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Early morning salivary cortisol and cortisone, and adrenal responses to a simplified low dose short Synacthen test in children with asthma

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Key words: asthma, adrenal, Synacthen, child

Abstract

Objective

To examine serum cortisol responses to a simplified low dose short Synacthen test (LDSST) in children treated with inhaled corticosteroids (ICS) for asthma and to compare these to early morning salivary cortisol (EMSC) and cortisone (EMSCn) levels.

Design

EMSC and EMSCn samples were collected for three consecutive days. On day three Synacthen 500ng/1.73m² was administered intravenously. Samples were collected at 0, 15, 25, 35 minutes.

Results

269 subjects (160 M: 109F), median (range) age 10.0 (5.1 – 15.2) years were studied. Peak cortisol in the LDSST was <500nmol/L in 101 subjects (37.5%) and <350nmol/L in 12 subjects (4.5%). Basal cortisol correlated with peak cortisol: $r=0.55$, (95% CI: 0.46, 0.63, $p<0.0001$). Time at which peak cortisol concentration was achieved was significantly related to the value of peak cortisol ($p<0.0001$), with higher cortisol peaks occurring later in the test and lower cortisol peaks occurring earlier. EMSC and EMSCn had no predictive value for the identification of patients with a peak cortisol <500nmol/L. EMSCn was superior to EMSC in identifying patients with a peak cortisol < 350nmol/L: a minimum EMSCn cut-off value of 12.5 nmol/L gave a negative predictive value of 99.2% and positive predictive value of 30.1%.

Conclusion

Our data illustrate that basal measures of cortisol are likely to be of value in screening populations for patients at greatest risk of adrenal crisis. EMSCn shows promise as a screening tool for the identification of patients with severe adrenal insufficiency.

Introduction

Asthma is the most common chronic disease of childhood (1). In the Health Survey for England 2010, a current diagnosis of asthma was reported in 11% of boys and 8% of girls and daily inhaled corticosteroid (ICS) use was reported in 31% of affected boys and 28% of affected girls (2).

Biochemical abnormalities of the hypothalamic-pituitary adrenal axis (HPA) are reported in 20–50% of children treated with ICS (3-8). Clinical episodes of adrenal crisis are rare, but may be fatal (3,9-15). Early morning salivary cortisol (EMSC) was a highly sensitive marker of adrenal suppression in a study of adult subjects treated with intra-nasal corticosteroids (16) and recent work suggests that salivary cortisone may be a better predictor of serum cortisol than salivary cortisol (17). This methodology is ideally suited to screening large paediatric populations in a non-invasive manner.

The low dose short Synacthen test (LDSST, 500nanograms/1.73m² Synacthen) is used widely in paediatric practice (18), however the protocol is technically challenging because blood samples are drawn at five minute intervals. We were keen to simplify the LDSST by reducing the intensity of sampling without compromising the sensitivity or specificity of the test. In this paper we report data from a large cohort of children with asthma treated with ICS who underwent a simplified LDSST and who collected early morning salivary samples for measurement of salivary cortisol and cortisone (EMSCn). The aims of the study were: (1) to compare cortisol responses to a simplified LDSST to results obtained from a similar cohort of asthmatic children studied using a more intensive sampling protocol (2) to investigate the utility of EMSC and EMSCn as a tool for the identification of children

with impaired responses to the LDSST, and (3) to describe the characteristics of children with impaired responses to the LDSST.

Subjects and methods

The study received approval from the Liverpool Children's Research Ethics Committee.

Patients aged 5 – 16 years with asthma treated with ICS for >3 months were eligible to participate. Consecutive children who satisfied the inclusion criteria were invited to participate: This included children with a wide range of severity of asthma who were on steps 2-5 of the British Thoracic Society/Scottish Intercollegiate Guidelines Network (BTS/SIGN) asthma management guidelines (19). Patients were excluded if they had co-existing pathology likely to affect serum cortisol or cortisol binding globulin (CBG) or if they were treated with additional medications likely to affect serum cortisol or CBG, with the exception of prednisolone. Patients taking prednisolone on alternate days were eligible for recruitment, but those taking prednisolone daily were excluded.

All steroid exposure was documented including ICS, intranasal, oral and corticosteroid creams. Doses of ICS and intranasal corticosteroids were converted to an equivalent dose of beclomethasone to permit analysis of pooled patient data (19). Informed, written consent, and where appropriate, assent was obtained from all participants and / or their parent or guardian.

Design of the LDSST

Paton et al reported plasma cortisol responses to a LDSST (Synacthen 500ng/1.73m² intravenously and sampling at 0, 15, 20, 25, 30 and 35 minutes) in 217 children treated with fluticasone ≥500 micrograms daily. (3). Raw data from this study were reviewed. Four samples, at time points 0, 15, 25, 35 minutes, identified all patients with a normal response to the LDSST, and were selected as the time points for our simplified LDSST.

LDSST protocol

On the day of the LDSST the morning dose of ICS was withheld until completion of the test. For patients treated with prednisolone on alternate days, the LDSST was performed on the day a dose was due to be given, but the dose was withheld until after the LDSST.

An indwelling venous catheter was sited following the application of local anaesthetic cream. A blood sample was collected (time 0). One hundred and twenty five micrograms (0.5ml) Synacthen (Alliance, Chippenham, UK) 0.25mg/ml solution was added to 500ml 0.9%NaCl (final concentration of 250nanograms/ml) and agitated. Five hundred nanograms/1.73m² Synacthen was administered as a bolus injection directly into the cannula and samples were collected 15, 25 and 35 minutes following administration.

The study was undertaken by designated research nurses trained by TM. Stimulated cortisol levels on the LDSST were not included in the analysis if there was uncertainty regarding the accuracy of sampling, or if there were missing samples.

Interpretation of cortisol response to the LDSST

A normal result was defined as: baseline cortisol value $\geq 100\text{nmol/L}$ and peak cortisol $\geq 500\text{nmol/L}$. If the baseline sample was not collected between 08.00 and 09.00 and was $< 100\text{nmol/L}$, but the peak was $\geq 500\text{nmol/L}$, then a repeat sample was collected at 09.00 on a subsequent day. If the repeat sample was $> 100\text{nmol/L}$ the patient was included in the 'normal' group.

It is widely accepted that peak cortisol values $< 500\text{ nmol/L}$ are abnormal, however the clinical significance of values close to 500nmol/L is uncertain. In the development of this study protocol we carefully reviewed case reports and case series of adrenal crisis during ICS therapy in childhood (9-15) (n=50 patients). Twenty nine patients were tested using the standard dose short Synacthen stimulation test (SDSST), in whom mean peak cortisol was 44 nmol/L , median 127 nmol , and maximum 387nmol/L . In two patients tested with the LDSST the peak cortisol values were 127 and

107 nmol/L, and in three patients tested with the glucagon stimulation test peak cortisol levels were < 70nmol/L in two, and 312 nmol/L in a third. In one exceptional patient, tested during an acute adrenal crisis, basal cortisol was 575nmol/L and peak cortisol was 600nmol/L. Peak cortisol responses to the SDSST are reported to be higher than those in the LDSST (6,20). In light of these data we considered two categories of patients: (1) all patients in whom peak cortisol was <500nmol/L and (2) those in whom peak cortisol was <350nmol/L.

Collection of Saliva samples

Participants provided an early morning sample of saliva for three consecutive days, and the LDSST was performed on day three. Saliva samples were collected 30 minutes after waking and before the patient brushed their teeth to avoid blood contamination. The patient rinsed their mouth with water and then collected a saliva sample using the Salivette sampling device (Sarstedt, Rommelsdorf, Germany).

Biochemical analyses

Serum cortisol was measured using the Immulite 2000, an automated immunoassay analyser using reagents supplied by the manufacturer (Siemens Healthcare, Camberley, UK). This assay has an intra- and inter-assay coefficient of variation of <5% and <7.7% respectively.

Salivary cortisol was measured by competitive immunoassay (Salimetrics LLC, State College, PA, USA). This is a highly sensitive assay with a detection of limit of 0.19nmol/l and an intra- and inter-assay coefficient of variation <6% and 10% respectively.

Salivary cortisone was measured by chemiluminescent immunoassay using reagents supplied by the manufacturer (Arbor Assays, Maidenhead, UK). This assay has an intra- and inter-assay coefficient of variation of <11% and <13% respectively.

Statistical analyses

Statistical analyses were performed using the statistical software package R (www.r-project.org).

The association between the peak cortisol concentration and the time at which this was achieved was tested using ANOVA. Factors affecting the peak cortisol response to LDSST were assessed using multiple linear regression. The association between basal and peak cortisol was assessed first by computing the Pearson correlation between the variables and also by including basal response as an additional covariate within the multiple linear regression for peak cortisol. Factors affecting basal cortisol were also investigated via regression models. A quantile regression method, assessing factors affecting 10th, 25th, 50th and 75th percentiles of basal cortisol, was adopted due to the basal cortisol measure being positively skewed.

Receiver operator characteristic (ROC) curves were computed to assess the predictive accuracy of EMSC and EMSCn to detect an abnormally low peak of LDSST. Predictive accuracy, measured through area under the ROC curve (AUC), was calculated for day 1, day 2, day 3, mean, min and maximum EMSC and EMSCn taking both an abnormal peak LDSST to be below 500nmol/L and below 350nmol/L. In addition, the possibility of age affecting diagnostic accuracy was investigated by computing separate ROC curves for those aged less than 12 years and those 12 years or over.

Two power calculations were performed using two different reported prevalences of HPA suppression of 25% (6) and 42% (7) in populations similar to the sample in this study. In order to estimate an area under the curve in a ROC analysis of 0.85 to within at least ± 0.06 using a 95% confidence interval a sample of 250 patients were required.

Results

Patient characteristics

269 patients (160M:109F), median (range) age 10 (5 – 15.2) years were studied to obtain 246 complete data sets. Details of ICS therapy are given in Table 1. Seventeen subjects (5.7%) were treated with oral prednisolone on alternate days.

Cortisol responses to the simplified LDSST

Baseline cortisol levels in all samples measured between 08.00 and 09.00 were >100nmol/L. Peak cortisol levels were <500nmol/L in 101 (37.5%) subjects, and <350nmol/L in 12 patients (4.5%). Nine of the 12 patients with peak cortisol levels < 350nmol/L were treated with fluticasone (median dose 500 micrograms / day, range 200 – 1000), two were treated with budesonide 800 micrograms daily, and one patient with beclomethasone 1000 micrograms daily.

Forty three out of 100 subjects (43.0%) treated with fluticasone in doses $\geq 500\text{mcg/day}$ had peak cortisol responses of <500nmol/L, compared to 88 / 194 (45.5%) subjects in the study from Paton et al ($p=0.142$).

Characteristics of subjects with normal and impaired cortisol response to the LDSST

There was no significant difference in age between patients with normal or impaired responses. ICS doses were higher in those with impaired cortisol responses ($752 \pm 375\text{mcg}$ vs $681 \pm 468\text{mcg}$, $p = 0.04$), and males were more likely to have an impaired response than females ($p=0.01$). Treatment with oral prednisolone did not increase the risk of an impaired response ($p=0.09$).

Basal cortisol levels

Basal cortisol correlated with peak cortisol ($r=0.55$, 95% CI: 0.46, 0.63, $p<0.0001$): A unit increase in basal cortisol corresponded to an expected increase of 0.63nmol/L in peak cortisol (95% CI: 0.52, 0.74, $p<0.001$) (Figure 1). To test whether basal and peak cortisol may be associated through other

factors we included basal cortisol as an additional covariate in the multiple linear regression for peak cortisol. The association between basal and peak cortisol remained statistically significant after adjusting for the other factors.

Basal cortisol levels were related to age, but not related gender, ICS dose or treatment with oral prednisolone. Median basal cortisol increased by 10nmol/L for every year of life, 95% CI = 6.54, 12.54.

Peak cortisol levels

A linear regression model demonstrated that peak cortisol was affected by age, (mean reduction of 6.0 nmol/L per year of life, 95% CI -10.9, -1.1, $p=0.017$) and gender (average peak cortisol of 51.9 nmol/L lower in boys than in girls, 95% CI -84.81, -18.89, $p = 0.002$). Peak cortisol was 96.8 nmol/L lower in those treated with oral prednisolone (95% CI -162.0,-31.6, $p = 0.004$).

Time at which peak cortisol concentration was achieved was significantly related to the value of peak cortisol ($p<0.0001$): Patients with higher peak cortisol values demonstrated a later peak cortisol than those in whom cortisol levels were low.

Comparison of EMSC and EMSCn and LDSST

1. ROC curve set to identify patients with peak cortisol < 500nmol/L

EMSC

The curves for EMSC day 1, EMSC day 2 and EMSC day 3 and minimum EMSC had areas under the curve (AUC) not significantly different from 0.5, suggesting no predictive ability above chance.

When the mean of the three measurements, or maximum (EMSC) was used, the ROC curves have AUCs were significantly greater than 0.5. The point that maximized sensitivity and specificity at a cut-off value of $\max(\text{EMSC}) < 8.7\text{nmol/L}$, gave a sensitivity of 58.6% (95% CI: 48.9%-68.3%) and a specificity of 60.8% (95% CI: 53.4%-68.3%).

When data were dichotomised into two age groups (5-11 years and 12-16 years) EMSC had no predictive ability in children aged less than 12 years. In children aged 12 years and above, EMSC day 3, maximum EMSC and mean EMSC showed statistically significant AUC : EMSC Day 3 AUC = 0.66 (95% CI: 0.55-0.77), maximum EMSC AUC = 0.62 (95% CI: 0.50-0.74) and mean EMSC AUC = 0.62 (95% CI: 0.50-0.74). The resulting diagnostic test from the best cut-off point of EMSC Day 3 < 6.2nmol/L gave a sensitivity of 64.9% and a specificity of 66.7%.

EMSCn

The AUCs were lower for EMSCn than EMSC, and ranged from 0.53 to 0.57. Unlike EMSC, there was no improvement in AUCs for the older group of patients.

2. ROC curve set to identify patients with peak cortisol < 350nmol/L

EMSC

The AUCs for days 1-3 were 0.73 (95% CI: 0.55, 0.90), 0.80 (95% CI: 0.65, 0.93), and 0.76 (95% CI: 0.60,0.91) respectively. For maximum EMSC AUC was 0.75 (95% CI: 0.56,0.90), for mean 0.76 (95% CI: 0.57,0.92) and for minimum 0.82 (95% CI: 0.66, 0.95) (Figure 1).

A mean EMSC cut-off value of 6 nmol/L gave a negative predictive value of 98.8% and a positive predictive value of 9.5%.

EMSCn

The AUCs for days 1-3 were 0.75 (95% CI: 0.52, 0.96), 0.88 (95% CI: 0.69, 0.98) and 0.80 (95% CI: 0.58,0.97) respectively. For maximum EMSCn AUC was 0.75 (95% CI: 0.55,0.93), for mean 0.87 (95% CI: 0.69,0.98) and for minimum 0.88 (95% CI: 0.69,0.98) (Figure 2).

A minimum EMSCn cut-off value of 12.5 nmol/L gave a negative predictive value of 99.2 % and a positive predictive value of 30.1%.

Discussion

The importance of recognising and treating adrenal suppression during ICS therapy for asthma has been of interest following reports of acute adrenal crisis and death in children treated with these medications (9-15). There is some evidence that the LDSST is the most sensitive test of adrenal function in children with asthma treated with ICS (6,20). However, the protocol requires sampling at 5 minute intervals (21) and this can be technically challenging, leading to mistimed samples and difficulty interpreting test results. In the design of this simplified LDSST protocol we selected the time points that identified all patients with peak cortisol $>500\text{nmol/L}$ from the raw data of the study by Paton et al (3).

Paton et al (3) reported data from 194 subjects treated with fluticasone in doses $\geq 500\text{mcg/day}$. When we compared the prevalence of an impaired response in our study in the 100 subjects treated with fluticasone $\geq 500\text{mcg/day}$ to that of the Paton study we found the prevalence of impaired responses was remarkably similar between test protocols and populations, suggesting that simplification of the test does not result in significant loss of sensitivity or specificity.

We observed a strong relationship between basal and peak cortisol levels, and this supports our hypothesis that basal measurements of cortisol secretion are useful in screening patients at risk of adrenal insufficiency. We also observed that patients with impaired adrenal function had peak cortisol levels early in the test which then fell, in contrast to patients in whom the peak cortisol value was $\geq 500\text{nmol/L}$ in whom cortisol levels increased throughout the test. This is a novel observation and is biologically plausible. In vivo work in healthy canines has found a positive correlation between the adrenal concentrations and the secretion rates of cortisol (22). We suggest that an atrophic adrenal gland would synthesise and secrete less cortisol in response to stimulation and have lower baseline values of circulating cortisol.

Accepted Article

There are some concerns about the test-retest repeatability of the LDSST in children. A recent study of 18 children with asthma treated with ICS reported only fair to good agreement between two LDSSTs performed at an interval of one month (23). However, it should be noted that in this study samples were collected at 20 and 30 minutes only. In our review of the Paton data, these time points would not have identified all patients with a normal response. Furthermore, significant adsorption of Synacthen to plastic giving sets has been reported and this may contribute to intra-individual variability between tests (24). In our study Synacthen contact with plastic was minimised by preparation of a dilute Synacthen solution for each patient immediately prior to injection and administration as a bolus directly into the patient cannula.

There is considerable discrepancy between the very high prevalence of biochemically abnormal results reported in this and previous studies, and the low prevalence of adrenal crisis. We speculate that the discordance in these data may be due in part, to the limited normative data available for interpretation of the test. In our study boys had lower cortisol levels than girls, although girls suppressed their peak cortisol to a much greater degree than males for a given increase in ICS dose. We speculate that sex steroids have a role in the regulation of the hypothalamic pituitary adrenal axis originating in the prenatal period and during early infancy, when sex steroids are known to be at near adult levels (25,26). Salivary cortisol levels increase with age and pubertal development in healthy children and adolescents and are higher in girls than in boys (27-29). Cortisol response to CRH stimulation increase during puberty girls while boys demonstrate a reduction in basal cortisol.(30). Collectively these data, and data from our study suggest that age and gender appropriate reference ranges should be developed to enable more meaningful interpretation of test results.

Within the range of ICS doses used by this cohort of patients, there is, at best, a weak association between ICS dose and adrenal reserve. When the patients were dichotomised (peak cortisol <

500nmol/L and peak cortisol $\geq$ 500nmol/L) we found a weak association with ICS dose ($p=0.04$): Patients treated with higher doses of ICS were more likely to have peak cortisol values of $<500\text{nmol/L}$. However, when the relationship between ICS dose and peak cortisol was examined for the entire cohort, this relationship was no longer observed. This may reflect the many influences on lung deposition of ICS, including inhaler technique, lung physiology, ICS delivery device and of course concordance with treatment.

Using the widely accepted threshold of peak cortisol $> 500\text{nmol/L}$ to define a normal response, more than a third of our patients had evidence of adrenal insufficiency, a figure in keeping with previous studies (3-8). When we set our ROC curves to identify patients with peak cortisol values $< 500\text{nmol/L}$, it was clear that neither EMSC or EMSCn in isolation or combination, could discriminate between those with normal and abnormal results.

When the ROC curves were set to identify patients with peak cortisol $< 350\text{ nmol/L}$, both EMSC and EMSCn showed promise as screening tools for the identification of patients at risk of adrenal crisis: Patients in whom mean EMSC is $\geq 6\text{nmol/L}$ have a 99% (NPV 98.8%) chance of having a cortisol peak of $\geq 350\text{nmol/L}$, and those in whom mean EMSC is $<6\text{nmol/L}$ have a 10% (PPV=9.5%) chance of having a peak cortisol $< 350\text{nmol/L}$ and patients in whom minimum EMSCn is $\geq 12.5\text{ nmol/L}$ have a 99% (NPV 99.2%) chance of having a cortisol peak of $\geq 350\text{nmol/L}$, and those in whom mean EMSCn is $<12.5\text{nmol/L}$ have a 30% (PPV=30.1%) chance of having a peak cortisol $< 350\text{nmol/L}$. In conclusion, a simplified LDSST can be used to assess the adrenal function of children with asthma. EMSC and EMSCn have promise as markers of severe HPA suppression, with EMSCn being superior to EMSC. Further studies are planned to improve the positive predictive value of these tests to a level that is clinically useful. Our literature review suggests that patients with peak cortisol levels $> 350\text{ nmol/L}$ are at very low risk of adrenal crisis. We aim to strengthen these data by reporting the prevalence of adrenal crisis in our cohort of patients since the study was completed. By completing

these extension studies we aim to develop practical guidelines for the screening of patients treated with ICS using EMSC and EMSCn, and revise the level at which adrenal function is considered to be impaired from 500 nmol/L to 350 nmol/L. Until these data are available we will continue to prescribe hydrocortisone for periods of acute illness or injury for patients with peak cortisol in the range 350 – 499 nmol/L. Those in whom peak cortisol is < 350nmol/L are advised to take hydrocortisone daily, increasing the dose during periods of acute illness or injury.

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References

1. www.who.int/respiratory/asthma/en/
2. www.dh.gov.uk/PublicationsAndStatistics/PublishedSurvey/HealthSurveyForEngland/fs/en
3. Paton J, Jardine E, McNeill E *et al*. Adrenal responses to low dose synthetic ACTH (Synacthen) in children receiving high dose inhaled fluticasone. *Arch Dis Child*. 2006 **91** 808-13.
4. Sim D, Griffiths A, Armstrong D *et al*. Adrenal suppression from high-dose inhaled fluticasone propionate in children with asthma. *Eur Respir J* 2003**21**633-6
5. Priftis KN, Papadimitriou A, Gatsopoulou E *et al*. The effect of inhaled budesonide on adrenal and growth suppression in asthmatic children. *Eur Respir J*. 2006 **27** 316-20.
6. Broide J, Soferman R, Kivity S *et al* Low-dose adrenocorticotropin test reveals impaired adrenal function in patients taking inhaled corticosteroids. *J Clin Endocrinol Metab* 1995 **80** 1243-6
7. Kannisto S, Korppi M, Remes K *et al* Adrenal suppression, evaluated by a low dose adrenocorticotropin test, and growth in asthmatic children treated with inhaled steroids *J Clin Endocrinol Metab*. 2000 **85** 652-7.

8. Mahachoklertwattana P, Sudkronrayudh K, Direkwattanachai C *et al.* Decreased cortisol response to insulin induced hypoglycaemia in asthmatics treated with inhaled fluticasone propionate. *Arch Dis Child.* 2004 **89**1055-8
9. Todd GR, Acerini CL, Ross-Russell R, *et al.* Survey of adrenal crisis associated with inhaled corticosteroids in the United Kingdom. *Arch Dis Child.* 2002 **87** 457-61.
10. Macdessi JS, Randell TL, Donaghue KC *et al.* Adrenal crises in children treated with high-dose inhaled corticosteroids for asthma. *Med J Aust.* 2003 **178** 214-6.
11. Drake AJ, Howells RJ, Shield JP *et al* Symptomatic adrenal insufficiency presenting with hypoglycaemia in children with asthma receiving high dose inhaled fluticasone propionate. *BMJ.* 2002 **324** 1081-2.
12. Carrel AL, Somers S, Lemanske RF Jr *et al* Hypoglycemia and cortisol deficiency associated with low-dose corticosteroid therapy for asthma. *Pediatrics.* 1996 **97** 921-4.
13. Patel L, Wales JK, Kibirige MS *et al* Symptomatic adrenal insufficiency during inhaled corticosteroid treatment. *Arch Dis Child.* 2001 **85** 330-4.
14. G.Todd, D Wright, M Ryan, Acute adrenal insufficiency in a patient with asthma after changing from fluticasone propionate to budesonide *J Allergy Clin Immunol* 1999 **103** 956-7.
15. Kennedy MJ, Carpenter JM, Lozano RA *et al* Impaired recovery of hypothalamic-pituitary-adrenal axis function and hypoglycemic seizures after high-dose inhaled corticosteroid therapy in a toddler *Annals of Allergy, Asthma & Immunology* 2002 **88** 523-526
16. Patel RS, Shaw SR, Wallace M *et al* Efficacy and systemic tolerability of mometasone furoate and betamethasone sodium phosphate. *J Laryngol Otol.* 2004 **118** 866-71.
17. Perogamvros I, Keevil BG, Ray DW *et al.* Salivary cortisol is a potential biomarker for serum free cortisol. *J Clin Endocrinol Metab.* 2010 **95** 4951-8.
18. Elder CJ, Sachdev P, Wright NP. The short Synacthen test: a questionnaire survey of current usage. *Arch Dis Child.* 2012 **97** 870-3.
19. <http://www.brit-thoracic.org.uk/Portals/0/Guidelines/AsthmaGuidelines/sign101%20Jan%202012.pdf>
20. Ozbek OY, Turktas I, Bakirtas A *et al* Evaluation of hypothalamic-pituitary-adrenal axis suppression by low-dose (0.5 microg) and standard-dose (250 microg) adrenocorticotrophic hormone (ACTH) tests in asthmatic children treated with inhaled corticosteroid. *J Pediatr Endocrinol Metab.* 2006 **19** 1015-23.
21. Agwu JC, Spoudeas H, Hindmarsh PC *et al.*, Tests of adrenal insufficiency. *Archives of Disease in Childhood*, 1999. 80(4): p. 330-333.
22. Holzbauer, M. and H.M. Newport, Adrenal secretion rates and adrenal tissue concentrations of pregnenolone, progesterone, 11 β OH-androstenedione and some other steroids in young pigs and dogs. *The Journal of Physiology*, 1969 **200** 821-848.
23. Gupta, A., Cheetham T, Jaffray C *et al.*, Repeatability of the low-dose ACTH test in asthmatic children on inhaled corticosteroids. *Acta Pædiatrica*, 2009 **98** 1945-1949.
24. Wade, M., Baid S, Calis K *et al.*, Technical details influence the diagnostic accuracy of the 1 {micro}g ACTH stimulation test. *European Journal of Endocrinology*, 2010 **162** 109-113.

25. Winter JSD, Hughes IA, Reyes FI *et al* 1976 Pituitary-gonadal relations in infancy. II. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. *J Clin Endocrinol Metab* **42** 679–686
26. Grumbach MM 2005 A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant. *J Clin Endocrinol Metab* **90** 3122–3127
27. Netherton C, Goodyer I, Tamplin A *et al*, Salivary cortisol and dehydroepiandrosterone in relation to puberty and gender. *Psychoneuroendocrinology*. 2004 **29** 125-40.
28. Gunnar MR, Wewerka S, Frenn K *et al*. Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: normative changes and associations with puberty. *Dev Psychopathol*. 2009 **21** 69-85.
29. Shirtcliff EA, Allison AL, Armstrong JM *et al*. Longitudinal stability and developmental properties of salivary cortisol levels and circadian rhythms from childhood to adolescence. *Dev Psychobiol*. 2012 **54** 493-502.
30. Stroud LR, Papandonatos GD, Williamson DE *et al* . Sex differences in cortisol response to corticotropin releasing hormone challenge over puberty: Pittsburgh Pediatric Neurobehavioral Studies. *Psychoneuroendocrinology*. 2011 **36** 1226-38.

Table 1: Inhaled corticosteroid therapy in 296 children and young people with asthma undergoing a modified low dose short synacthen test and three-day salivary cortisol and cortisone collection

Inhaled corticosteroid therapy (number of patients treated)	Median dose (range) mcg / day.
Budesonide (32)	800 (200, 1600)
Beclometasone Dipropionate (30)	400 (100, 800)
Fluticasone Propionate (201)	500 (100, 2000)
Clenil Modulite (3)	400 (400, 800)
Mometasone (2)	375 (250, 500)

Figure 1: Basal cortisol versus peak cortisol in a simplified low dose short Synacthen test in 246 children and young people with asthma

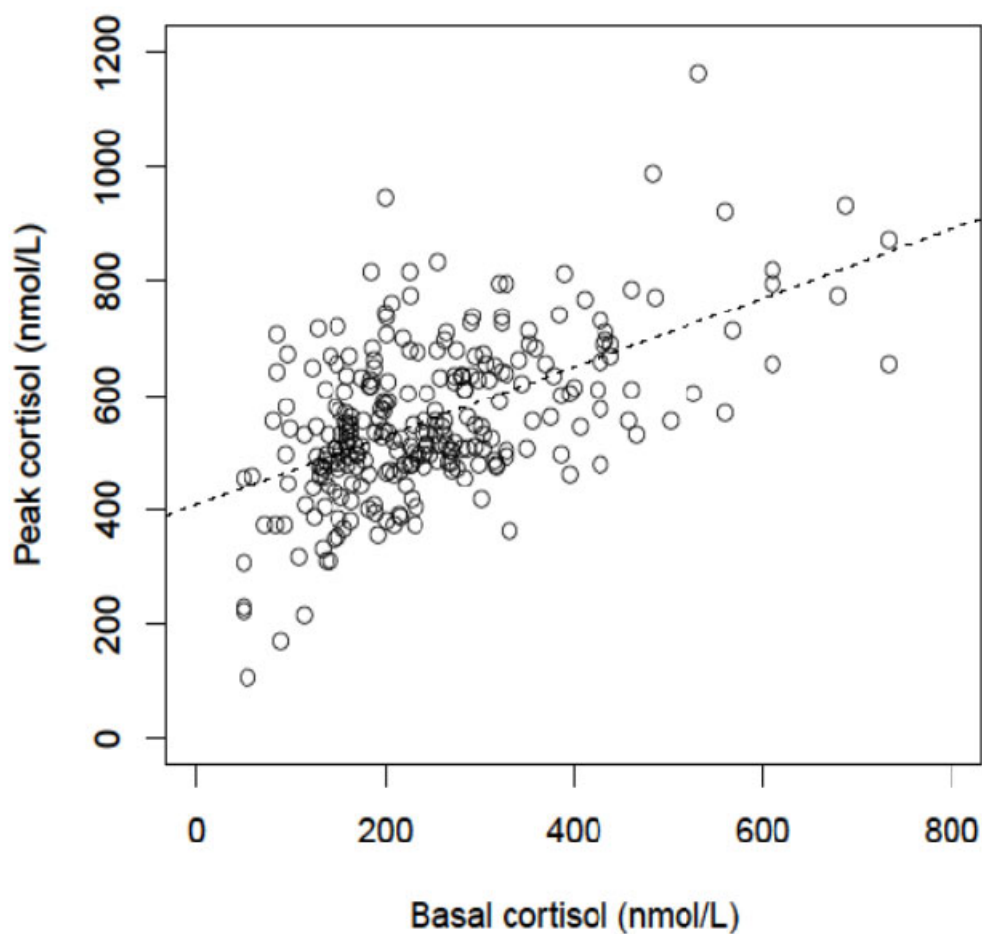


Figure 2: Receiver operating characteristic (ROC) curves for early morning salivary cortisol (EMSC) samples collected on three consecutive days versus peak serum cortisol below 350nmol/L on the low dose short synacthen test. Data are shown for each time point (EMSC 1-3), maximum, mean and minimum EMSCN.

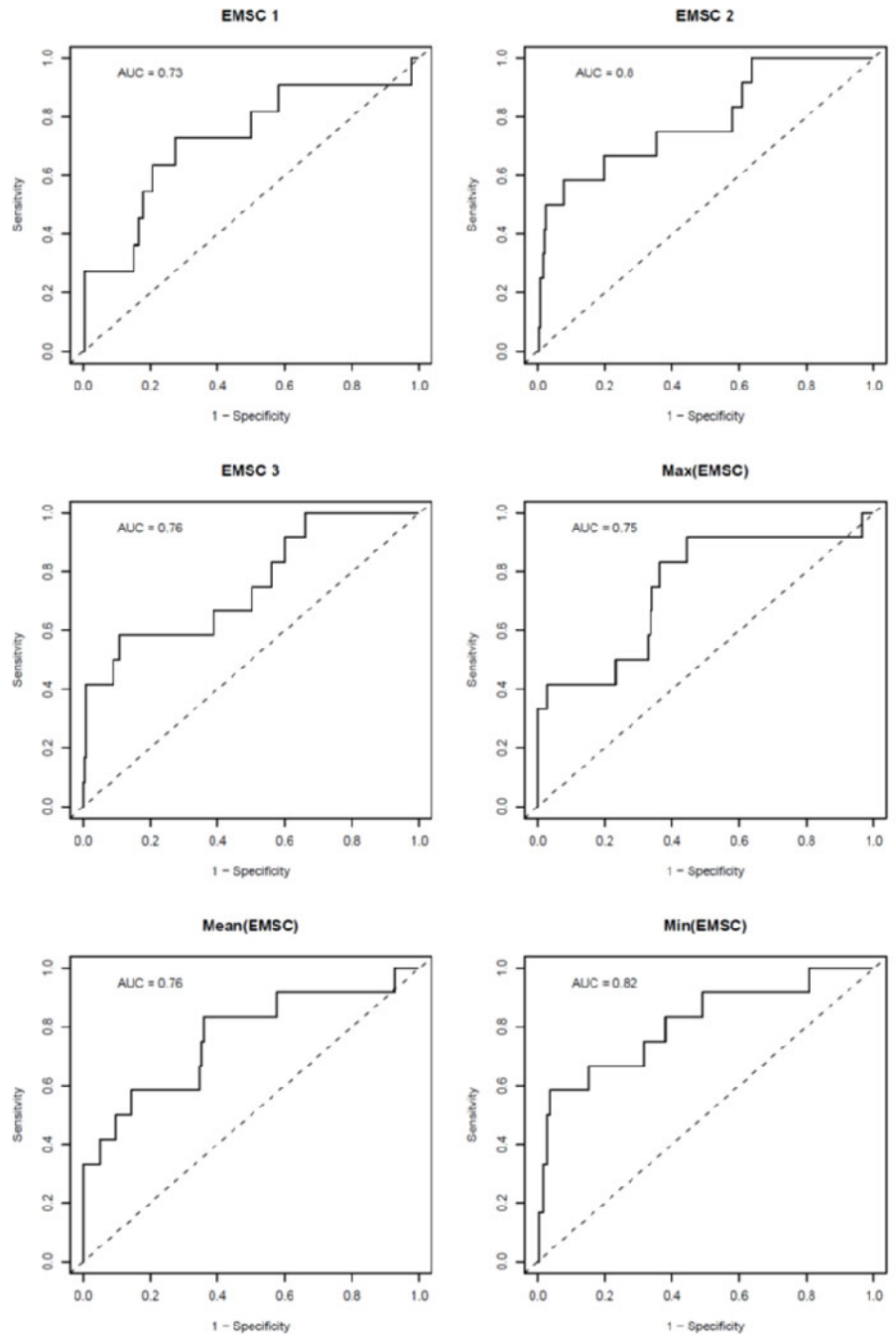


Figure 3: Receiver operating characteristic (ROC) curves for early morning salivary cortisone (EMSCN) samples collected on three consecutive days, (EMSCN 1-3), versus peak serum cortisol below 350nmol/L on the low dose short synacthen test. Data are shown for each time point, maximum, mean and minimum EMSCN.

