Monitoring of Therapy in Congenital Adrenal Hyperplasia

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BACKGROUND: Congenital adrenal hyperplasia is a group of disorders caused by defects in the adrenal steroidogenic pathways. In its most common form, 21-hydroxylase deficiency, patients develop varying degrees of glucocorticoid and mineralocorticoid deficiency as well as androgen excess. Therapy is guided by monitoring clinical parameters as well as adrenal hormone and metabolite concentrations.

CONTENT: We review the evidence for clinical and biochemical parameters used in monitoring therapy for congenital adrenal hyperplasia. We discuss the utility of 24-h urine collections for pregnanetriol and 17-ketosteroids as well as serum measurements of 17-hydroxyprogesterone, androstenedione, and testosterone. In addition, we examine the added value of daily hormonal profiles obtained from salivary or blood-spot samples and discuss the limitations of the various assays.

SUMMARY: Clinical parameters such as growth velocity and bone age remain the gold standard for monitoring the adequacy of therapy in congenital adrenal hyperplasia. The use of 24-h urine collections for pregnanetriol and 17-ketosteroids may offer an integrated view of adrenal hormone production but target concentrations must be better defined. Random serum hormone measurements are of little value and fluctuate with time of day and timing relative to glucocorticoid administration. Assays of daily hormonal profiles from saliva or blood spots offer a more detailed assessment of therapeutic control, although salivary assays have variable quality.

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Congenital adrenal hyperplasia (CAH) is a group of disorders characterized by defects in one of the enzymes of the adrenal steroidogenic pathway. More than 90% of cases are due to mutations in cytochrome P450, family 21, subfamily A, polypeptide 2 (CYP21A2), the gene coding for the 21-hydroxylase enzyme. Depending on the severity of the mutation, 21-hydroxylase deficiency ultimately leads to variable degrees of glucocorticoid and mineralocorticoid deficiency due to the inability to produce cortisol and aldosterone, respectively (Fig. 1). Cortisol deficiency results in an increase in adrenocorticotropic hormone (ACTH) concentrations due to a lack of negative feedback to the pituitary. Increased ACTH concentration, as its name implies, stimulates hyperplasia of the adrenal gland along with increased production of adrenal steroids proximal to the enzyme block. These increased concentrations of progesterone and 17-hydroxyprogesterone (17OHP) are shunted into the adrenal androgen pathway and lead to increased concentrations of dehydroepiandrosterone and androstenedione, which are then peripherally converted to testosterone.

The clinical symptoms of CAH directly result from either the deficiencies in mineralocorticoid or glucocorticoid production or from the overproduction of adrenal androgens. Mineralocorticoid deficiency leads to renal salt wasting, which results in severe dehydration if left untreated. Glucocorticoid deficiency has many clinical ramifications, but most importantly it can lead to adrenal crises with hypoglycemia and hypotension in the setting of an intercurrent illness or physiological stress. Androgen excess causes virilization of females, resulting in increased hirsutism, acne, menstrual abnormalities, ditoromegaly, and in its most severe form masculinization of the external genitalia. In both sexes, increased androgen production leads to growth acceleration as well as premature maturation of the growth plates, ultimately resulting in reduced final height. Traditionally, CAH is divided into classical and nonclassical forms. The classical form presents in infancy, and the nonclassical form presents later in childhood, leading to growth acceleration, premature adrenarche, or menstrual abnormalities. The presentation in patients with the classical form of CAH is further subdivided into salt-wasting and simple virilizing categories, depending on the presence or absence of mineralocorticoid deficiency, respectively.

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Nonstandard abbreviations: CAH, congenital adrenal hyperplasia; ACTH, adrenocorticotropic hormone; 17OHP, 17-hydroxyprogesterone; PRA, plasma renin activity; PT, pregnanetriol; 17-KS, 17-ketosteroids; LC-MS/MS, liquid chromatography-tandem mass spectrometry.
The goal of therapy in CAH is to replace the missing glucocorticoids and mineralocorticoids, thereby suppressing ACTH concentrations and normalizing adrenal androgens. Although this goal seems rather straightforward, in practice it is quite difficult to achieve. The inherent enzyme block leads to increased adrenal precursor concentrations despite normalization of ACTH; thus glucocorticoids must be replaced at a supraphysiologic concentration to fully normalize adrenal androgens (1). Glucocorticoids themselves have potent growth-suppressive effects. Practitioners must strike a fine balance, because overtreatment with glucocorticoids can lead to poor growth and undertreatment to androgen excess and premature epiphyseal maturation. A metaanalysis of the published CAH literature revealed that the mean adult height of patients with 21-hydroxylase deficiency is $-1.37$ SDs despite treatment (2). Over the years, practitioners have employed various methods of monitoring and treating CAH to optimize long-term outcomes. Medications commonly used for glucocorticoid replacement include hydrocortisone, prednisone, and dexamethasone, with hydrocortisone favored in children because of its short half-life and lower likelihood of suppressing growth (3). If mineralocorticoid replacement is necessary, fludrocortisone, a potent mineralocorticoid with minimal glucocorticoid effect, is prescribed. Treatment may not be necessary in the late-onset form and in selected adult patients, depending on the desired therapeutic goal (4). This report provides an overview of the clinical and biochemical parameters used to monitor therapy for 21-hydroxylase deficiency.

Monitoring of Mineralocorticoid Replacement

**CLINICAL MONITORING**

Mineralocorticoids are typically replaced by using fludrocortisone. Inadequate mineralocorticoid re-
placement leads to renal salt wasting, hyperkalemia, and hyponatremic dehydration. Patients may complain of salt craving and lightheadedness due to dehydration. Excessive fludrocortisone treatment can lead to hypertension that is often asymptomatic in the pediatric population. Blood pressure should be monitored at every clinical visit and compared to age- and height-adjusted norms.

**BIOCHEMICAL MONITORING**

Although inadequate mineralocorticoid replacement in CAH patients may lead to electrolyte abnormalities such as hyponatremia and hyperkalemia, these rarely occur after infancy. Plasma renin activity (PRA) is a sensitive marker of mild volume depletion and can be increased in patients who are receiving inadequate doses of fludrocortisone, even if the patients have electrolyte concentrations within reference intervals (5). CAH is typically dichotomized into “salt-wasting” and “non–salt-wasting” varieties based on initial presentation and electrolyte abnormalities in patients. In reality, however, there is a spectrum of mineralocorticoid deficiency similar to the spectrum of glucocorticoid deficiency that depends on the severity of the underlying CYP21A2 mutation. Even patients with non–salt-wasting” illness can have improvements in their overall adrenal suppression and decreased requirement for exogenous glucocorticoid with administration of fludrocortisone (5). Direct measurement of renin concentration has been shown to correlate with PRA in patients with CAH (6). PRA or renin concentrations should be measured periodically to ensure that patients are receiving optimal mineralocorticoid replacement. It is critical to the proper interpretation of data from these assays to understand the differences between the activity assays and immunoassays used to measure renin.

Activity assays measure the production of angiotensin I from angiotensinogen, during which the patient’s endogenous renin serves as the enzyme to catalyze the reaction. Typically the angiotensin I produced is then quantified with an immunoassay. Several variations of the assay exist. The advantages and disadvantages of PRA and renin immunoassays are discussed in the recent review by Campbell et al. (7). In the classic PRA assay the angiotensinogen and renin are endogenous, thus it is not possible to use a renin calibrator in this format. It is important that monitoring be conducted by using the same assay in the same laboratory over time to minimize variability in results.

Correct sample processing is crucial for accurate assessment of renin activity or concentration. In vitro conversion of prorenin to renin can occur via cryoactivation when samples are stored at temperatures between −5 °C and 4 °C (7–9). Although several investigators have found that room temperature storage for up to 24 h did not result in significant conversion, samples are typically frozen because few laboratories have the capability to perform the assay immediately. Most investigators recommend that blood be centrifuged within 10 min of collection and the plasma frozen if not immediately assayed; however, sample processing is still a controversial topic (7, 10). Brossard and Corcuff have demonstrated that in the physiologic range the clinical impact of 4 °C handling is not significant, and sample processing at this temperature is much more practical in the clinical setting (10).

In addition to standardized sample processing, conditions for the patient must be standardized before sample collection. Because changes in the patient’s dietary intake of salt and position of the patient during sample collection (supine vs standing) will cause fluctuations in renin production, collection should be conducted with the patient on a stable diet (100–200 mEq Na/day), and the patient must be ambulatory for at least 60 min before sample collection. Significant variation in results can be attributed to this normal physiologic variation (11). Reference intervals are based on postural state and time of day, and data should be evaluated accordingly. Clinicians should also be aware that activity assays are also affected by conditions affecting angiotensinogen concentrations, such as pregnancy and estrogen administration. Measurement of renin concentration by immunoassay in patients receiving renin inhibitors such as Aliskiren will differ depending on the assay used, and will typically lead to overestimation of the renin concentration (7).

**Monitoring of Glucocorticoid Replacement and Adrenarche Androgen Suppression**

**CLINICAL MONITORING**

Clinical parameters remain the gold standard for adequate control of CAH, because maximal adult height and decreased virilization are the ultimate goals of therapy. Height should be carefully measured at each clinical visit, and growth velocity should be calculated and compared to normal values for age (12). Children should be seen every 3–4 months for monitoring, especially during periods of rapid growth such as infancy and the pubertal years. X-rays of the hand should be performed annually, at a minimum, for evaluation of bone age (maturation of the epiphyses). In addition, signs of androgen excess should be evaluated at each visit, including development of acne, hirsutism, or oily skin. Increased growth velocity with advancement of bone age is a clear indicator of undertreatment, even in the absence of other physical signs of androgen excess. Conversely, decreased growth velocity may be a sign of overtreatment, especially if it is observed to occur in concert with weight gain or retardation of bone age.
Medication dose should be adjusted accordingly. One group reported that good height outcomes were obtained by adjusting glucocorticoid doses to maintain a growth velocity at the 50th percentile without any other biochemical monitoring (13). Einaudi et al. (14) found that growth velocity was a better predictor of the ratio of bone age to chronologic age than 24-h urine pregnanetriol (PT) or single morning 17OHP concentrations (see below).

BIOCHEMICAL MONITORING: URINE STUDIES
Before the development of serum steroid immunoassays, urine assays were the only available means of diagnosing and monitoring patients with CAH. The 2 most commonly used assays measure urinary excretion of PT, a urinary metabolite of 17OHP, or 17-ketosteroids (17-KS), a urinary metabolite of androgens. In theory, 24-h urine collections provide insight into the overall state of adrenal suppression throughout the day. Despite their widespread clinical use, there are few data to support the efficacy of adjusting glucocorticoid dose based on these urinary assays, and the American Academy of Pediatrics Technical Report on CAH states that 24-h urinary concentrations of 17-KS and PT are unnecessary (15). Brook et al. (16) commented that urinary 17-KS concentrations were significantly correlated with growth, whereas PT concentrations were not. They concluded that maintaining urinary steroid concentrations close to mean reference interval values leads to improved growth. Hendricks et al. (17) found no correlation between either PT or 17-KS and growth velocity and a modest correlation between 17-KS and bone age advancement. Bailey et al. (18) also did not find a correlation between 17-KS or PT and height velocity. They reported poor height outcomes after adjusting glucocorticoid dosage to normalize 17-KS and PT concentrations, likely owing to overtreatment. Both Hendricks and Bailey concluded that repeated careful clinical examination and measurement of bone age are the best criteria for determining glucocorticoid dose.

SERUM MONITORING
Because urine-based monitoring is cumbersome, often inaccurate owing to poor collection technique, and possibly of limited value, there has been great interest in using serum markers to monitor control in CAH. Various adrenal steroids have been measured as markers of control, but the 3 most commonly used are 17OHP, androstenedione, and testosterone. The concentrations of these hormones may vary widely in an individual patient depending on the time of day and the interval since the patient’s last glucocorticoid dose (19–22). The type of glucocorticoid will influence this response, depending on the pharmacokinetics of individual drugs (21, 23). ACTH has an inherent diurnal rhythmicity that influences the diurnal variation in each of these hormones. Although this variation is more marked for 17OHP, it is also present in androstenedione and testosterone (19–21, 24).

Given the influence of these factors, one must interpret the available medical literature carefully because in many of the study reports the timing of sample collection is not rigorously defined, especially in regard to the time interval since the last glucocorticoid dose. In addition, the majority of reported studies used urine markers or subjective assessments of clinical control as the gold standard for CAH control. Few longitudinal studies have compared serum markers to outcomes such as growth velocity or bone age acceleration (17, 25). With these caveats in mind, there are a few important lessons regarding serum monitoring that are clear in the medical literature. First, the majority of studies have shown good correlation between 17OHP, androstenedione, and testosterone concentrations in a single blood sample, indicating that these hormone concentrations are all under similar influences (26).

Second, random measurement of a single value of 17OHP is generally not beneficial and does not correlate with any other marker of control (14, 17, 19, 20, 26, 27). A concentration that is extremely high (>40 nmol/L) indicates undertreatment. Third, androstenedione concentrations correlate best with 24-h urine 17-KS (27–29), but single values are difficult to interpret and do not necessarily reflect clinical control, with overlap between values in well-controlled and poorly controlled individuals (29). Fourth, it is extremely difficult to assess overtreatment from a single serum value of any of these hormones, because values within the “normal range” may reflect either good control or overtreatment (26). In general, one should not attempt to normalize 17OHP concentrations, because this will certainly lead to overtreatment. Fifth, testosterone concentrations are not useful markers in male infants or pubertal males owing to testicular production of testosterone under the influence of gonadotropins (27–30). Finally, if one does wish to use serum concentrations to assess control, they should be obtained at a standardized time before the administration of the morning glucocorticoid dose. Hughes and Read (25) performed a longitudinal study of 16 patients with CAH in which they showed that 9 AM serum 17OHP concentrations <40 nmol/L or testosterone concentrations <0.8 nmol/L, drawn before hydrocortisone administration and measured by using an RIA, correlated well with adequate growth. One must be careful in implementing this approach because assays vary and these cutoffs may not apply depending on the assay used. Also, these concentrations would not apply to individ-
uals receiving long-acting glucocorticoids in the evening.

The gold standard method for analysis of 17OHP, androstenedione, and testosterone is widely considered to be liquid chromatography–tandem mass spectrometry (LC-MS/MS). LC-MS/MS offers superior analytical sensitivity and specificity compared to immunoassay but is not without its own issues. In particular, there is very little harmonization of methods between laboratories, necessitating site-specific reference intervals (31). As with plasma renin it is important that the same method and laboratory be used over time to assure that observed changes reflect physiologic change and are not due to differences in method. It is also important to note that although LC-MS/MS is highly specific, it is not free from interference. The use of ion ratios is an important tool to detect possible interference (31). MS methods are becoming more common in laboratories, and it can be expected that many institutions will switch to LC-MS/MS–based analysis of steroids in the near future. It is critical that clinical chemists keep clinicians informed when changes in methods occur to assure correct interpretation of results when monitoring CAH patients.

**BLOOD SPOT AND SALIVARY MONITORING**

Because serum monitoring provides a snapshot of adrenal hormonal production at only 1 point in time, some researchers advocate obtaining 24-h hormonal profiles by using serial blood spot or salivary samples (30). While it is possible to accomplish the same goal with multiple serum samples, this procedure is often not logistically feasible, and the stress of frequent phlebotomy may influence hormonal levels. Both blood spots and salivary 17OHP concentrations have been found to correlate well with serum values (32, 33). Measurement of blood spot 17OHP concentrations is the standard method for newborn screening for CAH, but blood spots are also useful for obtaining daily profiles in young children who are not able to reliably provide salivary samples (34). Blood spots require only a few drops of blood applied to filter paper. 17OHP concentrations are stable for weeks when samples are kept at room temperature, and the samples can be conveniently mailed back to providers (35).

Androstenedione concentrations can also be reliably measured in saliva and have been found to correlate with overall control (32). Young et al. created nomograms to interpret daily profiles of salivary or blood spot 17OHP and salivary androstenedione (24, 36). Daily profiles allow for an assessment of overall control and of medication compliance, as well as for fine-tuning of therapy, taking into consideration hormonal diurnal variation and response to various glucocorticoid regimens (33, 37). Some researchers have advocated for measurement of salivary cortisol concentrations as well to better understand hydrocortisone pharmacokinetics in individual patients (38, 39).

Logistically, the use of blood spots for CAH monitoring is ideal, given the ease of collection and simple sample storage and processing requirements as well as a solid body of knowledge related to the conduct of assays from blood spots. Measurement of steroids from blood spots by LC-MS is a fairly mature field of analysis and avoids the issues of cross-reactivity seen with many immunoassays (40). Application for monitoring in CAH does not require changes in analytical methods if blood spots are used. Although researchers who conducted the studies reported in the above-noted references have found utility in quantitative measurement of steroids in dried blood spots, it should be noted that most did not investigate the impact of differing hematocrits, showing a correlation only between venous samples and the dried blood spot samples. Clinicians should be cautious in comparing results from dried blood spots against expected values obtained from venous blood samples. The hematocrit of the sample applied to filter paper for dried blood spot sampling will have a significant impact on results. Mei et al. demonstrated that a 30% hematocrit provided approximately 47% more plasma than a punch taken from blood spot with a 70% hematocrit, resulting in falsely increased analyte concentrations (41). Ideally, interpretation should be based on reference values that have been determined with dried blood spots. If these are not available, one can assess relative changes against previous dried blood spot values obtained from a patient.

Although salivary collection seems logistically simpler compared to blood collection, proper preparation and collection are critical to obtaining quality samples that will provide reliable results. Gröschl has provided a nice review of the status of salivary hormone analysis (42). Approximately 1% of saliva is derived from plasma present in the mouth due to gingival leakage and abrasions to oral tissue (43). Individuals with poor oral health will have even more plasma mixed with their saliva. Swartz and Granger have recommended that measurement of saliva transferrin concentrations be used to assess the quantity of blood contamination in a saliva sample that samples with blood present be rejected (44). Patients who collect saliva must do so at least 1 h after eating, brushing, flossing, or other activities that may irritate the gums. Patients should avoid high sugar and acidic foods and thoroughly rinse their mouths before collection to prevent assay interference. Individuals with regular bleeding from their gums are not candidates for using saliva to monitor CAH.

Common methods for saliva collection, such as Salivettes, cotton rope, or polyester sponges, cannot be
used when collecting samples for 17OHP, androstenedione, or testosterone analysis. Chemicals present in these collection devices will interfere with the measurement of hormone concentrations when measured by immunoassay (45). Gröschl found that the polyester version of the Salivette was suitable for analysis of salivary steroids by LC-MS, but cotton rope or other versions of the Salivette were unsuitable (46). Unstimulated, passive drool is the preferred method for collection of saliva when measuring the above analytes. To simplify the collection process, patients can be provided with large-bore straws to direct the saliva into collection containers. The use of gum or wax for patients to chew in an effort to stimulate saliva production is to be avoided because this may introduce interferents or result in a sample pH that interferes with analysis (47, 48).

In contrast to blood spots, saliva samples must be refrigerated within 30 min of collection and frozen at −20°C within 4 h of collection to minimize bacterial growth. For both blood spot and saliva collection, patients must be reminded of proper sampling times in relation to medication dosage, and that recording the time and date of collection is critical to proper interpretation of the results.

It is also important that laboratories that accept saliva samples have validated the saliva matrix in their assay. Simple substitution of saliva into assays primarily designed to measure plasma concentrations of steroids usually results in poor assay precision and limits of quantification (49). The significantly different pH and protein content of saliva requires that most steroid immunoassays be redesigned to assure accuracy and precision. There are a few LC-MS–based steroid assays validated for use with saliva, but the majority of available assays are immunoassay based. Validated immunoassays for 17OHP and androstenedione have little cross-reactivity with other steroids, whereas cross-reactivity issues with exogenous steroids are problematic when measuring testosterone and cortisol by immunoassay. As such, if testosterone and cortisol concentrations are desired, analysis by LC-MS–based methods is preferred.

MONITORING FOR CATECHOLAMINE DEFICIENCY

Several investigators have documented epinephrine deficiency and impaired glucose regulation in patients with CAH (50–52), suggesting the possibility that the combination of epinephrine and cortisol deficiency might result in hypoglycemia during stress. Adrenomedullary function may predict the clinical severity of CAH, and the measurement of plasma free metanephrine has been suggested for this purpose (53), although this technique has not come into common practice.

Treatment of congenital adrenal hyperplasia requires frequent monitoring to optimize long-term outcomes. Clinical parameters such as growth velocity and epiphyseal maturation, as measured by bone age x-ray, are the most reliable markers of control and adequate treatment. However, clinical monitoring alone allows only for a reactive approach to treatment as opposed to a proactive approach, whereas 24-h urine collections for 17-KS provide an integrated view of androgen production and may be a useful indicator of control even though target values are not well defined. Single random measurements of serum hormonal values are not particularly useful except in the extremes. More information is provided by 24-h hormonal profiles obtained via saliva or blood spot collection, and their use allows for fine-tuning of medical therapy. A lack of long-term data, however, prevents us from concluding that 24-h hormonal profiles will improve clinical outcomes. Standardized sample collection and handling are critical to proper interpretation and comparability of results over time. Practitioners must become familiar with the hormonal assays used in their laboratories, because there is significant variability between the results of different types of assays.

References


