Adrenal insufficiency – causes and laboratory diagnosis

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Adrenal insufficiency (AI) manifests as a clinical syndrome arising from either the direct impairment of adrenal glands, leading to primary AI characterized by deficiencies in glucocorticoids and mineralocorticoids, or adrenal cortex atrophy due to diminished adrenocorticotropic hormone (ACTH) stimulation, a consequence of hypothalamic and/or pituitary damage, resulting in secondary AI. The diagnosis of AI is based on clinical assessment and biochemical tests, including basal hormone level measurements and stimulation tests. In evaluating the results of laboratory tests, it is necessary to consider factors that may influence both pre-analytical and analytical phases, as well as the chosen methodology. Correct diagnosis of adrenal insufficiency and timely initiation of suitable replacement therapy are paramount. These steps are crucial not only for managing the condition but also to avert potentially life-threatening adrenal crises.

Key words: adrenal insufficiency, cortisol, adrenocorticotropic hormone, stimulation test

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INTRODUCTION

Adrenal insufficiency (AI) is a medical condition characterized by decreased secretion of steroid hormones in the adrenal cortex. It may occur due to disorders affecting the adrenal glands, impairment of the pituitary gland and/or hypothalamus, or specific drugs. AI is a rare condition, with a prevalence of all forms being approximately 300 cases/million population[1,2]. Adrenal insufficiency poses diagnostic challenges due to its non-specific symptoms that develop gradually. Chronic adrenal insufficiency manifests with symptoms such as fatigue, diminished energy levels, reduced muscle strength, anorexia, nausea, weight loss, dizziness, myalgia, and joint pain. Given its gravity and associated increased morbidity and mortality, early and correct diagnosis is necessary.

CAUSES OF ADRENAL INSUFICIENCY

Primary adrenal insufficiency

The prevalence of primary adrenal insufficiency is estimated to be in the range of 110–140 cases per million, with an incidence of 4.7–6.2 per million[3]. Primary or peripheral adrenal insufficiency (PAI) is commonly referred to as Addison's disease. In developed countries, autoimmune adrenalitis accounts for 80–90% of primary adrenal insufficiency cases[4]. Autoimmune adrenalitis can occur in isolation (30–40%) or as part of autoimmune polyglandular syndrome type 1(APS) (10–15%) or type 2 (50–60%) (ref.[5-7]). Autoimmune polyglandular syndrome type 1, also known as APECED (autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy) is caused by a mutation in the autoimmune regulator (*AIRE*) gene and is defined as the combination of two of the three following components: PAI, hypoparathyroidism, and chronic mucocutaneous candidiasis[8]. APS type 2, is polygenic, connected to mutations in *DR3-DQ2, DR4-DQ8,55 or CTLA4*. APS 2 predominantly manifests as PAI and autoimmune thyroid disease, with a broader spectrum including primary gonadal failure, type 1 diabetes mellitus, and other autoimmune conditions like vitiligo, chronic atrophic gastritis, or coeliac disease[9,10]. Adrenal cortex autoantibodies or antibodies against 21-hydoxylase are present in more than 80% of patients with recent onset autoimmune adrenalitis[4,5,11].

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder resulting from mutations in genes that encode enzymes responsible for the production of glucocorticoids, mineralocorticoids, and sex steroids by the adrenal glands. CAH is most frequently caused by mutations in the *CYP21A* gene, which encodes enzyme 21-hydroxylase (P450c21) (ref.[12]). Clinical manifestations correlate with the severity of enzyme deficiency, encompassing "classic-salt-wasting" (inadequate aldosterone production), "simple virilizing" (inadequate cortisol production), and "non-classic" forms (features variable degrees of postnatal androgen excess or asymptomatic) (ref.[13,14]).

X-linked adrenoleukodystrophy is caused by a mutation in the *ABCD1* gene, which is responsible for encoding a peroxisomal membrane protein (adrenoleukodystrophy protein) (ref.[15,16]). This mutation leads to the accumulation of very-long-chain fatty acids (>24 carbon atoms). The clinical picture comprises adrenal insufficiency and neurological impairment due to white matter demyelination.

The two major forms are cerebral adrenoleukodystrophy and adrenomyeloneuropathy, with adrenal insufficiency being the sole manifestation in 15% of all cases[1,17].

Congenital lipoid adrenal hyperplasia (LCAH) is a rare autosomal recessive disorder. It is characterized by a disturbance of steroidogenesis of the adrenal cortex and gonads, resulting in impaired conversion of the cholesterol to pregnenolone[18]. LCAH is caused by mutations in the gene encoding steroidogenic acute regulatory protein (StAR), which facilitates the entry of cholesterol into mitochondria to initiate steroidogenesis. Affected patients typically present with signs of severe adrenal failure in early infancy, but 46 XY genetic males are phenotypic females due to disrupted testicular androgen secretion[19].

Congenital adrenal hypoplasia (AHC) is a rare adrenal cortex disorder caused by deletion or mutation of the *DAX-1* gene. Clinical signs and symptoms (poor feeding, failure to thrive, frequent vomiting, dehydration, hyperpigmentation) in infants with AHC, as well as biochemical findings (hyponatremia, hyperkalemia, metabolic acidosis, hypoglycemia), are characteristic of combined glucocorticoid and mineralocorticoid deficiencies[20]. *DAX-1* gene mutations are also responsible for the frequent occurrence of hypogonadotropic hypogonadism in AHC patients[21].

ACTH insensitivity syndromes encompass a rare group of disorders, which include familiar glucocorticoid deficiency (FGD) and triple A syndrome. FDG patients present with isolated glucocorticoid deficiency and normal mineralocorticoid production. They are characterized by undetectable serum cortisol and extremely high plasma ACTH (ref.[22]). The triple A syndrome is a distinct clinical syndrome marked by alacrima, achalasia, and a diverse range of neurological disorders in addition to ACTH insensitivity[23]. FDG is caused by mutations in the ACTH receptor (melanocortin 2 receptor, *MC2R*) and melanocortin 2 receptor accessory protein (*MRAP*) genes, and triple A syndrome is associated with mutations in the *AAAS* gene[24].

Various microbial pathogens, including viruses, fungi, and bacteria, can directly infect the adrenal gland. Immunocompromised individuals in particular are at the most significant risk of either primary adrenal infection or disseminated infection involving the adrenal gland. Attention should be paid to Waterhouse-Friderichsen syndrome, a rapidly progressive condition in which bacterial sepsis appears to cause bilateral adrenal haemorrhage[25].

Bilateral adrenalectomy is rarely used to treat patients with refractory Cushing's disease with persistent hormonal activity that is refractory to other treatment modalities. Other possible indications for bilateral adrenalectomy are bilateral adrenocortical tumours, bilateral pheochromocytoma in patients with hereditary paraganglioma-pheochromocytoma syndromes (PPS/PGL), or bilateral adrenal metastases.

Steroidogenesis inhibitors are a group of drugs that block one or more enzymes in the steroid synthesis pathway. They are usually effective in reducing hypercortisolism, but regular monitoring for adverse effects, such as adrenal insufficiency, is necessary[26]. Causes of primary adrenocortical insufficiency are listed in Table 1.

Central (secondary and tertiary) adrenal insufficiency

The estimated prevalence of secondary adrenal insufficiency is approximately 150–280 per million[2].

Central adrenal insufficiency is often a part of hypopituitarism, which is characterized by inadequate production and secretion of adenohypophysis hormones. Hypopituitarism may result from congenital or acquired causes due to disorders at the pituitary gland or hypothalamus level.

Pituitary tumours and their treatment are the most common causes of hypopituitarism in adults. Traumatic brain injury (TBI) is also a frequent cause[27]. In childhood, hypopituitarism may result from congenital anomalies, perinatal trauma, or defects acquired postnatally. The most common genetic cause of panhypopituitarism is mutations in the *PROP1* gene[28].

Table 1. Causes of primary adrenal insufficiency.

Major causes of primary adrenal insufficiency
Autoimmune adrenalitis
Isolated autoimmune adrenalitis or autoimmune adrenalitis as a part of autoimmune polyglandular syndrome (APS)
Infectious adrenalitis
Tuberculosis, HIV/AIDS, CMV, herpes simplex, fungus (mostly in immunosuppressed patients)
Genetic disorders
Congenital adrenal hyperplasia (21-hydroxylase deficiency, 11β-hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase type 2 deficiency, 17α-hydroxylase deficiency) Adrenoleukodystrophy Congenital lipoid adrenal hyperplasia Congenital adrenal hypoplasia ACTH insensitivity syndromes
Bilateral adrenal haemorrhage
Adrenal infiltration
Bilateral adrenal metastasis or lymphoma, amyloidosis, haemochromatosis
Bilateral adrenalectomy
Drug-induced adrenal insufficiency
Mitotane, ketoconazole, osilodrostat, metyrapone, aminoglutethimide

Hypopituitarism can also result from the use of certain medications. In patients treated with glucocorticoids (GCs), suppression of the hypothalamic-pituitary-adrenal (HPA) axis may occur. The likelihood of HPA suppression depends on factors such as the received dose, dosing time (morning vs evening), and treatment duration. Not only oral corticoids but also those delivered through oral inhalation and highly potent topical corticoids may induce HPA axis suppression when administered long-term[29,30]. Megestrol acetate may cause suppression of the pituitary-adrenal axis due to its affinity for the glucocorticoid receptor[31]. Similarly, medroxyprogesterone acetate affects the hypothalamic-pituitary-adrenal axis, inhibiting ACTH release and causing adrenal insufficiency. Additionally, medroxyprogesterone acetate may exert a mild glucocorticoid effect[32].

Opioid-induced adrenal insufficiency (OIAI) may develop in patients undergoing chronic opioid therapy, leading to the suppression of the hypothalamic-pituitary-adrenal axis. The reported prevalence of OIAI ranges from 15% to 24% (ref.[33]). Causes of central adrenal insufficiency are listed in Table 2.

BIOCHEMICAL DIAGNOSIS OF ADRENAL INSUFFICIENCY

Primary adrenal insufficiency (PAI)

The most common biochemical aberration in AI is hyponatremia, observed in 70–80% of AI cases. The hyponatremia results from aldosterone deficiency, leading to renal sodium loss and concurrent water retention caused by increased antidiuretic hormone release induced by cortisol deficiency[34]. Hyperkalemia, another frequent finding in 30–40% of cases, is a consequence of reduced potassium secretion caused by aldosterone and cortisol deficiency[35]. The combination of low sodium and hyperkalemia is a strong indicator of primary adrenal insufficiency (PAI) (ref.[36,37]). Other biochemical findings include hypoglycemia, mild normocytic anaemia, mild eosinophilia, lymphocytosis and increased creatinine[37-39]. Hypercalcemia is detected in approximately 5.5–6% of cases at the time of PAI diagnosis[40]. The mechanisms contributing to hypercalcemia include increased calcium renal reabsorption in the proximal tubule, increased intestinal reabsorption, and increased skeletal calcium efflux into circulation[41,42].

Central adrenal insufficiency

Comparable to PAI, hyponatremia is the most common biochemical finding, occurring mainly in elderly patients[43]. The cause is an inappropriate increase in vasopressin secretion due to cortisol deficiency and the inability to excrete free water[34]. Cortisol deficiency leads to an upsurge in hypothalamic corticotropin-releasing hormone (CRH) secretion, acting as an antidiuretic hormone (ADH) secretagogue. Moreover, the usual direct inhibitory effect of cortisol on ADH secretion is also absent. Causes of central adrenal insufficiency are listed in Table 2.

Assessment of basal serum cortisol level and diagnosis of AI

Cortisol exhibits both circadian and ultradian rhythms. Nadir cortisol levels are reached around midnight, followed by a gradual rise at around 02:00 to 03:00, and the peak is reached at around 08:30. Cortisol levels then slowly decrease back to the nadir to complete the cycle over 24h (ref.[44]). Approximately 90% of serum cortisol is bound to serum proteins, leaving only 10% circulating as free cortisol, representing the biologically active fraction. The cortisol binding protein (CBG) is the main transport

Table 2. Causes of central adrenal insufficiency.

Major causes of central (secondary and tertiary) adrenal insufficiency
Tumor of hypothalamic-pituitary region
Primary (pituitary adenoma, craniopharyngioma, meningioma, glioma, other)
Metastatic (breast, lung, melanoma)
Pituitary surgery, radiation
Central nervous system infection
Hypophysitis
primary, secondary
Infiltrative lesions
sarcoidosis, histiocytosis
Hemochromatosis
Head trauma
Pituitary apoplexy/Sheehan's syndrome
Empty sella syndrome
Isolated ACTH deficiency
idiopathic, autoimmune hypophysitis, *TRIT* gene mutation, gene mutation for POMC, POMC post-translational modification disorders
Genetic disease
PROP1 mutation, *TBX19* mutation
Inhibition of CRH and ACTH synthesis
Glucocorticoids (topical or systemic), megestrol acetate, medroxyprogesterone, opiates

protein for cortisol. Alterations in CBG levels, whether increased or decreased, correlate with corresponding changes in protein-bound cortisol levels. This, in turn, leads to analogous changes in total cortisol values while the levels of free cortisol remain unchanged[45]. Conditions associated with changes in CBG levels are listed in Table 3.

In cases where adrenal insufficiency is suspected, morning cortisol levels should be assessed, as this may either confirm or rule out AI without requiring additional testing.

Morning basal cortisol level indicating adrenal insufficiency

A morning basal cortisol level below 100 nmol/L indicates adrenal insufficiency, confirming the diagnosis[46,47]. Moreover, levels below 140 nmol/L strongly suggest the presence of AI (ref.[48,49]). It is crucial to emphasize the importance of accurate collection timing, ideally performed between 8–9 a.m.

Basal morning cortisol value excluding adrenal insufficiency

The morning cortisol level cut-off that enables the exclusion of adrenal insufficiency is not clearly defined, varying between 285 and 469 nmol/L across different studies[47-49]. The choice of a higher cut-off prevents dismissing the diagnosis of a partial AI (false negative) and the potentially severe consequences of such an error.

When evaluating the concentration of circulating cortisol determining total cortisol (i.e. free and bound to albumin and binding globulin (CBG) is standard practice in most cases. Immunoassays are most commonly used to measure total cortisol; other options include methods based on chromatographic separation and mass spectrometry[50,51]. The first-generation cortisol immunoassays use a polyclonal antibody and have a certain degree of cross-reactivity to other endogenous and exogenous steroids. The second-generation cortisol assays use a monoclonal antibody and are therefore characterized by increased specificity and reduced cross-reactivity[52,53]. Cortisol concentrations are approximately 20% lower compared to values determined with less specific assays[54]. The reference interval for cortisol is dependent on the analytical method used.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is considered the gold standard for assessing cortisol levels. It is a non-antibody, structural assay highly specific for cortisol[55]. An undeniable advantage of this method is the exclusion of interference with other endogenous or exogenous steroids and an opportunity to conduct simultaneous multiplex steroid analysis within a given sample. The main limitation of mass spectrometry in routine practice is the time-consuming and complex sample preparation, which requires skilled staff. Another limitation, besides the high purchase price of the equipment, is the limited number of samples that can be analysed in one measurement[55-57].

Table 3. Conditions associated with changes in CBG level.

Decreased CBG synthesis
Liver disease
Hypothyroidism
Sepsis
Increased CBG losses
Nefrotic syndrome
Increased CBG synthesis
Oral contraceptives
Pregnancy
Hyperthyroidism
Mitotane

Differentiation between peripheral and central adrenal insufficiency

Plasma ACTH levels are the main factor in distinguishing between peripheral and central AI. Peripheral AI is characterized by increased ACTH levels, whereas the central form typically exhibits decreased or normal ACTH levels.

An elevated ACTH concentration in the presence of cortisol within its normal range can be the first sign of early-stage PAI (ref.[45,58]). Establishing a specific cut-off for ACTH suggestive of PAI proves challenging because the measurement is noticeably affected by analytical bias demonstrated in the ACTH assay[59]. To address this, the Endocrine Society recommends considering a threshold of two times the upper limit of the reference interval.

To determine the presence of mineralocorticoid deficiency in PAI, the measurement of plasma renin and aldosterone simultaneously is recommended[60]. In the early phase of evolving PAI, mineralocorticoid deficiency may predominate and may be the sole manifestation. An elevated plasma renin concentration in combination with an (inappropriately) normal or low serum aldosterone concentration raises suspicion of PAI (ref.[61]).

Salivary cortisol

Serum and saliva cortisol profiles are synchronous with equal amplitudes, rendering saliva a representative medium for assessing serum cortisol levels across the 24-hour period[62]. Salivary samples offer the advantage of accessible collection, and salivary cortisol remains unaffected by changes in the concentration of binding proteins, such as CBG and albumin[63]. Consequently, the salivary cortisol reflects the free fraction of total serum cortisol, although it may be altered by 11β-hydroxysteroid dehydrogenase in the parotid gland[64]. While immunological methods for assessing salivary cortisol are sensitive, they may exhibit cross-reactivity with other steroids like serum cortisol assessment[65]. This problem can be eliminated by LC-MS/MS evaluation. In addition, the LC-MS/MS allows the simultaneous measurement of cortisol and cortisone in human saliva[66].

When measured by immunoassay, various cut-off points have been proposed to either exclude or confirm AI with 100% sensitivity. The most widely employed is a morning salivary cortisol level greater than 16 nmol/L to rule out AI and a level less than 5 nmol/L to strongly indicate AI (ref.[67]) However, this test is not universally accepted as a diagnostic tool for AI.

Urinary free cortisol

Roughly 20% of patients with AI have normal values of urinary-free cortisol (UFC) (ref.[68]). It is therefore not a suitable test for the diagnosis of AI and is not recommended for this purpose.

THE ROLE OF SIMULATION TESTS IN THE DIAGNOSIS OF ADRENAL INSUFFICIENCY

Stimulation tests are used to confirm or rule out the diagnosis of adrenal insufficiency.

Synacthen test

For the diagnosis of peripheral adrenal insufficiency, the short Synacthen (ACTH-(1-24), tetracosactide) test (SST) is recommended, i.e. administration of Synacthen intravenously followed by the measurement of serum cortisol 30 and 60 min after administration. Synacthen is an ACTH analogue which activates MC2 receptors in the adrenal cortex. The test is easy to perform and is not associated with developing side effects. The Synacthen test can also be used in patients with secondary (ACTH) deficiency, provided it is severe and prolonged, i.e. leading to adrenal atrophy. The minimum duration of presumed ACTH deficiency required, after which adrenal atrophy occurs, is 6 weeks[69].

The Synacthen test is performed with different doses of Synacthen, including the conventional dose of 250 μg (HDST) or the low dose of 1 μg (LDST). Another alternative is the administration of 10 μg of Synacthen (MDST), a version of the test performed at our department[70]. The maximum stimulated cortisol response occurs at 30 min during the LSDT and at 60 min during the HDST (ref.[71-73]). Traditionally, a peak cortisol cut-off value of 500 nmol/L can be considered the threshold for normal adrenal function[74]. However, the cut-off values may vary based on the immunoassays used. When interpreting the test results, it is again necessary to consider the factors influencing the total cortisol levels, i.e., the factors influencing the CBG and albumin levels. In diagnosing peripheral adrenal insufficiency, both the HDST and the LDST demonstrate equal predictive value[75], so that the LDST does not provide further sensitivity or specificity compared to the HDST. However, the LDST test may be more sensitive than HDST in diagnosing secondary adrenal insufficiency[49].

Insulin tolerance test

The insulin tolerance test (ITT) assesses the integrity of the entire hypothalamic-pituitary-adrenal axis. This test is considered the gold standard for diagnosing cortisol deficiency as well as growth hormone deficiency[76]. The standard dose of intravenous insulin administered in an ITT is 0.1–0.15 IU/kg body weight. Various protocols for blood sampling can be used, but sampling at 20, 30, 40, 60, 90 and 120 min after insulin administration shows the highest specificity[77]. Adequate hypoglycaemia is defined as a blood glucose nadir below 2.2 mmol/L (ref.[78]). In healthy subjects, hypoglycemia triggers the secretion of counterregulatory hormones (glucagon, epinephrine, norepinephrine, cortisol, GH, and ACTH) approximately 20 to 60 min after insulin administration.

The ITT must be performed with caution and under experienced medical supervision. The test is contraindicated in patients with a history of seizure disorder, coronary artery disease, or in elderly individuals.

Various cut-off values for peak cortisol levels have been proposed for ITTs. Cut-off values correspond to normative cortisol levels that were high when determined by fluorescence assay and resulted in high cut-off values of either greater than 550 nmol/L or 580 nmol/L and above[79]. However, peak cortisol levels are lower when measured by modern immunoassays. The study by Hurel et al. showed that peak serum cortisol levels greater than 500–520 nmol/L indicate a normal stress response[80]. Conversely, the study by Cho et al. found a significantly lower cut-off value for peak serum cortisol, namely 410 nmol/L (ref.[81]). While it would be ideal for laboratories to establish their own cortisol cut-off values for ITT, this is a challenging task. Consequently, the cut-off value of 500 or 550 nmol/L is currently well established[82].

Other tests that assess HPA axis function include the glucagon, CRH and metopirone tests. The glucagon test serves as an alternative to the insulin tolerance test in the diagnosis of secondary adrenal insufficiency, especially when the ITT is contraindicated, as in the cases of young children or older patients. The test involves the administration of glucagon at the dose 1–1.5 mg sc., based on body weight (1 mg for patients who weigh <90 kg and 1.5 for patients who weigh >90 kg), and subsequent measurement of serum cortisol every 30 min based on various recommendations from 3 to 5 hours. Most peak responses occur within three hours[83,84]. The exact pathophysiological mechanism of the test remains unknown.

Peak cortisol values during the glucagon test are lower than those during the ITT test. The glucagon test has low sensitivity compared to ITT. The recommended cut-off values for the glucagon test fall within the range of 300–420 nmol/L (ref.[85,86]).

Although the test is time-consuming and associated with side effects such as nausea, vomiting, abdominal cramps and hunger, the major advantage lies in its safety.

The CRH test, which involves stimulating the pituitary-adrenal axis with synthetic CRH, has low sensitivity and is thus not recommended as a secondary test to rule out adrenal insufficiency[47].

SPECIFIC SITUATIONS

Oestrogen administration and pregnancy

The administration of oestrogen and pregnancy, during which a physiological increase in oestrogen levels occurs, are known factors that increase CBG levels, subsequently increasing total blood cortisol levels. Furthermore, pregnancy represents a state of physiological hypercortisolism, where hyperestrogenism is just one facet of multifactorial mechanisms. During the second and third trimesters of gestation, total cortisol levels experience a two- to three-

fold increase. Free cortisol levels also increase during gestation, indicating an up-regulation of the HPA axis[86,87]. It is, therefore, important to bear this in mind when evaluating cortisol levels in these contexts.

Adrenal insufficiency in pregnancy is relatively rare but is associated with significant maternal and foetal morbidity and mortality if left untreated during pregnancy and the puerperium[88].

Laboratory diagnosis

Due to the physiological increase in cortisol levels during pregnancy, morning cortisol levels below 300, 450 and 600 nmol/L during the first, second, and third trimesters, respectively, should raise the suspicion of AI (ref.[82, 88]).

To confirm the diagnosis of adrenal insufficiency, the recommended approach is the Synacthen (ACTH-(1–24), tetracosactide) stimulation test (SST). The peak cortisol response to 250 μg Synacthen is increased by 60–80 % of the non-pregnant response peak. A peak cortisol cut-off of 700 nmol/L, 800 nmol/L and 900 nmol/L for the first, second and third trimesters, respectively is indicative of a normal response in SST (ref.[89]). It is important to note that cortisol concentrations can vary significantly depending on the assay used, making all cut-offs assay specific. Compared with LC-MS/MS, commercial immunoassays underestimate mean total plasma concentration during pregnancy[89]. There is a need to develop a pregnancy-specific reference range to enhance accuracy in diagnostic assessments.

Cross-reactivity of steroids compounds in cortisol immunoassays

The cause of cross-reactivity is the similar chemical structure of specific molecules. Immunoassays use antibodies with limited specificity, which can lead to a false positive result. Both endogenous and exogenous steroids, such as prednisolone, prednisone, corticosterone, cortisone, 11-deoxycortisol, 11-deoxycorticosterone, 21-deoxycortisol, tetrahydrocortisol, tetrahydrocortisone, 6-methylprednisolone, and 17-hydroxyprogesterone, may induce cross-reactivity in the determination of total cortisol[53,90]. The risk of false positive results can be mitigated when total cortisol is determined by LC-MS/MS.

Treatment with exogenous steroids is used in several indications.

We share our experience with a 55-year-old patient diagnosed with pulmonary fibrosis who had been on long-term glucocorticoids, specifically taking prednisone 10 mg daily at the time of the examination in the endocrinology outpatient clinic. Morning cortisol (obtained from a single blood sample taken at 8 am before prednisone intake) was measured both by immunoassay, yielding a cortisol concentration of 379 nmol/L, and by LC-MS/MS, revealing a cortisol concentration of 45.2 nmol/L. The morning cortisol concentration determined by LC-MS/MS clearly demonstrated suppression of the hypothalamic-pituitary-adrenal axis with long-term glucocorticoid therapy. Therefore, the patient has been informed about the necessity of increasing the glucocorticoid dose in situations of stress.

Congenital adrenal hyperplasia

Congenital adrenal hyperplasia (CAH) is caused by an enzymatic disorder in adrenal steroidogenesis, most commonly attributed to a deficiency in 21-hydroxylase (21-OH). The disorder is associated with impaired glucocorticoid and mineralocorticoid synthesis, coupled with the hypersecretion of steroid hormones (androgens) prior to the enzymatic block (deficiency) (ref.[12]). These steroids can interfere with the determination of total cortisol by immunoassays.

Unrecognised CAH in infants is a life-threatening event, and therefore, based on an Endocrine Society Clinical Practical Guideline, newborn screening based on immunoassays measuring 17-hydroxyprogesterone (17-OHP) in dried blood spots on filter paper should be used as a first-tier screening test. To confirm the diagnosis, biochemical screening of serum 17-OHP by immunoassays or with higher sensitivity by LC-MS/MS has been proposed. Molecular genetic screening is more relevant as a second-tier test[91]. Most cases of 21-OH deficiency cases can be identified by screening for the ten most common variants affecting 21-OH function. The test that covers the most common variants is a “strip test” or a Real-Time-PCR-based assay, which is commercially available. Comprehensive genotyping, including whole-genome sequencing in combination with MLPA (multiplex ligation-dependent probe amplification) or quantitative PCR, is more expensive but allows the detection of rare and novel variants and is therefore important for appropriate genetic counselling[92,93].

We present our experience with the case of a 42-year-old female patient, with a medical history of urogenital reconstruction surgery in childhood and corticosteroid therapy in the past due to 21-hydroxylase deficiency, specifically the classic “simple virilisation” form. As the patient stopped therapy a long time ago, clinical findings counting severe hirsutism, breast atrophy and amenorrhea were present. Laboratory tests using immunological methods revealed the expected elevation in 17-OH progesterone (1496 nmol/L [range 0.3–7 nmol/L]), 4-androstenedione (>139 nmol/L [range 1.4–11.9 nmol/L]) and testosterone (14.83 nmol/L [range 0.3–5.8 nmol/L]) levels, while the morning cortisol levels were within the normal range. The morning serum cortisol concentration determined by chemiluminescence immunoassay (Atellica Siemens) was 617 nmol/L (range 118–618 nmol/L). Steroid hormone measurements by LC-MS/MS confirmed the elevation of steroid hormone levels prior to enzymatic block. However, plasma cortisol concentration, determined to be 178 nmol/L, fell at the lower limit of the reference interval (160–600 nmol/L). The morning cortisol level detected by LC-MS/MS was indicative of AI.

Monitoring medical treatment for Cushing's disease

Endogenous Cushing's syndrome (CS) is characterised by excessive cortisol production. Administration of adrenal steroidogenesis inhibitors is one of the op-

tions for medical treatment. This group of medications includes metopirone (metyrapone), an 11β-hydroxylase inhibitor. In patients with altered steroid metabolism due to metopirone treatment, serum cortisol measurement by immunoassay is susceptible to positive interference due to cross-reactivity with precursor steroids such as 11-deoxycorticosterone, which accumulate due to metopirone blocking the adrenal steroidogenic pathway[94]. Therefore, the use of LC-MS/MS is recommended when monitoring serum cortisol in patients receiving metopirone to ensure proper dose titration and to reduce the risk of unrecognised hypocortisolism, the clinical signs of which may mimic the adverse effects of metopirone treatment.

We present our experience with the case of a 53-year-old man with Cushing's syndrome caused by a cortisol-producing carcinoma of the left adrenal gland. The patient underwent orthotopic adrenalectomy, and 11 months after surgery, a generalisation of the process was demonstrated. Palliative chemotherapy and adrenolytic therapy (mitotane) were indicated. Due to severe florid hypercortisolism, administration of the steroidogenesis blocker metopirone was also recommended to mitigate metabolic complications. Metopirone was administered at a daily dose of 1500 mg. Morning cortisol levels were measured at 1123 nmol/L by immunoassay and 350 nmol/L by LC-MS/MS. There were significant differences between the morning cortisol levels detected by immunoassay and those determined by LC-MS/MS.

CONCLUSION

In conclusion, correct diagnosis of adrenal insufficiency and subsequent initiation of appropriate replacement therapy are critical steps and are the only way to avert a potentially life-threatening adrenal crisis.

Search strategy and selection criteria

Our search strategy aimed to evaluate current studies and reviews published about adrenal insufficiency causes and pitfalls present in diagnostic process of AI. Scientific articles were searched using the PubMed and Web of Science databases. All searches were up to May 2024. The search terms included “adrenal insufficiency”, “cortisol level measurement”, “adrenocorticotropic hormone”, “stimulation test in endocrinology”, “LC-MS/MS”. Only the full texts of the articles in English were reviewed.

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