in the evaluation and management of adrenal diseases.

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