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REVIEW

Therapy of adrenal insufficiency: an update

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Abstract Adrenal insufficiency may be caused by the destruction or altered function of the adrenal gland with a primary deficit in cortisol secretion (primary adrenal insufficiency) or by hypothalamic-pituitary pathologies determining a deficit of ACTH (secondary adrenal insufficiency). The clinical picture is determined by the glucocorticoid deficit, which may in some conditions be accompanied by a deficit of mineralcorticoids and adrenal androgens. The substitutive treatment is aimed at reducing the signs and symptoms of the disease as well as at preventing the development of an addisonian crisis, a clinical emergency characterized by hypovolemic shock. The oral substitutive treatment should attempt at mimicking the normal circadian profile of cortisol secretion, by using the lower possible doses able to guarantee an adequate quality of life to patients. The currently available hydrocortisone or cortisone acetate preparations do not allow an accurate reproduction of the physiological secretion pattern of cortisol. A novel dual-release formulation of hydrocortisone, recently approved by EMEA, represents an advancement in the optimization of the clinical management of patients with adrenal insufficiency. Future clinical trials of immunomodulation or immunoprevention will test the possibility to delay (or prevent) the autoimmune destruction of the adrenal gland in autoimmune Addison's disease.

Keywords 21-Hydroxylase autoantibodies · ACTH · Addison's disease · Congenital adrenal hyperplasia · Cortisone acetate · Fludrocortisone · Hydrocortisone

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Introduction

Primary adrenal insufficiency (PAI) is the consequence of the destruction or impaired function of adrenocortical cells [1, 2]. The disease is also known as Addison's disease (AD), after the description of 11 cases published by Dr Thomas Addison of Guy's Hospital in London in 1856 [3]. Dr Addison was the first to recognize the clinical picture of lassitude, fatigue, weight loss and skin hyperpigmentation as caused by a disease of the adrenal glands. Since the middle of the nineteenth century, the denomination of AD has been widely used as a synonymous of PAI, and in some cases even of secondary hypocortisolism due to a deficit of ACTH secretion (so-called "white AD"). In this large sense, AD is a definition of hypocortisolism independently of the cause. During the last decades, however, it has become evident that the ethiology of adrenal insufficiency is much broader than initially believed by Dr. Addison, and many additional causes of PAI have been recognized that were not described in the 11 cases originally described in the paper published in 1856 [4, 5]. Accordingly, we will refer to AD only for those ethiologies included in the original cases described by Thomas Addison, namely post-tuberculosis (post-TBC AD) and autoimmune Addison's disease (AAD), on the basis of the description of one of the cases who presented with clinical and histological characteristics strongly suggestive for the immune-mediated form of the disease. For the other forms of hypocortisolism, we will refer either to PAI or to secondary hypocortisolism due to ACTH deficiency.

Epidemiological studies indicate that PAI is much more common than initially thought from studies carried on in the 1970s [6, 7]. A study performed by our group in the Umbria region in central Italy that combined a prescription registry with databases from endocrinological centres

taking care of patients with PAI showed a prevalence of the disease of 117 cases/million, corresponding to 1 case every 8,500 persons [8]. In that study [8], the prevalence resulted higher among females (127 cases/million) as compared to males (106 cases/million). Those figures resulted higher than those previously observed in the UK (at about 100/million) using simple clinical databases with no independent second source of enrolment [9, 10]. Subsequent studies performed in Norway revealed a even higher prevalence at about 140 cases/million, corresponding to 1 case every 7,150 persons [11, 12]. Only scarce data from a single Norwegian study [11] are available regarding incidence of the disease which is currently estimated at six new cases/million/year. Based on the prevalence numbers currently available we can estimate that the total number of individuals with PAI in Europe is around 90,000–100,000.

In Europe, US and Japan the most common cause of PAI is the autoimmune destruction of the adrenal cortex (AAD) [12, 13]. By using measurement of 21-hydroxylase autoantibodies (21OHAb) the gold immune marker of human adrenal autoimmunity [14–19], approximately 85–90 % of PAI cases are classified as AAD, at onset (Table 1). This does not appear to be the case in developing countries, in which most of the cases remain idiopathic. In a study performed in Indian patients [20], 21OHAb were detected in only 21 % of patients with clinically idiopathic, non-granulomatous PAI. It remains at present unclear whether other forms of adrenal autoimmunity or yet unidentified novel ethiologies may be responsible for many cases of adrenal insufficiency in Asia.

The second most common forms of PAI remains post-TBC AD which accounts for approximately 10 % of new cases of adrenal insufficiency in western countries and is still the most common cause of hypocortisolism in developing countries [21] (Table 1).

The clinical spectrum of adrenal insufficiency has, however, considerably expanded in the last decades to include rare genetic diseases, such as X-linked adrenoleukodystrophy (present in approximately 10 % of males with PAI), adrenal hypoplasia congenita, triple A syndrome, familiar glucocorticoid deficiency, Smith-Lemli-Opitz syndrome or Kearns-Sayre syndrome [22–28], but these cases are more prevalent in children population [29]. Other causes of PAI include: adrenalectomy, sarcoidosis, amyloidosis, mycosis (paracoccidiomycosis, coccidiomycosis, histoplasmosis, blastomycosis, cryptococcosis), infection by Cytomegalovirus or atypical Mycobacteria in AIDS patients, adrenal hemorrhages, the anti-phospholipid syndrome, drugs (such as ketoconazole, rifampicin, barbiturates, mitotane, metyrapone, aminoglutethimide) or toxics (paraquat).

PAI often has a progressive evolution that can even last for years. In the early phases, clinical signs are evident only in stressful conditions. With the progression of the glandular damage, reduction of glucocorticoid secretion is accompanied by an increased hypophyseal release of ACTH and POMC-derived peptides. In parallel, the insufficient production of mineraloactive steroids implies an increased plasmatic renin activity (PRA) with an excessive generation of angiotensin II [30]. Unfortunately, the clinical signs and symptoms of PAI are non-specific and a correct diagnosis may dangerously be delayed. Typically, in many cases, symptoms appear years prior to diagnosis, while in others, disease progression is more rapid and adrenal insufficiency manifests acutely. Many patients are diagnosed only after an acute life-threatening adrenal insufficiency (addisonian crisis). The detection of 21OHAb before clinical diagnosis identifies subjects with the so-called pre-clinical AAD in whom the overall risk to develop clinical AAD is 32–40 % within 10 years [30–35].

Table 1 Classification of primary adrenal insufficiency

Aetiology	Frequency
Autoimmune Addison's (isolated or APS2)	85–90 % in western countries/Japan
	20–30 % in developing countries
APS 1	Rare
Post-tuberculosis Addison's	10–15 % in western countries/Japan
	40–60 % in developing countries
Adrenoleukodystrophy	5–10 % of males in western countries
Post-mycosis	Frequent in developing countries
Other forms[a]	Rare

Adrenal hypoplasia congenital, triple A syndrome, familiar glucorticoid deficiency, Smith-Lemli-Opitz syndrome, Kearns-Sayre syndrome, sarcoidosis, amyloidosis, infection by Cytomegalovirus or athypical Mycobacteria in AIDS patients, adrenal hemorrhages, the anti-phospholipid syndrome, drugs or toxics

Thus, screening for 21OHAb may enable identification of at-risk subjects in whom start an early treatment to prevent an addisonian crisis [36]; however, no cost-benefit analyses are currently available and screening of patients with other endocrine autoimmune diseases, as well as relatives of patients with AAD, for adrenal autoantibodies is not yet considered a routine clinical procedure, with the exception of specific conditions such as primary ovarian insufficiency (POI) or chronic hypoparathyroidism.

Furthermore, a typical feature of AAD is the high prevalence of other autoimmune diseases in the context of the so-called autoimmune polyendocrine syndromes (APS) [12, 13, 37–39]. Approximately, two-thirds of patients with AAD have other autoimmune conditions, more often thyroid autoimmune disease, type 1 diabetes mellitus, and/or autoimmune gastritis. In women with AAD, autoimmune POI occurs in approximately 10–20 % of cases [40]. APS-1 is caused by mutations of the AutoImmune REgulator (AIRE) gene and is defined by the combination of two of three components, namely AAD, hypoparathyroidism and chronic mucocutaneous candidiasis [41, 42]. Given the high diagnostic accuracy of IFNϖ-Ab for APS-1 (close to 100 %) [43], it has been proposed that a diagnosis of APS-1 can be formulated also in patients with only one disease component, such as chronic candidiasis or hypoparathyroidism or AD, in the presence of this immune marker [44]. The most common APS-2 is a polygenic disorder linked to the polymorphism of the HLA class II region [45], defined by the presence of AAD with another endocrine autoimmune disorder different than hypoparathyroidism.

As for other endocrine autoimmune diseases, AAD can start at any age, but more often presents in young and middle-aged subjects. Although, the disease is more frequent among females than males, no gender difference is observed below the age of 30 years, and isolated AAD is more prevalent in males than in females.

Clinical signs of adrenal insufficiency are also present in congenital adrenal hyperplasia (CAH) due to steroid 21-hydroxylase deficiency [46]. The incidence of CAH ranges from 1:10,000 to 1:20,000 births [47–50], being more prevalent in some ethnic groups. Most cases of CAH, with only partial reduction of 21-hydroxylase activity, are defined "non classical" and no clinical signs of adrenal insufficiency are evident. Clinical signs of adrenal insufficiency are present in the so-called "classical" forms of salt-wasting or simple virilizing in which the enzymatic activity of 21-hydroxylase is reduced to zero or almost zero.

On the other hand, adrenal insufficiency is also present in secondary hypocortisolism due to ACTH deficiency [51]. Several pituitary and hypothalamic diseases, including tumours, surgical hypophysectomy, head traumas, radiotherapy, autoimmune hypophysitis [52], empty sella, Shehaan's syndrome, infiltrative and granulomatous diseases may determine a reduced secretion of ACTH, especially in stressful conditions, which leads to reduced stimulation of the fasciculata layer of adrenal cortex and hypocortisolism.

Finally, tertiary adrenal insufficiency may be caused by iatrogenic suppression of the hypothalamus–hypophysis–adrenal axis (for instance as a consequence of high-dose steroid treatment abruptly stopped).

Following the clinical suspect of adrenal insufficiency, the diagnosis is confirmed by the demonstration of reduced basal cortisol levels (<3 μg/dl) associated with increased basal ACTH (>100 pg/ml) in the case of PAI or with low/undetectable basal ACTH in the case of secondary hypocortisolism. In stressful conditions, a cortisol serum concentration <15 μg/dl associated with increased ACTH plasma concentration is diagnostic for PAI [53]. In the case of known pituitary disease, or in the suspect of secondary hypocortisolism, diagnosis cannot ruled out simply by detecting normal basal cortisol levels, but, in the case of low-normal cortisol concentrations (e.g. <10 μg/dl) by performing an insulin tolerance test (ITT) or, as a second choice, a stimulation test with low-dose (1 μg) synthetic ACTH. If the 1 μg ACTH test (low dose test—LDT) is considered a valid diagnostic test for the diagnosis of secondary hypocortisolism, even though it shows a lower diagnostic accuracy than the ITT, more controversial is its use in the diagnosis of clinical or pre-clinical PAI, as compared to the classical high-dose 250 μg test (high dose test—HDT). The rationale for using the LDT is based on the observation that 1 μg cosyntropin is inducing a maximal stimulation of the adrenal cortex, similar to that observed with the supra-maximal 250 μg dose [54]. Studies from our group have shown that the LDT has a diagnostic accuracy for pre-clinical adrenal insufficiency similar to that observed with the classical HDT [55, 56]. However, at present, there is not yet conclusive evidence that the LDT has a diagnostic accuracy for PAI higher than the classical HDT. Ongoing studies in subjects with pre-clinical adrenal insufficiency are currently testing the diagnostic sensitivity and specificity of several doses of synthetic ACTH in revealing an adrenal cortex dysfunction. In many cases of PAI (especially those of autoimmune origin, less commonly in post-TBC PAI), hypoaldosteronism with increased PRA and low DHEA-S serum concentrations coexist. During the natural history of AAD, increase of PRA is the earliest hormonal sign of adrenal dysfunction which is detectable in subjects with pre-clinical adrenal insufficiency, but its positive predictive value for future clinical adrenal insufficiency is low.

Once the diagnosis is formulated, it is mandatory to establish the aetiology. The Italian Addison Network developed a few years ago a comprehensive flow-chart taking into consideration immunological and biochemical determinations and imaging data [57]. Based on this flow-chart, the adrenal

autoantibody analysis (and most specifically 21OHAb) is the first assay to be performed. If 21OHAb are positive at medium–high levels (or if both 21OHAb and adrenal cortex autoantibodies—ACA—are positive, irrespective of titre) a diagnosis of AAD is made [57]. If 21OHAb are negative or if the antibody titre is very low in the absence of ACA, an adrenal imaging (such as MR or CT) is needed to exclude sign of infiltrative diseases (such as those typical for post-TBC PAI) [57]. In male patients negative for 21OHAb and with normal adrenal imaging, determination of plasmatic concentrations of very long chain fatty acids (VLCFA) is mandatory to exclude ALD [57, 58]. The importance of formulating a correct aetiological diagnosis is several-fold. Firstly, in AAD patients screening for other autoimmune diseases, such as thyroid diseases, type 1 diabetes or autoimmune gastritis is needed given the high-risk for APS. Secondly, the possibility that AAD may be part of an uncommon form of APS1 should always be considered [59]. Thirdly, women with AAD have an increased risk for POI and early menopause and must be informed of this possibility and offered autoantibody analyses to exclude an ongoing ovarian autoimmunity [40, 60, 61]. Furthermore, an early diagnosis of ALD is important, as in adults this disease may initially manifest only as adrenal insufficiency with no initial involvement of the CNS. Finally, treatment may differ in different forms of PAI as mineralocorticoid deficiency is almost invariably observed in AAD, but is less common in post-TBC PAI or in ALD.

Because of the central role of 21OHAb analysis in the aetiological classification of PAI and in identification of subjects with an ongoing adrenal autoimmune process who are at-risk for future development of clinical signs of the disease and in the attempt of standardizing this immunoassay among different laboratories, an international serum exchange study was performed that showed a high concordance in positive/negative score among four independent laboratories [62]. The EU Consortium Euradrenal has completed a standardization programme in which 14 independent laboratories have analyzed coded serum samples from AAD patients or healthy control subjects. The study confirmed a high concordance among laboratories that used assays based on immunoprecipitation of in vitro translated recombinant human 21OH, but revealed discrepancies in autoantibody levels, that demonstrated that results from different laboratories could not be used interchangeably (unpublished data).

Conventional treatment of adrenal insufficiency: past and present

Patients with adrenal insufficiency require lifelong glucocorticoid replacement and in many cases of PAI or CAH also therapy with mineralocorticoids [63] (Table 2).

Cortisol and other glucocorticoids are secreted by the adrenal gland in a pulsatile and circadian fashion, with a peak release in the morning at wakening and a nadir at midnight [64]. In normal conditions, the adrenal glands produce between 5 and 10 mg/m^2 body surface of cortisol a day [65], which is equivalent to an oral substitutive dose of 15–25 mg hydrocortisone a day.

In many countries, hydrocortisone is available as 10 or 20 mg tablets or 2.5 mg pellets. In some countries, however, oral hydrocortisone preparations are not easily available and the preferred choice of glucocorticoid substitution is cortisone acetate available as 25 mg tablets. Cortisone acetate is a pro-hormone which is converted to hydrocortisone after the first liver passage and with glucocorticoid activity equivalent to 0.8 that of hydrocortisone: a tablet of cortisone acetate 25 mg provides the equivalent glucocorticoid activity of 20 mg of hydrocortisone.

Independently of the aetiology of adrenal insufficiency, the aim of the substitutive therapy with glucocorticoids is to reproduce as much as possible the physiological pattern of cortisol secretion by the normal adrenal gland. Accordingly, the highest dose of hydrocortisone/cortisone acetate must be administered in the morning at wakening, before breakfast, and the total daily dose should be subdivided in 2 or 3 doses [64, 66, 67] (Table 2). It is recommendable to administer the second dose not later than 6 h after the first to minimize the reduction of cortisol serum concentrations below the normal range. If a three-dose daily regimen is chosen, the last dose should be administered 5–6 h before bedtime, to avoid an overexposure to cortisol during the night when cortisol secretion is barely detectable, in physiological conditions.

Other glucocorticoids should not be used for the substitutive therapy of adrenal insufficiency. However, if the patient is in treatment with prednisone or metilprednisolone for other concomitant diseases, it must be remembered that these drugs have four- and fivefold higher glucocorticoid activity than hydrocortisone, respectively.

Several drugs may affect efficacy of glucocorticoid treatment (Table 3) mostly interfering with transport of hydrocortisone or with the activity of CYP3A4, which is the key drug metabolising enzyme modulating hydrocortisone clearance.

A major unresolved problem of the substitutive therapy of adrenal insufficiency is monitoring the adequacy of the replacement with glucocorticoids. Unlikely hypothyroidism in which the single TSH measurement is sufficient to assess adequacy of L-thyroxine therapy ACTH cannot be used in PAI to adjust the posology of hydrocortisone/cortisone acetate. In a study by Feek et al. [68], morning plasma ACTH in PAI patients was either elevated, normal, or suppressed the day when patients omitted to take their doses. In another study of five patients with presumed adequate therapy [69], ACTH was elevated in the morning

Table 2 Glucocorticoid and mineralocorticoid replacement therapy in adrenal insufficiency

Drug	Dose range (mg/day)	Dose regimen (mg)
Glucocorticoids[a]		
Hydrocortisone	15–25	Three-doses (07h00, 12h00, 17h00 ± 1 h)
		15 + 5 + 5
		10 + 5 + 5
		10 + 5 + 2.5
		7.5 + 5 +2.5
		Two-doses (07h00, 12h00 ± 1 h)
		15 + 5
		10 + 10
		10 + 5
Cortisone acetate	25–43.5	Three-doses (07h00, 12h00, 17h00 ± 1 h)
		25 + 12.5 + 6.5
		18.75 + 12.5 + 6.25;
		12.5 + 12.5 + 6.25;
		12.5 + 6.25 + 6.25;
		Two-doses (07h00, 12h00 ± 1 h)
		25 + 12.5
		18.75 + 6.25
Mineralcorticoid[b]		
Fludrocortisone	0.05–0.2	Once a day in the morning (07h00–08h00)

Adapted and modified from UK Addison's disease self help group; www.addisons.org.uk

[a] Avoid other glucocorticoids for substitutive therapy

[b] Avoid other mineralcorticoids for substitutive therapy

Table 3 Drugs and food components interfering with glucocorticoid and mineralcorticoid treatment

Anti-epilepsy	Increase glucocorticoid need
Anti-tuberculosis	Increase glucocorticoid need
Barbiturates	Increase glucocorticoid need
Colestipol	Reduces CBG levels
Drospirenone	May increase fludrocortisone need
Estrogens	Increase CBG levels
Etomidate	Increases glucocorticoid need
Grapefruit juice	Reduces glucocorticoid need
Liquorice	Reduces glucocorticoid need
	Avoid during fludrocortisone treatment
Tamoxiphene	Increases CBG levels
Topiramate	Increases glucocorticoid need

at 08h00 but similar to controls between 12h00 and 14h00. Typically, glucocorticoid therapy must not be increased because of basal high ACTH levels, while a normal (or suppressed) ACTH concentration is considered the sign of excess of glucocorticoid therapy [70]. In a study from our group of 34 PAI patients [71], the subdivision of cortisone acetate in three daily doses was associated with a reduction of ACTH levels as compared to the classical two daily doses, but this finding has not been confirmed in other studies. The reasons why ACTH cannot be used as an accurate biomarker of glucocorticoid replacement are unclear. It is possible that a reduced sensitivity of the pituitary gland to cortisol inhibition, or, more likely, the unability of the standard hydrocortisone/cortisone acetate preparations to induce normal cortisol levels throughout the 24 h be responsible for this phenomenon. Similarly, the 24-h urine cortisol (urinary free cortisol, UFC) measurement has limited value to monitor the glucocorticoid substitutive therapy in patients with adrenal insufficiency. Indeed, UFC is a mere representation of total exposure to cortisol, without providing information on peaks and nadir [72]. In our study of 34 PAI patients [71], UFC was significantly higher in subjects treated with 3-daily doses of cortisone acetate as compared to 2-daily doses. This result, rather than indicating a more adequate treatment regimen with 3-doses, is probably related to the rapid saturation of cortisol-binding globulin (CBG) after absorption of glucocorticoids [73] and a consequence of the number of subdivided doses rather than a sign of adequate physiological substitution. Furthermore, CBG levels vary widely among individuals [74].

Several studies have used plasma cortisol concentrations as a measure of adequacy of glucocorticoid replacement therapy [69, 72, 73, 75–78]. Analysis of peak cortisol levels after 2 h of oral administration of the morning dose has always revealed supraphysiological concentrations, followed by subnormal cortisol levels before administration of the following dose.

Initial studies of plasma cortisol day curves revealed higher plasma concentrations in the morning hours and between 17h00 and 18h00 [69] compared to control

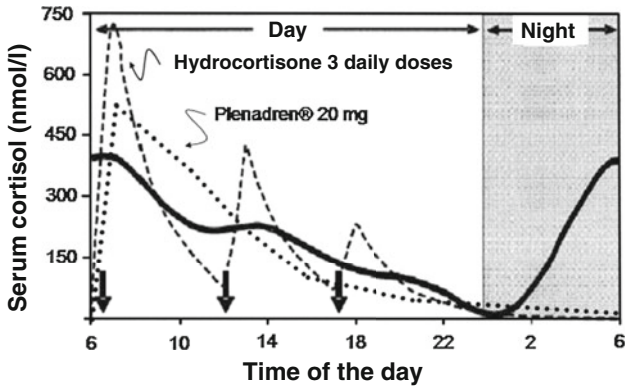

Fig. 1 Schematic representation of serum cortisol concentrations after administration of either three daily doses of hydrocortisone or a single dual-release hydrocortisone preparation (Plenadren® 20 mg) (*dotted line*) as compared to physiologic average concentration (*continuous line*). Adapted from Refs. [57, 153, 154]

subjects in patients with adrenal insufficiency treated with two daily doses of hydrocortisone. Subdivision of the glucocorticoid substitutive therapy in three daily doses corrected in part the nadir of cortisol levels after the initial post-administration peak observed with the two daily doses [76–78] (Fig. 1).

Mah et al. [73] showed that it was possible to adjust the hydrocortisone dose so to obtain an area under the curve of cortisol serum levels between 08h00 and 14h00 which was similar between patients and healthy control subjects. A reduction in variability among different profiles was obtained when using a 0.12 mg/kg dose of hydrocortisone. In the same study, a single blood sample taken 4 h after the first morning dose of hydrocortisone was able to predict the area under the curve and could potentially be used as a single biomarker for adjustment of the hydrocortisone dose. On the other hand, another study showed a significant inter-individual variability of the area under the curve of cortisol serum levels in the time period between 15 min and 8 h [79] after the ingestion of hydrocortisone. Both studies [73, 79] and another [80] showed that serum cortisol was undetectable after 6 h from the oral administration of hydrocortisone.

Alternative biomarkers are cortisol content of human hair [81] and salivary cortisol [82–89]. Several studies have addressed the diagnostic use of salivary cortisol in Cushing's syndrome [90–92] or in special conditions, such as pregnancy or in patients using oral contraceptives or hormonal replacement therapy [93]. Indeed, salivary cortisol can be considered an ultra-filtrate of plasma cortisol and useful to estimate free cortisol, being saliva free of CBG. Some studies have addressed the issue of using salivary cortisol in the monitoring of hydrocortisone substitutive therapy [79, 94–98]. Although an excellent correlation between salivary and serum concentration of cortisol was generally observed, a major concern remains the wide variability of salivary cortisol concentration which strongly limits the use of this analysis with the aim of adjusting glucocorticoid substitutive dose. In addition, several factors such as smoking [99], some chemicals of the diet [100] or lemon juice [101] may interfere with salivary cortisol determination. In addition, salivary cortisol concentration may vary according to the method of collection of saliva (by Salivettes or passive drool) [87, 88] and by number of freezing and thawing [102]. It must also be kept in mind that salivary cortisol correlates with an individual degree of stress and socio-economic status [103, 104]. Hence, even though more extensive investigations are needed, salivary cortisol is not at present a better biomarker than serum cortisol for monitoring of hydrocortisone/cortisone acetate therapy.

Though useful for research applications, cortisol day curves have severe limitations in routine clinical practice because of the need for frequent blood sampling and hospitalization. In addition, plasma cortisol is depending on the systemic absorption of hydrocortisone and equilibration with CBG, and the measure of total cortisol serum concentration is not an expression of the actual concentration of biologically active free cortisol. Determination of salivary cortisol is not yet standardized, and salivary cortisol concentrations appear to be too variable to be useful as a biomarker of the adequacy of the substitutive therapy.

In addition, one should always keep in mind that the biological effect of cortisol is exerted by inducing or suppressing protein synthesis and there is a time gap between the actual measure of cortisol level and the final effect of cortisol exposure on target tissues.

Still today, monitoring of glucocorticoid replacement predominantly relies mostly on clinical assessment. Weight gain, recurrent infection, insomnia, peripheral oedema are clinical sign of over-replacement, while nausea, loss of appetite, lethargy, pigmentation and weight loss are sign of under-replacement. Accordingly, the fine-tuning of daily hydrocortisone/cortisone acetate dosage is obtained only by a detailed investigation of patients feeling and analysis of clinical parameters such as blood pressure, weight, well-being, mental concentration, normal sexual activities, sleep disturbances, or daytime somnolence.

In summary, serum sodium and potassium determinations must be included in annual routine laboratory analyses. Serum cortisol is generally not informative. Cortisol determination prior and 2, 4 and possibly 6 h following the oral administration of hydrocortisone/cortisone acetate may be performed in subjects with signs or symptoms of glucocorticoid deficiency. Daily UFC is only useful to document an excess in the daily dose of glucocorticoids. Similarly, hydrocortisone/cortisone acetate dose must not be adjusted on the basis of ACTH concentration, with the

only exception of ACTH concentrations within the normal range that are generally a sign of an excessive treatment. PRA is useful to adjust treatment with fludrocortisone. An optimal dose of fludrocortisone should maintain sodium/potassium concentrations, plasmatic renin concentration and arterial blood pressure within the normal ranges.

Since the adrenals are stress glands, the glucocorticoid replacement dose must be increased in stressful conditions, such as high body temperature, trauma, surgical interventions or intercurrent illness. Accordingly, patients must be advised to double their oral dose of glucocorticoids in the case of fever or other inter-current illness. A triple dose may be needed for very high body temperature (above 40 °C). In the case of vomiting or diarrhoea, glucocorticoids must be administered i.m. or i.v. (e.g. hydrocortisone 50 mg i.m. twice daily). Similarly, in the case of surgical interventions or invasive diagnostic tests, the dose of hydrocortisone/cortisone acetate must be increased (Table 4). With the exception of the delivery, which requires an increase in dose similar to that of minor surgery with general anaesthesia, pregnancy does not normally require major adjustment of the glucocorticoid substitutive therapy.

At diagnosis, PAI manifests often as an addisonian crisis, with exacerbation of all typical symptoms and signs of adrenal hormone deficiency, with severe dehydration and hypovolemic shock. The emergency therapy is based on administration of saline solution (2–4 l/day) and intravenous administration of hydrocortisone as bolus (100 mg) followed by continuous infusion of 400–500 mg during the first 24 h, with subsequent progressive tapering. An addisonian crisis may be the consequence of the missed administration of oral hydrocortisone for more than 12–18 h or the inadequate reduction of the daily dose or a stressful condition without an adequate increase of the glucocorticoid dose [105, 106].

Along with the need for glucocorticoids, most patients with PAI, and those with salt-wasting classical CAH, but not those with secondary adrenal insufficiency, requires also treatment with mineralocorticoids. The major adrenal autoantigen is expressed in all layers of the adrenal cortex and the autoimmune process attacks both glomerulosa and fasciculata regions. Accordingly, a mineralocorticoid deficiency is almost invariably observed in patients with AAD, who require treatment with fludrocortisone. In some forms of PAI, such as post-TBC, the need for treatment with fludrocortisone is more variable, while in others, such as familiar glucocorticoid deficiency due to mutations of the ACTH receptor, as well as most patients with ALD, only glucocorticoid replacement is necessary. The chosen drug is fludrocortisone normally administered at 0.05–0.2 mg/day. Fludrocortisone is given once a day in the morning, at breakfast. If the treatment with fludrocortisone is stopped, the patients will often develop hypotension and hyperkalemia [107].

During pregnancy, because of the increase of progesterone levels, that peak in the last trimester, dose of fludrocortisone needs often to be increased substantially [108, 109]. In addition, several drugs and substances interfere with hydrocortisone/cortisone acetate and/or fludrocortisone. Thus, patients in therapy with anti-epilepsy/barbiturates, anti-tuberculosis, topiramate or etomidate require higher doses of glucocorticoids. On the other hand, large use of grapefruit juice or licorice may reduce the need for glucocorticoids [110, 111]. Women who use contraceptive pills containing drospirenone may need higher doses of fludrocortisone.

Controversial results are currently available for the substitutive therapy with DHEA. Although a deficit in adrenal androgens is constantly observed in AAD, there is not yet a general consensus on therapy with DHEA in all patients, in routine clinical practice. Epidemiological studies have

Table 4 Doses of hydrocortisone in the case of surgical interventions

Type of surgery	Pre-surgery	Post-surgery
Major surgery with general anaesthesia	i.v. or i.m. 100 mg before anaesthesia	i.v. infusion of 300 mg in 2–3 l saline in the first 24 h
		i.v. or i.m. 100 mg × 2 the 2nd day and 50 mg × 2 the 3th day
		Double oral dose the 4th and 5th day and subsequent progressive tapering
Minor surgery with general anaesthesia	i.m. 100 mg before anaesthesia	Double oral dose for 48 h
		Subsequent progressive tapering
Local anaesthesia	Not required	Double oral dose for 24 h after surgery
Major dental surgery with general anaesthesia	i.m. 100 mg before anaesthesia	Double oral dose for 48 h
		Subsequent progressive tapering
Minor dental surgery with local anaesthesia	Double oral dose before surgery	Double oral dose for 24 h

documented an association between reduced circulating levels of DHEA-S and the development of age-related diseases such as cardiovascular diseases, tumours and osteoporosis [112–114]. Furthermore, in some studies, therapy with DHEA was associated with an improvement of psychic well-being, body composition or bone mineral density [115–117]. In a randomised, double blind clinical trial of 24 women with PAI, DHEA supplementation determined an improvement of the subjective well-being and of parameters related to sexuality [116]. However, other studies have failed to confirm such effects on cognitive and sexual functions, though confirming a beneficial effect on bone mineral density and body composition [118].

An yet unresolved issue is the long-term effects of the substitutive therapy with glucocorticoids. Until a few years ago, the general opinion of physicians was divided between two leading hypothesis: that long-term therapy with hydrocortisone had no negative effects due to its substitutive nature or that may induce the same side effects of anti-inflammatory steroid therapy observed with other, more potent glucocorticoids. None of the two hypothesis was, however, substantiated by strong evidence-based data. During the last 6–7 years, some studies have addressed the issue of mortality, cardiovascular risk and risk for osteoporosis in patients with PAI in life-time substitutive therapy.

By using the National Swedish Hospital and Cause of Death Registers, that covered the period from 1987 to 2001, Bergthorsdottir et al. [119] reported an increased mortality in patients with PAI as compared to the general population. In that study, PAI patients had a risk for premature mortality more than double than that expected, with a significant increase of risk for cardiovascular diseases and malignancies [119]. The presence of diabetes mellitus as comorbidity further increased that risk. Though not supported by any data, authors hypothesized that an excess of hydrocortisone dose as well as the non-physiological pattern of cortisol peaks and nadir during the day may be responsible for the increased mortality observed in PAI patients [120]. Another Swedish study, linking the hospital discharge diagnosis to the National Death Register and Swedish Cancer Register also reported an overall standardised mortality rate of 2.7, with a peak of 4.6 among patients with APS1 [121]. The increased mortality in APS1 patients was also observed in a third Scandinavian study from Norway [121]. The two Swedish studies presented some limitations related to the use of registers in which it was not possible to confirm the accuracy of the diagnosis of PAI and in which very limited information was provided on aetiology of adrenal insufficiency. Interestingly, the Norwegian study [121] was based on the identification of the entire population of patients with adrenal insufficiency in Norway in which mortality rate was then analyzed. By using this method (which appears methodologically more correct because is based on a definitive diagnosis of PAI and includes both hospitalized and not hospitalized individuals), no major increase in mortality rate was observed among patients as compared to the general population, with the exception of young patients below the age of 40 years [121]. Accordingly, at present, no definitive information is indisputably supporting the notion of an increased mortality rate in patients with PAI under substitutive therapy. Patients with hypopituitarism seem to have a double standardized mortality rate [122, 123] and in young adults, adrenal insufficiency accompanying a hypopituitarism have a sevenfold increase in mortality as compared to the general population [124].

In theory, overtreatment with glucocorticoids may have negative health effects, such as cardiovascular and metabolic complications (weight gain, diabetes mellitus type 2, dyslipidemia), hypertension or osteoporosis [125–132]. A positive correlation exists between plasma LDL-cholesterol and endogenous plasma cortisol in healthy men [133]. Some reports have shown that hypopituitary patients on replacement therapy with hydrocortisone, thyroxine and sex steroids have an increased risk of morbility due to accelerated atherosclerosis [134, 135]. Hypopituitary patients in optimal replacement therapy have adverse lipid profiles of increased triglycerides and increased LDL, as compared to healthy controls [134]. Similarly, in hypopituitary patients reduction of total hydrocortisone replacement dose determined a beneficial effect on lipid profiles [134]. In an Italian study [136] an increased, though not statistically significant, risk for impaired glucose tolerance and dyslipidemia was observed in patients with PAI. Ongoing studies are testing the possible influence of polymorphisms of the glucocorticoid receptor on the risk for metabolic complications in PAI patients [137]. Controversial data are available regarding bone mineral density [138–150], mostly because of very low number of subjects studied in most investigations. A large study including patients from Norway, UK and New Zealand reported an increased risk for reduced bone mineral density in PAI patients with an inverse correlation between bone mass density and hydrocortisone dose [148]. Recently, a higher frequency of hip fractures was reported in patients with AAD [149], but it must be noted that a recent study in 81 German patients who received low replacement doses of glucocorticoids found no reduction in BMD [150]. In patients with untreated growth hormone deficiency (GHD), glucocorticoid replacement therapy of secondary hypocortisolism was associated with increased prevalence of vertebral fractures [151]. However, in treated GHD, the prevalence of vertebral fractures was not influenced by the glucocorticoid substitutive dose [151].

More consistent data are available regarding quality of life and general well-being. Questionnaires administered to patients with adrenal insufficiency have shown reduced

vitality and perception of general well-being, with increased concern for health status [152–154]. A limit of these studies is the fact that questions included were not specifically constructed for patients with adrenal insufficiency thus reducing the power of the analysis. Recently, an AddiQoL disease-specific questionnaire was developed as an evaluative disease-specific tool in patients with PAI [155, 156]. The use of this specific questionnaire may facilitate in the future the detection of changes in well-being in both clinical trials and during regular follow-up of patients.

Though not conclusively, the available, published data suggest that the substitutive therapy used so far may be inadequate because it does not avoid the potential risk for an addisionian crisis in stressful conditions and may determine a chronic overexposure to glucocorticoids, as the currently used doses of 20–25 mg/day of hydrocortisone are largely higher than the expected daily cortisol production (7–12 mg/day). In addition, it has been hypothesized that an altered circadian cortisol profile may be associated with an increased risk for abdominal obesity and metabolic syndrome [157]. Indeed, it is likely that the majority of patients currently in substitutive therapy with hydrocortisone/cortisone acetate are overtreated, but the large interpersonal variability in daily need, intestinal absorption and CBG concentrations, as well as glucocorticoid receptor polymorphism make it difficult to translate the biochemical data to the clinical setting.

Nevertheless, it is clear that the available hydrocortisone/cortisone acetate preparations have a limited effect after oral administration (around 5–6 h). Accordingly, patients require several administrations during the day (which limits compliance) and experience up and downs in both cortisol levels and subjective symptoms. A typical cortisol profile performed in a patient in three daily doses of hydrocortisone/cortisone acetate shows reduced or undetectable levels in the morning at wakening, supraphysiological peaks 90–120′ after each oral administration and cortisol levels below the normal range at 12h00–14h00, before the administration of the second dose. Although no conclusive evidence is available, the altered circadian rhythm, much more than the total daily dose may be responsible for both the impairment of the quality of life and the long-term metabolic and bone effects observed in patients with adrenal insufficiency under conventional treatment.

Modified hydrocortisone preparations in the treatment of adrenal insufficiency: the future

There is a strong need for the development of novel pharmaceutical preparations and/or novel strategies of administration to obtain a more physiological serum cortisol concentration time-profile and to improve outcome of glucocorticoid substitutive therapy.

Infusion pumps have been used for subcutaneous deliver of hydrocortisone in seven patients with PAI [97]. The continuous subcutaneous infusion of hydrocortisone has indeed enabled the reconstitution of normal serum levels and of the expected circadian rhythm, which allowed a significative reduction of total daily dose in most patients, with no significative adverse reactions. In clinical practice, this approach may have a natural application in those complicated cases in which the oral administration is not able to prevent addisonian crisis or guarantee a sufficient degree of quality of life because of concomitant diseases or specific subjective needs related to life style and/or genetic factors. Cost considerations certainly represent yet another complicating factor in the large use of this strategy, that, at present, is exclusively experimental and requires larger clinical studies before to be included among the strategies used in routine clinical practice.

A hydrocortisone preparation with delayed-release (Chronocort®) was studied in clinical trials [158, 159]. The rationale of this preparation was to provide a peak in cortisol levels at wakening, also reproducing a more physiological cortisol serum profile. Accordingly, this preparation required two daily administration, but the larger dose was to be administered at evening. Chronocort® induced an increase in cortisol levels after 4 h from oral administration and a peak at 8 h. In clinical trials, 20 mg of this preparation with delayed-release was administered between 22h00 and 23h00. A second, lower dose (10 mg) is to be assumed in the morning for the daily need of late morning and afternoon. Studies on healthy volunteers, treated with dexamethasone to suppress endogenous production of cortisol, have documented an adequate reproduction of the physiological circadian cortisol rhythm when using Chronocort® [158]. A phase II study with patients with CAH treated with a single dose of 30 mg Chronocort® demonstrated a single cortisol peak at 6h00 and 17OH-progesterone concentrations significantly lower than those observed in patients treated with the conventional hydrocortisone preparation [159]. However, high concentrations of 17OH-progesterone where observed in the afternoon [159], which confirms the need for a second smaller dose in the morning. Although the use of this delayed hydrocortisone preparation was shown to provide a more physiological cortisol serum profile, as compared to the conventional preparations of hydrocortisone/cortisone acetate, an important limit was a slightly higher cortisol night exposure, due to the fact that cortisol levels started to raise after 4 h from the evening dose (around 2h00–3h00) to reach the peak at wakening. No line of evidence is supporting the hypothesis that low cortisol exposure during the night may be a safety issue [160], while elevated

cortisol levels in the late evening and at night (between midnight and 4h00) may have detrimental effects on sleep quality and well-being [161] and may provide the basis for long-term metabolic effects.

A dual-release preparation of hydrocortisone (Plenadren®) has been developed as 20 mg or 5 mg tablets. This novel preparation of hydrocortisone is formulated to have an external layer that guarantee a rapid release of the drug and an internal core with delayed-release. This formulation has been developed to combine the two major requests of glucocorticoid replacement therapy: a rapid increase of cortisol levels in the morning and a prolonged lower release of hydrocortisone, to mimic the physiological circadian cortisol rhythm. Thanks to its formulation this dual-release tablet may be administered only once a day in most patients and in the every-day life, in the absence of concomitant illnesses or stressful events. A phase I study showed the safety of this drug and the ability to induce adequate increase of morning cortisol levels in all treated subjects [162]. In addition, it also showed a more gradual decline in cortisol concentrations that reduced or avoided the subnormal serum levels observed between 12h00 and 14h00 with the conventional hydrocortisone preparation (Fig. 1). Furthermore, the single day administration prevented the supraphysiological afternoon cortisol peaks observed with standard hydrocortisone/cortisone acetate tablets and was associated with undetectable cortisol levels at night [162].

A phase II open, randomized, two-period, 12-week crossover multicenter trial confirmed an improved serum cortisol profile with the dual-release formulation, as compared to conventional hydrocortisone, in 64 adult patients with PAI [163]. Patients referred an improvement of the quality of life as psychosocial functioning, cognitive functioning, and positive well-being when taking the dual-release formulation. In the extension phase of the trial, 92 % of randomized patients chose to continue the treatment with the dual-release formulation. Interestingly, a significant reduction in HbA1c was observed in both the entire population of 64 PAI patients (−0.1 % vs. standard hydrocortisone) and in 11 patients with concomitant type 1 diabetes (−0.6 %) [163]. Less pronounced were the effects on lipid metabolism: total cholesterol and LDL-cholesterol were not significantly different between the dual-release and the standard hydrocortisone preparations; administration of the dual-release preparation slightly reduced HDL in the total population, but not in the subgroup of diabetic patients, slightly increased triglycerides in the total population, but decreased them in diabetic patients. A beneficial effect was also observed on systolic and diastolic blood pressure that declined from baseline to 12 weeks treatment with the dual-release, but not with standard hydrocortisone. At the end of the 12-week treatment period, body weight resulted significantly reduced during administration of the dual-release preparation [163].

Thanks to the above mentioned results, the dual-release preparation (Plenadren®) was recently approved by the EMEA for the replacement therapy of PAI, CAH, and secondary adrenal insufficiency, after having received the orphan drug denomination in 2006. Plenadren® is commercially available in Europe from Fall 2012.

After over 50 years of hydrocortisone/cortisone acetate treatment, a novel drug is entering the routine practice of clinical management of adrenal insufficiency. The dual-release hydrocortisone preparation combines several interesting characteristics as it guarantees a rapid release of hydrocortisone early in the morning, when mostly needed, with a prolonged lower effect during the day, thus making it possible a single administration a day, which is certainly important to improve patient compliance. The more physiological serum cortisol profile observed with the dual-release preparation as compared to the conventional hydrocortisone treatment is likely at the basis of the positive effects on quality of life and metabolic parameters. Nevertheless, because of the design of the Phase II trial (open) and the low number of patients, larger and more detailed analyses of the impact of the change in cortisol profile on quality of life, glucose and lipid metabolism, bone and sleep disturbances need to be carried out. No information is currently available on the use of the dual-release preparation in secondary adrenal insufficiency or in CAH, as well as in special conditions such as childhood or pregnancy. In addition, an accurate study of the impact of gastrointestinal pathologies and/or drugs (such as pump inhibitor) on the pharmacodynamics of the dual-release hydrocortisone preparation is needed.

Immunomodulation and immunoprevention in AAD

Extremely scarce data are available on immunotherapy of AAD. The unavailability of an animal model of spontaneous autoimmune adrenalitis has so far limited studies of immunomodulation or immunoprevention of AAD. Interestingly, oral dehydroepiandrosterone (DHEA) replacement in patients with AAD had a bimodal effect on naturally occurring regulatory (CD4 + CD25hiFoxP3+) T cells and lymphocyte FoxP3 expression [164]. Oral DHEA replacement restored normal levels of regulatory T cells and led to increased FoxP3 expression, thus, paving the way to future studies of the impact of this treatment on the human immune system and adrenal autoimmunity, in particular.

More recently, a study of 6 patients with recent-onset AAD showed that treatment with i.v. rituximab was associated with function recovery in one patient who was able to discontinue the substitutive treatment 15 months after

treatment [165]. In another study, of a patient with subclinical AAD, with impaired response to ACTH Synacthen test, high-dose i.v. metilprednisolone treatment, carried out because of a concomitant severe Graves' ophthalmopathy was associated with disappearance of 21OHAb and was able to revert the adrenal dysfunction for over 10 years [166]. Taken together, these limited experiences suggest that both subclinical and new-onset AAD is a potentially reversible condition in some patients, who may benefit of immunomodulatory therapies.

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