Knemometry is more sensitive to systemic effects of inhaled corticosteroids in children with asthma than 24-hour urine cortisol excretion

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Background: Pharmacodynamic assessment of the systemic effect of inhaled corticosteroids (ICSs) is often done by measuring 24-hour urine free cortisol (UFC) excretion. Knemometry assessing short-term lower-leg growth rate (LLGR) is a more rarely used alternative.
Objective: The primary aim of this study was to compare the sensitivity of LLGR and 24-hour UFC excretion for evaluating systemic exposure to ICSs in prepubertal children with asthma. The secondary aim was to evaluate factors influencing the precision of LLGR calculated by the traditional 1 leg nonparametric method versus a new 2 leg parametric method.
Methods: The study evaluated 60 children with mild asthma aged 5 to 12 years participating in a randomized controlled trial of ICSs with longitudinal concomitant assessments of LLGR and 24-hour UFC excretion. The sensitivity of the safety assessments was analyzed by comparing LLGR and 24-hour UFC in the placebo run-in period with values in the ICS treatment period by using paired *t* tests. Factors with a potential influence on LLGR were analyzed by means of ANOVA and the Levene test of homogeneity.
Results: The mean LLGR was significantly reduced during the ICS versus placebo run-in periods: 0.18 mm/wk (SD, 0.55 mm/wk) versus 0.45 mm/wk (SD, 0.39 mm/wk), with a mean difference of 0.27 mm/wk (95% CI, 0.05-0.48 mm/wk; $P = .02$). In contrast, there was no difference in 24-hour UFC excretion: 6.91 nmol/mmol (SD, 4.67 nmol/mmol) versus 7.58 nmol/mmol (SD, 6.17 nmol/mmol), with a mean difference of 0.67 nmol/mmol (95% CI, −1.13 to 2.48 nmol/mmol; $P = .46$). We observed no significant difference in parametric determined LLGR caused by the child's age or sex, investigator, or season of measurement, whereas some differences were observed for the nonparametric LLGR.
Conclusion: These findings suggest that knemometry is a more sensitive pharmacodynamic measure of systemic effects of ICSs than 24-hour UFC excretion and that a parametric determination of LLGR increases the sensitivity of the method. These findings should be considered by legislative authorities in the future. (J Allergy Clin Immunol 2017;140:431-6.)

Key words: *Asthma, children, inhaled corticosteroids, knemometry, urine cortisol excretion*

Regular treatment with inhaled corticosteroids (ICSs) leads to systemic steroid effects.[1,2] Therefore the US Food and Drug Administration (FDA) and the Pediatric Committee of the European Medicines Agency (EMA) require pharmacokinetic and pharmacodynamic evaluation for approving ICS use in children. Guidelines require pharmacodynamic comparisons to be done by means of assessment of 24-hour urine free cortisol (UFC) excretion, which has been shown to be a sensitive measure of the systemic effect of exogenous steroids on the hypothalamic-pituitary-adrenocortical (HPA) axis.[3-5] Knemometry is an alternative method to assess pharmacodynamics from ICSs by measuring short-term lower-leg growth rate (LLGR) in prepubertal children,[6] even in toddlers,[7,8] and has been suggested as a useful physiologic alternative to measurement of 24-hour UFC excretion.[9] However, there are to our knowledge no previously published studies comparing these 2 assessments of the pharmacodynamics of ICSs in children, and it is unknown which method is the most sensitive.

The primary aim of this study was to compare short-term LLGR and 24-hour UFC excretion for evaluating systemic exposure to ICSs in 60 children aged 5 to 12 years with mild asthma participating in a clinical trial of ICSs. The secondary aim was to evaluate the effect of sex, age, season, and investigator on the precision of LLGR assessments. The latter was done evaluating LLGR calculated by the traditional nonparametric method on 1 leg versus a parametric method, where we increased the number of measurements and included data from both legs.

METHODS

Study population, design, and medication

Children 5 to 12 years of age in Tanner stage I with a diagnosis of mild asthma with an FEV_1 of greater than 80% of predicted value were eligible for enrollment in the study. After a screening visit (visit 1), eligible patients entered a 2-week placebo run-in period followed by two 2-week treatment periods of either the fixed or free combination of beclomethasone dipropionate extrafine particle pressurized metered-dose inhaler (pMDI; 50 μg × 2 twice daily; total daily dose, 200 μg) and formoterol fumarate pMDI (6 μg × 2 twice daily; total daily dose, 24 μg) delivered through the AeroChamber Plus spacer device (Trudell Medical International, Ontario, Canada). Treatment periods were separated by a 2-week washout period in a crossover design. Clinic visits were performed at the beginning and end of each period, during which

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The data used in the study originate from a randomized controlled trial, which was conducted as a phase III study for Chiesi Farmaceutici S.p.A.

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Abbreviations used
FDA: US Food and Drug Administration
HPA: Hypothalamic-pituitary-adrenocortical
ICS: Inhaled corticosteroid
IQR: Interquartile range
LLGR: Lower-leg growth rate
pMDI: Pressurized metered-dose inhaler
UFC: Urine free cortisol

systemic exposure to ICSs was evaluated based on LLGR and 24-hour UFC excretion.

The study was conducted in agreement with the guidelines for good clinical practice and was approved by the local ethics committee (clinicaltrials.gov: CCD-1012-PR-0051). Informed consent was obtained from the parents before any study-related procedures were undertaken.

Knemometry

A Valk knemometer (see Fig E1 in this article's Online Repository at www.jacionline.org) was used to measure the child's right and left lower-leg length at each visit, adhering to a predefined protocol.[10,11] The child was dressed in underwear and placed on the knemometer chair, which was subsequently adjusted to an angle of no more than 90° between the lower and upper legs. We applied talcum on the knees of the child and ensured the arms were kept crossed and held in front of the body to avoid any pressure on the legs. Seat height, depth, and distance to the measuring plates were adjusted by using 3 fixed rulers. The feet of the child were placed on cardboard, on which the position of the child's feet was marked. The knemometer chair settings and the cardboard were reused at all visits to ensure identical positioning of the child.

All knemometry assessments were done by 4 trained investigators (A, B, C, and D), who performed the measurements without reference to previous measurements on that child. At each visit, a total of 16 knemometry measurements were performed. Four measurements of the left and 4 measurements of the right lower leg were performed and noted. The child was asked to stand up and walk a short distance before being reseated, and another 4 measurements of the left and 4 measurements of the right legs were done.[12] The children were instructed not to perform heavy physical exercise on the day of knemometry, and the measurements were performed at the same time of day at every visit (±1 hour).

We only included children in the analyses if they had not taken inhaled, nasal, or systemic corticosteroids 2 weeks before the placebo run-in period. In analyses of factors with a possible influence on the precision of LLGR, we only included measurements performed by the same investigator at all visits for each child.

Twenty-four-hour UFC excretion

Families were instructed to collect urine over 24 hours at the end of the placebo run-in period and each treatment period. Initially, the child was instructed to empty the bladder. Thereafter, all urine during the next 24 hours was collected in a plastic collection container, which was kept in a refrigerator in the home. At the clinic, the total volume of urine was noted, and aliquots of 10 mL were stored at −80°C before shipment on cold dry ice to LabCorp Clinical Trials (Hamburg, Germany). At LabCorp, the cortisol and creatinine contents of the samples were analyzed by means of liquid chromatography/tandem mass spectrometry to determine the 24-hour UFC/creatinine excretion ratio.

Statistical analyses

Treatment compliance was defined and calculated as the percentage of days on which the test drug was inhaled based on daily diary recordings.

The lower-leg length at each visit was calculated by a parametric method as the average of the median of the 4 assessments (right and left and right and left, each with 4 repeats, equaling 4 × 4 single measurements). Thereafter, LLGR (in millimeters per week) was calculated as the lower-leg length at the end of the period minus the lower-leg length at the beginning of the period adjusted for period length, which is hereafter termed $LLGR_{4x4}$.

The sensitivity of LLGR was analyzed by comparing LLGR during the placebo run-in period versus that during the ICS treatment periods with paired *t* tests. The sensitivity of the 24-hour UFC/creatinine excretion ratio (in nanomoles per millimole) was also investigated by comparing results from the placebo run-in and ICS treatment periods with paired *t* tests.

Thereafter, we analyzed the effect of age (stratified as 5-8 years, >8-11 years, or >11 years), sex, investigator, and season of measurement (spring, summer, autumn, or winter) on LLGR during the placebo run-in period by using 1- and 2-way ANOVA and the Levene test of homogeneity.

Lastly, we performed power analyses for 2-sided paired *t* tests of LLGR based on the SD of the baseline LLGR during the baseline placebo run-in period to estimate the number of children needed in future trials to obtain an 80% power to detect a range of mean differences in LLGR per week.

The ANOVA and power analyses were also conducted with LLGR calculated by using the conventional nonparametric method, in which lower-leg length is defined as the mean of the 2 first measurements of the left leg before and after the child has been reseated and for which the most deviant measurement is discarded (ie, the mean of the 3 most alike measures, hereafter termed $LLGR_{2x2}$).[13]

A significance level of .05 was used in all analyses. Data processing was conducted with SPSS and SAS software (version 9.4 for Windows; SAS Institute, Cary, NC) and R version 3.2.2 for Windows.

RESULTS

Baseline characteristics

A total of 60 children were included in the trial, but 17 children were excluded from the sensitivity analyses of LLGR and 24-hour UFC excretion (13 because of use of corticosteroids within 2 weeks of the placebo run-in period and 4 because of missing 24-hour urine collection). A further 11 children were excluded from the variance analyses (10 because of different investigators at the visits and 1 child who was the only one measured by investigator D, see the study flowchart in Fig 1).

The median age of the 43 children (18 [42%] boys) included in the sensitivity analyses was 11.3 years (interquartile range [IQR], 9.6-11.8). The mean FEV_1 at visit 1 was 2.01 L (SD, 0.45 L; Table I). The median treatment compliance (percentage of days during which the test drug was inhaled) was 98.4% (IQR, 96.3% to 100.0%). The included versus excluded children were older (median, 11.3 years [IQR, 9.6-11.8 years] vs 9.5 years [IQR, 7.6-10.5 years]; $P = .03$) and less often of male sex (18 [42%] vs 12 [71%], $P = .04$) but showed no other differences in baseline characteristics (see Table E1 in this article's Online Repository at www.jacionline.org).

Sensitivity of knemometry and 24-hour UFC excretion (n = 43)

The mean $LLGR_{4x4}$ during the baseline placebo run-in period versus the ICS period was 0.45 mm/wk (SD, 0.39 mm/wk) and 0.18 mm/wk (SD, 0.55 mm/week), respectively. Thus the $LLGR_{4x4}$ was significantly reduced during the ICS periods, with a mean difference of 0.27 mm/wk (95% CI, 0.05-0.48 mm/wk; $P = .02$; Fig 2). In contrast, there was no significant difference in the mean 24-hour UFC excretion during the baseline placebo run-in period versus the ICS period: 7.58 nmol/mmol (SD, 6.17 nmol/mmol) versus 6.91 nmol/mmol (SD, 4.67 nmol/mmol), with a mean difference of 0.67 nmol/mmol (95% CI, −1.13 to 2.48 nmol/mmol; $P = .46$; Fig 3).