The MOSAIC study: **M**onitoring **O**f **S**alivary cortisol in **A**nogenital skin **I**nflammation treated with topical **C**orticosteroids

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Background and relevant literature:

Steroid medications are effective and often lifesaving medications in a range of childhood conditions. However, they are associated with a wide range of side effects, including adrenal suppression which may be life threatening. While there is little doubt that high doses of systemic steroids given for prolonged periods of time result in adrenal suppression, the degree of risk for topical steroid medications applied to the skin for shorter periods of time is uncertain.

Foreskin problems in boys:

Foreskin symptoms frequently cause concern for boys and their parents, and many boys attend paediatric surgical clinics for this reason. Phimosis, where the foreskin cannot be retracted over the glans, can be pathological or physiological and is a common finding (1). It is important to differentiate between pathological phimosis which may require surgical management, and physiological phimosis which is usually managed non-operatively (2). Physiological phimosis is normal in younger boys and resolves spontaneously in nearly all boys by the time they reach puberty (3).

Most boys with phimosis can be reassured and discharged with advice about good preputial hygiene. For boys with symptomatic physiological phimosis, topical corticosteroids are the mainstay of treatment, thinning the preputial skin and thereby improving retraction of the

foreskin (4). Application of potent topical steroids, such as 0.05% betamethasone dipropionate, result in a retractile foreskin in around 80% of boys with physiological phimosis (5). Around 100 boys a year are given a prescription for topical steroids in the surgical clinics at Alder Hey Children's Hospital.

Pathological phimosis is typically due to lichen sclerosus et atrophicus (LS), a chronic inflammatory skin disorder which is also known as balanitis xerotica obliterans (BXO) when it affects the penis. LS nearly always results in a non-retractile foreskin since the opening becomes increasingly narrow due to circumferential scarring (6). Topical corticosteroids can also be prescribed to boys with pathologic phimosis to reduce inflammation and can resolve phimosis when the condition is treated in its early stages.

Girls with lichen sclerosus

LS is also seen in young girls who present with vulval pain and itching. The prevalence is estimated to be around 1:900 prepubertal girls. Again, the mainstay of treatment is topical corticosteroids; there is no surgical management option in this patient group. Girls are treated with ultra-potent topical steroids, typically over a three-month period. In our service, it is standard practice to measure blood cortisol levels three times during the course of treatment (after 4, 8 and 12 weeks of treatment). Over the last 3 years, early morning cortisol measurements were requested from 21 patients with LS, treated with clobetasol propionate. Results were available for 18 patients of whom four were found to have abnormally low cortisol levels of <100nmol/L. These 4 patients underwent a short synacthen test and one required steroid replacement therapy for 14 months.

Steroid Treatment Cards:

The risk of adrenal suppression during steroid therapy was highlighted by NHS England in August 2020 through a National Patient Safety Alert(7). To aid the identification of paediatric patients at risk of adrenal suppression, the Neonatal and Paediatric Pharmacists Group (NPPG) issued a position statement in May 2022 detailing which steroid medications and doses may cause adrenal suppression, and when Steroid Treatment Cards should be issued (8). Whilst a large amount of data has been analysed in order to make this recommendation, there is little information specifically relating to application of topical corticosteroids to the

genitalia. The position statement advises that all children receiving large quantities of potent or very potent topical steroids for ≥4 weeks should receive a Steroid Treatment Card. A large quantity in a 7-year-old boy is estimated to be in the region of 80g/week(8, 9). The typical prescription from the paediatric surgery clinic is for a 30g tube of cream which will usually be sufficient for two 6-week courses, so it might be anticipated that the risk of adrenal suppression is very low. However, genital skin is highly permeable, and absorption of steroids may be greater than following application to keratinised skin. The position statement advises that issue of a card should be considered for patients using potent or very potent topical steroids, regardless of quantity, where there are other risk factors including application to highly permeable areas such as the genitalia (8). The pharmacy at Alder Hey has confirmed that boys and girls receiving treatment for phimosis and LS should be issued with a Steroid Treatment Card.

The supporting information for the position statement acknowledges the sparsity of published evidence for the risk of drug induced adrenal suppression in the paediatric population, explaining how some of the recommendations are based on data extrapolated from adult studies. The strongest evidence for this recommendation was from a meta-analysis of 522 children treated with potent/very potent topical steroid for atopic dermatitis, of whom 3.8% had adrenal suppression (10). However, it is important to note that none of the children reported symptoms of adrenal insufficiency, and adrenal function was normal on repeat testing. Although the proportion of children with adrenal suppression increased when the potency of topical steroid increased, the relationship was not statistically significant. The authors concluded that routine testing of the adrenal function should not be performed unless children are symptomatic of adrenal insufficiency (10). Of note, none of the data in the meta-analysis were directly applicable to the cohort of patients who will be included in this study, specifically since the skin type (more keratinised) and surface area to which the topical steroids are applied are not comparable.

Issues with potentially unnecessary steroid cards:

Medication adherence is an issue in many spheres of medicine and one key reason for poor adherence is fear of side effects (11, 12). It is likely that issuing a Steroid Treatment Card might

cause undue concern for some families, and they may not use the cream as prescribed as a result. This is likely to result in poor outcomes and an increase in the number of boys undergoing foreskin surgery which could otherwise have been avoided.

What this study will involve:

This study will investigate if there is evidence of adrenal suppression when using potent or very potent topical steroids on the genital skin. This will help advise whether these children need to be given Steroid Treatment Cards.

Salivary cortisol and cortisone as a measurement of cortisol:

The measurement of cortisol in saliva has a number of advantages compared to the measurement of cortisol in blood. Only free, biologically active cortisol is measured in saliva, whereas measurements of cortisol made in blood include both the biologically active, free hormone and protein bound inactive hormone. Cortisol is rapidly metabolised to cortisone as it passes through the parotid gland. Cortisone concentrations in saliva are approximately four times higher than cortisol concentrations, and both hormones correlate strongly with serum cortisol.

Saliva samples can be timed more closely to the time of waking, to capture the early morning rise in cortisol concentrations, compared to blood tests which are timed to phlebotomy opening hours and frequently collected sometime after the peak in morning cortisol. Samples can be collected at home, minimising disruption to families and reducing cost to the NHS. The stress related rise in cortisol associated with venepuncture is avoided during saliva sampling. Of note, salivary cortisol and cortisone are very stable over time and a range of temperatures, and tolerate freezing/refreezing well (13-15).

Aim

To determine whether potent, topical steroid creams, applied to genital skin, result in significant changes in early morning salivary cortisol and cortisone concentrations from baseline measurements.

Objectives:

- To measure early morning salivary cortisol (EMSC) and cortisone (EMSCn) before the start of treatment, on completion of treatment and one month after treatment
- To report change in EMSC and EMSCn from baseline at each time point
- To compare EMSC and EMSCn in patients at each timepoint to measurements made in healthy child volunteers(16).

Research Setting:

- Paediatric surgery and urology clinics – boys prescribed potent steroids for phimosis (physiological and pathological)
- Paediatric gynaecology/dermatology clinics – girls prescribed very potent topical steroids for LS

Study Design:

[1] Design: A prospective, pilot study evaluating EMSC and EMSCn in children before, during and after treatment of LS and phimosis with topical steroids. If there is no evidence of HPA axis suppression (fall in EMSC or EMSCn more than twice the coefficient of variation of the assay) in the first 55 children recruited then the study will end. If there is evidence of HPA axis suppression in any child then the study protocol will be amended to continue recruitment and to include dynamic testing of adrenal function. Further funding will be requested at this stage.

[2] Patients: Patients will be recruited during attendance to paediatric surgery, urology and gynaecology/gynaecology-dermatology clinics at Alder Hey Children's Hospital as described above. The study will aim to recruit 50 boys over a 12 month period. Clinicians will be asked to use a standardised prescribing protocol so that all boys receive the same steroid for the same duration ('Synalar', 0.025% fluocinolone twice daily for 6 weeks).

Girls diagnosed with LS during the study window will also be invited to participate, though the number of girls will be 5-6 at the most. The girls will also be managed with a standardised prescribing protocol (Dermovate ointment, Clobetasol 17-propionate 0.05% every night for 4 weeks, alternate nights for 4 weeks then twice weekly for 4 weeks).

[3] Inclusion criteria:

BOYS: Males 5-15 years who are treatment naïve (no steroid medication of any kind in the previous 3 months) receiving potent topical steroid for pathological or physiological phimosis.

GIRLS: Pre-pubertal females aged 5-15 years who are treatment naïve (no steroid medication of any kind in the previous 3 months) receiving very potent topical steroid for LS following diagnosis made in the paediatric gynaecology/dermatology clinics.

Exclusion criteria:

1. Patients with oral conditions which could contaminate saliva samples with blood, such as current mouth ulcers or gingivitis.
2. Patients taking additional medications which are likely to impact on cortisol levels e.g. glucocorticoids, sex steroids, thyroxine, growth hormone, insulin, metformin, opiates, loperamide and azole compounds.
3. Children with a family history of adrenal insufficiency due to an inherited condition, including congenital adrenal hyperplasia.
4. Children <5 years of age and children at high risk of choking on the cotton wool roll used to collect the saliva.
5. The treating clinician does not consider it appropriate to delay treatment whilst the family consider the study and take the pre-treatment samples.
6. Recent (within 3 weeks) ingestion of liquorice

Methods:

Participants will be identified during their outpatient clinic appointment. Once a decision has been made that they will receive topical steroid treatment their treating clinician or one of the study team will inform them of the study, give them the age-appropriate information sheets and answer any questions that they may have. If they are happy to join the study at that time then consent will be obtained and they will be given the study pack (which will include instructions and the equipment required for sample collection) to take home. A follow up appointment will be arranged as per usual care requirements. If the patient and family wish to take some time to think about study participation, they will be asked for their contact

details and will be contacted no more than one week later to see if they do or do not want to take part. They will be advised that they should not start using the topical steroid until this contact has been made. If they are happy to take part in the study consent will be taken via an electronic system so the families will not need to return to the hospital. The sample collection packs will be posted or they can return to the hospital to collect them. Some families may be provided with the pack at the initial clinic appointment if sufficient packs are available, accepting some may go to waste if families opt not to join the study.

The first three samples will be collected on three consecutive days before the treatment starts, ideally starting the day after they have been seen in clinic or given consent following the post-clinic contact. The BOYS will be asked to provide nine early morning saliva samples in total: three before treatment starts, three at the end of treatment and three samples one month after completing treatment. The GIRLS will be asked to provide nine samples, three before treatment starts, three at the end of the first four weeks of treatment and three samples just after completing treatment (timeslot chosen for final batch of samples to fit with timing of clinic review to minimize burden on the families).

The samples will be collected 30 minutes after waking, before eating or drinking and before cleaning their teeth. Children will be asked rinse their mouth with tap water before collecting their saliva sample. They will use a Salivette sampling device (Sarstedt, Rommelsdorf, Germany), this contains a plastic sampling tube enclosing a sterile cotton wool swab. The swab will be chewed for approximately 45 seconds and then placed back into the tube carefully without touching the swab. All samples will be collected at home and stored in a domestic freezer until they return for their clinic appointment. A member of the study team will contact families by phone, text, whatsapp or email (according to family preference) before each set of samples is due to remind them to collect the samples. The families will also be contacted a few days prior to the follow up clinic appointment to remind them to bring the saliva samples with them. They will also be asked to bring back the tube of steroid cream. The tube will be weighed to calculate how approximately much cream has been used over the treatment course.

The samples will be labelled with the participants study number, i.e. pseudoanonymised, then stored at -80 degrees in research freezers at Alder Hey. Once a suitable sized batch of samples has been received, they will be sent to the biochemistry laboratory at Wythenshaw for analysis. Consent will be obtained for the samples to be gifted to the research team and used for further studies. If sufficient sample remains after analysis, samples will be returned to Alder Hey and stored at -80°C. Children identified to have EMSC >2 standard deviations below the mean (based on analysis of normative data) will be referred to the endocrinology service and a 9 am serum cortisol will be requested. Further management will be advised by the endocrinologists.

Children who return their samples to the study team will receive a £25 voucher as a 'thank-you 'for taking part in the study. They will be informed of this in the patient information leaflet.

Data to be collected:

1. Age, sex, height and weight (all collected as part of normal care)
2. For boys, foreskin retractility at the time of enrolment and at the post treatment review according to a standard grading(17) [Grade 1 = complete preputial retractility, Grade 2 = partial retractility with partial exposure of the glans, Grade 3 = partial retractility with exposure of the meatus only, Grade 4 = no retractility]
3. For girls, clinical findings at the time of enrolment and at the post treatment review with regard to examination findings (nil, stable or active LS) and symptoms (yes or no)
4. Weight of tube of cream at beginning and end of treatment
5. The child's stage of pubertal development will be defined according to the Tanner criteria (18).

Assays:

EMSC and EMSCn will be measured using liquid chromatography-electrospray tandem mass spectrometry (TMS). This technique has been reported and has shown to have a lower limit of detection of cortisol 1.38nmol/l, and an intra- and inter-assay coefficient of variation of 7.0% at 1.9nmol/l and 11.0% at a concentration of 6.9nmol/l respectively (19).

Outcome measures:

Primary outcome

1. BOYS - change in EMSC and EMSCn from baseline to end of treatment and one month following treatment and in GIRLS - change in EMSC and EMSCn from baseline to the end of the first 4 weeks of treatment and at the end of treatment

Secondary outcome measures

1. Number of patients with EMSC or EMSCn >2 standard deviations below the mean of a cohort of healthy child volunteers
2. Percentage change in EMSC/EMSCn
3. Amount of steroid used according to age and sex
4. Acceptability of and compliance with the study protocol
5. Protocol completion
6. Adequacy of samples
7. Number of children requiring referral to endocrinology for further evaluation

Data analysis:

The data will be collected in an Excel database and analysed using statistical software such as SPSS, v26.

Data file / data storage:

The site file will be maintained electronically in a password protected file on the Alder Hey computers. The data will be stored for 10 years.

Recruitment period:

The planned recruitment period will be 1 year with a further 3 months to collect the samples from patients recruited at the end of the recruitment window. The estimated start date for the study is 01/09/2023.

Ethical considerations:

Ethical approval will be sought for the study. Parent and patient information sheets and consent forms will be submitted for review following evaluation by parents and patients seen in clinics in the months preceding the application and who would be eligible for the study.

Costs and Funding:

Each patient recruited will require funding of £75: £25 for a reward voucher and £50 for the sample collection pack and sample analysis, 55 patients (50 boys, 5 girls) will require a minimum funding of £4,125. A further sum will be required for spare sample collection packs, postage of sample collection packs to families and to transport the samples to the laboratory in Wythenshaw.

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References:

1. Moreno G, Corbalán J, Peñaloza B, Pantoja T. Topical corticosteroids for treating phimosis in boys. Cochrane Database Syst Rev. 2014(9):Cd008973.
2. McGregor TB, Pike JG, Leonard MP. Pathologic and physiologic phimosis: approach to the phimotic foreskin. Can Fam Physician. 2007;53(3):445-8.
3. Oster J. Further fate of the foreskin. Incidence of preputial adhesions, phimosis, and smegma among Danish schoolboys. Arch Dis Child. 1968;43(228):200-3.
4. Dave S, Afshar K, Braga LH, Anderson P. Canadian Urological Association guideline on the care of the normal foreskin and neonatal circumcision in Canadian infants (full version). Can Urol Assoc J. 2018;12(2):E76-e99.
5. Huntley JS, Bourne MC, Munro FD, Wilson-Storey D. Troubles with the foreskin: one hundred consecutive referrals to paediatric surgeons. J R Soc Med. 2003;96(9):449-51.
6. Spilsbury K, Semmens JB, Wisniewski ZS, Holman CD. Circumcision for phimosis and other medical indications in Western Australian boys. Med J Aust. 2003;178(4):155-8.
7. England N. National Patient Safety Alert – Steroid Emergency Card to support early recognition and treatment of adrenal crisis in adults 2022 [2023 Feb 24]. Available from: https://www.england.nhs.uk/publication/national-patient-safety-alert-steroid-emergency-card-to-support-early-recognition-and-treatment-of-adrenal-crisis-in-adults/.
8. Neonatal & Paediatric Pharmacists Group (NPPG). Position statement 2021-02 Use of Steroid Medication Warning Cards for Children and Young People [internet] 2021 [[cited Feb 2023 10]]. Available from: http://nppg.org.uk/wp-content/uploads/2021/12/Position-Statement-Steroid-Cards-V1.pdf.
9. Erskine D and Simpson H oboSfESECwg. Exogenous steroids treatment in adults. Adrenal insufficiency and adrenal crisis - who is at risk and how should they be managed safely. [internet] 2021 [[2023 Feb 11]]. Available from: https://www.endocrinology.org/media/4091/spssfe_supporting_sec_-final_10032021-1.pdf.
10. Wood Heickman LK, Davallow Ghajar L, Conaway M, Rogol AD. Evaluation of Hypothalamic-Pituitary-Adrenal Axis Suppression following Cutaneous Use of Topical Corticosteroids in Children: A Meta-Analysis. Horm Res Paediatr. 2018;89(6):389-96.
11. Smith LE, Webster RK, Rubin GJ. A systematic review of factors associated with side-effect expectations from medical interventions. Health Expect. 2020;23(4):731-58.
12. Kvarnström K, Westerholm A, Airaksinen M, Liira H. Factors Contributing to Medication Adherence in Patients with a Chronic Condition: A Scoping Review of Qualitative Research. Pharmaceutics. 2021;13(7).
13. Garde AH, Hansen AM. Long-term stability of salivary cortisol. Scand J Clin Lab Invest. 2005;65(5):433-6.
14. Wood P. Salivary steroid assays - research or routine? Ann Clin Biochem. 2009;46(Pt 3):183-96.
15. Tonge JJ, Keevil BG, Craig JN, Whitaker MJ, Ross RJ, Elder CJ. Salivary Steroid Collection in Children Under Conditions Replicating Home Sampling. J Clin Endocrinol Metab. 2022;107(11):3128-36.
16. Titman A, Price V, Hawcutt D, Chesters C, Ali M, Cacace G, et al. Salivary cortisol, cortisone and serum cortisol concentrations are related to age and body mass index in healthy children and young people. Clin Endocrinol (Oxf). 2020;93(5):572-8.

17. Meuli M, Briner J, Hanimann B, Sacher P. Lichen sclerosus et atrophicus causing phimosis in boys: a prospective study with 5-year followup after complete circumcision. J Urol. 1994;152(3):987-9.
18. Gunnar MR, Wewerka S, Frenn K, Long JD, Griggs C. Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: normative changes and associations with puberty. Dev Psychopathol. 2009;21(1):69-85.
19. Jönsson BA, Malmberg B, Amilon A, Helene Garde A, Orbaek P. Determination of cortisol in human saliva using liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2003;784(1):63-8.