

ORIGINAL ARTICLE

Hypothalamic–pituitary–adrenal axis suppression in asthmatic children on inhaled and nasal corticosteroids: is the early-morning serum adrenocorticotrophic hormone (ACTH) a useful screening test?

Ekkehard W. Zöllner¹, Carl Lombard², Ushma Galal², Stephen Hough³, Elvis Irusen⁴ & Eugene Weinberg⁵

¹Paediatric Endocrine Unit, Tygerberg Children's Hospital, University of Stellenbosch, Cape Town, South Africa (formerly Endocrine Unit, Red Cross Children's Hospital, University of Cape Town, Cape Town, South Africa); ²Biostatistics Unit, Medical Research Council, Cape Town, South Africa; ³Department of Medicine, Endocrine Unit, Tygerberg Hospital, University of Stellenbosch, Cape Town, South Africa;

⁴Pulmonology Division, Department of Medicine, Tygerberg Hospital, University of Stellenbosch, Cape Town, South Africa; ⁵Allergy Unit, Red Cross Children's Hospital, University of Cape Town, Cape Town, South Africa

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Correspondence

Ekkehard Werner Zöllner, Endocrine & Diabetes Unit, Faculty of Health Sciences, Department of Paediatrics, P.O. Box 19063, 7505 Tygerberg, South Africa.
Tel.: +27219389506
Fax: +27219389138
E-mail: zollner@sun.ac.za

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Abstract

Background: Hypothalamic–pituitary–adrenal axis suppression (HPAS) in asthmatic children treated with inhaled corticosteroids (ICS), with or without nasal steroids (NS), may be more common than previously thought. Only dynamic testing will identify children at risk of adrenal crisis. It is impractical to test all asthmatic children for HPAS with a gold standard adrenal function test, i.e. the metyrapone or insulin tolerance test.

Objective: To determine which clinical or biochemical parameter is the most useful screening test for HPAS in asthmatic children.

Methods: Twenty-six asthmatic children, 5–18 yr old, on ICS $\pm$ NS, not treated with oral or topical steroids in the preceding year were recruited. Height, weight, height velocity, weight velocity and a change in systolic blood pressure from the recumbent to the standing position (Δ SBP) were recorded. Early-morning urine for urinary free cortisol (UFC) and urinary cortisol metabolites (UCM) was collected. UFC was analysed by both a chemiluminescent assay and gas chromatography/mass spectrometry (GC-MS). Morning serum cortisol and adrenocorticotrophic hormone (ACTH) levels were measured. The overnight metyrapone test was performed if the fasting morning serum cortisol was >83 nmol/l. HPAS was diagnosed if the ACTH failed to rise >100 pg/ml after metyrapone. Spearman correlation coefficients (r) were calculated between the post-metyrapone ACTH and each variable. A receiver-operating characteristics (ROC) curve was drawn for the most promising test, and the diagnostic performance was calculated.

Results: All clinical and biochemical parameters investigated were weakly and non-significantly correlated with the post-metyrapone ACTH, except for the morning serum ACTH ($r = 0.68$; $p < 0.001$). The best discrimination between those who have and those who do not have HPAS is a morning serum ACTH level of 11.7 pg/ml. This corresponds to a sensitivity of 0.89 (0.57–0.98), a specificity of 0.77 (0.53–0.90), a positive predictive value of 0.67 (0.39–0.87), a negative predictive value of 0.93 (0.69–0.99), an accuracy of 0.81 (0.61–0.94), a positive likelihood ratio of 3.78 (1.68–9.49) and a negative likelihood ratio of 0.15 (0.03–0.60).

Conclusions: The morning serum ACTH level was found to be the most useful screening test to detect HPAS in this sample of children receiving ICS $\pm$ NS. A larger study should be undertaken to refine the diagnostic precision of the morning serum ACTH level.

Hypothalamic–pituitary–adrenal axis suppression (HPAS) may be more common in asthmatic children on inhaled corticosteroids (ICS) than previously thought (1). As a rule, HPAS can only be detected clinically once the children present with an adrenal crisis during a time of stress. Symptoms of chronic adrenal insufficiency on the other hand are non-specific and can thus easily be overlooked or misinterpreted. Only dynamic testing will identify which children are at risk of adrenal crisis. It is, however, impractical to test all children with a gold standard adrenal function test [insulin tolerance test (ITT), metyrapone test]. A suitable screening test should therefore be identified.

Before a decision to screen for any disease is made, strict criteria pertaining to the disease, the test and the intervention policy have to be met (2, 3). Children on ICS and/or nasal steroids (NS) should be screened for HPAS, because an adrenal crisis is associated with significant morbidity and mortality. Overt adrenal insufficiency would be preceded by a period of suppression in which the patient appears to be entirely asymptomatic. The prevalence of HPAS has been established: roughly every third child attending an allergy clinic for asthma and allergic rhinitis may develop HPAS (in press). The suppression can easily be reversed by decreasing the dose of ICS and/or NS and substituting it with a steroid alternative, i.e. a long-acting β agonist (LABA), a leukotriene receptor antagonist (LTRA) or a long-acting (LA) theophylline (at an anti-inflammatory low dose). Whenever the dose cannot be reduced, a Medic Alert bracelet should be provided on which the condition is clearly stated and the attending clinician is instructed to provide additional steroid therapy during times of stress such as an acute asthma exacerbation, intercurrent illness or trauma. A suitable screening test for HPAS should first and foremost be valid, i.e. it should accurately distinguish between those who do and those who do not have HPAS. Furthermore, it should be cost-effective, easy to perform, safe, reliable, and the cut-off value between pathology and normality should be reasonably well defined.

Screening tests to be considered include clinical indicators (e.g. anthropometric measurements, detection of orthostatic hypotension) or basal adrenal function tests. The limitations of the latter have previously been discussed in detail (4). Cortisol profiles, for example, are only useful 'when equi-systemic effects of various ICS are evaluated or the bioactivity of the ICS given by various delivery devices is determined (4).' These are very complex to perform (thus unsuitable for clinical practice) and cannot predict which children would respond appropriately to stress. Furthermore, there is a marked interindividual variability in cortisol rhythm making a comparison between patterns difficult, if not impossible. Given these limitations, cortisol profiles are unlikely to be suitable as clinically useful screening tests.

Height velocity (HV) was assessed in a descriptive study of 35 asthmatic children on ICS, utilizing the $0.5 \mu\text{g}/1.73 \text{ m}^2$ adrenocorticotrophic hormone (ACTH) stimulation test to assess HPAS (5). Its utility approaches that of a gold standard adrenal function test, provided it is performed correctly (4). The results of the study showed that growth in asthmatic

children treated with ICS does not predict HPAS. The investigators, however, may have missed the cortisol peak post-stimulation, as they did not follow the recommended protocol regarding the timing of the test, the sampling interval and the most appropriate cortisol cut-off (4, 6–8).

Fundamentally, the usefulness of dehydroepiandrosterone sulphate (DHEAS) as a screening test is problematic in that DHEAS production is not solely controlled by ACTH (4). Furthermore, DHEAS increases during adrenarche, necessitating different cut-offs at different ages, Tanner stages and sexes (9). When DHEAS was compared to the $0.5 \mu\text{g}/\text{m}^2$ ACTH stimulation test as a screening test for HPAS in a study of 22 asthmatic children on ICS, the optimal trade-off between sensitivity and specificity seemed to be at 60% and 75%, respectively. With the published data, it is unfortunately not possible to identify the optimal DHEAS SDS cut-off at this pair of sensitivity and specificity (10). Furthermore, by taking only one single post-ACTH at 30 min, the peak cortisol level may have been missed 80% of the time (7, 8), not considering that both the time of day and the peak cortisol cut-off during this particular study were unconventional.

A study of 78 asthmatic adults on ICS used a back-to-front approach to determine the best screening test for HPAS (11). If the 09:00-h serum cortisol, the 24-h urinary free cortisol (24-h UFC) excretion or the standard-dose ACTH stimulation test yielded a result thought to be consistent with HPAS, an ITT was performed. The authors claim that 24-h UFC was a useful screening test based on a sensitivity of 87% and a specificity of >95%. However, neither the ACTH stimulation test nor the 24-h UFC excretion could qualify as appropriate screening test especially in so far as children are concerned. The validity and reliability of the UFC when analysed by radioimmunoassay in the presence of ICS, as in this study, have been questioned (12). Furthermore, the 95% confidence intervals (CI) were wide (calculated by us, not by the authors).

Because 24-hourly urine collections are impractical, spot or early-morning UFC or urinary cortisol metabolites (UCM) have been used (13–15). On the basis of similar UFC suppression while on ICS, it was claimed that a so-called 'fractionated' urine collection was as 'sensitive' as the 24-h UFC (13). This claim has not been substantiated by a proper comparison between a spot urine and a 24-hourly urine collection nor has it been validated against a gold standard adrenal function test. Early-morning UFC would certainly be the screening test most suitable for paediatric use, because it is cost-effective, non-invasive, easily performed, and measured in most routine laboratories. The same cannot be said for UCM, because gas chromatography–mass spectrometry (GC-MS) has to be used to measure the levels. Although it makes sense to rather determine UCM than UFC, because most cortisol is converted to its metabolites before it is excreted, neither UCM nor UFC is expected to detect mild HPAS (4).

Given these uncertainties as to the most suitable method to readily assess HPAS, the current study was undertaken in an attempt to determine which clinical or biochemical

parameter would be the most useful screening test for HPAS in asthmatic children treated with ICS and NS.

Subjects and methods

Twenty-six asthmatic children, 5–18 yr old, who were on ICS $\pm$ NS, but did not receive any oral or dermatological steroids during the preceding year, were recruited from the allergy unit of Red Cross Children's Hospital (RCCH) and its satellite clinics. All eligible children were recruited over a period of 6 months from spring to autumn after obtaining prior Research Ethics approval and written informed consent from parents and children. Height (ht), weight (wt) and change in systolic blood pressure (Δ SBP), as measured with a manual sphygmomanometer, were recorded by a trained nursing sister. The annualized height and weight velocity (HV, WV) were calculated by extracting the height and weight data of approximately 1 yr earlier from the records. The following growth standards were used: the UK 1990 weight and height standards for weight and height, the UK 1966 HV standards for HV and the Gerver 2001 WV standards for WV. Anthropometric measurements and velocities were recorded as standard deviation scores (SDS). Early-morning urine for UFC and UCM was collected between 06:00 and 07:00h. The results were expressed both in nmol/m² body surface area (BSA) and in nmol/mmol creatinine (Cr). Fasting blood for cortisol and ACTH was obtained between 08:00h and 09:00h. Frank hypocortisolaemia was thought to be present if the fasting morning cortisol was <83 nmol/l. The overnight metyrapone test was performed if the fasting morning cortisol was >83 nmol/l. An adequate response was defined by a rise of ACTH from baseline to >100 pg/ml (16).

Assays

Serum cortisol and UFC were measured with the ADVIA automated chemiluminescent assay (CMA) from Bayer. The analytical sensitivity was 5.5 nmol/l. At 107.05 nmol/l, the intra-assay CV was 3.69% while the inter-assay CV was 5.45%. No cross-reactivity to fluticasone or budesonide was identified during a preliminary assessment. Furthermore, no cross-reactivity is known to occur with either beclomethasone or prednisone. Because of the possible unreliability of the CMA, UFC was also measured by GC-MS with selective ion monitoring (12).

Intact ACTH was measured by an automated sequential chemiluminescent immunometric assay (Immulite 2000). Its analytical sensitivity was 5 pg/ml, while its precision at 23 pg/ml ranged from 8.7 (within run) to 10% (run-to-run). No cross-reactivity has been reported. Serum cortisol was measured on the day of the venipuncture. Serum ACTH was collected appropriately in a cold EDTA tube, transported on ice to the laboratory, processed and snap-frozen within 20 min of collection. It was stored at -20°C and assayed in batches once every 30 days.

Urinary creatinine (UCr) was measured on the Roche Modular analyser that measures creatinine photometrically.

The method is based on the Jaffe reaction, with rate blanking and compensation. There is no significant interference from bilirubin, haemolysis, lipaemia or ketones. At an UCr level of 1.10 mmol/l, the intra-assay CV was 2.1%, while at an UCr level of 1.08 mmol/l, the inter-assay CV was 2.2%.

The following UCM were measured by GC-MS with selective ion monitoring: tetrahydrocortisone (THE), tetrahydrocortisol (THF), allo-tetrahydrocortisol (5 α -THF), α cortolone, α cortol, β cortolone, β cortol. No cross-reactivity was expected, because separation of the compounds preceded the analysis. UCr, UFC and UCM were all measured at the end of the study.

Statistical analysis

Resource and recruitment issues (requiring that no oral and dermatological steroids had been prescribed for 1 yr) necessitated the design of a pilot rather than a bigger study as determined by sample size calculations based on the anticipated prevalence of HPAS. Growth velocities were analysed by Growth Analyser, Version 3.0. Spearman correlations (r) were calculated between the post-metyrapone ACTH (which was transformed by taking its square root to make it more symmetrical) and each screening variable to assess its potential. A receiver-operating characteristics (ROC) curve was drawn for the most promising test. Diagnostic statistics and their 95% CIs were calculated. The score method was used to calculate the confidence intervals of the likelihood ratios.

Results

Twenty-six patients were enrolled in the study. Their ages ranged from 5 yr 2 months to 16 yr 11 months (mean 10.2 yr, median 10.4 yr). There were 15 girls and 11 boys. Of these, 22 children were of mixed ancestry, 3 blacks and 1 Caucasian. All children were on budesonide metered dose inhalers (MDIs). Nineteen (73%) of these used a MDI with a hydrofluoroalkane (Hfa) propellant and the rest a MDI with a chlorofluorocarbon (CFC) propellant. Twenty-one (81%) used a spacer. The dose administered ranged from 200 to 800 $\mu\text{g/day}$ (mean 454 $\mu\text{g/day}$; median 400 $\mu\text{g/day}$). The duration of therapy varied from 5 to 314 months (mean 51 months, median 35 months). Twenty-two children (85%) were treated with NS (virtually all with beclomethasone dipropionate) for allergic rhinitis. The daily dose was 100–400 $\mu\text{g/day}$ (mean 213 $\mu\text{g/day}$, median 200 $\mu\text{g/day}$) or 43–556 $\mu\text{g/m}^2/\text{day}$ (mean 209 $\mu\text{g/m}^2/\text{day}$, median 207 $\mu\text{g/m}^2/\text{day}$), and the duration of therapy varied between 3 and 90 months (mean 34.9 months, median 34 months). The combined daily dose of ICS and NS (budesonide/budesonide equivalent) varied between 200 and 1200 $\mu\text{g/day}$ (mean 667 $\mu\text{g/day}$, median 600 $\mu\text{g/day}$) or 147–1667 $\mu\text{g/m}^2/\text{day}$ (mean 649 $\mu\text{g/m}^2/\text{day}$, median 588 $\mu\text{g/m}^2/\text{day}$).

No child was frankly hypocortisolaemic. Nine of 26 children were diagnosed with HPAS on the basis of a failed metyrapone test. Spearman correlations between post-metyrapone ACTH and selected clinical and biochemical parameters are shown in Table 1. Of these, only the morning (fasting)

Table 1 Spearman correlations between post-metyrapone ACTH and screening variables (n = 26)

Screening variable (n)	r	p-value
Height SDS (25)	0.02	0.918
Weight SDS	0.14	0.482
HV† SDS (23)	0.08	0.718
WV‡ SDS (22)	-0.32	0.143
ΔSBP	-0.03	0.901
Morning cortisol	0.33	0.102
Morning ACTH	0.68*	<0.001*
UFC§(CMA*) nmol/m ²	0.17	0.395
UFC(GC-MS**) nmol/m ²	-0.01	0.927
UFC(CMA) nmol/mmolCr	0.20	0.329
UFC(GC-MS) nmol/mmolCr	0.01	0.971
UCM†† μmol/m ²	-0.17	0.433
UCM μmol/mmolCr	-0.03	0.889

*Significant result. †height velocity. ‡weight velocity. §urinary free cortisol. ¶chemiluminescent assay. **gas chromatography/mass spectrometry. ††urinary cortisol metabolites.

ACTH is moderately correlated ($r = 0.68$; $p < 0.001$) to the post-metyrapone ACTH. The scatter plot confirms that the morning ACTH is a reasonable potential screening test for HPAS (Fig. 1). All plots indicating HPAS (below the square root of 100 pg/ml) are associated with a low morning ACTH. Of these, all but one are below the serum ACTH level of 12 pg/ml. The ROC curve of the morning ACTH determines the best discrimination between those who do and those who do not have HPAS to be at a morning ACTH level of 11.7 pg/ml (Fig. 2). At this point, the true-positive fraction is 0.89 (sensitivity of 89%) and the false-positive fraction is 0.23 (specificity of 77%). The diagnostic performance of the

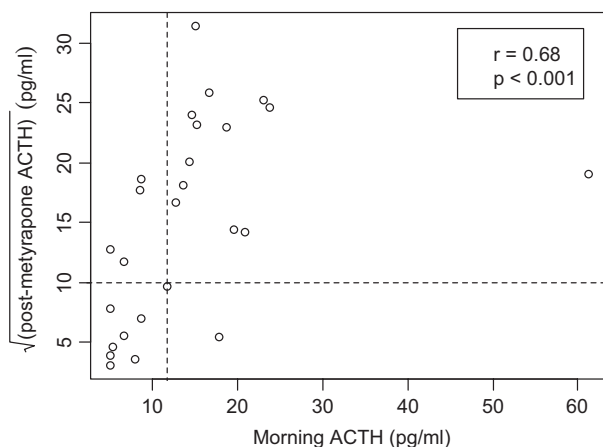


Figure 1 Scatter plot of post-metyrapone ACTH vs. morning ACTH. The horizontal dotted line indicates the cut-off between an adequate and inadequate post-metyrapone ACTH response. The vertical dotted line is drawn at 11.7 pg/ml, denoting the best trade-off between sensitivity and specificity as established by the receiver-operating characteristics curve.

morning ACTH at 11.7 pg/ml is shown in Table 2. The prevalence or pre-test probability of having HPAS has been established separately and is known to be 35% (in press). Given the positive likelihood ratio of 3.78 (1.68–9.49), the post-test probability of having HPAS as determined by a low morning ACTH (< 11.7 pg/ml) is 67 (45–80) %. Conversely, the prevalence or pre-test probability of not having HPAS has been established and is known to be 65% (in press). The negative likelihood ratio of 0.15 (0.03–0.60) determines the post-test probability of not having HPAS by a high (> 11.7 pg/ml) morning serum ACTH to be 93 (72–98) %.

Discussion

The morning ACTH was the only parameter we evaluated that was moderately and significantly correlated with HPAS. It was thus the only variable that is worth considering as a screening test. The optimal cut-off of 11.7 pg/ml is in the lower range of the normal ACTH levels provided by the supplier for the particular assay employed for this study (reference range ≤ 46 pg/ml). From the limited evidence of one small study of children and adolescents (aged 3–15.5 yr), it appears that ACTH secretion is not influenced by either pubertal status or gender (17). ACTH secretion, however, seems to be lower in children than in adult males. The recommended cut-off of 11.7 pg/ml may therefore be applicable throughout the paediatric age group, provided the same analyser is used to measure ACTH in a similar asthmatic childhood population on exogenous steroids. This decision level may not be applicable to adrenal insufficiency of any other cause. Whether it can be used to identify adrenal insufficiency caused by asthma per se (6, 18, 19) still needs to be

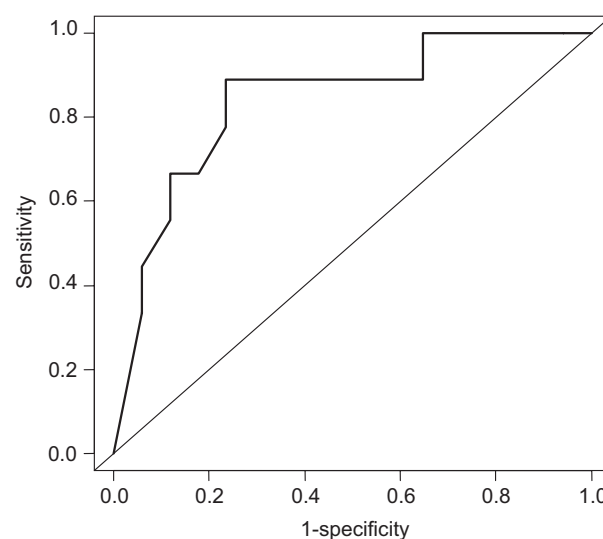


Figure 2 Nonparametric receiver-operating characteristics (ROC) plot of morning ACTH. The point of the ROC curve closest to the upper left hand corner indicates the best pair of sensitivity (0.89) and specificity (0.77). It corresponds to a diagnostic cut-off of 11.7 pg/ml.

Table 2 Diagnostic performance of morning ACTH at 11.7 pg/ml

Index of test validity	Value	95% CI
Sensitivity	0.89 (8/9)*	0.57–0.98
Specificity	0.77 (4/17)†	0.53–0.90
Positive predictive value	0.67	0.39–0.87
Negative predictive value	0.93	0.69–0.99
Accuracy	0.81	0.61–0.94
Positive likelihood ratio	3.78	1.68–9.49
Negative likelihood ratio	0.15	0.03–0.60

*Screening frequencies for HPAS cases. †screening frequencies for non-HPAS cases.

established. If another assay is used, a cut-off for this specific assay should ideally also be established. Failing this a comparative assessment between the Immulite 2000 assay used in this study and the ACTH assay to be used in another laboratory should be performed to establish the bias of the second assay. Furthermore, it should be remembered that ACTH has a very short half-life (4). This means that during sample collection and processing a strict protocol needs to be followed to obtain valid ACTH measurements. The screening test would thus only be suitable for centres that have a laboratory on site familiar with the processing of such samples. This will also ensure that ACTH results are reproducible. Repeatability of ACTH measurements has, however, not been assessed in this study. A normal physiological variation in ACTH levels can be expected (4). Based on the inter-assay CV of ACTH of the Immulite 2000 assay, a repeated ACTH measurement might deviate from the initial result by about 10%. For levels to be comparable, measurements need to be performed during the same time of day, there should be no additional stressor, dosing of and adherence to ICS/NS should be consistent, and HPAS should be maintained by using the same delivery device with the correct technique. Whenever venipunctures are performed in succession, ACTH levels obtained from the second serum specimen might be lower because of an attenuated stress response during the second venipuncture.

The diagnostic performance of the morning ACTH compares very favourably to the one of both insulin growth factor one (IGF-1) and insulin growth factor-binding protein three (IGFBP-3), which have been used as screening tests for growth hormone deficiency. The sensitivity and specificity for IGF-1 are 0.75 and 0.32 and for IGFBP-3 0.67 and 0.50, respectively (20). The likelihood ratios, when used in conjunction with a nomogram to determine the post-test probability, provide the best information about the value of the morning ACTH level as a screening test (21, 22). These ratios indicate that it would indeed be advantageous to use the morning ACTH as such. The probability of identifying HPAS by a morning ACTH level of below 12 pg/ml is virtually doubled by using the test compared to not using the test (by measuring the serum ACTH level, the pre-test probability or prevalence of 35% is increased to a post-test probability of 67%). The probability of missing HPAS by performing the screening test (i.e. of having a high morning ACTH level

in the face of HPAS) is reduced considerably when compared to not doing the screening test (the pre-test probability or prevalence of children without HPAS increases from 65% to a post-test probability of 93%). The small study sample, however, limits the test's clinical usefulness as demonstrated by the wide CIs of the post-test probabilities. Realistically speaking, the post-test probability of having HPAS when finding a low morning ACTH could vary between 45% and 80%, while the post-test probability of having HPAS when measuring a high morning ACTH could vary between 2% and 28%. Only a larger study could narrow the range of the post-test probability of this proposed screening test. By changing the inclusion criteria, more subjects could be enrolled in a future study. A fourfold increase in sample size should reduce the CI by approximately half. A sample size of 100 would therefore yield a sensitivity of 0.89 with a CI of 0.70–0.95 assuming the same prevalence as in the current study. The CIs of the positive and negative likelihood ratios would thus be 2.42–5.89 and 0.06–0.37, respectively. The post-test probability of having HPAS in the face of a low morning ACTH would thus only vary between 55% and 72%, while the post-test probability of having HPAS when measuring a high morning ACTH would vary between 4% and 14%.

Morning cortisol was only weakly and non-significantly correlated with the post-metyrapone ACTH. This is not surprising as its value is determined by both physiological phenomena, not related to the hypothalamic–pituitary–adrenal axis (HPA), and methodological issues (4). As confirmed by this study, a morning cortisol in the normal reference range does not exclude HPAS. It is thus also unsuitable as a screening test.

The UFC assay was primarily developed for the detection of cortisol excess. In the absence of the latter, cortisol-binding globulin (CBG) binds most of the circulating cortisol, resulting in <1% of free cortisol being filtered by the kidneys (4). UFC will only increase if the binding capacity of CBG is exceeded at a serum cortisol level of 690 nmol/l (4). Use of UFC to detect HPAS is therefore fundamentally and conceptually erroneous. Furthermore, UFC measurements when established by immunoassays in the setting of adrenal insufficiency caused by ICS may be both invalid and unreliable (4, 12). We therefore measured UFC by both CMA and GC-MS. GC-MS is, however, not freely available making it less suitable for screening purposes. As mentioned above, 24-h urine collections are impractical in the paediatric age group and are too cumbersome to even be considered as a screening test. Hence, a early-morning urine (the first of the day) was collected to measure both UFC and UCM. The measurements of both are best expressed in relation to body surface area, because this ratio was found to be independent of age or pubertal development while there was a weak negative correlation when UFC was related to Cr excretion (23). As virtually all cortisol is converted to its metabolites, total cortisol excretion would be expected to be a better indicator of cortisol production than UFC alone (4). UCM (as measured by GC-MS) were weakly negatively correlated to HPAS (when UCM excretion was corrected for BSA), and UFC (as measured by CMA) was weakly positively

correlated to HPAS. The unexpected discrepancy in the direction of the correlation of UFC and UCM can probably be explained by the inaccuracy of the CMA. This finding would thus confirm the previously well-described inadequacy of radioimmunoassays in diagnosing HPAS (12). Cross-reactivity to the metabolites of the exogenous steroids could result in erroneous results depending on the assay. UFC measured by GC-MS would be a far better option to consider as a screening test. However, even when total cortisol excretion (i.e. when both UFC and UCM are measured by GC-MS) is determined, its correlation with HPAS is so poor that we would discourage its consideration as a screening test. It has been argued that UCM excretion is highest in the first half of the morning and that a urine collection during this time would be more appropriate. However, it has also been shown that in the face of an administered exogenous steroid such as prednisolone, UCM excretion is virtually identical throughout the course of the day (John Honour – unpublished observation). Liquid chromatography–tandem mass spectrometry (LC-MS/MS) has been found to be superior to any other method in detecting UFC (24). Its general unavailability, however, would make it impractical as a screening tool.

All clinical parameters investigated in this study were found to be unsuitable as screening tests. Inter alia, this study

thus confirms the finding by Dunlop et al. that growth does not predict HPAS (5). It is also clear that the identification of orthostatic hypotension by using a manual sphygmomanometer is unnecessarily time-consuming.

Conclusion

The morning ACTH level, as measured by the Immulite 2000 assay, was found to be the most useful screening test. No clinical parameter or other basal adrenal function test investigated merited any further consideration. A larger study should be undertaken to refine the diagnostic precision of the proposed screening test.

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Potential conflict of interest

None.

References

1. Zöllner EW. Hypothalamic-pituitary-adrenal axis suppression in asthmatic children on inhaled corticosteroids (Part 2) – the risk as determined by gold standard adrenal function tests: a systematic review. *Pediatr Allergy Immunol* 2007; **18**: 469–74.
2. Grimes DA, Schulz KF. Uses and abuses of screening tests. *Lancet* 2002; **359**: 881–4.
3. Hammond M, Gear J. Measuring Community Health, Workbook 1. Cape Town: Oxford University Press, 1986.
4. Zöllner EW. Hypothalamic-pituitary-adrenal axis suppression in asthmatic children on inhaled corticosteroids: part 1. Which test should be used? *Pediatr Allergy Immunol* 2007; **18**: 401–9.
5. Dunlop KA, Carson DJ, Steen HJ, McGovern V, McNaboe J, Shields MD. Monitoring growth in asthmatic children treated with high dose inhaled glucocorticoids does not predict adrenal suppression. *Arch Dis Child* 2004; **89**: 713–6.
6. Zöllner EW. Author's reply to 'Testing for hypothalamic-pituitary-adrenal axis suppression in asthmatic children'. *Pediatr Allergy Immunol* 2008; **19**: 468–70.
7. Crowley S. Adrenal function in asthmatic children. *Thorax* 1999; **54**: 92.
8. Crowley S, Hindmarsh PC, Holownia P, Honour JW, Brook CGD. The use of low doses of ACTH in the investigation of adrenal function in man. *J Endocrinol* 1991; **130**: 475–9.
9. Forest MG. Adrenal function tests. In: Ranke MB, ed. *Diagnostics of Endocrine Function in Children and Adolescents*. Basel: Karger, 2003: 372–426.
10. Dorsey MJ, Cohen LE, Phipatanakul W, Denufrio D, Schneider LC. Assessment of adrenal suppression in children with asthma treated with inhaled corticosteroids: use of dehydroepiandrosterone sulphate as a screening test. *Ann Allergy Asthma Immunol* 2006; **97**: 182–6.
11. Brown PH, Blundell G, Greening AP, Crompton GK. Screening for hypothalamic-pituitary-adrenal axis suppression in asthmatics taking high dose inhaled corticosteroids. *Respir Med* 1991; **85**: 511–6.
12. Fink RS, Pierre LS, Daley-Yates PT, Richards DH, Gibson A, Honour JW. Hypothalamic-pituitary-adrenal axis function after inhaled corticosteroids: unreliability of urinary free cortisol estimation. *J Clin Endocrinol Metab* 2002; **87**: 4541–6.
13. Wilson AM, Lipworth BJ. 24 hour and fractionated profiles of adrenocortical activity in asthmatic patients receiving inhaled and intranasal corticosteroids. *Thorax* 1999; **54**: 20–6.
14. McIntyre HD, Mitchell CA, Bowler SD, Armstrong JG, Wooler JA, Cowley DM. Measuring the systemic effects of inhaled beclomethasone: timed morning urine collections compared with 24 hour specimens. *Thorax* 1995; **50**: 1280–4.
15. Contreras LN, Hane S, Tyrrell JB. Urinary cortisol in the assessment of pituitary-adrenal function: utility of 24-hour and spot determinations. *J Clin Endocrinol Metab* 1986; **62**: 965–9.
16. Staub JJ, Noelp B, Girard J, Baumann JB, Graf S, Ratcliffe JG. The short metyrapone test: comparison of the plasma ACTH response to metyrapone and insulin-induced hypoglycaemia. *Clin Endocrinol* 1979; **10**: 595–601.
17. Wallace WHB, Crowns EC, Shalet SM, et al. Episodic ACTH and cortisol secretion in normal children. *Clin Endocrinol* 1991; **34**: 215–21.
18. Barr J, Rapoport MJ, Tauber T, Hertzianu I, Cafri Y, Bistrizter T. The relation between adrenal function and the severity of bronchial hyperresponsiveness in children as measured by the metacholine provocation test. *J Pediatr Endocrinol Metab* 2002; **15**: 307–12.
19. Priftis KN, Papadimitriou A, Gatsopoulou E, et al. Adrenal function improves in asthmatic children on inhaled steroids: a longitudinal study. *Neuroimmunomodulation* 2006; **13**: 56–62.
20. Ranke MB, Schweizer R, Elmlinger MW, et al. Significance of basal IGF-I, IGFBP-3 and IGFBP-2 measurements in the diagnostics of short stature in children. *Horm Res* 2000; **54** (2): 60–8.

21. Fagan TJ. Nomogram for Baye's theorem. *N Eng Med* 1975; **293**: 257.
22. Jaeschke R, Guyatt G, Lijmer J. Diagnostic tests. In: Guyatt G, Drummond R, ed. *Users' Guides to the Medical Literature*. Chicago: Ama Press, 2002: 187–217.
23. Gomez MT, Malozowski S, Winterer J, Vamvakopoulos NC, Chrousos GP. Urinary free cortisol values in normal children and adolescents. *J Pediatr* 1991; **118**: 256–8.
24. Taylor RL, Machacek D, Singh RJ. Validation of a high-throughput liquid chromatography-tandem mass spectrometry method for urinary cortisol and cortisone. *Clin Chem* 2002; **48** (9): 1511–9.