RESEARCH

Pilot study shows suppression of mineralocorticoid precursors under high-dose glucocorticoid therapy in pediatric acute lymphoblastic leukemia

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Abstract

Glucocorticoids represent a key element in the treatment of pediatric acute lymphoblastic leukemia (ALL) and lead to adrenal suppression. We aimed to assess the differential response profile of adrenal steroids in children with ALL during BFM (Berlin–Frankfurt–Münster) induction treatment. Therefore, we performed liquid chromatography tandem–mass spectrometry (LC–MS/MS)-based steroid profiling of up to seven consecutive leftover morning serum samples derived from 11 patients (pts) with ALL before (day 0) and during induction therapy at days 1–5, 6–12, 13–26, 27–29, 30–35 and 36–40. 17-hydroxyprogesterone (17OHP), 11-deoxycortisol (11S), cortisol, 11-deoxycorticosterone (DOC), corticosterone and aldosterone were determined in parallel. Subsequently, steroid concentrations were normalized by multiples of median (MOM) to adequately consider pediatric age- and sex-specific reference ranges. MOM-cortisol and its precursors MOM–11S and MOM–17OHP were significantly suppressed by glucocorticoid treatment until day 29 ($P < 8.06 \times 10^{-10}$, $P < 5.102 \times 10^{-5}$, $P < 0.0076$, respectively). Cortisol recovered in one of four pts at days 27–29 and in two of five pts at days 36–40. Among the mineralocorticoids, corticosterone was significantly suppressed ($P < 3.115 \times 10^{-6}$). Aldosterone and DOC showed no significant changes when comparing day 0 to the treatment time points. However, two ALL patients with ICU treatment due to the sepsis showed significantly lower MOM–DOC ($P = 0.006436$) during that time and almost always the lowest aldosterone compared to all other time points. Suppression of mineralocorticoid precursors under high-dose glucocorticoid therapy suggests a functional cross talk of central glucocorticoid regulation and adrenal mineralocorticoid synthesis. Our data should stimulate prospective investigation to assess potential clinical relevance.

Key Words

- acute lymphoblastic leukemia
- glucocorticoids
- mineralocorticoids
- LC–MS/MS
- steroid profiling

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Introduction

High-dose glucocorticoids are indispensable in the treatment of childhood acute lymphoblastic leukemia (ALL) (1). Two different highly potent synthetic glucocorticoids are used in the current standard protocols, namely prednisolone and dexamethasone (2). The hypothalamic–pituitary–adrenal axis is generally suppressed during and following glucocorticoid treatment in ALL (3). The duration of adrenal suppression is highly variable. While some studies reported persistence for more than 1 week, others reported durations ranging from 10 to 20 weeks and even up to several months after the end of the therapy (4, 5, 6, 7). Inadequate function of the hypothalamic–pituitary–adrenal axis, and hence inadequate adrenal response, is a significant health risk for affected children during acute febrile illness and may lead to increased morbidity and mortality (3, 6). Glucocorticoid replacement regimens during stress episodes, for example, during infection and sepsis, have therefore been recommended (3, 4, 7, 8).

Prednisolone and dexamethasone are highly potent glucocorticoids with only a little mineralocorticoid effect. Consequently, the regulation of glucocorticoids has been the main focus to investigate adrenal gland-related toxicity in ALL (3, 4, 5, 6, 7, 8). However, adrenal insufficiency may affect different steroids differently and each effect may have distinct clinical consequences. Global adrenal insufficiency affects both glucocorticoids and mineralocorticoids. The most important acquired example of global adrenal insufficiency is Addison's disease (9). Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21OHD) is a genetically determined block of glucocorticoid and mineralocorticoid biosynthesis (10). Both are typically at risk for adrenal crises in stress episodes. Interestingly, rare genetic subtypes of CAH like 11β hydroxylase deficiency (11βOHD) and 17 alpha/17–20 lyase deficiency block cortisol synthesis but allow for certain mineralocorticoid synthesis and have only little or absent risk for adrenal crises (11, 12).

It has been shown earlier that not only glucocorticoids but also mineralocorticoids may be blocked by dexamethasone treatment (14, 15). We hypothesize that adrenal suppression using high-dose prednisolone or dexamethasone during ALL treatment might affect mineralocorticoid biosynthesis. Since mineralocorticoids are key regulators of blood pressure and fluid and salt balance in the body (16) and since deterioration in blood circulation and blood pressure are key characteristics of clinical sepsis (17), differences in suppression of mineralocorticoids may be assumed to influence adrenal crisis severity. Liquid chromatography tandem mass spectrometry (LC–MS/MS) is today's gold standard for determining serum steroids. We aimed to investigate LC–MS/MS steroid profiles of childhood ALL serum samples, encompassing cortisol and aldosterone as well as their precursors. Samples were taken from routine blood samples of leftover material before and during glucocorticoid treatment. To our knowledge, this is the first study assessing iatrogenic adrenal insufficiency in high-dose glucocorticoid-treated ALL at the level of LC–MS/MS-based steroid profiling.

Materials and methods

Patients and ALL treatment protocol

Twelve patients with ALL (five girls and seven boys) were included in the study after signed informed consent was given. The study was approved by the local ethics committee of the University of Lübeck (reference number 17-080). ALL was treated according to the AIEOP-BFM ALL 2009 protocol (18). After a 7-day prednisolone prephase, patients received either 60 mg/m^2 prednisolone or 10 mg/m^2 dexamethasone per day (in three doses) for 28 days. Only 1 of the 12 patients received dexamethasone. From day 29, steroids were tapered by 50% every 3 days and terminated on treatment day 37. Two patients had to be treated in the pediatric intensive care unit (ICU) due to infection, including the 1 patient treated with dexamethasone, and two patients suffered from infection without needing ICU treatment. Eight patients had no infection and needed no ICU treatment.

Steroid measurements

Serum samples for steroid measurements were collected from ALL patients during morning routine blood sampling on day 0 before starting the glucocorticoid prephase and on days 1–5, 6–12, 13–26, 27–29, 30–35 and 36–40 of induction therapy (six time points per patient). They were stored at –80°C and sample leftovers were used for the study. Up to six serum samples per patient could be included. It should be noted that it was not possible to obtain a blood sample from each of the 12 patients at each time point. Unfortunately, no EDTA-plasma had been stored and therefore, neither adrenocorticotropic hormone (ACTH) nor renin could be measured.

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The number of patients per time point is shown in the graphical display of steroid multiples of medians (MOMs).

Three steroids representing glucocorticoid synthesis, namely 17-hydroxyprogesterone (17OHP), 11-deoxycortisol and cortisol as well as three steroids representing mineralocorticoid synthesis namely 11-deoxycorticosterone (DOC), corticosterone and aldosterone were measured in a parallel assay by as previously described (19, 20). Thus, a total of six steroids were measured in each sample after extraction of 0.1 mL patient's serum using an Oasis SPE system (Waters, Milford, MA, USA). Steroid hormone determination was then carried out using a UPLC Quattro Premier/Xe system (Waters). Intra- and inter-assay CVs were <5% for all LC–MS/MS methods.

Normalization by multiples of median transformation

Pediatric reference ranges for steroid hormones and their precursors change significantly with increasing age and they differ according to the male and female sex (19). In order to achieve comparability of steroid data in a pediatric cohort with different ages and sexes, we normalized steroid data according to the MOM method as previously described (21). Calculation of each subject's MOM followed the equation by Wald (22). A measured single-plasma steroid value (individual patient's result) is divided by the population median derived from the corresponding age- and gender-specific reference group. MOM calculation in this manuscript is based on our previously published pediatric reference ranges, using the same accredited LC–MS/M–S machine and the identical evaluation protocol (19).

Statistical analysis

Statistical analysis was carried out using R 4.1.0 (https://www.r-project.org/). Data were analyzed to detect significant differences between the three groups (without infection and ICU treatment; with infection and without ICU treatment; ICU treatment) as well as between the MOM values before and during the treatment. In both cases, normal distribution was tested with a Kolmogorov–Smirnov test with Lilliefors' correction for each hormone. The nonparametric Wilcoxon rank sum test was used to evaluate statistical differences between the groups. A *P*-value of <0.05 was considered to be statistically significant.

Results

Glucocorticoids

Cortisol MOMs were mostly within the normal range, or they were already elevated at day 0 before the start of the therapy (Fig. 1). Cortisol dropped below the reference range following prednisolone or dexamethasone treatment, starting at days 1–5 and lasting until days 27–29. At days 30–35 in the so-called tapering phase and at days 36–40 at the end of treatment, one of four patients for whom plasma samples were available and two of five other patients, respectively, showed normal cortisol levels again. Comparing day 0 to all days under therapy up to day 29, there was a significant decrease in cortisol ($P = 8.06e{-}10$) (Table 1). MOMs of the cortisol precursors 17OHP and 11-deoxycortisol were more variable and lay in the normal MOM range in some ALL patients. However, they also dropped significantly when comparing day 0 to the treatment days up to day 29 (17OHP, $P = 0.007622$; 11-deoxycortisol, $P = 8.06e{-}10$) (Fig. 1; Table 1). There was no significant difference in cortisol and 11-deoxycortisol between the two ALL patients who needed ICU treatment due to infection and all other patients considering all time points together after day 0. However, the 17OHP MOM was significantly lower in the two ICU patients ($P = 0.03629$) (Table 2). Supplementary Table 1 (see section on supplementary materials given at the end of this article) shows the corresponding non-normalized original steroid concentrations (nmol/L) also showing the initial drop of glucocorticoid synthesis after initiation of therapy with later recovery in the tapering phase (Supplementary Table 1). We have also determined cortisone in all patients at all available time points (not displayed in figures). The initial mean MOM cortisone was 1.3 (47 nmol/L) and decreased to 0.08 (2.8 nmol/L) under therapy ($P = 8.06e{-}10$).

Mineralocorticoids

The greatest changes were observed for corticosterone. At day 0, corticosterone MOMs were mostly elevated or in the normal MOM range (Fig. 2). At days 1–5, corticosterone MOMs dropped down to the lowest normal MOM range or even below. At days 12–26 and subsequent time points, corticosterone MOMs lay more frequently in the middle of the MOM reference range (Fig. 2). Comparing day 0 to all days under therapy up to day 29, the drop of corticosterone was significant ($P = 3.115e{-}06$) (Table 1). In contrast, MOMs for DOC and aldosterone did not show significant changes (DOC,

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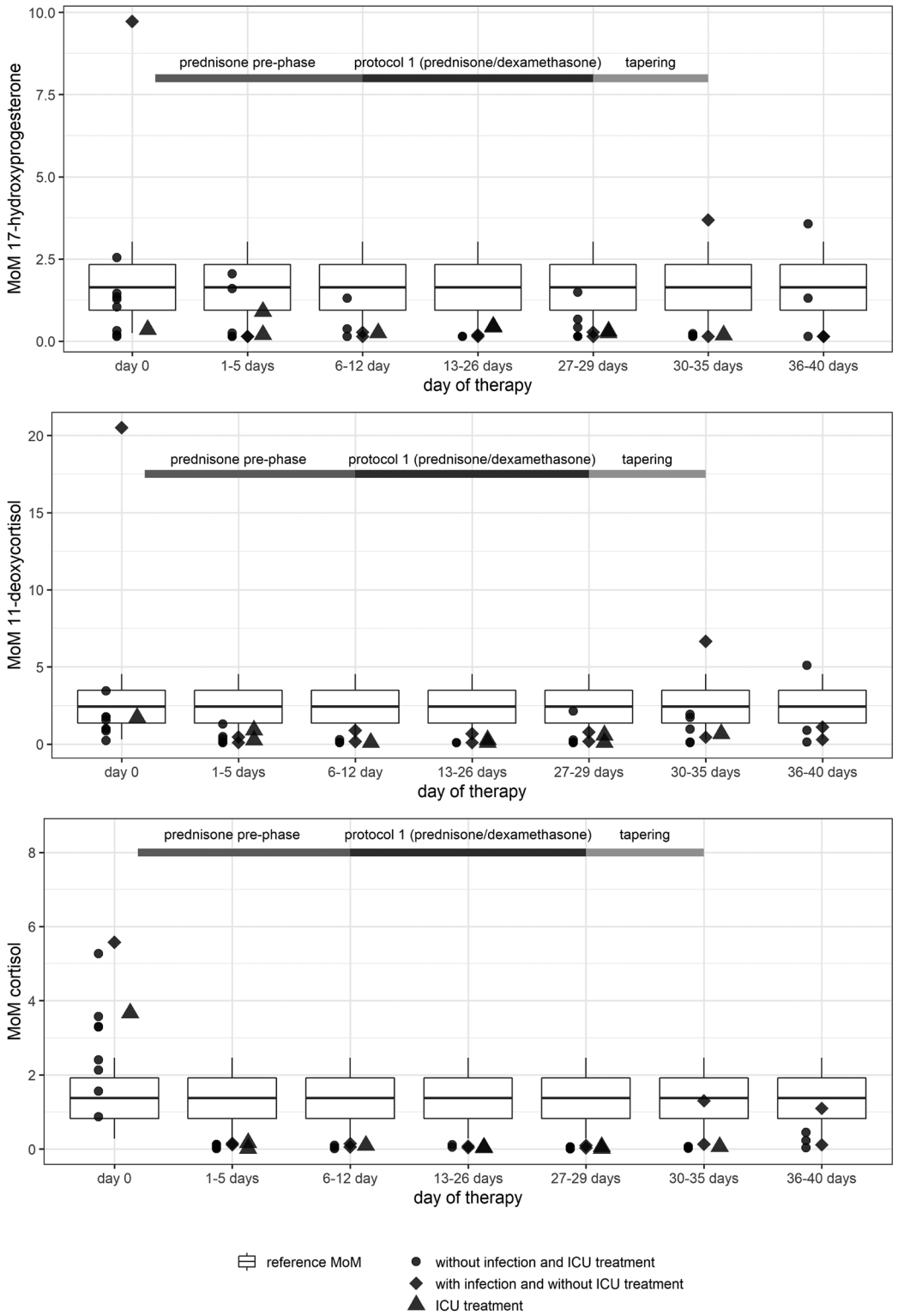

Figure 1

The MOMs of 17-hydroxyprogesterone (top panel), 11-deoxycortisol (middle panel) and cortisol (bottom panel). MOMs are represented in the *y*-axes. The *x*-axes represent the different time points starting before therapy, under therapy, the tapering phase and one time point at the end of tapering. The therapy phase is further indicated by a bar at the top of the figure. The normal ranges for the MOMs (21) are represented by gray background. Circles represent patients who did not suffer from sepsis and who were not admitted to ICU. Squares represent patients who suffered from sepsis but without ICU treatment. Triangles represent patients who were admitted to ICU during sepsis.

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Table 1 Statistical calculation (Wilcoxon rank test) to test whether the MOMs for 17-hyroxyprogesterone, 11-deoxycortisol, cortisol, 11-deoxycorticosterone, corticosterone and aldosterone differed between time point 1 before therapy (day 0) compared with all other time points under therapy (except days 30–35 and days 36–40 during and after tapering, respectively). Data from all available patients per time point were considered.

	P-value
17OHP	**0.007622**
11-desoxycortisol	**5.102e−10**
Cortisol	**8.06e−10**
11-desoxycorticosterone	0.359
Corticosterone	**3.115e−6**
Aldosterone	0.8441

Table 2 *P*-values of statistical calculation (Wilcoxon rank test), comparing the degree of suppression of the named steroids in the two ALL patients who needed ICU treatment due to infection with all other patients, considering all time points except time point 1 before therapy (day 0).

	P-value
17OHP	**0.03629**
11-desoxycortisol	0.8082
Cortisol	0.5877
11-desoxycorticosterone	**0.006436**
Corticosterone	0.3149
Aldosterone	0.4887

$P = 0.359$; aldosterone, $P = 0.8441$) (Table 1). DOC MOM was significantly lower ($P = 0.006436$) in the two ALL patients who underwent ICU treatment due to infection compared with all other patients at all time points except on day 0. In contrast, corticosterone and aldosterone were not significantly lower. However, aldosterone MOMs were mostly lower compared to the other patients at the time points between days 1–5 up to days 27–29 under high doses of glucocorticoid (Fig. 2). Supplementary Table 1 shows the corresponding non-normalized original steroid concentrations (nmol/L) also visualizing the striking drop of mean corticosterone following initiation of glucocorticoid therapy. In addition, the decrease of 11-deoxycorticostrone concentrations in patients with infections is well visible at the level of non-normalized data and the two ICU children demonstrate the lowest DOC plasma concentrations (Supplementary Table 1).

Discussion

To the best of our knowledge, without exception, previous studies on the suppressive effects of high-dose glucocorticoid treatment in pediatric ALL focused on cortisol as the only readout for adrenal function (3, 4, 5, 6, 7, 23). This disregards several aspects of the complex endocrine biochemistry of adrenal steroid biosynthesis. First, the importance of mineralocorticoids for salt and water balance has not been considered to be potentially affected. This is probably because classical endocrinology in the textbooks and in current reviews usually separates discussion of CRH–ACTH-driven regulation of glucocorticoids on the one hand and renin–angiotensin-driven regulation of mineralocorticoids on the other hand (24, 25). Secondly, the focus on only one steroid, cortisol, ignores the considerably high intrinsic activity of variably abundant so-called adrenal precursor steroids, both in their role as glucocorticoids (26) – potentially even contributing to the survival of untreated CAH patients – and in their role as mineralocorticoids, protecting children with rare subtypes of CAH from life-threatening salt wasting crises (10, 11, 13). Our study is the first to investigate the differential suppressive effects of high-dose glucocorticoid treatment on steroid metabolism in children with ALL by using a state-of-the-art steroid profiling LC–MS/MS method, enabling analysis of cortisol, aldosterone and their relevant steroid precursors in parallel.

In line with many previous studies in ALL (3, 4, 5, 6, 7, 8, 23), we found significant suppression of cortisol in our dataset, starting at treatment days 1–5. At days 30–35 in the tapering phase and at days 36–40 at the end of tapering, most of the patients still showed suppressed cortisol levels (three of four patients for whom plasma samples were available and three of five patients, respectively). This is again well in line with the literature, confirming the variable and in part sustained normalization of cortisol biosynthesis in many patients (4, 5, 6, 7). In addition to cortisol, 11-deoxycortisol, the direct precursor of cortisol, as well as 17OHP dropped significantly when comparing day 0 values with treatment days. This shows that suppression of endogenous glucocorticoid biosynthesis is not only reflected by cortisol but also visible, as would be expected, at the level of its endogenous precursors. Interestingly, cortisone concentrations also decreased significantly when comparing high-dose glucocorticoid treatment days with pre-treatment conditions supporting endogenous glucocorticoid suppression (27). We suggest that this may be due to the massively reduced cortisol as a substrate for the 11βOHD type 2 enzyme usually inactivating cortisol to cortisone.

Among the most striking observations in our study is the significant suppression of the mineralocorticoid

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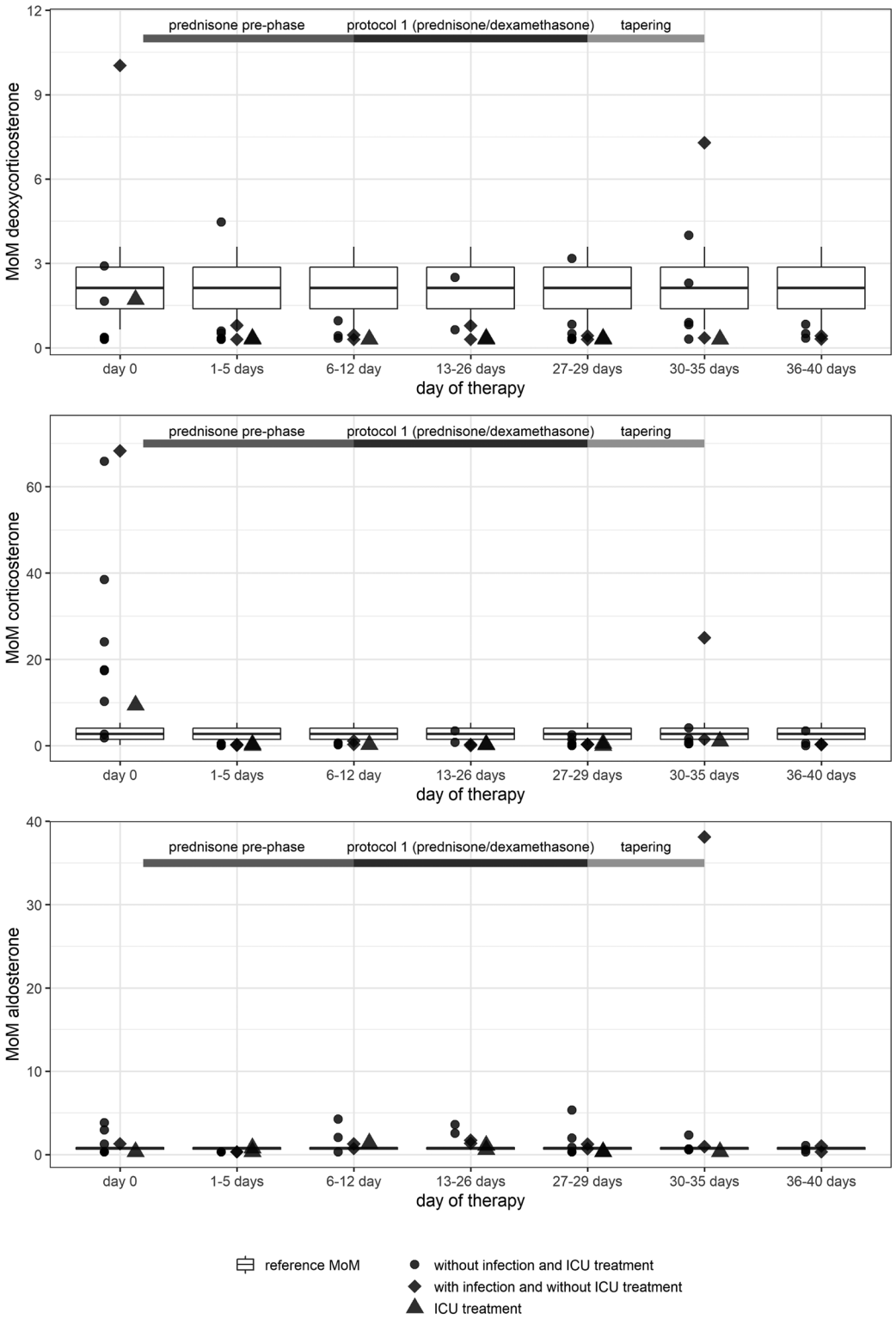

Figure 2

The MOMs of 11-deoxycorticosterone (top panel), corticosterone (middle panel) and aldosterone (bottom panel). MOMs are represented in the *y*-axes. The *x*-axes represent the different time points starting before therapy, under therapy, the tapering phase and one time point at the end of tapering. The therapy phase is further indicated by a bar in the top of the figure. The normal ranges for the MOMs (21) are represented by gray background. Circles represent patients who did not suffer from sepsis and who were not admitted to ICU. Squares represent patients who suffered from sepsis but without ICU treatment. Triangles represent patients who were admitted to ICU during sepsis.

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precursor corticosterone. DOC showed a similar pattern in part, but the drop was not statistically significant, possibly due to the small number of patients enrolled. Both DOC and corticosterone have an intrinsic partial mineralocorticoid activity (10, 11, 13). Potential clinical relevance of these two mineralocorticoid precursors in other situations than ALL has been discussed in patients with 11βOHD, where high DOC is thought to protect affected children from severe salt wasting and to contribute to hypertension (10, 11). In CYP 17 deficiency, both DOC and corticosterone potentially prevent salt wasting or at least make it unlikely, also contributing to high blood pressure (10, 13).

The suppression of mineralocorticoids by pharmacological therapy with prednisolone and dexamethasone has only very rarely been reported in the literature. More than 40 years ago, Kinoshita and co-workers reported dexamethasone-induced suppression of aldosterone in CAH (14). Holsboer and co-workers reported dexamethasone-induced suppression of DOC and corticosterone in female patients with endogenous depression (15). The endocrine mechanism underlying this high-dose glucocorticoid-mediated cross talk to mineralocorticoid suppression is unclear. Interestingly, aldosterone-producing adenomas may respond to dexamethasone treatment by suppressing aldosterone synthesis (28, 29). However, the mechanism of glucocorticoid-remediable hyperaldosteronism involves a specific gene duplication (28) and therefore cannot serve as an explanation outside this specific disease.

Both untreated 11βOHD and untreated CYP17 deficiency demonstrate that mineralocorticoid synthesis can be upregulated significantly by ACTH. ACTH is elevated in these conditions as a result of the lack of sufficient cortisol biosynthesis (10, 11, 13). Accordingly, DOC, corticosterone and, to a lesser extent, aldosterone increase in a standard synacthen test (30). The lesser response of aldosterone to ACTH stimulation compared to DOC and corticosterone is most probably due to the exclusive expression of CYP11B2 in the adrenal zona glomerulosa. This enzyme is mainly regulated by the renin–angiotensin–aldosterone system, while DOC and corticosterone are also secreted within the zona fasciculata, being mainly controlled by ACTH (24). In a recent review on adrenal insufficiency, Husebye and co-workers reported some central regulation of aldosterone via ACTH (25). In essence, mineralocorticoid synthesis seems in part to be controlled by ACTH, and some activity of the CRH–ACTH axis appears to be necessary for full adrenal potential for mineralocorticoid biosynthesis, which is blocked by high-dose prednisolone and dexamethasone therapy. In our dataset, aldosterone did not decrease significantly during treatment as compared to day 0. On the one hand, this may be influenced by the fact that defined orthostasis conditions are needed for meaningful determination of aldosterone, which we cannot prove for all the patients. This may have induced a bias. On the other hand, this observation indicates that the different mineralocorticoids DOC, corticosterone and aldosterone depend differently on a functioning CRH–ACTH axis, probably due to their partly different origins in the zona glomerulosa and/or the zona fasciculata. Interestingly, the aldosterone concentrations in the ALL children who were admitted to ICU with sepsis were among the lowest in all the study individuals (Fig. 2) and they also showed significantly suppressed DOC levels (Fig. 2). This suggests that downregulation of mineralocorticoids in addition to the glucocorticoids, as observed in our study, may not only be a biochemical phenomenon. However, specific clinical conclusions are currently difficult, because the most potent mineralocorticoid aldosterone was not significantly downregulated in our pilot dataset and we did not determine important biochemical end points like plasma renin, sodium and potassium. In addition, we could only use minimal leftover samples of 100–150 µL for each steroid profile due to the pilot character of our study.

An important weakness of this study is the limited number of ALL patients and the fact that we were not able to receive plasma samples from all patients at all time points. This may have resulted in an overestimation or underestimation of the biological effects of the suppressive glucocorticoid treatment on specific steroids at particular time points. Another weakness is the fact that one patient received dexamethasone instead of prednisolone as in all other patients. It cannot be excluded that prednisolone in contrast to dexamethasone may have had minimal separate effects on aldosterone biosynthesis via its very low intrinsic mineral corticoid activity. However, since our study was primarily planned as a pilot study to gain first insights into the endocrine effects of high-dose glucocorticoid treatment on the global steroid profile, we decided to keep this patient in the study cohort. As stated earlier, the lack of renin – and ACTH determinations and also the lack of clinical data on circulation in the moment of blood sampling impair functional understanding of the glucocorticoid – induced changes of the biochemical steroid profile. Considering the sometimes very long time periods for recovery of the adrenals (4, 5, 6, 7), the limited long-term observation period in our study with only one time point at the end of the glucocorticoid

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tapering phase is another potential weakness. We are therefore not able to provide insight into the potentially different long-term recovery patterns of glucocorticoids and mineralocorticoids. One might also discuss whether ACTH stimulation should have been included in our study after the tapering phase. However, evaluation of classical glucocorticoid sufficiency and insufficiency using low-dose and regular-dose ACTH-tests has been reported in many excellent studies in the past (4, 5, 6, 7). In fact, this was not the aim of our pilot study, but it might be interesting to prospectively investigate whether ACTH-stimulated LC–MS/MS steroid profiles can provide a more comprehensive functional understanding of the differential effects of glucocorticoid treatment on adrenal glucocorticoids, mineralocorticoids and adrenal recovery.

An important strength of our study is the use of state-of-the-art tandem mass spectrometry multi-steroid analysis, enabling us to visualize the differential effects of high-dose glucocorticoid treatment on the adrenal steroid spectrum in children with ALL for the first time. Another strength is that our LC–MS/MS method is accredited (ilac-MRA, DAkkS, D-ML-13069-16-00 and D-ML-13314-04-00) and based on comprehensive published pediatric reference ranges (19). Data were MOM normalized (21) to enable comparison of children, independent of age and sex. All children with ALL were treated according to the same AIEOP-BFM ALL 2009 protocol, ensuring a standardized glucocorticoid regimen in the study group (18).

In summary, this is the first study to show by LC–MS/MS steroid profiling that high-dose glucocorticoid treatment of children with ALL leads to differential suppression of both glucocorticoids and mineralocorticoids. Looking at the mineralocorticoids, this is statistically significant for corticosterone, less obvious for 11-deoxycortisosterone and currently not clear for aldosterone. Since the loss of water and salt is an important feature of adrenal crisis, our data should stimulate prospective follow-up investigations of steroid profiling in high-dose glucocorticoid treatment regimens in children involving higher numbers of patients and a combination with a thorough assessment of the clinical and hormonal readouts of mineralocorticoid action.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EC-23-0002.

Declaration of interest

The authors have nothing to disclose.

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