Towards beating dexamethasone-induced side effects in children with acute lymphoblastic leukemia

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Op Weg naar het Overwinnen van Dexamethason-Geïnduceerde Bijwerkingen bij Kinderen met Acute Lymfatische Leukemie
(met een samenvatting in het Nederlands)

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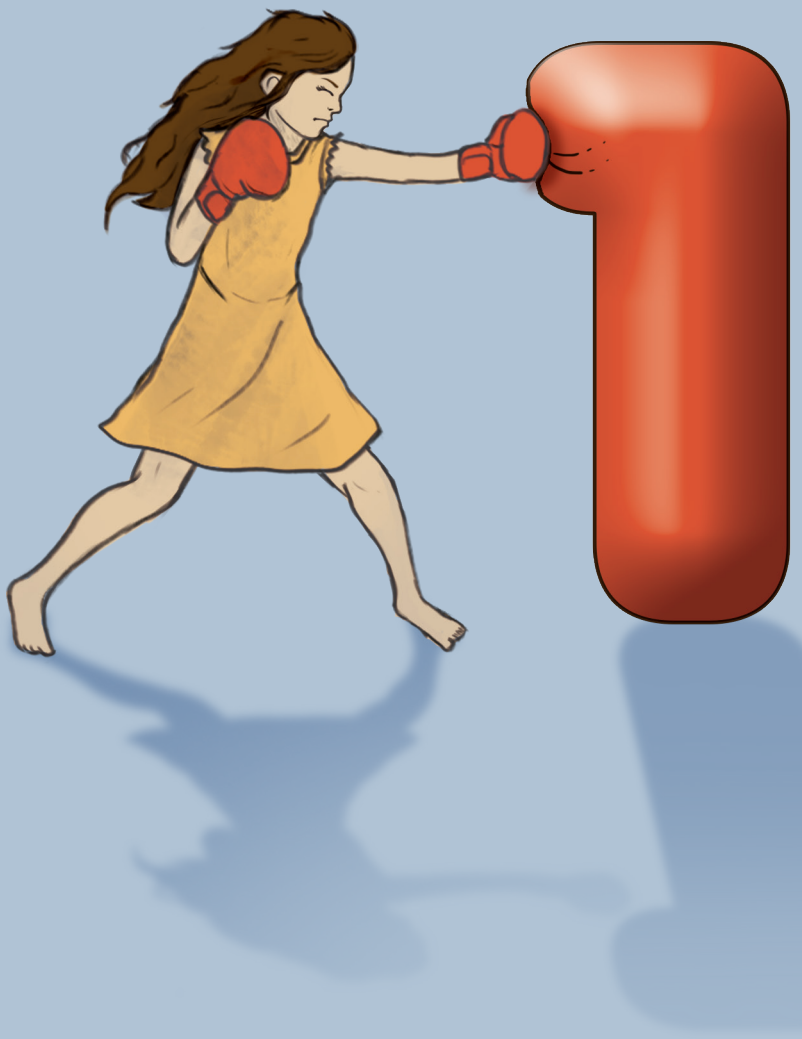
"It's the repetition of affirmations that leads to belief.
And once that belief becomes a deep conviction,
things begin to happen."

- Muhammad Ali

CONTENT



General introduction

ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN

Pediatric acute lymphoblastic leukemia (ALL) is the most common childhood cancer type, with approximately 120 new patients each year in The Netherlands.[1] This hematologic malignancy originates from the bone marrow, where under normal circumstances hematopoietic stem cells produce all lineages of blood and immune cells (Figure 1). In ALL, normal hematopoiesis is interrupted by maturation arrest of one of the lymphatic cell lines, followed by uncontrolled growth of malignant immature monoclonal lymphoid cells. This expansion of leukemic cells leads to a decreased production of erythrocytes, platelets and functional leukocytes.[2] Both precursor B-cell and T-cell leukemia can occur in children, with precursor B-cell ALL being the most common variant (85%). The peak incidence of ALL in children is between the age of two and five years and boys are slightly more often affected than girls.[1]

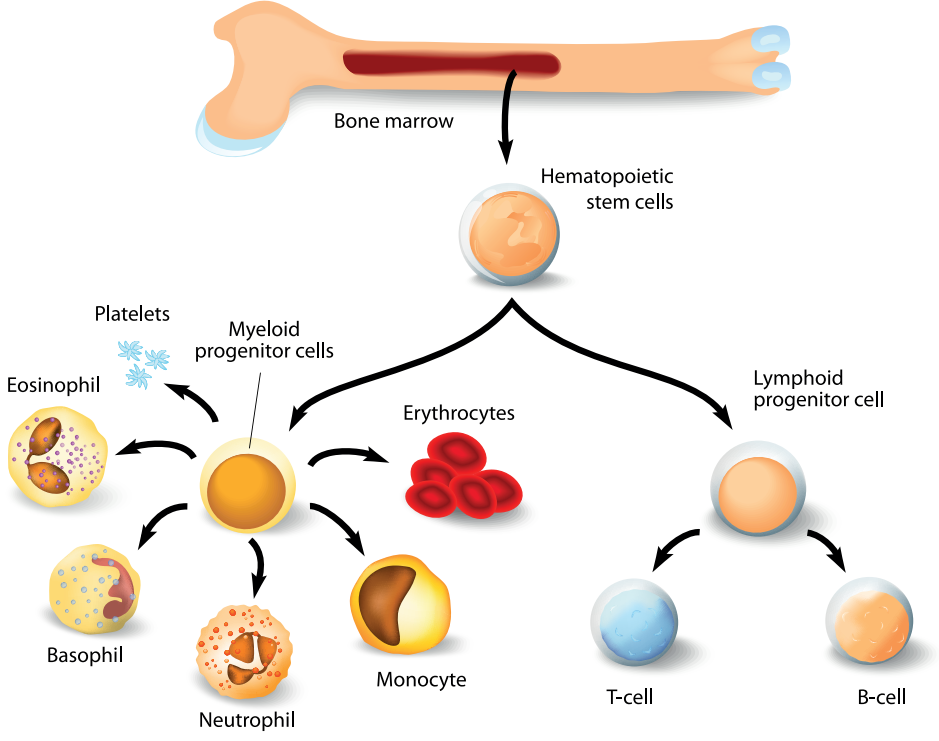


Figure 1. Normal hematopoiesis

TREATMENT AND SURVIVAL

Survival of ALL has increased tremendously over the past decades. In high-income countries, the five-year event free survival rose from around 35% in the 1970's to more than 90% in current treatment protocols (Figure 2).[3,4] This improvement was due to optimization of chemotherapy regimens as well as improved response based risk stratification and supportive care. From 2011 to 2020, children with ALL were treated according to the Dutch Childhood Oncology Group (DCOG) ALL-11 protocol, and the studies described in this thesis were all conducted under this protocol.

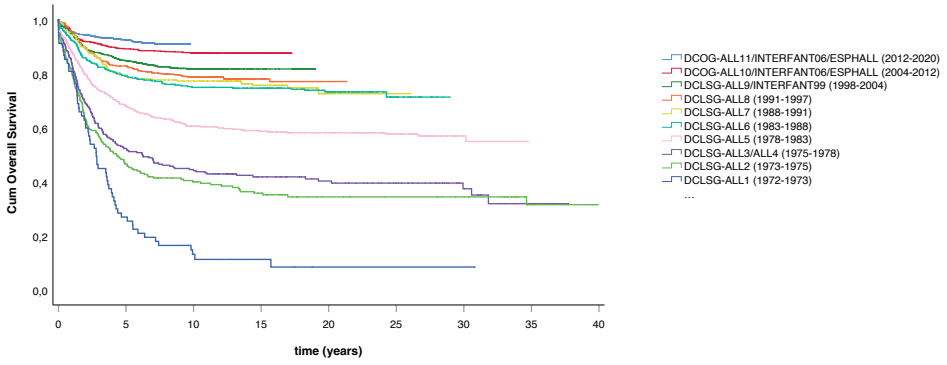


Figure 2. DCOG Registration: Outcome ALL 1997-2020 by protocol period

Courtesy dr. H. de Groot-Kruseman

Treatment in ALL-11 consisted of four phases: induction, consolidation, intensification and maintenance. During induction treatment, patients received high doses chemotherapy and prednisolone. The intensity of treatment after induction therapy was based on therapy response and specific chromosomal abnormalities. In the ALL-11 protocol, patients were stratified to standard, medium or high risk treatment. Medium risk (MR) maintenance treatment plays a key role in this thesis. MR maintenance treatment contained 28 three weekly treatment cycles. Patients received doxorubicin on the first day of the first four treatment cycles, vincristine once every three weeks, methotrexate once per week and 6-mercaptopurine once per day, as well as dexamethasone for five consecutive days at the beginning of each treatment cycle (Figure 3). Depending on randomization, patients also received asparaginase once every three weeks until week 15 or 27 of maintenance treatment. Apart from curative treatment and associated supportive care, standard care includes systematic psychosocial support for both the child and family, support from social work and physical activity recommendations.

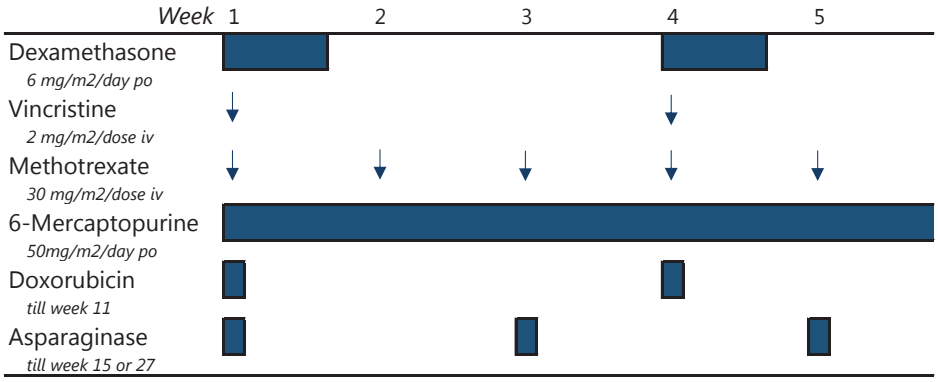


Figure 3. ALL-11 medium risk maintenance treatment schedule

CORTICOSTEROIDS

Glucocorticoids, such as dexamethasone and prednisone, are important components in the treatment of ALL. Glucocorticoids regulate numerous biological processes such as metabolism, immunity, inflammation and the stress response.[5] Mineralocorticoids, such as aldosterone, are another corticosteroid type, which regulate electrolyte and fluid balance.[5] The naturally occurring glucocorticoid in humans is cortisol, which is produced by the adrenal cortex and which exerts a negative feedback through the hypothalamic-pituitary-adrenal (HPA) axis upon endogenous production (Figure 4).[5] Hydrocortisone is the medical equivalent of cortisol and is among others used as substitution therapy for patients who lack endogenous cortisol due to adrenal insufficiency.[5] Prednisone and dexamethasone are synthetic glucocorticoids, a drug class that was used as the first treatment of (childhood) leukemia in the late 1940s due to the cytotoxic effect on leukemic cells.[6] Prednisone, which first requires hepatic conversion to its biologically active form (prednisolone), was the preferred steroid in the treatment of ALL during many different treatment protocols. However, since the introduction of dexamethasone led to a decrease in central nervous system (CNS) relapses, and a higher event-free survival in most ALL patients, dexamethasone has been increasingly used in current treatment protocols.[7-11]

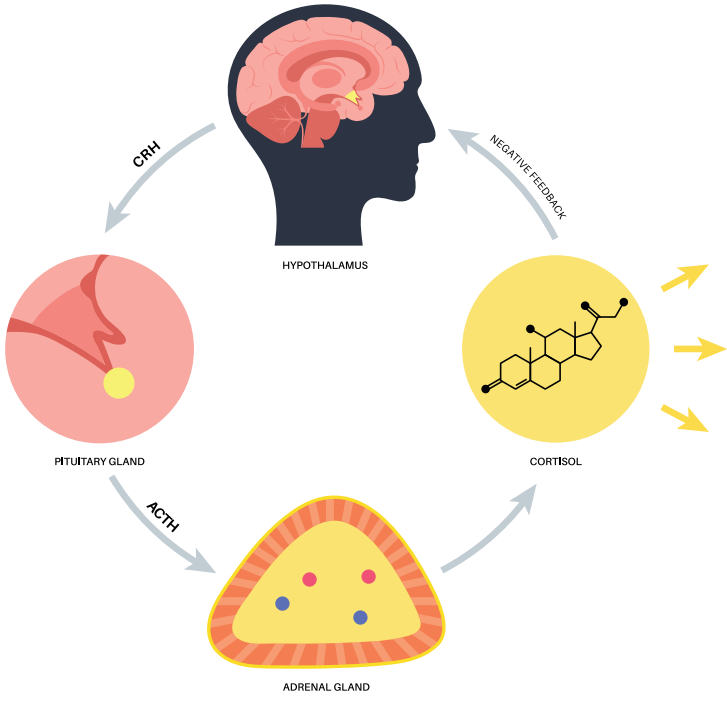


Figure 4. Hypothalamic-pituitary-adrenal (HPA) axis

CORTICOSTEROID RECEPTORS AND GLUCOCORTICOID AFFINITY

Glucocorticoids can bind and activate two receptor types: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Both are members of the steroid receptor superfamily and are encoded by the *NR3C1* and *NR3C2* gene respectively. The GR and MR are structurally and functionally related. They are localized in the cytosol and upon ligand binding translocate into the nucleus, where they exert their actions through transcriptional activation or repression.[12,13] The MR is expressed in numerous tissues: in epithelial tissues such as the kidney it is aldosterone selective and its main function is sodium and water retention, alongside potassium secretion.[14] In other tissues such as heart, muscle, liver and brain, the MR is also present but its function is more complex.[12] The GR is expressed in nearly all tissue types and is above all essential in maintaining physiological balance.[13] Depending on the expressing tissue, the GR and MR show different affinities for cortisol which can lead to distinct effects.[15,16] This effect is influenced by two key enzymes: 11beta-hydroxysteroid dehydrogenase (11β−HSD) 1 and 11β−HSD 2. 11β−HSD 2 metabolizes cortisol to inactive cortisone, therefore favoring binding of aldosterone to the MR. Conversely, 11β−HSD 1 converts cortisone to the active cortisol. The presence or absence of both enzymes in peripheral tissue mostly determines the effect of corticosteroids on both receptors.

Glucocorticoids exert their cytotoxic effect on leukemic cells predominantly through activation of the GR. After binding of glucocorticoids to the GR, the complex is translocated to the nucleus where it can induce cell-cycle arrest and apoptosis through multiple pathways.[17-20] Different synthetic glucocorticoids have different affinities for the GR and MR. Conventionally, dexamethasone is reported to be the most potent glucocorticoid with a sevenfold higher glucocorticoid activity than prednisolone, and no mineralocorticoid activity.[5] Prednisolone exerts an effect through both receptors, although with higher affinity for the GR.[5] However, these reports are based on anti-inflammatory and Na^+-retaining potency. In pediatric ALL samples, previous studies showed that the in vitro anti-leukemic (cytotoxic) activity of dexamethasone is seventeen fold higher than prednisolone.[21,22] However, different studies, using various models and evaluating diverse effects of glucocorticoids, show wide ranges of glucocorticoid and mineralocorticoid activity when comparing either dexamethasone, prednisolone or hydrocortisone.[22-25] The anti-leukemic activity of these glucocorticoids and the role of the GR and MR in this cytotoxic effect therefore remains unclear.

GLUCOCORTICOID-INDUCED SIDE EFFECTS

Besides the anti-leukemic effect of dexamethasone and prednisolone, both glucocorticoids can also induce various undesirable side effects. These side effects involve almost every organ system and range from acute side effects, to side effects that become apparent later in life. Overall, dexamethasone is associated with more (severe) side effects than prednisolone.[26] Both glucocorticoid-induced adverse psychological reactions and somatic side effects may occur during ALL treatment.

Adverse psychological reactions

Adverse psychological and neurocognitive reactions due to glucocorticoids may include neurobehavioral problems (e.g. increased distress, compulsive behavior or altered emotions), psychiatric deterioration (e.g. psychosis, depression), cognitive decline, changes in sleep, increased fatigue or preoccupation with food.[27-30] All these side effects may potentially impact quality of life during treatment of childhood ALL for both the patient and family, for a substantial period of time, since ALL treatment lasts 2-3 years.[31,32] Reports on estimated frequencies of glucocorticoid-induced neurobehavioral problems in children range from 5% to 75%,[28,33-36] whereas sleep problems are reported in 19% to 87%.[35,37]

In this thesis, the emphasis lies on dexamethasone-induced neurobehavioral and sleep problems, as well as the feeling of hunger children experience during dexamethasone treatment.

Patients at risk

The inter-individual variation in the severity of glucocorticoid-induced side effects is high. For better understanding of this inter-individual variation, more insight in contributing factors that may influence neurobehavioral and sleep problems during dexamethasone treatment would be of value. The risk factors for developing neurobehavioral side effects during dexamethasone treatment are multi-dimensional and therefore gathering insight in the full scope of possible determinants is important.

Patient and treatment characteristics

In adults (both with and without cancer diagnosis), a higher steroid dose as well as psychiatric history increased the risk of neurobehavioral side effects.[38,39]. In children, dexamethasone, as compared to prednisolone, as well as a younger age appear to increase the risk of steroid-induced neurobehavioral problems.[40,41] Previously established risk factors for sleep problems in healthy children are female sex, a difficult temperament and unhealthy sleep behavior.[42] In childhood cancer survivors, female sex, co-morbidities and lower educational level were associated with insomnia.[43]

Psychosocial and environmental factors

It has been previously shown that the child's distress during procedures in childhood cancer treatment is associated with parental distress and parental stress on its own is associated with behavioral problems in children.[44,45] Moreover, parents of a child with cancer appear to have higher stress levels than parents of children with physical disabilities.[46] Overall, the degree of parental stress may be a factor in the occurrence of dexamethasone induced behavioral or sleep problems.[47] Besides parenting stress, other family or environmental risk factors such as familial predisposition,[48] parenting strategies,[49-51] or psychosocial support may affect parents' perceptions of the side effects which occur during dexamethasone.

Genetic and pharmacokinetic factors

Genetic variation may contribute to the differences in dexamethasone-induced side effects as well, therefore studying single nucleotide polymorphisms (SNPs) which may contribute to differences in neurobehavioral and sleep problems would be of value. Two previous studies suggest an association between the *BcI-1* polymorphism *(NR3C1* gene) and depressive symptoms.[52,53] The *rs4918* polymorphism (*Alpha2-HS glycoprotein (AHSG)* gene) was suggested to be associated with impaired sleep during dexamethasone treatment in pediatric ALL patients.[54] However, replication of these results is still pending. Genetic variants that have been shown to be associated with psychopathology or sleep problems, may give further insight in the pathophysiology and risk of these side effects caused by dexamethasone.

In addition, dexamethasone pharmacokinetics may play a role in the occurrence of neurobehavioral side effects. Dexamethasone clearance is higher in younger children, so there may be an inter-patient variability in dexamethasone levels during maintenance phase, which may explain the differences in side effects.[55]

In summary, many different factors may contribute to the inter-patient variability of both dexamethasone-induced neurobehavioral and sleep problems. Some of these factors have been described before, however, findings are often conflicting or focus on only one possible determinant or outcome. It would be of interest to review the complete literature regarding risk factors for dexamethasone-induced neurobehavioral and sleep problems and to prospectively study these possible determinants.

Somatic side effects

Somatic side effects of glucocorticoids include increased risk of infections, osteonecrosis, osteopenia and consequent fractures, thromboembolisms, metabolic changes such as hyperglycemia, hyperlipidemia, weight gain, hypertension and myopathy.[11,26,56-58] Previous research in children with ALL showed that merely four or five days of dexamethasone

treatment induced metabolic toxicity on three components of the metabolic syndrome as well as significant insulin resistance in 45-85% of all cases.[58,59] This implies that the high dose glucocorticoid pulses which are frequently administered in ALL treatment trigger significant metabolic changes. In survivors of childhood ALL, obesity is a well-known late side effect, and glucocorticoid use is an independent risk factor for obesity in survivors.[60] Leptin is an adipokine which is mainly produced by adipose tissue, and is among others involved in regulating food intake, which is also disturbed during glucocorticoid treatment.[61] The effect of five days of dexamethasone on leptin, fat mass and feeling of hunger has not been studied before. By exploring these acute side effects of dexamethasone, new insights in the pathophysiological mechanisms of important late side effect may arise.

PATHOPHYSIOLOGY OF NEUROBEHAVIORAL SIDE EFFECTS

The proposed pathophysiology behind neurobehavioral side effects of glucocorticoids commences in the brain, where both the GR and MR are present. Both receptors are expressed in different areas of the brain: the MR is mostly present in limbic areas whereas the GR is present in nearly every brain region.[62,63] Because dexamethasone, through negative feedback on the HPA axis, suppresses the endogenous production of cortisol which has a high affinity for the MR, an imbalance between activation of the GR and MR occurs during high dose dexamethasone treatment.[62,64] Dexamethasone-induced neurobehavioral problems may be due to overactivation of the GR, underactivation of the MR or an imbalance between activation of both receptors.[65] Still, in animals as well as humans, it has been shown that the MR plays an important role in behavior and cognition. For instance, in MR knockout mice, an increased anxiety behavior has been observed due to the absence of functional MR.[66] Conversely, overexpression of MR in the brain of mice resulted in decreased anxiety.[67,68] In healthy humans, treatment with the MR antagonist spironolactone has been associated with impaired attention, memory and sleep.[69,70] Furthermore, in patients with psychiatric disorders such as depression, schizophrenia or bipolar disorder, a decreased expression of MR in parts of the brain has been established.[71,72] In contrast, treatment with MR agonist fludrocortisone showed a beneficial effect as add-on to standard depression treatment.[73] The MR therefore may play an important role in the development of dexamethasone-induced neurobehavioral problems.

TREATMENT OF DEXAMETHASONE-INDUCED NEUROBEHAVIORAL PROBLEMS

Based on the previously mentioned studies in mice and human, it has been hypothesized that addition of a physiological dose of hydrocortisone during dexamethasone treatment would diminish the neurobehavioral side effects of dexamethasone through refilling of the brain MR.[65] This was explored in a randomized clinical trial in 50 pediatric ALL patients: the DexaDays-1 study.[35] The safety of addition of a physiological dose of hydrocortisone to dexamethasone treatment was first ensured in a preclinical study, which showed that hydrocortisone did not interfere with the anti-leukemic efficacy of dexamethasone.[74] In the total group of pediatric ALL patients, no beneficial effect of hydrocortisone on neurobehavioral or sleep problems was observed (Figure 5). However, in a subgroup of patients with clinically relevant dexamethasone-induced neurobehavioral problems (38%) or clinically relevant sleep problems (19%), hydrocortisone addition showed a significant decrease of the side effects (Figure 5). These results implicated that behavioral and sleep problems may be reduced in children who are most affected. However, despite the significance, since these results were based on a relatively small subgroup, validation in a larger targeted cohort was desired.

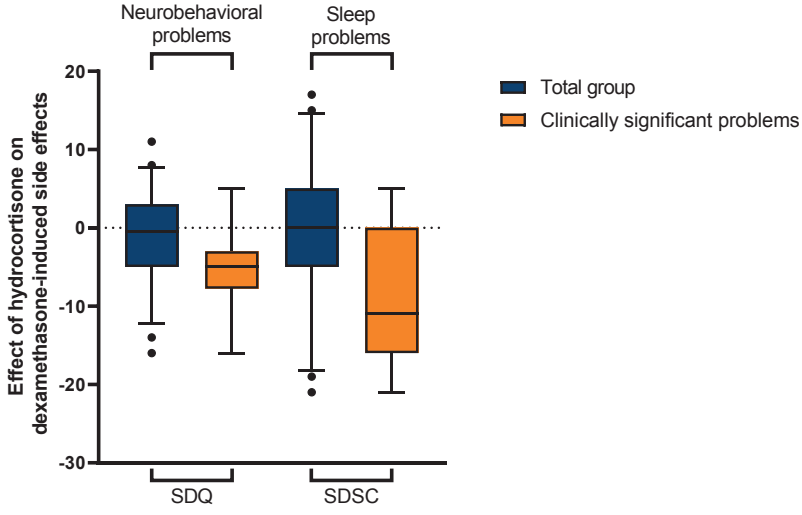


Figure 5. Results of the DexaDays-1 study. The effect of hydrocortisone addition in the total group (blue) and in patients with clinically relevant dexamethasone-induced behavioral (left) or sleep (right) problems (orange). Behavioral problems were measured with the Strengths and Difficulties Questionnaire (SDQ) and sleep problems were measured with the Sleep Disturbance Scale for Children (SDSC).

Adapted from Warris et al. Journal of Clinical Oncology 2016.[35]

SCOPE AND OUTLINE OF THIS THESIS

In this thesis, we aim to increase existing knowledge on the prevalence and determinants of dexamethasone-induced side effects in children with ALL. Moreover, we aim to validate the finding that hydrocortisone addition to dexamethasone treatment leads to a significant reduction of clinically relevant dexamethasone-induced neurobehavioral and sleep problems. Furthermore, we aim to describe the role of the mineralocorticoid receptor in steroid-induced cytotoxicity.

Chapter 2 provides a systematic review of the literature regarding the risk factors for glucocorticoid-induced neurobehavioral and sleep problems. In **Chapter 3**, we describe the design of the DexaDays-2 study, which consists of two parts. First, we measured which patients experience clinically relevant dexamethasone-induced neurobehavioral problems. The risk factors for developing these problems are described in **Chapter 4.** Related somatic effects were studied in this cohort by measuring the influence of a five-day dexamethasone course on leptin levels, fat mass and feeling of hunger (**Chapter 5**). The core part of the DexaDays-2 study comprised a randomized placebo-controlled trial, which evaluated the beneficial effect of hydrocortisone addition to dexamethasone treatment on neurobehavioral and sleep problems, as well as quality of life. The results of this study are described in **Chapter 6.** Finally, in addition to studying glucocorticoid related side effects in children with ALL, in **Chapter 7** we describe our study that was designed to get insight into biological mechanisms of the *in vitro* effect of various steroids on glucocorticoid-induced cytotoxicity through glucocorticoid and mineralocorticoid receptor activation.

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Risk factors for steroid-induced adverse psychological reactions and sleep problems in pediatric acute lymphoblastic leukemia:

A systematic review

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ABSTRACT

Objective

Steroids play an essential role in treating pediatric acute lymphoblastic leukemia (ALL). The downside is that these drugs can cause severe side effects, such as adverse psychological reactions (APRs) and sleep problems, which can compromise health-related quality of life. This study aimed to systematically review literature to identify risk factors for steroid-induced APRs and sleep problems in children with ALL.

Methods

A systematic search was performed in six databases. Titles/abstracts were independently screened by two researchers. Data from each included study was extracted based on predefined items. Risk of bias and level of evidence were assessed, using the Quality In Prognosis Studies tool and the Grading of Recommendations Assessment, Development and Evaluation tool, respectively.

Results

Twenty-four articles were included. APR measurement ranged from validated questionnaires to retrospective record retrieval, sleep measurement included questionnaires or actigraphy. Overall, quality of evidence was very low. Current evidence suggests that type/dose of steroid is not related to APRs, but might be to sleep problems. Younger patients seem at risk for behavior problems and older patients for sleep problems. No studies describing parental stress or medical history were identified. Genetic susceptibility associations remain to be replicated.

Conclusions

Based on the current evidence, conclusions about risk factors for steroid-induced adverse psychological reactions or sleep problems in children with ALL should be drawn cautiously, since quality of evidence is low and methods of measurement are largely heterogeneous. A standardized registration of steroid-induced APRs/sleep problems and risk factors is warranted for further studies in children with ALL.

INTRODUCTION

Glucocorticoids, such as prednisone and dexamethasone, were among the first drug classes successfully used in the treatment of childhood acute lymphoblastic leukemia (ALL) and are still regarded as cornerstones of ALL therapy.[1] These drugs have contributed substantially to the current 5-year overall survival of more than 90% in developed countries.[2] However, glucocorticoids can also cause severe side effects, such as osteonecrosis, hyperlipidemia, hyperglycemia, altered body composition, and thromboembolisms.[3] Besides these physical toxicities, steroid treatment can cause severe adverse psychological reactions (APRs). These include mood swings, behavioral changes, but also anxiety, psychosis and depression.[4,5] Steroid related APRs in ALL are experienced as the most detrimental contributor to impaired health-related quality of life (HRQoL) by both patients and parents.[6] Reports on estimated frequencies of steroid-induced APRs in children range from 5% to 75%.[5,7-10]

Closely related to APRs and also common in children with ALL, are sleep problems, with an estimated prevalence of 19% to 87%.[9,11] Steroid-induced APRs and sleep problems are often studied and reported as separate phenomena in pediatric ALL literature.[9,12,13] However, sleep problems interrelate with APRs by being both a symptom of certain APRs, such as depression or psychosis, as well as a risk factor to develop APRs.[14] Additionally, during ALL steroid-treatment sleep problems significantly impact the quality of life of children.[15]

An important step to handle both APRs and sleep problems is to identify potential risk factors, making early recognition of susceptible patients possible. This may allow implementation of early intervention strategies to potentially prevent or overcome APRs and sleep problems and their related HRQoL impairments. This was recently acknowledged by the International Psycho-Oncology Society Pediatrics Special Interest Group, which published a call for awareness of sleep problems in pediatric oncology. One of their recommendations was to identify risk factors.[16] In adults (both with and without cancer diagnosis), a higher steroid dose as well as past psychiatric history increases the risk of APRs.[17,18] In children, only the use of dexamethasone (in comparison to prednisone) appears to influence the occurrence of steroid-induced APRs.[19] Known risk factors for sleep problems in the general population are female sex, familial (genetic) predisposition, history of sleep problems, personality type or having a parent with depression.[20-23] Although some possible risk factors for APRs and sleep problems have been described, findings in pediatric oncology are often conflicting or not specific for steroid-induced problems.[5,24,25]

Therefore, this systematic review aimed to summarize all available literature to identify potential risk factors for steroid treatment-induced APRs and sleep problems in children with ALL. APRs and sleep problems are closely linked and may influence each other, however since both phenomena are often described separately, we reviewed them individually as well.

To address our aim, we formulated several research questions (with reference to patient population, interventions, comparisons, and outcomes [PICO]). Our patient population encompassed children (0 till 18 years old) with ALL receiving steroid treatment. The outcome parameters were either APRs or sleep problems (or both). Based on previous literature, we hypothesized that the following risk factors might contribute to APRs and/or sleep problems (interventions and comparisons): sociodemographic factors (age and sex),[5,24] treatment factors (type and dose of steroid),[5,10,19,24,26] parental factors (coping strategies, stress),[27-29] (medical) history,[20,30] and genetic predisposition.[24,31] However, we did not limit our search on these risk factors. See Supplemental Table 1 for an overview of the PICOs.

METHODS

The protocol of this study was based on the PRISMA statement.[32] The study was registered in PROSPERO international prospective register of systematic reviews during the data extraction phase (registration number CRD42020167173).

Search strategy and information sources

A comprehensive search was performed using the bibliographic databases PubMed, Embase.com, Scopus, the Cochrane Library, Cinahl (via Ebsco) and PsycINFO (via Ebsco) from inception to 15 August 2019 in collaboration with a medical librarian (Linda J. Schoonmade, Annelienke M. van Hulst and Shosha H.M. Peersmann). Search terms included controlled terms (MeSH in PubMed, Emtree in Embase, Thesaurus terms in Cinahl and PsycINFO) as well as free text terms. The following search terms were used (including synonyms and closely related words) as index terms or free-text words: "ALL" and "children" and 'steroids" and "adverse effects" or "APR" or "sleep problems." The search was performed without date or language restrictions. Duplicate articles were excluded. The full search strategy for all databases can be found in Appendix 1. In addition, reference lists of all included studies and relevant reviews were manually searched (cross-reference check) for potential additional studies by two authors (Annelienke M. van Hulst and Shosha H.M. Peersmann).

Eligibility criteria and study selection

All studies were independently screened by two researchers (Annelienke M. van Hulst and Shosha H.M. Peersmann). First, studies were screened on title and abstract using reference program Rayyan.[33] Studies that met the following predefined inclusion criteria were included: (*a) study population of children aged 0–18 years old, (b) diagnosed with ALL, (c) receiving steroids (e.g., dexamethasone, prednisone) as part of their leukemia treatment, (d) including an APR or sleep outcome*. All types of outcome measurements (questionnaires, observational, chart review, and actigraphy) were deemed eligible.

Studies were excluded if they only entailed adults or animals, were nonpeer reviewed (congress abstract/poster), only reported neurocognitive measures or nonacute behavioral or sleep outcomes (late effects). Second, full-texts were screened and included if any of the risk factors of behavior and sleep mentioned above were evaluated. As stated before, risk factors that were not predefined could also be included. Studies were excluded if no original data was reported (reviews), it entailed a duplicate, a case report (series) or if full-text was unavailable. Case reports and relevant reviews were set aside to check references. In addition, articles that reported on outcomes of ALL trials were kept apart, as these articles were not designed to meet

aforementioned inclusion criteria, but were regarded as potentially discussing APRs or sleep problems as part of toxicity registration during trials. Therefore, the full texts of these articles were reviewed as well.

Data extraction

Data from each study were extracted independently by two authors (Annelienke M. van Hulst and Shosha H.M. Peersmann) based on predefined items: study design, number of participants, mean age, type and dose of steroids, type of APR/sleep outcome, method of measuring APR/sleep outcome, risk factors, method of measuring risk factors and results (often descriptive/percentages). Disagreements were resolved by consensus (Annelienke M. van Hulst and Shosha H.M. Peersmann). If necessary, a third reviewer was consulted (Raphaële R. L. van Litsenburg).

Assessment of risk of bias of individual studies

To assess risk of bias, the Quality In Prognosis Studies (QUIPS) tool was used. The QUIPS systematically appraises risk of bias in individual studies of prognostic (risk) factors.[34] The Cochrane Prognosis Methods Group recommends the use of this instrument.[35] The QUIPS ascertains high, moderate or low risk of bias on six domains: (*1) study participation, (2) study attrition, (3) prognostic factor measurement, (4) outcome measurement, (5) study confounding, and (6) statistical analysis and reporting*. Each study was independently rated using the QUIPS tool by Annelienke M. van Hulst and Shosha H.M. Peersmann after which the scores were discussed to resolve any disagreements. A third reviewer was available when necessary (Raphaële R. L. van Litsenburg). In line with the recommendations of Hayden and colleagues (2013), we assessed each domain and did not rate a summated risk of bias score for individual studies based on the six domains.[34] See Supplemental Table 2 for definitions and application of the QUIPS domains.

To summarize the quality of individual study results, we took into account: the number of QUIPS domains scoring high on risk of bias, the sample size of APRs/sleep outcomes and whether a study was a priori designed to study risk factors of steroid-induced APRs or sleep problems. We considered a study of lower quality when it entailed more high risk of bias domains, was not a priori designed and had a small sample size. A color-coding was used to indicate our considerations: red (lower quality), orange (medium quality), and green (higher quality).

Assessment of grading evidence across studies and synthesis of results

To systematically evaluate the quality of summated evidence for each study question and to identify the level of evidence for each risk factor of either APR or sleep problems, we used the Grading of Recommendations Assessment, Development and Evaluation

(GRADE) tool.[36] This tool is recommended by the Cochrane Prognosis Methods Group.[35] The GRADE includes a synthesis of results (combined number of participants, studies, cohort phase study and either a positive, negative or no effect) and scores each factor of the GRADE framework: (*a) study limitations, (b) inconsistency, (c) indirectness, (d) imprecision, (e) publication bias, (f) effect sizes, and (g) dose effect*. See Supplemental Table 3 for definitions and application of the GRADE domains.

All evidence for each PICO (Supplemental Table 1) was independently assessed by Annelienke M. van Hulst and Shosha H.M. Peersmann. Besides the predefined PICOs, we also identified new risk factors from literature. Taking into account the combined GRADE synthesis and ratings, the overall level and quality of evidence was determined: + very low, ++ low, +++ moderate, or ++++ high quality. Individual synthesis and ratings (Annelienke M. van Hulst and Shosha H.M. Peersmann) were discussed until consensus was reached. If necessary, a third reviewer was consulted (Raphaële R. L. van Litsenburg). The results of the grading provide an overview of the results per risk factor and the (gaps of) evidence for each risk factor of developing either APRs or sleep problems.

RESULTS

Our search yielded 8626 unique records after duplicate removal (Supplemental Figure 1: PRISMA Flow diagram). Hundred and ninety full texts were screened of which 23 articles were included. Furthermore, 245 ALL trial papers were screened of which one article was eligible, resulting in a total of 24 articles included in this review.

Nineteen studies reported on risk factor(s) for steroid-induced APRs, whereas seven studies reported on risk factor(s) for steroid-induced sleep problems. Two studies described risk factors for both APRs and sleep problems. See Tables 1 and 2 for all study characteristics, results and quality of each individual study based on risk of bias. Supplemental Table 4 depicts the risk of bias domain scoring within the separate studies. The summated evidence for each identified risk factor of either APRs or sleep problems and the evaluation of evidence using GRADE is shown in Tables 3 and 4 respectively.

Adverse psychological reactions

Different APRs were described in the included articles: neuropsychiatric signs, toxicities, or adverse events, personality or behavioral change, steroid psychosis, child difficulties, psychiatric disorders and (neuro)behavioral problems. The measurement of these APRs ranged from using validated questionnaires to retrospective collection from patient files. Eleven studies collected any information of APRs without the use of a validated questionnaire.[37-47] The other eight studies used five different parent reported questionnaires: Conners rating scale,[48,49] Child Difficulties questionnaire,[50,51] Child Behavior Checklist,[4,25,49,52] Children's Depression Inventory,[49] and the Strength and Difficulties Questionnaire.[9,53] Assessment of the different risk factors depended on the nature of the risk factor. For example, sociodemographic factors were retrieved from patient records, whereas treatment factors usually were per protocol. APRs were measured during (remission-)induction[4,37-40,43-47] or maintenance phase[9,25,37,41,46,48,49,51-53] (unclear in one study[42]). Overall, the quality of evidence regarding risk factors for APRs was very low (Table 3).

Sociodemographic factors (age and sex)

Nine studies evaluated age as a risk factor for steroid-induced APRs. Three studies found younger age (0–6 years old) to be a risk factor for behavioral problems of which two were of higher quality.[25,41,52] One study of lower quality comparing patients aged 10–15 years with 16–24 years old described an increased frequency of steroid-induced psychosis in the older age group.[42] Five studies of lower quality found no significant impact of age on the development of steroid-induced behavior problems or psychosis.[9,40,46,48,49] Two studies used age as interval variable,[9,49] but most studies used age group categories with variable

ranges to compare differences.[25,40-42,46,48,52] Regarding sex, four out of five studies (of which two high quality) did not find a significant difference between boys and girls.[40,46,49,52] Only one lower quality study found an effect on one of their measured domains: listlessness. Girls seem to be at risk for listlessness; however no effect on all other domains (attention/hyperactivity, emotional liability, and depressed mood) was found.[48] All analyses regarding age and sex were univariate, no multivariate analyses were conducted. Overall, sex seems no risk factor for APRs, but certain age groups might be at risk for specific APRs. The evidence that younger children (0–6 years old) are at risk for behavioral problems is stronger, than the evidence that teenagers are at risk for psychosis. The latter needs to be confirmed in higher quality studies.

Treatment factors (type of steroid, steroid dose, and cumulative dose)

Six out of eight studies did not find more APRs when comparing dexamethasone to prednisone treatment, including four higher quality articles.[4,25,37,38,43,51] Although the majority reports that steroid type is not a risk factor, evidence is not undisputed: two high quality studies did report more APRs during dexamethasone treatment.[46,52]

Steroid dose was investigated in four studies (one of higher quality).[25,47-49] Three report no increased risk of APRs with increasing dose, one low quality study reports an effect on one of their measures domains (listlessness), but not on all other APR domains.[48] Steroid dose seems no risk factor based on current evidence, which is overall of low quality. Only one study evaluated the risk of cumulative steroid dose and did not find an increased risk on APRs with a higher dose of prednisone nor dexamethasone.[25] All studies on the risk of APRs by steroid type and dose were univariate, no multivariate analyses were used.

Parental factors

We did not identify any studies describing steroid-induced APRs and parental factors with our search.

Medical history

With our search, we did not find any studies describing medical history as a risk factor for steroid-induced APRs.

Genetic predisposition

Five articles studied the influence of genetic variation on steroid induced APRs,[4,39,40,44,45] of which Eipel et al. described the same patient cohort twice.[39,40] This was the largest patient cohort, consisting of 346 patients. The other studies included 37, 47, and 36 participants, respectively.[4,44,45]

Table 1. Results per Adverse Psychological Reaction (APR) study

Study	Study design	A priori design for risk factors	N=	n= APR outcome	Age	Steroid	APR outcome
Bostrom et al (2003)[37]	RCT	No	1060	6	1-10 years	Dex (6 mg/m²/d) Pred (40 mg/m²/d)	Neuropsychiatric toxicities
Domenech et al (2014)[38]	RCT	No	1947	53	0-18 years	Dex (6 mg/m²/d) Pred (60 mg/m²/d)	Personality change
Drigan et al (1992)[48]	Prospec-tive	Yes	38	38	SR: 51.4 months (29-94) HR: 49.1 months (25-63)	Pred (40 mg/m²/d or 120 mg/m²/d)	Behavior changes
Eipel et al (2013)[40]	Retro-spective	No	346	29	1-18 years	Dex (10 mg/m²/d) Pred (60 mg/m²/d)	Seriously altered behavior or steroid psychosis
Eipel et al (2016)[39]	Retro-spective	No	346	29	0,2-17,9 years (median 4.95)	Dex (10 mg/m²/d) Pred (60 mg/m²/d)	Seriously altered behavior or steroid psychosis
Eiser et al (2006)[51]	RCT	Yes	45	41	7,2 (3.8 SD) years	Dex Pred	Child difficulties
Felder-Puig et al (2007)[4]	Prospec-tive	Yes	37	20	9,27 (3,96 SD) years	Dex (10 mg/m²/d) Pred (60 mg/m²/d)	Adverse psychological reactions

	Measurement outcome	Risk factor	Measurement risk factor	Results	Risk of bias: QUIPS domains
	Toxicity questions	Type of steroid	Assigned by protocol	Dex: 6 events (dysesthesia and agitation) Pred: 0 events (not tested)	**4/6 high**
	Reported grade III/IV toxicity (WHO criteria)	Type of steroid	Assigned by protocol	Dex: 2.5% Pred: 3.0% (NS)	**2/6 high**
	Conners Parent-Teacher Hyperkinesis Index	- Steroid dose - Age (<4 / >4) - Sex	- Assigned by protocol - Patient record - Patient record	High dose pred: higher listlessness (p<.04). No other disturbances. Age: no difference Sex: girls listlessness (p<.01). No other disturbances.	**4/6 high**
	Collected retrospectively	- SNP: N363S - Age (<2 / 2–11 / 12–18) - Sex	- Allele specific PCR - Patient record - Patient record	N363S: 8.6% vs 6.3% (carriers vs non-carriers) (p=1.0) Age and sex: NS	**4/6 high**
	Collected retrospectively	SNPs: - N363S - BclI - ER22/23EK	- Allele specific PCR - Allele specific PCR - Melting curve analysis	N363S: NS (p=1.0) BclI: NS (p=.405) ER22/23EK: NS (p=.695)	**4/6 high**
	Child Difficulties Questionnaire	Type of steroid	Assigned by protocol	No significant difference	**3/6 high**
	CBCL	- Type of steroid -Hormone levels -Neuronal cell destruction - SNPs: ER22/23EK N363S BclI	- Per protocol - Venous blood samples - 14-3-3 protein level in CSF - TaqMan PCR	Dex: OR 2.2 (CI 0.5-9.1) High cortisol and/or ACTH: OR 5.0 (CI 0.9-28.1) Neuronal cell destruction: no evidence SNPs: no correlation	**3/6 high**

Table 1. Continued

Study	Study design	A priori design for risk factors	N=	n= APR outcome	Age	Steroid	APR outcome
Harris et al (1986)[41]	Prospective	No	16	16	4-16 years	Pred (60 mg/m^2/d)	Behavior
Hough et al (2016)[42]	RCT	No	3126	18	1-25 years (median 5 years)	Dex (6 or 10 mg/m^2/d)	Steroid induced psychosis
Igarashi et al (2005)[43]	RCT	No	359	3	1-10 years	Pred (40-60 mg/m^2/d) Dex (6-8 mg/m^2/d)	Neuropsychiatric adverse event
Kaymak Cihan et al (2017)[44]	Retrospective	No	49	13	1.4-17 years	Pred (40-60 mg/m^2/d) Dex (10 mg/m^2/d)	Depression symptoms (according to CTCAE 4.0)
Marino et al (2009)[45]	Retrospective	No	36	25	5,3 (1,3-16) years	Dex (10 mg/m^2/d) Pred (60 mg/m^2/d)	Neuropsychiatric signs
Messina et al (1989)[49]	Prospective	No	26	23	8 (3-16) years	Pred (60 mg/day)	Mood, activity level and behavior
Mitchell et al (2005)[46]	RCT	No	1603	58	1-18 years	Pred (40 mg/m^2/d) Dex (6,5 mg/m^2/d)	Acute behavioral toxicity (grade III/IV)
Mrakotsky et al (2011)[25]	Prospective repeated measures	Yes	60	60	2-17 years	Pred (40 mg/m^2/d) Dex (6 mg/m^2/d)	Neurobehavioral problems

	Measurement outcome	Risk factor	Measurement risk factor	Results	Risk of bias: QUIPS domains
	Corticosteroid symptom inventory	Age (4-5 / 7-10 / 12-16)	Patient record	Trend of more behavioral symptoms in younger children (<7 years)	**5/6 high**
	Prospectively collected SAE	Age (<16 / ≥16)	Patient record	<16 years: 0,4%, ≥16 years 2,2% (p<.05)	**4/6 high**
	Observation (collected retrospectively)	Type of steroid	Assigned by protocol	Dex: 3 neuropsychiatric adverse events Pred: 0 events (p=.24)	**4/6 high**
	Collected retrospectively	SNPs: - N363S - BclI	PCR-RFLP	N363S: no SNP present BclI: depression symptoms more frequent among carriers (40.7% vs 11.8%, p=.040)	**4/6 high**
	Collected retrospectively	Polymorphisms in - *ABCB1* - *NR3C1* - *GST* - *IL-10* genes	- PCR-RFLP - PCR-RFLP - PCR-ASO - PCR-RFLP	No correlation between neuropsychiatric toxicity and genotype	**4/6 high**
	- CBCL - CDI - Conners Parent Questionnaire	- Platelet MAO activity - Age - Sex	-Radiochemical assay - Prior pred exposure - Patient record - Patient record	MAO activity: correlated with steroid induced changes in CBCL and Connors, not CDI. Prior pred exposure: NS Age: NS Sex: NS	**4/6 high**
	Reported by clinician	- Type of steroid - Age (<2 / 2–9 / ≥10) - Sex	- Assigned by protocol - Patient record - Patient record	Dex: 6% vs pred 1% (p<0.0001) Age: NS Sex: depression in girls, aggression in boys (both trend)	**2/6 high**
	CBCL	- Type of steroid - Age (2-<6 / ≥6-17) - Sex -Cumulative steroid dose	- Assigned by protocol - Patient record - Patient record - Patient record - Per protocol	Dex vs pred: NS Age: 2-<6 years more problems than ≥6-17 years. Sex: NS Cumulative steroid dose: NS	**1/6 high**

Table 1. Continued

Study	Study design	A priori design for risk factors	N=	n= APR outcome	Age	Steroid	APR outcome
Pound et al (2012)[52]	Prospec-tive	Yes	43	43	7 (SD 4.1) years	Pred Dex	Behavioral problems
Warris et al (2016)[9]	RCT	No	46	46	3-16 years	Dex (6 mg/ m^2/d)	Behavior
Warris et al (2016)[53]	RCT	Yes	46	46	6,0 (4.0-9.8) years	Dex (6 mg/m2/ day)	Behavior
Yetgin et al (2003)[47]	RCT	No	205	3	5,5 years (11 months-16 years)	Pred (60mg/m2/ day) HDMP (600-900mg/m2/ day)	Behavioral disturbance

Note: Age reported as mean or range.
Color-coding: **red** (lower quality), **orange** (medium quality), **green** (higher quality).
Abbreviations: ABCB1: ATP-Binding Cassette B1, ACTH: Adrenocorticotropic Hormone, APR: Adverse Psychological Reaction, ASO: allele-specific oligonucleotide, AUC: Area Under the Curve, CBCL: Child Behavior Checklist, CDI: Children's Depression Inventory, CI: Confidence Interval, CLIA: Chemiluminescence-based Immunoassay, CSF: Cerebrospinal Fluid, CTCAE: Common Terminology Criteria for Adverse Events, Dex: Dexamethasone, DST: Dexamethasone Suppression Test,

	Measurement outcome	Risk factor	Measurement risk factor	Results	Risk of bias: QUIPS domains
	CBCL	- Type of steroid - Age (<5, >5) - Sex	- Assigned by protocol - Patient record - Patient record	Dex: ≤5 years no difference >5 years: more total (p=.041), affective (p=.015) and anxiety problems (p=.050) Age: ≤5 years: higher internalizing (p=.003), externalizing (p=.005) and total problems (p=.003). >5 years: higher externalizing (p=.021), aggressive behavior (p=.017) and oppositional defiant problems (p=.036) Sex: NS	0/6 high
	SDQ	Age	Patient record	No relation between age and dex induced behavior problems	1/6 high
	SDQ	- Cortisol - Dex PK	- DST, CLIA - Trough levels	Cortisol: Baseline and AUC not correlated with behavior. Cortisol suppression correlated with SDQ conduct and impact score. Trough levels: no correlation with behavior	2/6 high
	Unknown	Type and dose of steroid	Per protocol	HDMP: 3 behavioral disturbances, Pred: 0. NS	4/6 high

GST: glutathione and glutathione-S-transferase, HDMP: High Dose Methylprednisolone, HR: High Risk, IL-10: interleukin-10, NS: Not Significant, MAO: monoamine oxidase, OR: Odds Ratio, PCR: Polymerase Chain Reaction, PK: Pharmacokinetics, Pred: Prednisone, RCT: Randomized Controlled Trial, RFLP: restriction fragment length polymorphism, RR: Relative Risk, SAE: Serious Adverse Event, SD: Standard Deviation, SDQ: Strength and Difficulties Questionnaire, SNP: Single Nucleotide Polymorphism, SR: Standard Risk, WHO: World Health Organization.

Table 2. Results per Sleep study

Study	Study design	A priori design for risk factors	N=	n= Sleep outcome	Age	Steroid	Sleep outcome
Daniel et al (2016)[12]	Prospective	Yes	81	61	6.21 (SD 2.22) years	Dex Pred	Sleep parameters
Drigan et al (1992)[48]	Prospective	Yes	38	38	SR: 51.4 months (29-94) HR: 49.1 months (25-63)	Pred (40 mg/m^2/d or 120 mg/m^2/d)	Sleep disturbance
Hinds et al (2007)[13]	Prospective	Yes	100	88	9.24 (SD 3.23) years	Dex (6-12 mg/m^2/d)	Sleep parameters
Rogers et al (2014)[54]	Prospective	No	82	82	8.8 (SD 3.3) years	Dex (6, 8 or 12 mg/m^2/d)	Circadian activity rhythms
Sanford et al (2008)[55]	Prospective	No	88	88	9.15 (SD 3.24) years	Dex (6, 8 or 12 mg/m^2/d)	Sleep parameters
Vallance et al (2010)[56]	Prospective	No	88	88	9.24 (SD 3.23) years	Dex (6, 8 or 12 mg/m^2/d)	Sleep parameters

	Measurement outcome	Risk factor	Measurement risk factor	Results	Risk of bias: QUIPS domains
	28-day sleep diary	Type of steroid	Assigned by protocol	Pred better sleep quality (p=.014) and fewer night awakenings (p=.013)	0/6 high
	Additional 1 item rating sleep disturbance	- Steroid dose - Age (<4 / >4) - Sex	- Assigned by protocol - Patient record - Patient record	High dose pred: no difference Age: no difference Sex: girls more sleep disturbance (p<.05)	4/6 high
	Actigraphy and sleep diary	- Age (<7 / 7-12 / ≥13) - Sex - Steroid dose	All patient record	Age: older children less sleep duration (p=.018), less sleep minutes/24 hours (p=.002) Sex: boys more awakenings (p=.020), girls more naps (p=.027) Steroid dose: higher dose associated with: sleep efficiency (p=.012), sleep minutes (p=0.13) and nocturnal awakenings (p=.034)	1/6 high
	Actigraphy and sleep diary	- Dex dose - Age (5-12 / 13-17) - Sex	- Per protocol - Patient record - Patient record	High dose: NS for circadian parameters Age: NS Sex: NS	2/6 high
	Actigraph and sleep diary	- Sex	- Patient record	Boys increased WASO Girls decreased WASO	1/6 high
	Actigraphy and sleep diary	- Dex PK - Dex dose - Serum albumin - Genotyping	- Liquid chromatography - Standard testing method - Per protocol / DNA-Print Genomics	PK: increased time above 100nM dex increase in sleep time (p=.05). Higher AUC (univariate) is less sleep efficiency and sleep time. Multivariable NS. Dose: higher dex dose, less sleep efficiency (p=.0015) Albumin: NS AHSG SNP: sleep duration and actual sleep time increased IL6 G174C SNP: NS IL6 C634G SNP: NS	1/6 high

Table 2. Continued

Study	Study design	A priori design for risk factors	N=	n= Sleep outcome	Age	Steroid	Sleep outcome
Warris et al (2016)[53]	RCT	Yes	47	47	6,0 (4.0-9.8) years	Dex (6 mg/m²/d)	Sleep

Note: Age reported as mean or range.
Color-coding: **red** (lower quality), **orange** (medium quality), **green** (higher quality).
Abbreviations: AUC: Area Under the Curve, CLIA: Chemiluminescence-based Immunoassay, Dex: Dexamethasone, DST: Dexamethasone Suppression Test, HR: High Risk, NS: Not Significant,

All studies used a candidate gene approach, usually focusing on the glucocorticoid receptor gene *(NR3C1*; Supplemental Table 5). One study also included three other genes: the ATP-Binding Cassette B1 (*ABCB1*) gene, glutathione and glutathione-S-transferase (*GST*) gene and interleukin-10 (*IL-10*) gene.[45] None of the studies adjusted for multiple testing or controlled for confounding variables. Furthermore, none of the studies included a replication cohort. One study used a validated questionnaire to measure APRs,[4] the other studies used retrospectively collected toxicity data.[39,40,44,45]

The *Bcl-1* polymorphism on the GR gene was studied in four patient cohorts,[4,39,44,45] and only Kaymak Cihan et al. found a positive association between the homozygous CC genotype and the occurrence of depression symptoms during steroid treatment (Supplemental Table 5). This result has not been replicated in another cohort. The *ER22/23EK* and *N363S* GR gene polymorphisms were studied in respectively three[4,39,45] and four patient cohorts[4,39,40,44,45] of which none found a significant association with an APR outcome. The SNPs in the three additional genes described by Marino et al. (*ABCB1, GST and IL-10)* were studied in 36 patients; no significant association with the occurrence of neuropsychiatric signs was found.[45]

Other factors

Several additional possible risk factors were identified during our literature search. Only a serum elevated monoamine oxidase was correlated with steroid-induced behavioral changes.[49] However, monoamine oxidase changes appears to be an effect of stress, rather than a risk factor.[57,58] Cortisol levels,[4,53] adrenocorticotropic hormone

Measurement outcome	Risk factor	Measurement risk factor	Results	Risk of bias: QUIPS domains
SDSC	- Cortisol - Dex PK	- DST, CLIA - Trough levels	Cortisol: Baseline and AUC not correlated with sleep. Cortisol suppression not correlated with sleep. Trough levels: no correlation with sleep	**2/6 high**

PK: Pharmacokinetics, Pred: Prednisone, RCT: Randomized Controlled Trial, SD: Standard Deviation, SDSC: Sleep Disturbance Scale for Children, SNP: Single Nucleotide Polymorphism, SR: Standard Risk, WASO: Wake time After Sleep Onset.

levels,[4] dexamethasone pharmacokinetics,[53] and neuronal cell destruction[4] were studied but not confirmed as significant risk factors for APRs, possibly due to small sample sizes (n = 37 and n = 46).

Sleep problems

Risk factors for steroid-induced sleep problems were evaluated in seven studies of which three[54-56] reported secondary analyses of the cohort originally collected by Hinds et al.[13] Four papers used an objective measuring method: actigraphy.[13,54-56] Three papers used (parent-reported) subjective methods: 28-day sleep diary,[12] the Sleep Disturbance Scale for Children (SDSC) questionnaire,[12,53] and a self-constructed item rating sleep disturbance.[48] All studies measured sleep problems during maintenance phase of treatment. Overall, the quality of evidence regarding risk factors for sleep problems was very low to low (Table 4), mostly due to the limited amount of studies conducted in this area.

Sociodemographic factors (age and sex)

The influence of age on sleep problems was investigated in three studies (two cohorts). A higher quality study found that age was associated with sleep duration. Older children were in bed less during dexamethasone treatment and older age was also associated with less total daily sleep minutes, however other sleep parameters did not differ significantly between age groups.[13] In the same cohort, Rogers et al. reports no difference in age on actigraphic circadian parameters,[54] in coherence with Drigan et al. who also did not find a difference in age on sleep disturbances.[48] However, this is a low quality evidence paper, using subjective measurement for sleep. Evidence for age as a risk factor is limited to

only one high quality paper on well-defined sleep parameters. Older children might have a higher risk of impaired sleep duration during steroid use, but age as risk factor for impaired circadian parameters is not found. Replication studies are needed to confirm which age group is particularly at risk for specific sleep problems.

Sex as risk factor was investigated in four studies (two cohorts) of which three of high quality[13,54,56] and one of lower quality.[48] Two studies in the same cohort[13,56] reported no sex difference on most actigraphic sleep parameters, but boys did experience more nocturnal awakenings, whereas girls napped more in univariate analyses.[13] In a multivariable analysis, only the parameter wake time after sleep onset (WASO) was decreased in girls and increased in boys during dexamethasone treatment.[55] In the same cohort, Rogers et al. also did not find significant sex differences in the circadian rhythm parameters.[54] Drigan et al. described that parents reported girls to have an increased risk for steroid-induced sleep disturbance.[48] However, their 1-item parent reported question to assess sleep is not a validated questionnaire, making this evidence of lower quality. The quality of evidence investigating sex as a risk factor is overall low. It suggests that sex does not impact most sleep parameters (e.g., sleep quality), however some parameters (nocturnal awakenings, napping, WASO) may be impacted differently for boys and girls.

Treatment factors (type of steroid and steroid dose)

Only one study compared type of steroid as a risk factor for sleep problems. Using multivariate analyses, it was found that children receiving prednisone experienced better sleep quality and fewer night awakenings during steroid treatment in comparison with dexamethasone.[12] Although this single study is of higher quality, evidence regarding type of steroid as a risk factor is scarce and therefore rated as very low quality in the GRADE.

Four studies (two cohorts) compared the effect of steroid dose on sleep problems. Three of the studies in the same cohort drew the conclusion that a higher steroid dose gave rise to more sleep problems.[13,54,56] Only one other study with a different cohort evaluated steroid dose and found that steroid dose was not related to sleep disturbance.[48] However, this study is of lower quality partly due to methodological problems with the validity of the measurement method. Overall, the review suggests, without clear evidence, that steroid type and dose might have an impact on sleep problems, but this is only based on one cohort of patients and therefore of low to very low quality.

Parental factors

We did not find any studies describing steroid-induced sleeping problems and parental factors with our search.

Medical history

With our search, we did not identify any studies describing medical history as a risk factor for steroid-induced sleeping problems.

Genetic predisposition

Only one study (n = 72) investigated genetic variation as possible risk factor for steroid-induced sleep problems in ALL.[56] Vallance et al. studied 99 polymorphic loci in candidate genes associated with glucocorticoid metabolism. They included actigraphy data of 72 Caucasian patients, no replication cohort was used. They did not adjust for multiple testing and did not describe controlling for confounding variables (Supplemental Table 6).

Three different SNPs in two genes were described in relation to dexamethasone induced sleeping problems. A homozygous variant in the α2-Heremans-Schmid glycoprotein *(AHSG)* gene was associated with longer sleep time and longer sleep duration during dexamethasone treatment.[56] Carriership of two SNPs in the Interleukin-6 (*IL-6)* gene was not significantly associated with sleep problems during dexamethasone treatment (Supplemental Table 6).[56]

Other factors

We identified two additional studied risk factors for sleep problems. Dexamethasone pharmacokinetics was investigated in two ALL studies. One study (n = 24) did not find an association of higher dexamethasone levels (trough levels following four days of dexamethasone) with sleep problems.[53] Another study (n = 100) described that a decrease of the cumulative time above a threshold of 100 nM dexamethasone was associated with increased actual sleep time. Furthermore, in a univariate analysis wake after sleep onset (WASO) increased and sleep efficiency and sleep time decreased as the dexamethasone area under the curve increased. However, multivariate analysis did not reveal statistical evidence independent of the dexamethasone area under the curve level.[56] The same group studied albumin levels and the occurrence of sleep problems and did not find a significant relation between both.[56]

Table 3. GRADE Adverse Psychological Reactions

Potential Risk Factors				Univariable			Multivariable		
	Number of patients	Number of studies	Number of cohorts	+	0	-	+	0	-
Age (younger age)	331	9	9	3[25,41,52]	5[9,40,46,48,49]	1[42]	-	-	-
Sex (boys)	191	5	5	0	4[40,46,49,52]	1[48]	-	-	-
Type of steroid (Dex)	284	8	8	2[46,52]	6[4,25,37,38,43,51]	0	-	-	-
Steroid dose (higher)	124	4	4	1[48]	3[25,47,49]	0	-	-	-
Cumulative steroid dose (higher)	60	1	1	0	1[25]	0	-	-	-
Parental coping strategy	0	0	0	-	-	-	-	-	-
Parental stress	0	0	0	-	-	-	-	-	-
History of psychiatric problems	0	0	0	-	-	-	-	-	-
Genetic predisposition									
N363S	87	5	4	0	5[4,39,40,44,45]	0	-	-	-
Bcl1	67	4	4	1[44]	3[4,39,45]	0	-	-	-
ER22/23EK	67	3	3	0	3[4,39,45]	0	-	-	-
ABCB1 gene	25	1	1	0	1[45]	0	-	-	-
GST gene	25	1	1	0	1[45]	0	-	-	-
IL-10 gene	25	1	1	0	1[45]	0	-	-	-
Platelet MAO activity (higher)	23	1	1	1[49]	0	0	-	-	-
Cortisol levels (higher)	66	2	2	0	2[4,53]	0	-	-	-
ACTH level (higher)	20	1	1	0	1[4]	0	-	-	-
Dex kinetics	46	1	1	0	1[53]	0	-	-	-
Neuronal cell destruction	20	1	1	0	1[4]	0	-	-	-

Notes: Phase= phase of investigation. For uni- and multivariable analyses: += number of significant effects with a positive value; 0= number of non-significant effects; -= number of significant effects with a negative value. Below the reference for each study is depicted. For GRADE factors: √= no serious limitations; x= serious limitations (or not present for moderate/large effect size, dose effect); unclear= unable to rate item based on available information. For overall quality of evidence: += very low; ++= low, +++= moderate, ++++= high.

	GRADE Factors[36]							
Phase	Study limitations	Inconsistency	Indirectness	Imprecision	Publication bias	Moderate/large effect sizes	Dose effect	Overall quality
1,2	x	x	✓	unclear	x	unclear	x	**+**
1,2	x	✓	✓	unclear	x	unclear	NA	**+**
1,2	✓	x	✓	unclear	✓	unclear	unclear	**+**
1,2	x	✓	✓	unclear	x	unclear	unclear	**+**
2	✓	NA	✓	✓	x	unclear	unclear	**++**
NA	NA	NA	NA	NA	NA	NA	NA	**None existing**
NA	NA	NA	NA	NA	NA	NA	NA	**None existing**
NA	NA	NA	NA	NA	NA	NA	NA	**None existing**
1,2	x	✓	✓	unclear	✓	unclear	NA	**+**
1,2	x	x	✓	unclear	✓	unclear	NA	**+**
1,2	x	✓	✓	unclear	✓	unclear	NA	**+**
1	x	NA	✓	unclear	x	unclear	NA	**+**
1	x	NA	✓	unclear	x	unclear	NA	**+**
1	x	NA	✓	unclear	x	unclear	NA	**+**
1	x	NA	✓	unclear	x	unclear	x	**+**
2	x	✓	✓	x	x	unclear	x	**+**
2	x	NA	✓	x	x	unclear	x	**+**
2	✓	NA	✓	unclear	x	unclear	unclear	**+**
2	x	NA	✓	unclear	x	unclear	x	**+**

Abbreviations: ABCB1: ATP-Binding Cassette B1, ACTH: Adrenocorticotropic Hormone, Dex: Dexamethasone, GRADE: Grading of Recommendations Assessment, Development and Evaluation GST: glutathione and glutathione-S-transferase, IL-10: interleukin-10, MAO: Monoamine Oxidase, NA: not applicable

Table 4. GRADE Sleep problems

Potential Risk Factors				Univariable			Multivariable		
	Number of patients	Number of studies	Number of cohorts	+	0	-	+	0	-
Age (younger age)	208	3	2	0	2[48,54]	1[13]	0	0	0
Sex (girls)	208	4	2	2[13,48]	1[54]	1[13]	0	0	1[55]
Type of steroid (Dex)	61	1	1	-	-	-	1[12]	0	0
Steroid dose (higher)	208	4	2	3[13,54,56]	1[48]	0	-	-	-
Parental coping strategy	0	0	0	-	-	-	-	-	-
Parental stress	0	0	0	-	-	-	-	-	-
History of sleep problems	0	0	0	-	-	-	-	-	-
Genetic predisposition									
AHSG	88	1	1	1[56]	0	0	-	-	-
IL-6 (G174C)	88	1	1	0	1[56]	0	-	-	-
IL-6 (C634G)	88	1	1	0	1[56]	0	-	-	-
Dex kinetics	134	2	2	1[56]	1[53]	0	0	1[56]	0
Albumin level (higher)	88	1	1	0	1[56]	0	-	-	-

Notes: Phase= phase of investigation. For uni- and multivariable analyses: + = number of significant effects with a positive value; 0= number of non-significant effects; - = number of significant effects with a negative value. Below the reference for each study is depicted. For GRADE factors: √= no serious limitations; x= serious limitations (or not present for moderate/large effect size, dose effect); unclear = unable to rate item based on available information. For overall quality of evidence: + = very low; ++ = low, +++ = moderate, ++++ = high.

	Grade Factors[36]							
Phase	**Study limitations**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Moderate/large effect sizes**	**Dose effect**	**Overall quality**
1,2	x	x	√	unclear	x	unclear	unclear	**+**
1,2,3	√	x	√	unclear	x	unclear	NA	**++**
2	√	NA	√	√	x	unclear	unclear	**++**
1,2	√	√	x	√	x	unclear	x	**+**
NA	NA	NA	NA	NA	NA	NA	NA	**None existing**
NA	NA	NA	NA	NA	NA	NA	NA	**None existing**
NA	NA	NA	NA	NA	NA	NA	NA	**None existing**
2	√	NA	√	unclear	x	unclear	NA	**+**
2	√	NA	√	unclear	x	unclear	NA	**+**
2	√	NA	√	unclear	x	unclear	NA	**+**
2	√	√	√	unclear	x	unclear	x	**++**
2	√	NA	√	unclear	x	unclear	x	**+**

Abbreviations: AHSG: α2-Heremans-Schmid glycoprotein, Dex: Dexamethasone, GRADE: Grading of Recommendations Assessment, Development and Evaluation, IL-6: interleukin-6, NA: not applicable.

DISCUSSION

Overall, evidence regarding risk factors for steroid-induced APRs and sleep problems in children with ALL is low, studies are scarce and the quality of summated evidence is low to very low. Therefore, the current summary should be interpreted with caution. Nevertheless, acquired data suggest that sex, type of steroid and (cumulative) steroid dose are no clear risk factors for steroid-induced APRs. A younger age (0-6 years old) seems to be a risk factor for behavioral problems. Older age seems more a risk factor for sleep problems. Sex does not seem a risk factor for overall sleep disturbance, but might be for specific sleep parameters. Steroid dose and type appear the be a risk factor for steroid-induced sleep problems, although these findings are only based on one patient cohort. We did not find any studies which analyzed parental stress/coping or medical or sleep history as risk factor for APRs/sleep problems. Genetic susceptibility associations are weak and not replicated, therefore no conclusions can be drawn. Overall, more high quality evidence and replication studies are needed to confirm our identified findings.

In this review, APRs and sleep were evaluated as two independent phenomena. Indeed, both are usually described separately in literature. However, sleep problems can also be either an effect of or a trigger for APRs.[14] The exact mechanism of how behavior and sleep are impacted by steroids is unknown but is thought to be caused by their effect on the glucocorticoid receptor and by their disruptive nature on the diurnal rhythm of the hypothalamic-pituitary-adrenal (HPA-) axis, and to suppression of endogenous cortisol production.[59] Cortisol has a high affinity for the mineralocorticoid receptor (MR) in the brain, whereas exogenous steroids such as dexamethasone have a higher affinity for the glucocorticoid receptor (GR).[60] In patients treated with steroids, the hypothesis is that the GR in the brain is stimulated, whereas the MR is not activated. This disturbance of GR:MR balance is thought to deregulate the stress-system and enhance vulnerability to stress-related disorders.[60] Furthermore, disruption of the diurnal rhythm at any level of the HPA-axis can disturb the regulation of the sleep-wake rhythm. Cortisol is secreted in a circadian rhythm which has its nadir in the night, important for falling asleep, and a peak when waking up.[61] Glucocorticoid replacement therapy has been shown to be permissive for rapid eye movement sleep and sleep consolidation in patients with adrenal insufficiency who experience disturbed sleep phases.[62]

The heterogeneity of studied APRs and sleep problems makes it difficult to generalize conclusions regarding risk factors. For example, young children seem to be at risk for behavioral problems,[25,41,52] whereas older children seem to experience more steroid-induced psychosis.[42] These are two different outcomes within the spectrum of APRs, and it is possible that for each APR different risk factors exist. Another explanation is that

some APRs are better recognized in different age groups, or that younger children might not have developed the skills necessary to control their behavior. Age differences also differ per investigated domain of sleep problems, for example when measured in circadian parameters no differences were found, but when measured in sleep parameters, older children appear to have more sleep problems.

Another source of heterogeneity complicating the generalization of conclusions, is the methodology of measuring APRs and sleep problems, which differed considerably between studies. Several large randomized controlled trials[37,38,42,46] reported APRs as part of toxicity registration. This could potentially give an underestimation of the problem, since usually only extreme cases (toxicity grade III or IV) are reported. Nevertheless, grade II/IV toxicities include side effects that are clinically relevant. These studies found an APR incidence of 0,1-6,0% in their population, remarkably lower than the reported 19-86% in prospective studies which used validated questionnaires to measure APRs as primary outcome parameter.[4,9,41,48,52] Sleep problems were not registered as toxicity in any of the trials, which recently led to a call for action to start screening for sleep problems.[16]

Since dexamethasone is more potent and penetrates the central nervous system better than prednisone,[63] and as dexamethasone has a higher affinity for the GR, it is conceivable that more APRs or sleep problems may be expected with dexamethasone treatment. Contrary to this expectation, results were conflicting. Most (6/8) studies of which four of higher quality did not find a difference between dexamethasone and prednisone with regard to developing APRs.[4,25,37,38,43,47,51] This is in line with a previous review investigating neuropsychological side effects of dexamethasone versus prednisone.[10] Oppositely, two other high quality studies did find more APRs during dexamethasone treatment[46,52] and one described significantly more dexamethasone related sleep problems.[12] Despite being a possible risk factor, dexamethasone has a higher anti-leukemic activity and will probably remain the preferred steroid in the treatment of ALL. Although it was expected that a higher steroid dose might predispose for APRs or sleep problems as well, this was not reported. Steroid dose was not related to APRs in four studies of which one of high quality.[25,47-49] This is surprising, since in adults dosage appears to be the most significant risk factor.[64] Evidence is contradictory in children with chronic diseases, though dexamethasone levels and pharmacokinetics may play a role in the occurrence of steroid-induced toxicities. Dexamethasone clearance is known to be higher in younger children, which might explain the inter-patient variability.[65] Furthermore, even the lowest steroid dose children with ALL receive during their treatment is very high compared to adults or other pediatric patients with diseases such as asthma. This could possibly explain why we did not find a difference comparing steroid dose in the occurrence of APRs in children with ALL.

When looking into treatment related risk factors, it is important to realize that not only steroids can cause APRs or sleep problems. Other ALL treatment components, such as methotrexate, might cause synergistic toxicity.[8] Also, a higher steroid dose and dexamethasone, both risk factors for sleep problems, are commonly used in treatment protocols for children with higher risk ALL. These children are treated with more chemotherapy compared to lower risk groups, which could explain a higher occurrence of sleep problems as well. Furthermore, the distress associated with being confronted with ALL and subsequent treatment regimen can cause both APRs and sleep problems on its own.[66,67].

We hypothesized that a (family) history of psychiatric or sleep problems might predispose for steroid-induced adverse events, since in the general or adult oncology population this factor increases the risk of developing APRs or sleep problems.[64,68] However, no studies assessed this risk factor for steroid-induced APR or sleep problems. Only case reports describing steroid-induced APRs in patients with a (family) history of psychiatric symptoms were found. However, case reports of patients with psychiatric deterioration without such histories were described as well. See Supplemental Tables 7 and 8 for an overview of these case reports. No case reports regarding steroid-induced sleep problems were found. Larger studies focusing on (medical) history and the occurrence of both APR and sleep problems are warranted. Besides a history of psychiatric or sleep problems, it is conceivable that certain family risk factors (e.g., family background, premorbid functioning), parenting stress, but also received psychosocial support can influence the coping strategies of parents and may thereby influence their perceptions of problems during steroid treatment.[27,66,69,70] None of these possible risk factors have been studied in steroid-induced APRs or sleep problems.

Genetic predisposition may contribute to the inter-individual differences in developing steroid-induced APRs or sleep problems. Several studies have identified relevant SNPs in the GR gene, which could contribute to differences in increased glucocorticoid sensitivity as well as APRs such as depression.[71,72] Only one of our included studies found a significant association between a SNP and APR: Kaymak Cihan et al. described that carriers of the *BclI* polymorphism were more susceptible for depression symptoms.[44] However, this result was not replicated, nor did the other included studies find this association.[4,39,45] No other SNPs were found to be associated with APRs. Genetic predisposition for sleep problems is complex and correlations depend on the definition of sleep outcome.[73,74] Vallance et al. studied several polymorphisms that may contribute to inter-patient variability of steroid-induced sleep problems, using a candidate gene approach.[56] Only one polymorphism (rs4918, *AHSG* gene) was associated with impaired sleep both on and off dexamethasone treatment in children with ALL.[56] *AHSG* is a hepatic protein, associated with type 2 diabetes.[75] During dexamethasone treatment, the rs4918 polymorphism may

be associated with longer sleep duration.[56] However, this finding remains to be replicated. In general, the quality of the included studies on the influence of genetic variation on steroid-induced APRs and sleep problems is very low (Supplemental Tables 5 and 6). Most patient cohorts were very small which could explain the inability to demonstrate significant differences between genetic profiles. Other limitations include the lack of adjustments for multiple testing and confounding variables, as well as the absence of a replication cohort. This makes it impossible to provide evidence based recommendations regarding genetic susceptibility. Larger studies with proper replication are warranted.

Study limitations

Some strengths and limitations should be discussed. For our systematic review, we used six different search engines and did not limit our search on our predefined risk factors (PICO's). This generated an extensive and complete search result and cross reference check did not reveal any new evidence. Furthermore, two high quality tools (QUIPS and GRADE) were used. Both tools complementarily facilitate a structured assessment and interpretation of results. All evidence screening, data extraction and assessment was performed by two independent researchers, limiting inter-individual differences. A limitation includes that the interpretability of the results of this review is overall of very low quality of evidence, partly due to the average high risk of bias within single studies. This indicates that more extensive research designed to primarily investigate steroid-induced APRs and sleep is warranted.

We included a screen of 245 papers that reported on outcomes of clinical pediatric ALL trials. Of these 245, only six mentioned either APRs or sleep problems as a steroid-induced toxicity, of which one was included in our review.[42] Numerous large trial papers which included (randomization for) steroids did not report APRs or sleep problems as adverse events, even though other toxicities such as osteonecrosis or infections were prospectively collected.[76-78] These trials are mainly designed to improve (event free) survival, and/or to a lesser extent to decrease treatment induced toxicity. APRs and sleep problems are common (steroid-induced) toxicities, which can influence HRQoL substantially. An integrated system to measure and report both toxicities should be implemented in upcoming treatment protocols. Integration of patient-reported outcome measures (PROMs) could be valuable to establish a systematic approach.[79,80]

Clinical implications and conclusions

Based on this systematic review of literature, we conclude that there is no high level of evidence for risk factors for developing steroid-induced APR or sleep problems in children with ALL. There are few high quality prospective studies and patient numbers are small. Methods of measurement are heterogeneous and evidence is weak. However, current evidence suggests that type and dose of steroids are not related to APRs, but may be related

to sleep problems. Younger patients seem at risk for behavioral problems and older patients for sleep problems. Overall, these conclusions should be interpreted with caution. We made recommendations to improve evidence for findings regarding risk factors for steroid-induced APRs and sleep problems (Table 5). One important recommendation is to implement a standardized prospective registration of both steroid-induced APRs and sleep problems and risk factors in future studies in children with ALL, since identifying children at risk and determining effective care can improve health-related quality of life during treatment.

Table 5. Summary of findings, gaps of knowledge and recommendations

Summary of findings	
Age	APR: younger patients (0-6 years old) seem more at risk for behavioral problems Sleep: adolescent patients seem at risk for more sleep problems (less sleep duration)
Sex	APR: boys and girls do not differ in risk Sleep: most sleep parameters are not differently impacted, however WASO, napping and number of nocturnal awakening may differ for boys and girls
Steroid type	APR: no clear difference between dexamethasone versus prednisone Sleep: receiving dexamethasone increased sleep problems compared to prednisone
Steroid dose	APR: higher dose does not increase risk for APRs Sleep: higher dose does increase risk for sleep problems

Gaps of knowledge	**Recommendations**
Scarce evidence on prospectively measured steroid-induced APR and sleep problems and related risk factors (only 6 out of 245 c	Systematically monitor psychological and sleep toxicities in new studies and specifically in clinical pediatric ALL trials.
Lack of high quality studies investigating steroid-induced APR and sleep problems Current evidence is of very low quality.	Develop larger studies that are a priori designed to investigate risk factors for steroid-induced APR and sleep problems. Use validated measures to study APR and sleep, e.g. validated questionnaires, sleep diary or actigraphy Replication studies, particularly for sleep problems, to increase quality of evidence.
Studies investigating parental coping, stress, family and medical history are currently lacking.	Include parental coping, stress, family and medical history in new studies, since they are potentially risk factors.
Genetic susceptibility studies are scarce, patient cohorts are small, no adjustments for multiple testing or confounding variables are made and findings remain to be replicated	Larger studies on the influence of genetic variation are needed, including appropriate replication cohorts

Abbreviations: ALL: acute lymphoblastic leukemia, APR: adverse psychological reaction

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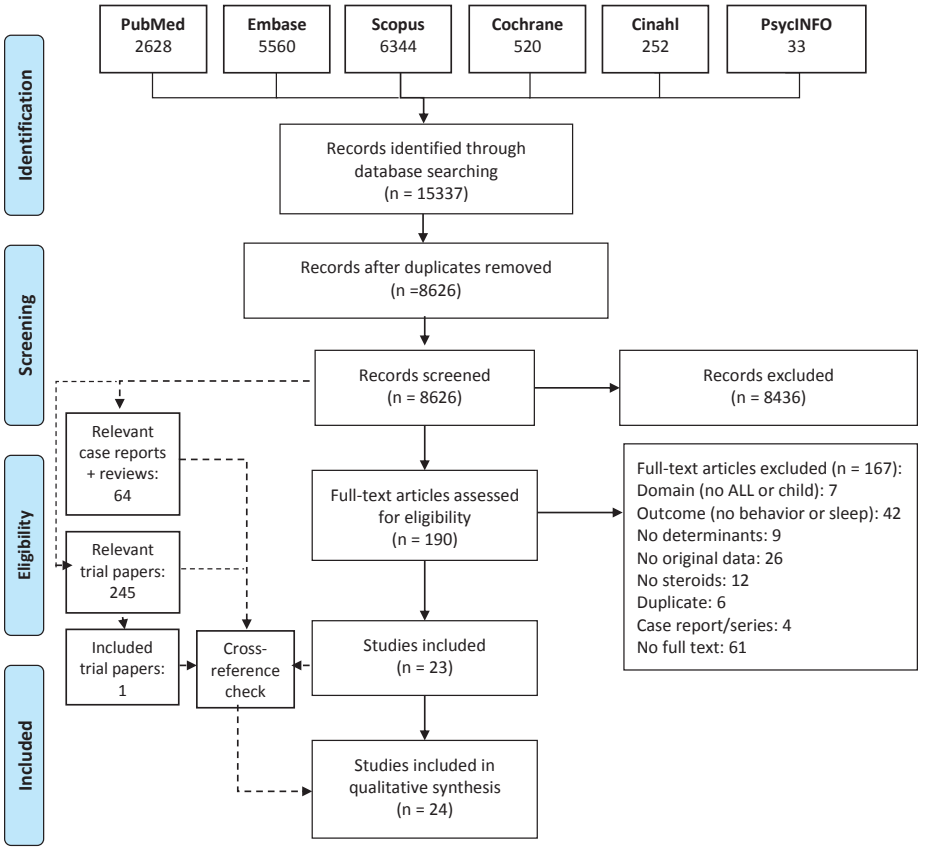
SUPPLEMENTAL FIGURE



Supplemental Figure 1. PRISMA Flow diagram

SUPPLEMENTAL TABLES

Supplemental Table 1. Patient, Intervention, Comparison and Outcome (PICO)

Patient population	
Children (0-18 years old) with acute lymphoblastic leukemia receiving glucocorticoid treatment	
Intervention	**Comparison**
Sociodemographic factors	
Young childhood age	Older childhood age
Girl	Boy
Treatment factors	
Dexamethasone	Prednisone
High dose steroid	Low dose steroid
Parental factors	
Unhealthy parental coping strategy	Healthy parental coping strategy
Parental stress	No parental stress
Medical history	
History of APR / sleep problem	No history of APR / sleep problem
Genetic predisposition	
SNP present	SNP absent
Outcome	
Adverse psychological reaction *and/or* Sleep problem	

Abbreviations: APR: Adverse Psychological Reaction, SNP: Single Nucleotide Polymorphism

Supplemental Table 2. Definition and application of QUIPS (Quality in Prognosis Studies) domains

Biases	Issues to consider for judging overall rating of "Risk of bias"
1. Study Participation	**Goal: To judge the risk of selection bias (likelihood that relationship between *PF* and *outcome* is different for participants and eligible non-participants).**
Source of target population	The source population or population of interest is adequately described for key characteristics.
Method used to identify population	The sampling frame and recruitment are adequately described, including methods to identify the sample sufficient to limit potential bias (number and type used, e.g., referral patterns in health care)
Recruitment period	Period of recruitment is adequately described
Place of recruitment	Place of recruitment (setting and geographic location) are adequately described
Inclusion and exclusion criteria	Inclusion and exclusion criteria are adequately described (e.g., including explicit diagnostic criteria or "zero time" description).
Adequate study participation	There is adequate participation in the study by eligible individuals
Baseline characteristics	The baseline study sample (i.e., individuals entering the study) is adequately described for key characteristics.
Summary Study participation	**The study sample represents the population of interest on key characteristics, sufficient to limit potential bias of the observed relationship between PF and outcome.**
2. Study Attrition	**Goal: To judge the risk of attrition bias (likelihood that relationship between *PF* and *outcome* are different for completing and non-completing participants).**
Proportion of baseline sample available for analysis	Response rate (i.e., proportion of study sample completing the study and providing outcome data) is adequate.
Attempts to collect information on participants who dropped out	Attempts to collect information on participants who dropped out of the study are described.
Reasons and potential impact of subjects lost to follow-up	Reasons for loss to follow-up are provided.

	Rating of reporting	Rating of "Risk of bias" - Application
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
		High: ≥3x 'no' or <3 'yes' **Moderate:** everything in between **Low:** ≥4x 'yes' and 'inclusion and exclusion criteria' must be 'yes'
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	

Supplemental Table 2. Continued

Biases	Issues to consider for judging overall rating of "Risk of bias"
Outcome and prognostic factor information on those lost to follow-up	Participants lost to follow-up are adequately described for key characteristics.
	There are no important differences between key characteristics and outcomes in participants who completed the study and those who did not.
Study Attrition Summary	**Loss to follow-up (from baseline sample to study population analyzed) is not associated with key characteristics (i.e., the study data adequately represent the sample) sufficient to limit potential bias to the observed relationship between PF and outcome.**
3. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how PF was measured (differential measurement of PF related to the level of outcome).**
Definition of the PF	A clear definition or description of 'PF' is provided (e.g., including dose, level, duration of exposure, and clear specification of the method of measurement).
Valid and Reliable Measurement of PF	Method of PF measurement is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).
	Continuous variables are reported or appropriate cut-points (i.e., not data-dependent) are used.
Method and Setting of PF Measurement	The method and setting of measurement of PF is the same for all study participants.
Proportion of data on PF available for analysis	Adequate proportion of the study sample has complete data for PF variable.
Method used for missing data	Appropriate methods of imputation are used for missing 'PF' data.
PF Measurement Summary	***PF* is adequately measured in study participants to sufficiently limit potential bias.**
4. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome (differential measurement of outcome related to the baseline level of PF).**
Definition of the Outcome	A clear definition of outcome is provided, including duration of follow-up and level and extent of the outcome construct.

	Rating of reporting	Rating of "Risk of bias" - Application
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
		High: ≤1x 'yes' **Moderate:** 2x 'yes' **Low:** ≥3x 'yes'
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
		High: ≥3x 'no' or <3 'yes' **Moderate:** everything in between **Low:** ≥4x 'yes' and '*Valid and Reliable Measurement of PF'* must be 'yes'.
	Yes / Partial / No / Unsure	

Supplemental Table 2. Continued

Biases	Issues to consider for judging overall rating of "Risk of bias"
Valid and Reliable Measurement of Outcome	The method of outcome measurement used is adequately valid and reliable to limit misclassification bias (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and confirmation of outcome with valid and reliable test).
Method and Setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants.
Outcome Measurement Summary	***Outcome of interest* is adequately measured in study participants to sufficiently limit potential bias.**
5. Study Confounding	**Goal: To judge the risk of bias due to confounding (i.e. the effect of PF is distorted by another factor that is related to PF and outcome).**
Important Confounders Measured	All important confounders, including treatments (key variables in conceptual model: LIST), are measured.
Definition of the confounding factor	Clear definitions of the important confounders measured are provided (e.g., including dose, level, and duration of exposures).
Valid and Reliable Measurement of Confounders	Measurement of all important confounders is adequately valid and reliable (e.g., may include relevant outside sources of information on measurement properties, also characteristics, such as blind measurement and limited reliance on recall).
Method and Setting of Confounding Measurement	The method and setting of confounding measurement are the same for all study participants.
Method used for missing data	Appropriate methods are used if imputation is used for missing confounder data.
Appropriate Accounting for Confounding	Important potential confounders are accounted for in the study design (e.g., matching for key variables, stratification, or initial assembly of comparable groups).
	Important potential confounders are accounted for in the analysis (i.e., appropriate adjustment).
Study Confounding Summary	**Important potential confounders are appropriately accounted for, limiting potential bias with respect to the relationship between *PF* and *outcome*.**

	Rating of reporting	Rating of "Risk of bias" - Application
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
		High: <1 'yes' **Moderate:** everything in between **Low:** 3x 'yes' or 2x 'yes' + 1x 'partial' and '*Valid and Reliable Measurement of Outcome*' must be 'yes'
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
		High: ≤1 'yes' **Moderate:** everything in between **Low:** ≥3x 'yes'

Supplemental Table 2. Continued

Biases	Issues to consider for judging overall rating of "Risk of bias"
6. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results.**
Presentation of analytical strategy	There is sufficient presentation of data to assess the adequacy of the analysis.
Model development strategy	The strategy for model building (i.e., inclusion of variables in the statistical model) is appropriate and is based on a conceptual framework or model.
	The selected statistical model is adequate for the design of the study.
Reporting of results	There is no selective reporting of results.
Statistical Analysis and Presentation Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results.**

Modified from: Hayden JA, Côté P, Bombardier C. Evaluation of the Quality of Prognosis Studies in Systematic Reviews. Annals of Internal Medicine. 2006;144:427-437.

	Rating of reporting	Rating of "Risk of bias" - Application
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
	Yes / Partial / No / Unsure	
		High: ≤1x 'yes' **Moderate:** everything in between **Low:** ≥3x 'yes' and '*Reporting of results*' must be 'yes'

Supplemental Table 3. Definition and application of GRADE (Grading of Recommendations Assessment, Development and Evaluation) domains

GRADE factor	Explanation
Phase of the study	Phase 3 study: cohort study design that seeks to generate understanding of the underlying processes for the prognosis of a health condition. Phase 2 study: cohort study design that seeks to confirm independent associations between the prognostic factor and the outcome Phase 1 study: Outcome prediction research or explanatory research aimed to identify associations between potential prognostic factors and the outcome.
Study limitations	Overall limitations based on the QUIPS assessment.
Inconsistency	Unexplained heterogeneity or variability in results across studies with differences of results not clinically meaningful.
Indirectness	The study sample, the prognostic factor, and/or the outcome in the primary studies do not accurately reflect the review question.
Imprecision	Within-study imprecision: sample size justification is not provided and there are less than 10 outcome events for each prognostic variable (for dichotomous outcomes) OR there are less than 100 cases reaching endpoint (for continuous outcomes), and no precision in the estimation of the effect size within each primary study Across study imprecision: there are few studies and small number of participants across studies.
Publication bias	Downgrade, unless the value of the risk/protective factor in predicting the outcome has been repetitively investigated
Moderate/large effect sizes	Moderate or large similar effect is reported by most studies
Dose effect	Possible gradient exists within and between primary studies
Overall quality	High / Moderate / Low / Very low

Modified from: Huguet A, Hayden JA, Stinson J, McGrath PJ, Chambers CT, Tougas ME, et al. Judging the quality of evidence in reviews of prognostic factor research: adapting the GRADE framework. Syst Rev. 2013;2:71

	Application
	Study phase was defined per included study. Phase 3 and 2 = high quality of evidence Phase 1 = moderate quality of evidence
	Present (x) when less than 50% of the included studies were of higher quality in the QUIPS assessment.
	Present (x) when studies report both positive and negative results. Not applicable (NA) when only one included study.
	Present (x) when the included studies do not reflect the predefined research question (as defined by Patient/ Intervention/ Comparison/ Outcome (PICO))
	Present (x) when more than 50% of included studies have imprecision. Unclear when not described in more than 50% of included studies.
	Do not downgrade when specifically investigated in multiple included studies
	Present (✓) when moderate or large effect is reported. Unclear when effect is reported without use of appropriate statistics.
	Present (✓) when higher levels of the risk factor lead to a larger effect size over lower levels of the factor. Unclear when no appropriate effect size reported. Not applicable (NA) when a gradient is not possible (e.g. boy/girl).
	1. Start with highest study phase and accompanying quality of evidence 2. Downgrade if: study limitations, inconsistency, indirectness, imprecision or publication bias are present (x) or unclear. 3. Upgrade if: moderate/large effect sizes or dose effect are present (✓)

Supplemental Table 4. QUIPS Risk of bias domain scoring

Author (year)	Study participation	Study attrition
Bostrom et al. (2003) [37]	○	●
Daniel et al. (2016) [12]	◐	◐
Domenech et al. (2014) [38]	○	◐
Drigan et al. (1992) [48]	●	●
Eipel et al. (2016) [39]	●	●
Eipel et al. (2013) [40]	●	●
Eiser et al. (2006) [51]	◐	●
Felder-Puig et al. (2007) [4]	●	●
Harris et al. (1986) [41]	●	●
Hinds et al. (2007) [13]	◐	●
Hough et al. (2016) [42]	◐	●
Igarashi et al. (2005) [43]	◐	●
Kaymak et al. (2017) [44]	●	●
Marino et al. (2009) [45]	◐	●
Messina et al. (1989) [49]	●	●
Mitchell et al. (2005) [46]	○	◐
Mrakotsky et al. (2011) [25]	◐	●
Pound et al. (2012) [52]	○	◐
Rogers et al. (2014) [54]	◐	●
Sanford et al. (2008) [55]	○	●
Vallance et al. (2010) [56]	○	●
Warris et al. (2016) [9]	◐	●
Warris, et al. (2016) [53]	○	●
Yetgin et al. (2003) [47]	○	●

Black= high risk of bias. Grey= moderate risk of bias. White= low risk of bias.

	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis and reporting
	○	●	●	●
	◌	○	◌	○
	○	●	●	◌
	○	◌	●	●
	○	●	●	◌
	○	●	●	◌
	○	●	●	◌
	○	○	●	◌
	○	●	●	●
	○	○	◌	◌
	○	●	●	●
	○	●	●	●
	○	●	●	◌
	○	●	●	●
	◌	○	●	●
	○	●	●	◌
	○	○	◌	◌
	○	○	◌	◌
	○	◌	●	◌
	○	○	◌	○
	○	○	◌	○
	○	○	◌	◌
	○	○	●	◌
	○	●	●	●

Supplemental Table 5. Studies on the influence of genetic variation on steroid induced adverse psychological reactions

	Study population					**Analyses**	
	Method	*Cohort size (cases / control)*	*Country of origin; ethnicity*	*Sex (% males)*	*Steroid treatment*	*Replication*	*Definition endpoint*
Eipel et al. (2013)[40]	Candidate gene	346 (29/317)	Hungary (ethnicity not specified)	57	Pred 60mg/m^2 and dex 10mg/m^2	No	Seriously altered behavior or steroid psychosis
Eipel et al (2016)[39]	Candidate gene	346 (29/317)	Hungary (ethnicity not specified)	60	Pred 60mg/m^2 and dex 10mg/m^2	No	Seriously altered behavior or steroid psychosis
		257 (33/224)		54			
Felder-Puig et al. (2007)[4]	Candidate gene	37 (20/17)	Austria (ethnicity not specified)	59.5	Pred 60mg/m^2 and dex 10mg/m^2	No	Steroid induced APR measured with CBCL questionnaire
Kaymak Cihan et al. (2017)[44]	Candidate gene	47 (13/34)	Turkey (ethnicity not specified)	47.8	Pred (40-60 mg/m^2/d) Dex (10 mg/m^2/d)	No	Depression symptoms (according to CTCAE 4.0)
Marino et al. (2009)[45]	Candidate gene	36 (25/11)	Italy (ethnicity not specified)	47.2	Pred (60mg/m^2/d) Dex (10mg/m^2/d)	No	Neuro-psychiatric signs

Abbreviations: APR: adverse psychological reaction, ABCB1: ATP-Binding Cassette B1, CBCL: Child Behavior Checklist, CTCAE: Common Terminology Criteria for Adverse Events, dex: dexamethasone, GR: glucocorticoid receptor,

	Studied no of SNPs (adj for multiple testing)	*Gene/ region*	*Variant*	*Effect allele / genotype*	*MVA adjust for:*	*OR*	*P-value*
	1 (no multiple testing)	*NR3C1* (GR gene)	N363S (rs6195)	Carrier vs noncarrier	None	0.7 (0.16-3.13)	1.0
	3 (no multiple testing)	*NR3C1* (GR gene)	N363S (rs6195)	Carrier vs noncarrier	None	0.7 (0.16-3.13)	1.0
			ER22/23EK (rs6189/rs6190)	Carrier vs noncarrier	None	NA	0.695
			Bcl-1 (rs41423247)	CC vs GC+GG, GG vs CC+GC	None	NA	0.785 0.209
	3 (no multiple testing)	*NR3C1* (GR gene)	N363S (rs6195)	Not stated	None	NA	n.s.
			ER22/23EK (rs6189/rs6190)	Not stated	None	NA	n.s.
			Bcl-1 (rs41423247)	Not stated	None	NA	n.s.
	2 (no multiple testing)	*NR3C1* (GR gene)	N363S (rs6195)	NA	NA	NA	NA
			Bcl-1 (rs41423247)	CC vs CG CC vs CG+GG	None	7.5 5.2	0.011 0.04
	8 (no multiple testing)	*NR3C1* (GR gene)	Bcl-1 (rs41423247)	Mutation vs wild type	None	NA	n.s.
			N363S (rs6195)				
			ER22/23EK (rs6189/rs6190)				
		ABCB1	T-129C				
			G2677T				
			C3435T				
		GST-P1	A2627G				
		IL-10	A1082G				

GST: glutathione and glutathione-S-transferase, IL-10: interleukin-10, MVA: multivariable analysis, NA: not applicable, n.s.: not significant, OR: odds ratio, PCR: polymerase chain reaction, pred: prednisone, SNP: single nucleotide polymorphism

Supplemental Table 6. Studies on the influence of genetic variation on steroid induced sleep problems

	Study population					**Analyses**	
	Method	*Cohort size (cases / control)*	*Country of origin; ethnicity*	*Sex (% males)*	*Steroid treatment*	*Replication*	*Definition endpoint*
Vallance et al. (2010)[56]	Candidate gene	72	USA (only white patients included for SNP analysis)	62	Dex (6, 8 or 12 mg/ m^2/d)	No	Sleep measured with actigraphy and sleep diary

Abbreviations: AHSG: α2-Heremans-Schmid glycoprotein, dex: dexamethasone, NS: not significant, OR: odds ratio, SNP: single nucleotide polymorphism

Supplemental Table 7. Case reports of patients with steroid-induced adverse psychological reactions with a (family) history of psychiatric symptoms.

Case report	**n=**	**Age (years)**	**Sex**	**Steroid**
Ducore et al (1983)[81]	1	15	Male	Prednisone (40mg/m2/day)
Hechtman et al (2013)[82]	1	14	Male	Prednisone
Hochhauser et al (2005)[8]	2	4 and 15	Male	Dexamethasone (6mg/m2/day)
Kramer et al (1999)[83]	1	14	Female	Dexamethasone (24mg/day)
Samsel et al (2017)[84]	3	2, 3 and 5	2 Male and 1 female	Dexamethasone (6mg/m2/day)
Tutkunkardas et al (2010)[85]	1	14	Male	Dexamethasone
Watanabe et al (1994)[86]	2	13 and 17	Female	Dexamethasone (15 mg/day)

Abbreviations: APR: adverse psychological reaction, ADHD: Attention deficit disorder, ASD: autism spectrum disorder, HFA: high functioning autism, PDD-NOS pervasive developmental disorder not otherwise specified, PTSD: post-traumatic stress disorder

	Studied no of SNPs (adj for multiple testing)	*Gene/ region*	*Variant*	*Effect allele / genotype*	*MVA adjust for:*	*OR*	*P-value*
	99 (no multiple testing)	*AHSG*	rs4918	GG vs CC+CG	Not stated	Not stated	0.023 (sleep duration) 0.005 (sleep time)
		IL-6	rs1800795 rs13447445	GG vs CG+CC CG vs CC			NS NS

	Type of APR	**History**
	Psychosis	Schizophrenia *(half-brother)*
	Psychosis	ADHD *(self)*
	Emotional and behavioral problems	PDD-NOS (*self)* Tic disorder + ADHD *(self)*
	Psychosis	Suicidal ideation *(grandfather)* Depression *(mother)*
	Behavior and mood symptoms Behavioral problems Mood problems	Anxiety disorder, substance dependence *(family)* ASD *(self)*, mood disorder *(parent)* Depression, anxiety, bipolar disorder *(family)*
	Mood disorder	HFA and ADHD *(self)* Depression *(mother)*
	Panic Mania	Mixed adjustment disorder *(self),* depression *(mother)*, PTSD *(father),* depression *(sibling).* Dysthymia *(mother),* panic attacks *(father),* depression and mania *(grandparents)*

Supplemental Table 8. Case reports of patients with steroid-induced APR without a (family) history of psychiatric symptoms

Case report	n=	Age	Sex	Steroid	Type of APR
Cassidy et al (2012)[87]	1	17	Male	Dexamethasone (10mg/day)	Mania with psychotic symptoms
Ducore et al (1983)[81]	1	13	Female	Prednisone (40mg/m2/day)	Psychosis
Ingram et al (2003)[88]	1	2	Male	Methylprednisolone 2 mg/kg/day	Mood and behavioral problems
Hochhauser et al (2005)[8]	2	4 and 6	Male	Dexamethasone (6mg/m2/day)	Emotional and behavioral problems
Sutor et al (1996)[89]	1	15	Female	Prednisone (90mg/day)	Depressive psychosis
Ularntinon et al (2010)[90]	3	8, 10 and 16	Male	Prednisone (2mg/kg/day) Dexamethasone (0.25mg/kg/day / 12mg/day)	Mood lability Agitation, irritability Mania with psychotic symptoms

Abbreviations: APR: adverse psychological reaction

Appendix 1. Search strings per database

PubMed History August 15, 2019

Search	PubMed Query – August 15, 2019	Items found
#8	#1 AND #2 AND #3 AND #7	2628
#7	#4 OR #5 OR #6	10710722
#6	"Sleep"[Mesh] OR "Sleep Wake Disorders"[Mesh] OR bed time[tiab] OR bedtime[tiab] OR circadian rhythm*[tiab] OR circadian activity rhythm*[tiab] OR dream*[tiab] OR hypersomnia*[tiab] OR insomnia*[tiab] OR night rest[tiab] OR night awakening*[tiab] OR nightmare*[tiab] OR parasomnia*[tiab] OR sleep*[tiab] OR somnolence[tiab] OR time in bed[tiab] OR night waking*[tiab]	242703
#5	"Behavior and Behavior Mechanisms"[Mesh] OR "Cognition"[Mesh] OR "Cognition Disorders"[Mesh] OR "Fatigue"[Mesh] OR "Mental Disorders"[Mesh] OR "Mental Health"[Mesh] OR "Mood Disorders"[Mesh] OR "Neurobehavioral Manifestations"[Mesh] OR "Psychophysiology"[Mesh] OR "Quality of Life"[Mesh] OR affect*[tiab] OR agitation[tiab] OR agressiv*[tiab] OR anxiet*[tiab] OR arousal[tiab] OR attention*[tiab] OR awareness[tiab] OR behavio*[tiab] OR bipolar[tiab] OR cognit*[tiab] OR compulsiv*[tiab] OR concentrat*[tiab] OR craving*[tiab] OR delirium[tiab] OR dementia[tiab] OR depress*[tiab] OR distress[tiab] OR emotion*[tiab] OR externaliz*[tiab] OR externalis*[tiab] OR fatigue*[tiab] OR HRQOL[tiab] OR hyperactiv*[tiab] OR hypomania*[tiab] OR impulsiv*[tiab] OR internaliz*[tiab] OR internalis*[tiab] OR mania*[tiab] OR mental*[tiab] OR mood*[tiab] OR neuroc*[tiab] OR neurol*[tiab] OR neurobehavio*[tiab] OR neurotox*[tiab] OR obsessi*[tiab] OR opposition*[tiab] OR panic*[tiab] OR psychi*[tiab] OR psychol* [tiab] OR psychop*[tiab] OR psychos*[tiab] OR QOL[tiab] OR quality of life[tiab] OR restless*[tiab] OR SDQ[tiab] OR stress*[tiab] OR toxicit*[tiab]	9199275
#4	"adverse effects" [Subheading] OR "Drug-Related Side Effects and Adverse Reactions"[Mesh] OR "Long Term Adverse Effects"[Mesh] OR cerebral effect*[tiab] OR adverse effect*[tiab] OR adverse reaction*[tiab] OR adverse drug reaction*[tiab] OR adverse event*[tiab] OR injurious effect*[tiab] OR side effect*[tiab] OR undesirable effect*[tiab] OR unwanted effect*[tiab]	2470648
#3	"Glucocorticoids"[Mesh] OR "Glucocorticoids" [Pharmacological Action] OR "Steroids"[Mesh] OR corticoid*[tiab] OR corticosteroid*[tiab] OR cortisol*[tiab] OR cortison*[tiab] OR dehydrocortison*[tiab] OR dexamethason*[tiab] OR dexason*[tiab] OR glucocorticoid*[tiab] OR hydrocortison*[tiab] OR prednisolon*[tiab] OR prednison*[tiab] OR steroid*[tiab]	1100955

PubMed History August 15, 2019 (Continued)

Search	PubMed Query – August 15, 2019	Items found
#2	"Child"[Mesh] OR "Infant"[Mesh] OR "Adolescent"[Mesh] OR "Minors"[Mesh] OR "Pediatrics"[Mesh] OR child*[tiab] OR schoolchild*[tiab] OR infan*[tiab] OR adolescen*[tiab] OR pediatri*[tiab] OR paediatr*[tiab] OR neonat*[tiab] OR boy[tiab] OR boys[tiab] OR boyhood[tiab] OR girl[tiab] OR girls[tiab] OR girlhood[tiab] OR youth[tiab] OR youths[tiab] OR baby[tiab] OR babies[tiab] OR toddler*[tiab] OR teen[tiab] OR teens[tiab] OR teenager*[tiab] OR newborn*[tiab] OR postneonat*[tiab] OR postnat*[tiab] OR perinat*[tiab] OR puberty[tiab] OR preschool*[tiab] OR suckling*[tiab] OR picu[tiab] OR nicu[tiab] OR kid[tiab] OR kids[tiab] OR kindergarten*[tiab] OR youngster*[tiab] OR juvenil*[tiab] OR minor*[tiab] OR schoolchild*[tiab] OR school child*[tiab] OR underage*[tiab] OR playgroup*[tiab] OR play-group*[tiab] OR playschool*[tiab] OR prepuber*[tiab] OR preadolescen*[tiab] OR junior high*[tiab] OR highschool*[tiab] OR senior high[tiab] OR young people*[tiab] NOT (animals[mh] NOT (humans[mh] AND animals[mh]))	4223171
#1	"Leukemia"[Mesh] OR leukemi*[tiab] OR leukaemi*[tiab] OR leucaemi*[tiab] OR leucemi*[tiab] OR leucocythaemi*[tiab] OR leucocythemi*[tiab] OR bloodcancer*[tiab] OR blood cancer*[tiab]	314869

Embase.com History August 15, 2019

Search	Embase.com Query – August 15, 2019	Items found
#10	#9 NOT ('conference abstract'/it OR 'conference review'/it)	5560
#9	#8 NOT ([animals]/lim NOT [humans]/lim)	6801
#8	#1 AND #2 AND #3 AND #7	6866
#7	#4 OR #5 OR #6	13016263
#6	'sleep'/exp OR 'sleep disorder'/exp OR 'bed time':ab,ti,kw OR bedtime:ab,ti,kw OR ((circadian NEAR/3 rhythm*):ab,ti,kw) OR dream*:ab,ti,kw OR hypersomnia*:ab,ti,kw OR insomnia*:ab,ti,kw OR 'night rest':ab,ti,kw OR ((night NEAR/3 awakening*):ab,ti,kw) OR nightmare*:ab,ti,kw OR parasomnia*:ab,ti,kw OR sleep*:ab,ti,kw OR somnolence:ab,ti,kw OR 'time in bed':ab,ti,kw OR ((night NEAR/3 waking*):ab,ti,kw)	444286
#5	'behavior'/exp OR 'cognition'/exp OR 'mental health'/exp OR 'mental disease'/exp OR 'fatigue'/exp OR 'psychophysiology'/exp OR 'quality of life'/exp OR affect*:ab,ti,kw OR agitation:ab,ti,kw OR agressiv*:ab,ti,kw OR anxiet*:ab,ti,kw OR arousal:ab,ti,kw OR attention*:ab,ti,kw OR awareness:ab,ti,kw OR behavio*:ab,ti,kw OR bipolar:ab,ti,kw OR cognit*:ab,ti,kw OR compulsiv*:ab,ti,kw OR concentrat*:ab,ti,kw OR craving*:ab,ti,kw OR delirium:ab,ti,kw OR dementia:ab,ti,kw OR depress*:ab,ti,kw OR distress:ab,ti,kw OR emotion*:ab,ti,kw OR externaliz*:ab,ti,kw OR externalis*:ab,ti,kw OR fatigue*:ab,ti,kw OR hrqol:ab,ti,kw OR hyperactiv*:ab,ti,kw OR hypomania*:ab,ti,kw OR impulsiv*:ab,ti,kw OR internaliz*:ab,ti,kw OR internalis*:ab,ti,kw OR mania*:ab,ti,kw OR mental*:ab,ti,kw OR mood*:ab,ti,kw OR neuroc*:ab,ti,kw OR neurol*:ab,ti,kw OR neurobehavio*:ab,ti,kw OR neurotox*:ab,ti,kw OR obsessi*:ab,ti,kw OR opposition*:ab,ti,kw OR panic*:ab,ti,kw OR psychi*:ab,ti,kw OR psychol*:ab,ti,kw OR psychop*:ab,ti,kw OR psychos*:ab,ti,kw OR qol:ab,ti,kw OR 'quality of life':ab,ti,kw OR restless*:ab,ti,kw OR sdq:ab,ti,kw OR stress*:ab,ti,kw OR toxicit*:ab,ti,kw	12311540
#4	'adverse event'/exp OR ((cerebral NEAR/3 effect*):ab,ti,kw) OR ((adverse NEAR/3 effect*):ab,ti,kw) OR ((adverse NEAR/3 reaction*):ab,ti,kw) OR ((adverse NEAR/3 event*):ab,ti,kw) OR ((injurious NEAR/3 effect*):ab,ti,kw) OR ((side NEAR/3 effect*):ab,ti,kw) OR ((undesirable NEAR/3 effect*):ab,ti,kw) OR ((unwanted NEAR/3 effect*):ab,ti,kw)	1299122
#3	'glucocorticoid'/exp OR 'steroid'/exp OR corticoid*:ab,ti,kw OR corticosteroid*:ab,ti,kw OR cortisol*:ab,ti,kw OR cortison*:ab,ti,kw OR dehydrocortison*:ab,ti,kw OR dexamethason*:ab,ti,kw OR dexason*:ab,ti,kw OR glucocorticoid*:ab,ti,kw OR hydrocortison*:ab,ti,kw OR prednisolon*:ab,ti,kw OR prednison*:ab,ti,kw OR steroid*:ab,ti,kw	1675623

Embase.com History August 15, 2019 (Continued)

Search	Embase.com Query – August 15, 2019	Items found
#2	'child'/exp OR 'adolescent'/exp OR 'minor (person)'/exp OR 'pediatrics'/exp OR child*:ab,ti,kw OR infan*:ab,ti,kw OR adolescen*:ab,ti,kw OR pediatri*:ab,ti,kw OR paediatr*:ab,ti,kw OR neonat*:ab,ti,kw OR boy:ab,ti,kw OR boys:ab,ti,kw OR boyhood:ab,ti,kw OR girl:ab,ti,kw OR girls:ab,ti,kw OR girlhood:ab,ti,kw OR youth:ab,ti,kw OR youths:ab,ti,kw OR baby:ab,ti,kw OR babies:ab,ti,kw OR toddler*:ab,ti,kw OR teen:ab,ti,kw OR teens:ab,ti,kw OR teenager*:ab,ti,kw OR newborn*:ab,ti,kw OR postneonat*:ab,ti,kw OR postnat*:ab,ti,kw OR perinat*:ab,ti,kw OR puberty:ab,ti,kw OR preschool*:ab,ti,kw OR suckling*:ab,ti,kw OR picu:ab,ti,kw OR nicu:ab,ti,kw OR kid:ab,ti,kw OR kids:ab,ti,kw OR kindergarten*:ab,ti,kw OR youngster*:ab,ti,kw OR juvenil*:ab,ti,kw OR minor*:ab,ti,kw OR schoolchild*:ab,ti,kw OR 'school child*':ab,ti,kw OR underage*:ab,ti,kw OR playgroup*:ab,ti,kw OR 'play-group*':ab,ti,kw OR playschool*:ab,ti,kw OR prepuber*:ab,ti,kw OR preadolescen*:ab,ti,kw OR 'junior high*':ab,ti,kw OR highschool*:ab,ti,kw OR 'senior high':ab,ti,kw OR 'young people*':ab,ti,kw	4862932
#1	'leukemia'/exp OR leukemi*:ab,ti,kw OR leukaemi*:ab,ti,kw OR leucaemi*:ab,ti,kw OR leucemi*:ab,ti,kw OR leucocythaemi*:ab,ti,kw OR leucocythemi*:ab,ti,kw OR bloodcancer*:ab,ti,kw OR 'blood cancer*':ab,ti,kw	438993

PsycINFO (Ebsco) History August 15, 2019

Search	PsycINFO (Ebsco) Query – August 15, 2019	Items found
S8	S1 AND S2 AND S3 AND S7	33
S7	S4 OR S5 OR S6	3003416
S6	DE (“Dreaming” OR “Nightmares” OR “Sleep Onset” OR “Sleep Wake Cycle” OR “Sleepiness” OR “Sleep Disorders” OR “Hypersomnia” OR “Insomnia” OR “Parasomnias” OR “Sleep” OR “Human Biological Rhythms”) OR TI (bed-time OR bedtime OR (circadian N3 rhythm*) OR dream* OR hypersomnia* OR insomnia* OR “night rest” OR (night N3 awakening*) OR nightmare* OR parasomnia* OR sleep* OR somnolence OR “time in bed” OR (night N3 waking*)) OR AB (bed-time OR bedtime OR (circadian N3 rhythm*) OR dream* OR hypersomnia* OR insomnia* OR “night rest” OR (night N3 awakening*) OR nightmare* OR parasomnia* OR sleep* OR somnolence OR “time in bed” OR (night N3 waking*)) OR KW (bed-time OR bedtime OR (circadian N3 rhythm*) OR dream* OR hypersomnia* OR insomnia* OR “night rest” OR (night N3 awakening*) OR nightmare* OR parasomnia* OR sleep* OR somnolence OR “time in bed” OR (night N3 waking*))	111145

PsycINFO (Ebsco) History August 15, 2019 (Continued)

Search	PsycINFO (Ebsco) Query – August 15, 2019	Items found
S5	DE ("Attachment Behavior" OR "Childhood Play Behavior" OR "Classroom Behavior" OR "Coping Behavior" OR "Eating Behavior" OR "Health Behavior" OR "Illness Behavior" OR "Aggressive Behavior" OR "Social Cognition" OR "Social Skills" OR "Antisocial Behavior" OR "Behavior" OR "Prosocial Behavior" OR "Social Behavior" OR "Behavior Disorders" OR "Mental Disorders" OR "Affective Disorders" OR "Mental Health" OR "Mental Disorders due to General Medical Conditions" OR "Childhood Psychosis" OR "Psychosis" OR "Cognition" OR "Fatigue" OR "Psychophysiology" OR "Quality of Life" OR "Agitation" OR "Stress" OR "Distress" OR "Anxiety Disorders" OR "Anxiety" OR "Depression (Emotion)" OR "Neurocognition" OR "Toxic Psychoses") OR TI (affect* OR agitation OR agressiv* OR anxiet* OR arousal OR attention* OR awareness OR behavio* OR bipolar OR cognit* OR compulsiv* OR concentrat* OR craving* OR delirium OR dementia OR depress* OR distress OR emotion* OR externaliz* OR externalis* OR fatigue* OR HRQOL OR hyperactiv* OR hypomania* OR impulsiv* OR internaliz* OR internalis* OR mania* OR mental* OR mood* OR neuroc* OR neurol* OR neurobehavio* OR neurotox* OR obsessi* OR opposition* OR panic* OR psychi* OR psychol* OR psychop* OR psychos* OR QOL OR "quality of life" OR restless* OR SDQ OR stress* OR toxicit*) OR AB (affect* OR agitation OR agressiv* OR anxiet* OR arousal OR attention* OR awareness OR behavio* OR bipolar OR cognit* OR compulsiv* OR concentrat* OR craving* OR delirium OR dementia OR depress* OR distress OR emotion* OR externaliz* OR externalis* OR fatigue* OR HRQOL OR hyperactiv* OR hypomania* OR impulsiv* OR internaliz* OR internalis* OR mania* OR mental* OR mood* OR neuroc* OR neurol* OR neurobehavio* OR neurotox* OR obsessi* OR opposition* OR panic* OR psychi* OR psychol* OR psychop* OR psychos* OR QOL OR "quality of life" OR restless* OR SDQ OR stress* OR toxicit*) OR KW (affect* OR agitation OR agressiv* OR anxiet* OR arousal OR attention* OR awareness OR behavio* OR bipolar OR cognit* OR compulsiv* OR concentrat* OR craving* OR delirium OR dementia OR depress* OR distress OR emotion* OR externaliz* OR externalis* OR fatigue* OR HRQOL OR hyperactiv* OR hypomania* OR impulsiv* OR internaliz* OR internalis* OR mania* OR mental* OR mood* OR neuroc* OR neurol* OR neurobehavio* OR neurotox* OR obsessi* OR opposition* OR panic* OR psychi* OR psychol* OR psychop* OR psychos* OR QOL OR "quality of life" OR restless* OR SDQ OR stress* OR toxicit*)	2959068

PsycINFO (Ebsco) History August 15, 2019 (Continued)

Search	PsycINFO (Ebsco) Query – August 15, 2019	Items found
S4	DE "Side Effects (Drug)" OR TI (cerebral N3 effect* OR adverse N3 effect* OR adverse N3 reaction* OR adverse N3 event* OR injurious N3 effect* OR side N3 effect* OR undesirable N3 effect* OR unwanted N3 effect*) OR AB (cerebral N3 effect* OR adverse N3 effect* OR adverse N3 reaction* OR adverse N3 event* OR injurious N3 effect* OR side N3 effect* OR undesirable N3 effect* OR unwanted N3 effect*) OR (cerebral N3 effect* OR adverse N3 effect* OR adverse N3 reaction* OR adverse N3 event* OR injurious N3 effect* OR side N3 effect* OR undesirable N3 effect* OR unwanted N3 effect*)	79732
S3	DE ("Dexamethasone" OR "Glucocorticoids" OR "Steroids" OR "Corticosterone" OR "Cortisone" OR "Prednisolone" OR "Hydrocortisone" OR "Corticosteroids") OR TI (corticoid* OR corticosteroid* OR cortisol* OR cortison* OR dehydrocortison* OR dexamethason* OR dexason* OR glucocorticoid* OR hydrocortison* OR prednisolon* OR prednison* OR steroid*) OR AB (corticoid* OR corticosteroid* OR cortisol* OR cortison* OR dehydrocortison* OR dexamethason* OR dexason* OR glucocorticoid* OR hydrocortison* OR prednisolon* OR prednison* OR steroid*) OR KW (corticoid* OR corticosteroid* OR cortisol* OR cortison* OR dehydrocortison* OR dexamethason* OR dexason* OR glucocorticoid* OR hydrocortison* OR prednisolon* OR prednison* OR steroid*)	39135

PsycINFO (Ebsco) History August 15, 2019 (Continued)

Search	PsycINFO (Ebsco) Query – August 15, 2019	Items found
S2	ZG (“adolescence (13-17 yrs)” OR “childhood (birth-12 yrs)” OR “infancy (2-23 mo)” OR “neonatal (birth-1 mo)” OR “preschool age (2-5 yrs)” OR “school age (6-12 yrs)”) OR DE “Pediatrics” OR TI (child* OR schoolchild* OR infan* OR adolescen* OR pediatri* OR paediatr* OR neonat* OR boy OR boys OR boyhood OR girl OR girls OR girlhood OR youth OR youths OR baby OR babies OR toddler* OR teen OR teens OR teenager* OR newborn* OR postneonat* OR postnat* OR perinat* OR puberty OR preschool* OR suckling* OR picu OR nicu OR kid OR kids OR kindergarten* OR youngster* OR juvenil* OR minor* OR schoolchild* OR ‘school child*’ OR underage* OR playgroup* OR “play-group*” OR playschool* OR prepuber* OR preadolescen* OR “junior high*” OR highschool* OR “senior high” OR “young people*”) OR AB (child* OR schoolchild* OR infan* OR adolescen* OR pediatri* OR paediatr* OR neonat* OR boy OR boys OR boyhood OR girl OR girls OR girlhood OR youth OR youths OR baby OR babies OR toddler* OR teen OR teens OR teenager* OR newborn* OR postneonat* OR postnat* OR perinat* OR puberty OR preschool* OR suckling* OR picu OR nicu OR kid OR kids OR kindergarten* OR youngster* OR juvenil* OR minor* OR schoolchild* OR ‘school child*’ OR underage* OR playgroup* OR “play-group*” OR playschool* OR prepuber* OR preadolescen* OR “junior high*” OR highschool* OR “senior high” OR “young people*”) OR KW (child* OR schoolchild* OR infan* OR adolescen* OR pediatri* OR paediatr* OR neonat* OR boy OR boys OR boyhood OR girl OR girls OR girlhood OR youth OR youths OR baby OR babies OR toddler* OR teen OR teens OR teenager* OR newborn* OR postneonat* OR postnat* OR perinat* OR puberty OR preschool* OR suckling* OR picu OR nicu OR kid OR kids OR kindergarten* OR youngster* OR juvenil* OR minor* OR schoolchild* OR ‘school child*’ OR underage* OR playgroup* OR “play-group*” OR playschool* OR prepuber* OR preadolescen* OR “junior high*” OR highschool* OR “senior high” OR “young people*”)	1235283
S1	DE “Leukemias” OR TI (leukemi* OR leukaemi* OR leucaemi* OR leucemi* OR leucocythaemi* OR leucocythemi* OR bloodcancer* OR “blood cancer*”) OR AB (leukemi* OR leukaemi* OR leucaemi* OR leucemi* OR leucocythaemi* OR leucocythemi* OR bloodcancer* OR “blood cancer*”) OR KW (leukemi* OR leukaemi* OR leucaemi* OR leucemi* OR leucocythaemi* OR leucocythemi* OR bloodcancer* OR “blood cancer*”)	2258

Cinahl (Ebsco) History August 15, 2019

Search	Cinahl (Ebsco) Query – August 15, 2019	Items found
S8	S1 AND S2 AND S3 AND S7	252
S7	S4 OR S5 OR S6	251981
S6	MH (“Sleep+” OR “Sleep-Wake Transition Disorders+” OR “Sleep Disorders+”) OR TI (bed-time OR bedtime OR (circadian N3 rhythm*) OR dream* OR hypersomnia* OR insomnia* OR “night rest” OR (night N3 awakening*) OR nightmare* OR parasomnia* OR sleep* OR somnolence OR “time in bed” OR (night N3 waking*)) OR AB (bed-time OR bedtime OR (circadian N3 rhythm*) OR dream* OR hypersomnia* OR insomnia* OR “night rest” OR (night N3 awakening*) OR nightmare* OR parasomnia* OR sleep* OR somnolence OR “time in bed” OR (night N3 waking*))	76960
S5	MH (“Child Behavior Checklist” OR “Behavior and Behavior Mechanisms+” OR “Cognition+” OR “Cognition Disorders+” OR “Behavioral and Mental Disorders+” OR “Mental Fatigue+” OR “Social Behavior Disorders+” OR “Mental Health” OR “Neurobehavioral Manifestations+” OR “Psychophysiology” OR “Psychophysiologic Disorders” OR “Quality of Life+”) OR TI (affect* OR agitation OR agressiv* OR anxiet* OR arousal OR attention* OR awareness OR behavio* OR bipolar OR cognit* OR compulsiv* OR concentrat* OR craving* OR delirium OR dementia OR depress* OR distress OR emotion* OR externaliz* OR externalis* OR fatigue* OR HRQOL OR hyperactiv* OR hypomania* OR impulsiv* OR internaliz* OR internalis* OR mania* OR mental* OR mood* OR neuroc* OR neurol* OR neurobehavio* OR neurotox* OR obsessi* OR opposition* OR panic* OR psychi* OR psychol* OR psychop* OR psychos* OR QOL OR “quality of life” OR restless* OR SDQ OR stress* OR toxicit*) OR AB (affect* OR agitation OR agressiv* OR anxiet* OR arousal OR attention* OR awareness OR behavio* OR bipolar OR cognit* OR compulsiv* OR concentrat* OR craving* OR delirium OR dementia OR depress* OR distress OR emotion* OR externaliz* OR externalis* OR fatigue* OR HRQOL OR hyperactiv* OR hypomania* OR impulsiv* OR internaliz* OR internalis* OR mania* OR mental* OR mood* OR neuroc* OR neurol* OR neurobehavio* OR neurotox* OR obsessi* OR opposition* OR panic* OR psychi* OR psychol* OR psychop* OR psychos* OR QOL OR “quality of life” OR restless* OR SDQ OR stress* OR toxicit*)	2429877
S4	(MH “Adverse Drug Event+”) OR TI (cerebral N3 effect* OR adverse N3 effect* OR adverse N3 reaction* OR adverse N3 event* OR injurious N3 effect* OR side N3 effect* OR undesirable N3 effect* OR unwanted N3 effect*) OR AB(cerebral N3 effect* OR adverse N3 effect* OR adverse N3 reaction* OR adverse N3 event* OR injurious N3 effect* OR side N3 effect* OR undesirable N3 effect* OR unwanted N3 effect*)	99762

Cinahl (Ebsco) History August 15, 2019 (Continued)

Search	Cinahl (Ebsco) Query – August 15, 2019	Items found
S3	MH (“Glucocorticoids+” OR “Steroids+”) OR TI (corticoid* OR corticosteroid* OR cortisol* OR cortison* OR dehydrocortison* OR dexamethason* OR dexason* OR glucocorticoid* OR hydrocortison* OR prednisolon* OR prednison* OR steroid*) OR AB (corticoid* OR corticosteroid* OR cortisol* OR cortison* OR dehydrocortison* OR dexamethason* OR dexason* OR glucocorticoid* OR hydrocortison* OR prednisolon* OR prednison* OR steroid*)	99762
S2	MH (“Child+” OR “Adolescence+” OR “Infant+” OR “Pediatrics+”) OR TI (child* OR schoolchild* OR infan* OR adolescen* OR pediatri* OR paediatr* OR neonat* OR boy OR boys OR boyhood OR girl OR girls OR girlhood OR youth OR youths OR baby OR babies OR toddler* OR teen OR teens OR teenager* OR newborn* OR postneonat* OR postnat* OR perinat* OR puberty OR preschool* OR suckling* OR picu OR nicu OR kid OR kids OR kindergarten* OR youngster* OR juvenil* OR minor* OR schoolchild* OR “school child*” OR underage* OR playgroup* OR “play-group*” OR playschool* OR prepuber* OR preadolescen* OR “junior high*” OR highschool* OR “senior high” OR “young people*”) OR AB (child* OR schoolchild* OR infan* OR adolescen* OR pediatri* OR paediatr* OR neonat* OR boy OR boys OR boyhood OR girl OR girls OR girlhood OR youth OR youths OR baby OR babies OR toddler* OR teen OR teens OR teenager* OR newborn* OR postneonat* OR postnat* OR perinat* OR puberty OR preschool* OR suckling* OR picu OR nicu OR kid OR kids OR kindergarten* OR youngster* OR juvenil* OR minor* OR schoolchild* OR “school child*” OR underage* OR playgroup* OR “play-group*” OR playschool* OR prepuber* OR preadolescen* OR “junior high*” OR highschool* OR “senior high” OR “young people*”)	1109291
S1	MH “Leukemia+” OR TI (leukemi* OR leukaemi* OR leucaemi* OR leucemi* OR leucocythaemi* OR leucocythemi* OR bloodcancer* OR “blood cancer*”) OR AB (leukemi* OR leukaemi* OR leucaemi* OR leucemi* OR leucocythaemi* OR leucocythemi* OR bloodcancer* OR “blood cancer*”)	26882

The Cochrane Library History August 15, 2019

Search	The Cochrane Library Query – August 15, 2019	Items found
#8	#1 AND #2 AND #3 AND #7	520
#7	#4 OR #5 OR #6	786707
#6	(bed-time or bedtime or circadian near/3 rhythm* or dream* or hypersomnia* or insomnia* or "night rest" or night near/3 awakening* or nightmare* or parasomnia* or sleep* or somnolence or "time in bed" or night near/3 waking*):ti,ab,kw (Word variations have been searched)	49212
#5	(affect* or agitation or agressiv* or anxiet* or arousal or attention* or awareness or behavio* or bipolar or cognit* or compulsiv* or concentrat* or craving* or delirium or dementia or depress* or distress or emotion* or externaliz* or externalis* or fatigue* or HRQOL or hyperactiv* or hypomania* or impulsiv* or internaliz* or internalis* or mania* or mental* or mood* or neuroc* or neurol* or neurobehavio* or neurotox* or obsessi* or opposition* or panic* or psychi* or psychol* or psychop* or psychos* or QOL or "quality of life" or restless* or SDQ or stress* or toxicit*):ti,ab,kw (Word variations have been searched)	603176
#4	(cerebral near/3 effect* or adverse near/3 effect* or adverse near/3 reaction* or adverse near/3 event* or injurious near/3 effect* or side near/3 effect* or undesirable near/3 effect* or unwanted near/3 effect*):ti,ab,kw (Word variations have been searched)	318023
#3	(corticoid* or corticosteroid* or cortisol* or cortison* or dehydrocortison* or dexamethason* or dexason* or glucocorticoid* or hydrocortison* or prednisolon* or prednison* or steroid*) ti,ab,kw (Word variations have been searched)	76768
#2	(child* or schoolchild* or infan* or adolescen* or pediatri* or paediatr* or neonat* or boy or boys or boyhood or girl or girls or girlhood or youth or youths or baby or babies or toddler* or teen or teens or teenager* or newborn* or postneonat* or postnat* or perinat* or puberty or preschool* or suckling* or picu or nicu or kid or kids or kindergarten* or youngster* or juvenil* or minor* or schoolchild* or (school NEXT child*) or underage* or playgroup* or (play NEXT group*) or playschool* or prepuber* or preadolescen* or (junior NEXT high*) or highschool* or "senior high" or (young NEXT people*)):ti,ab,kw (Word variations have been searched)	291062
#1	(leukemi* or leukaemi* or leucaemi* or leucemi* or leucocythaemi* or leucocythemi* or bloodcancer* or (blood NEXT cancer*)):ti,ab,kw (Word variations have been searched)	13934

Scopus History August 15, 2019

Search	Scopus Query – August 15, 2019	Items found
#8	#1 AND #2 AND #3 AND #7	6344
#7	#4 OR #5 OR #6	19481909
#6	TITLE-ABS-KEY (bed-time OR bedtime OR (circadian W/3 rhythm*) OR dream* OR hypersomnia* OR insomnia* OR "night rest" OR (night W/3 awakening*) OR nightmare* OR parasomnia* OR sleep* OR somnolence OR "time in bed" OR (night W/3 waking*))	481042
#5	TITLE-ABS-KEY (affect* OR agitation OR agressiv* OR anxiet* OR arousal OR attention* OR awareness OR behavio* OR bipolar OR cognit* OR compulsiv* OR concentrat* OR craving* OR delirium OR dementia OR depress* OR distress OR emotion* OR externaliz* OR externalis* OR fatigue* OR hrqol OR hyperactiv* OR hypomania* OR impulsiv* OR internaliz* OR internalis* OR mania* OR mental* OR mood* OR neuroc* OR neurol* OR neurobehavio* OR neurotox* OR obsessi* OR opposition* OR panic* OR psychi* OR psychol* OR psychop* OR psychos* OR qol OR "quality of life" OR restless* OR sdq OR stress* OR toxicit*)	18699373
#4	TITLE-ABS-KEY ((cerebral W/3 effect*) OR (adverse W/3 effect*) OR (adverse W/3 reaction*) OR (adverse W/3 event*) OR (injurious W/3 effect*) OR (side W/3 effect*) OR (undesirable W/3 effect*) OR (unwanted W/3 effect*))	1177803
#3	TITLE-ABS-KEY (corticoid* OR corticosteroid* OR cortisol* OR cortison* OR dehydrocortison* OR dexamethason* OR dexason* OR glucocorticoid* OR hydrocortison* OR prednisolon* OR prednison* OR steroid*)	1154904
#2	TITLE-ABS-KEY (child* OR schoolchild* OR infan* OR adolescen* OR pediatri* OR paediatr* OR neonat* OR boy OR boys OR boyhood OR girl OR girls OR girlhood OR youth OR youths OR baby OR babies OR toddler* OR teen OR teens OR teenager* OR newborn* OR postneonat* OR postnat* OR perinat* OR puberty OR preschool* OR suckling* OR picu OR nicu OR kid OR kids OR kindergarten* OR youngster* OR juvenil* OR minor* OR schoolchild* OR "school child*" OR underage* OR playgroup* OR "play-group*" OR playschool* OR prepuber* OR preadolescen* OR "junior high*" OR highschool* OR "senior high" OR "young people*")	6046290
#1	TITLE-ABS-KEY (leukemi* OR leukaemi* OR leucaemi* OR leucemi* OR leucocythaemi* OR leucocythemi* OR bloodcancer* OR "blood cancer*")	425097


3

Study protocol: DexaDays-2, hydrocortisone for treatment of dexamethasone-induced neurobehavioral side effects in pediatric leukemia patients: a double-blind placebo controlled randomized intervention study with cross-over design

Annelienke M. van Hulst, Emma J. Verwaaijen, Marta Fiocco, Saskia M.F. Pluijm, Martha A. Grootenhuis, Rob Pieters, Erica L.T. van den Akker, Marry M. van den Heuvel-Eibrink

BioMed Central Pediatrics. 2021 Sep;21(1):427-435

ABSTRACT

Background

Dexamethasone, a highly effective drug in treating pediatric acute lymphoblastic leukemia (ALL), can induce serious neurobehavioral side effects. These side effects are experienced by patients and parents as detrimental with respect to health related quality of life (HRQoL). Based on previous studies, it has been suggested that neurobehavioral side effects are associated to cortisol depletion of the mineralocorticoid receptor in the brain. Our previously reported randomized controlled trial, the Dexadagen study (NTR3280), suggests that physiological hydrocortisone addition during dexamethasone treatment may overcome clinically relevant neurobehavioral problems in patients who experience these problems during dexamethasone treatment. With our current study, we aim to replicate these results in a targeted larger sample before further implementing this intervention into standard of care.

Methods

In a national center setting, pediatric ALL patients between 3 and 18 years are enrolled in an Identification study, which identifies patients with clinically relevant dexamethasone-induced neurobehavioral side effects using the Strengths and Difficulties Questionnaire (SDQ). Contributing factors, such as genetic susceptibility, dexamethasone pharmacokinetics as well as psychosocial and family factors are studied to determine their influence in the inter-patient variability for developing dexamethasone-induced neurobehavioral side effects.

Patients with clinically relevant problems (i.e. a rise of ≥ 5 points on the SDQ Total Difficulties Score after 5 days of dexamethasone) are subsequently included in a randomized double-blind placebo-controlled trial with a cross-over design. They receive two courses placebo followed by two courses hydrocortisone during dexamethasone treatment, or vice versa, each time at least 16 days without study medication in between. The primary endpoint is change in SDQ score. The secondary endpoints are sleep (measured with actigraphy and the Sleep Disturbance Scale for Children) and HRQoL (Pediatric Quality of Life Questionnaire).

Discussion

The results of our current study may contribute to the management of future ALL patients who experience dexamethasone-induced neuropsychological problems as it may improve HRQoL for patients who suffer most from dexamethasone-induced neurobehavioral side effects. Furthermore, by investigating multiple risk factors that could be related to inter-patient variability in developing these side effects, we might be able to identify and treat patients who are at risk earlier during treatment.

BACKGROUND

Dexamethasone, a highly effective drug for the treatment of pediatric acute lymphoblastic leukemia (ALL),[1-3] can induce serious neurobehavioral side effects. These side effects are experienced as particularly detrimental to health-related quality of life (HRQoL) by patients and parents.[4] Recent studies emphasize that the mineralocorticoid receptor (MR) in the brain plays an important role in the regulation of mood, behavior and sleep.[5,6] Both the glucocorticoid receptor (GR) and MR are important for the binding of endo- and exogenous glucocorticoids.[5] In animals as well as humans it has been shown that the MR plays an important role in behavior, cognition and psychiatric diseases.[6-11] Besides MR expression in the brain, cortisol affinity and MR:GR balance are thought to be associated with behavior. It has been shown, that the MR has a tenfold greater affinity for endogenous cortisol than the GR.[12] Synthetic glucocorticoids mostly have the GR as their therapeutic target: dexamethasone has a high potency to activate GRs, but does not bind MRs.[13] In patients treated with glucocorticoids the production of endogenous cortisol is suppressed. Therefore, in patients treated with high doses dexamethasone, the hypothesis is that the GR in the brain is stimulated, whereas the MR is underactivated. The disturbance of this GR:MR balance conceivably deregulates the stress-system and enhances vulnerability to stress-related problems.[5]

Consequently, we previously hypothesized that pediatric ALL patients who receive dexamethasone treatment, cortisol depletion of the MR in the brain may be responsible for attendant neurobehavioral problems. We therefore performed a randomized controlled trial (RCT), the DexaDays-1 trial, to investigate whether these side effects could be ameliorated by adding a physiological dose of hydrocortisone which stimulates the MRs in the brain in a physiological way.[14] No beneficial effect of hydrocortisone on neurobehavioral problems could be shown in the complete group of 46 patients. However, in a small subgroup of patients with clinically relevant dexamethasone-induced neurobehavioral or sleeping problems (n = 16 and n = 9 respectively), hydrocortisone addition had a significant beneficial effect.[14] Our results suggest that neurobehavioral and sleeping problems can be reduced in children who are most affected. Before implementing this into standard clinical practice, we felt that the results require replication in a larger patient cohort with clinically relevant dexamethasone-induced neurobehavioral problems. Hence, we initiated the DexaDays-2 trial in 2018.

Several factors may be associated to neurobehavioral side effects during dexamethasone treatment which warrant further study.

Firstly, the role of genetic variation is evaluated. Several studies found single nucleotide polymorphisms (SNPs) in the MR and GR gene, which could contribute to inter-individual differences in increased glucocorticoid sensitivity and neurobehavioral and sleeping problems.[7,9,12,15-20] Carrier status of specific relevant SNPs which have been linked before to psychopathology or sleeping problems may be associated to dexamethasone-induced side effects.

Secondly, dexamethasone pharmacokinetics may play a role. Dexamethasone clearance is higher in younger children, hence taking an inter-patient variability in dexamethasone levels during maintenance phase into account is important.[21]

Thirdly, psychosocial and environmental factors may influence the severity of neurobehavioral side effects. It has been previously shown that the child's distress during procedures in childhood cancer treatment is associated with parental distress.[22] Parental stress is associated with behavioral problems in children.[23] Overall, parental stress could potentially accelerate the development of dexamethasone-induced behavioral problems.[24] Furthermore, some social (family) risk factors, but also psychosocial support can influence coping strategies of parents and may thereby influence their perceptions of the problems caused by dexamethasone.[25,26]

METHODS

General study design

The DexaDays-2 study is a Dutch national study and is coordinated from the Princess Máxima Center for pediatric oncology. The study consists of two parts: an *Identification study* (T1-T2) and a *Randomized Controlled Trial (RCT)* (T3-T11). Figure 1 gives a schematic overview of the complete study. Tables 1 and 2 depict the content of all measurements in the Identification study and RCT, respectively.

In- and exclusion criteria

Every Dutch ALL patient is screened on in- and exclusion criteria. After permission of their pediatric oncologist, eligible patients are approached by the study team. Patients are eligible if they fulfill the following criteria: age 3–18, confirmed diagnosis of acute lymphoblastic leukemia (ALL), inclusion in DCOG ALL MRG protocol and able to comply with scheduled follow-up. Only patients between 3 and 18 years can participate because our questionnaires are validated for these ages. Exclusion criteria are: patient or parent refusal, anticipated compliance problems, underlying conditions which affect the absorption of oral medication, pregnant or lactating patients, current uncontrolled infection or any other complications which may interfere with dexamethasone treatment, language barrier, pre-existing mental retardation, current hydrocortisone use or risperidone use.

In addition, to be eligible for the RCT, a patient has to show a rise of five or more points on the SDQ Total Difficulties scale after five days of dexamethasone treatment.

Randomized Controlled Trial

The main study is a prospective double-blind placebo-controlled randomized trial (RCT) with a cross-over design. The primary aim of the RCT is to replicate the finding that addition of physiological doses of hydrocortisone to standard dexamethasone treatment reduces neurobehavioral side effects in pediatric ALL patients who suffer from clinically relevant dexamethasone-induced neurobehavioral problems. Neurobehavioral problems are measured with the parent-reported Strengths and Difficulties Questionnaire in Dutch (SDQ)[27] at every time point (T3-T11) (Figure 1 and Table 2).

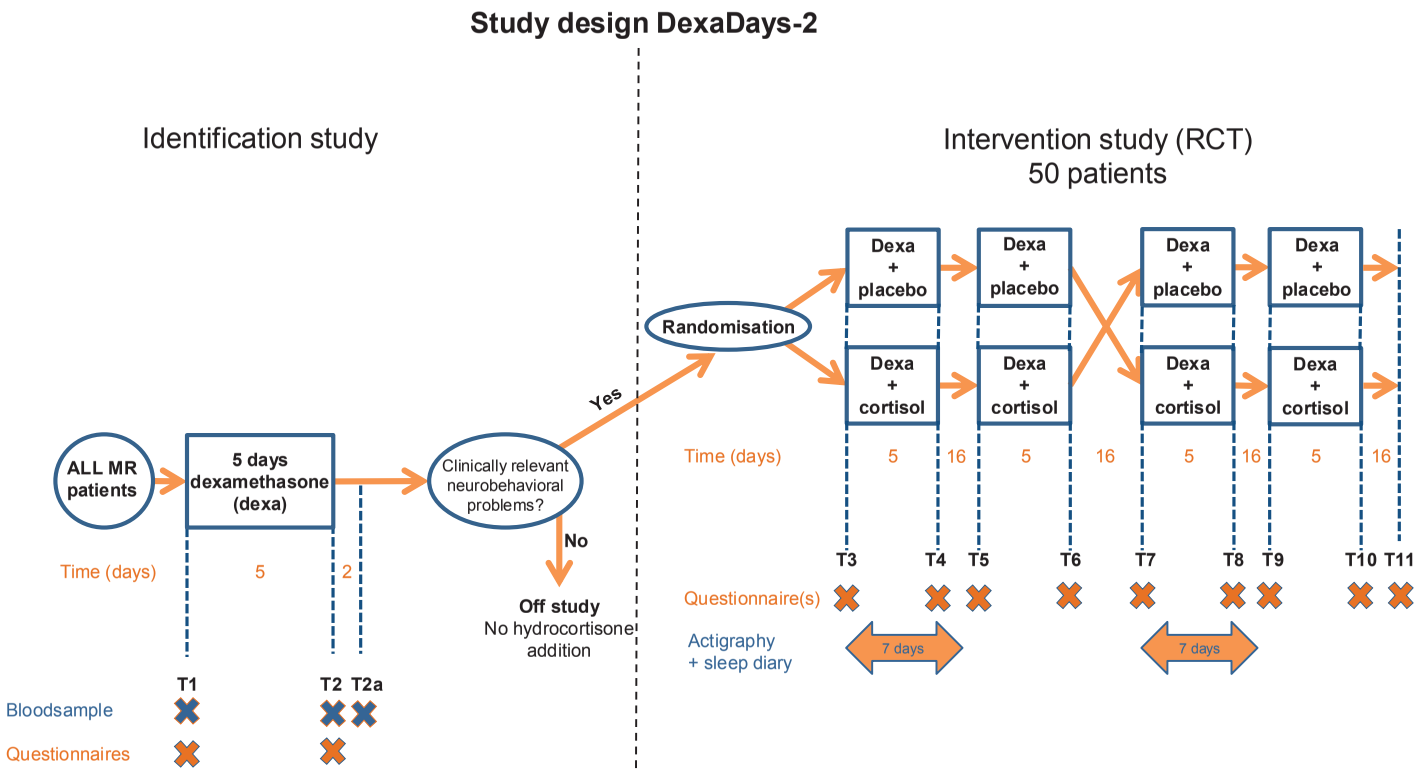


Figure 1. Study Design DexaDays-2. Schematic study design of the DexaDagen-2 study. See Table 1 (Identification study) and Table 2 (RCT) for the specification of blood samples and questionnaires.

Abbreviations: RCT: randomized controlled trial, ALL: acute lymphoblastic leukemia, MR: medium risk, Dexa: dexamethasone

Table 1. Procedures in the Identification Study

Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Timepoint	**T1**					**T2**		**T2a**
Procedure								
Standard medication								
- *Dexamethasone*	X	X	X	X	X			
- *Vincristine*	X							X
- *Methotrexate*	X							
Questionnaires								
- *SDQ*	X					X		
- *SDSC*	X					X		
- *PSI*	X					X		
- *DT*	X							
- *DT (short)*						X		
- *Support*	X							
- *Support (short)*						X		
- *Eating thermometer*	X					X		
Blood sample								
- *Genetics*	X							
- *Dexa peak level*	X					X		X
- *Dexa trough level*								
Somatic parameters	X					X		X
- *Weight*	X					X		X
- *Height*	X					X		X
- *Blood pressure*								
Done at (h= home / H= hospital)	H	h	h	h	h	H	h	H

The Identification study is the cohort from which eligible candidates for the Randomized Controlled Trial are selected. T1 = start dexamethasone. T2 = after 5 days of dexamethasone. T2a = 2 days after stop dexamethasone. See Figure 1 for a schematic overview
Abbreviations: SDQ: Strengths and difficulties questionnaire, SDSC: Sleep disturbance scale for children, PSI: Parenting stress index, DT: Distress thermometer, PedsQL: Pediatric quality of life questionnaire

Table 2. Procedures and intervention in the RCT

Day	**1 / 43**	**2 / 44**	**3 / 45**	**4 / 46**	**5 / 47**
Timepoint	**T3/T7**				
Procedure					
Standard medication					
- *Dexamethasone*	X	X	X	X	X
- *Vincristine*	X				
- *Methotrexate*	X				
Study medication					
- *Hydrocortisone or placebo*	X	X	X	X	X
Questionnaires					
- *SDQ*	X				
- *SDSC*	X				
- *PedsQL generic*	X				
- *DT (short)*	X				
- *Eating thermometer*	X				
Sleep diary	X	X	X	X	X
Actigraphy	X	X	X	X	X
Somatic parameters					
- *Weight*	X				
- *Height*	X				
- *Blood pressure*	X				
Done at (h= home / H= hospital)	H	h	h	h	h

T3/T7 & T5/T9 = start of dexamethasone and study medication. T4/T8 & T6/T10 = after 5 days of dexamethasone and study medication. T11 = closing visit. See Fig. 1 for a schematic overview

	6 / 48 T4/T8	7 / 49	22 / 64 T5/T9	23 / 65	24 / 66	25 / 67	26 / 68	27 / 69 T6/T10	85 T11
			X	X	X	X	X		X
			X						X
			X						X
			X	X	X	X	X		
	X		X					X	X
	X								
	X								
	X								
	X								
	X	X							
	X	X							
	X		X						X
	X		X						X
			X						X
	h	h	H	h	h	h	h	h	H

Abbreviations: RCT: Randomized controlled trial, SDQ: Strengths and difficulties questionnaire, SDSC: Sleep disturbance scale for children, PedsQL: Pediatric quality of life questionnaire, DT: Distress thermometer

The secondary aim is to estimate the percentage of patients with clinically relevant dexamethasone-induced sleeping problems and replicate our previous finding that addition of physiological doses of hydrocortisone to standard dexamethasone treatment reduces these sleeping problems. Sleeping difficulties are measured using the Sleep Disturbance Scale for Children (SDSC)[28,29] at T3+4 and T7+8, i.e. before and after one course hydrocortisone and one course placebo. Sleep is measured through actigraphy as well.[30] Patients wear a nonintrusive wrist actigraph for seven days.[31] Parents are asked to keep a sleep log during the actigraphy measurement to interpret the data. These measurements take place twice: once when a patient receives hydrocortisone and once during placebo.

We also evaluate whether hydrocortisone addition improves HRQoL in patients with dexamethasone-induced clinically relevant neurobehavioral problems. HRQoL is measured with the Pediatric Quality of Life questionnaire (PedsQL).[32] The PedsQL is filled in before and after a hydrocortisone and placebo course, at T3+4 and T7+8.

Randomization and blinding

Patients are allocated to start with hydrocortisone or placebo using the method of a prefixed randomization list. This randomization list is prepared by the pharmacy, independent of the clinical investigators. The study is double blinded. Blinding of subject, researchers and physicians is ensured through use of the investigational medicinal product (IMP) and an identical placebo solution. In case of problems regarding study medication, the randomization list is available 24 hours per day through the pharmacy.

Investigational treatment

The IMP is hydrocortisone solution, given orally. The drug is administered in physiological dosages of 10 mg/m2/day. Patients use hydrocortisone (1 mg/ml) or placebo three times daily divided in a ratio of 5:3:2, following the physiological circadian rhythm.

Patients receive hydrocortisone (two consecutive courses) or placebo (two consecutive courses) in a randomized order during a five-day dexamethasone treatment. A washout period of at least 2 weeks and 2 days is always present between courses to prevent carry-over effect. After 2 courses cross-over takes place (Figure 1). The washout period renders the carry-over effect in the next period negligible. The idea is to use each patient as his own control by trying both regiments at different times and comparing the results.

Pharmacovigilance is guaranteed by measuring the fluid volume in the medicine bottles after each 5-day course of study medication.

Power calculation for the primary outcome parameter

A sample size of 23 pairs with a correlation equal to 0 achieves 79% power to detect a difference of -5,2 between the null hypothesis mean difference of 0 and the actual mean difference of -5,2 at the 5% significance level (alpha) using a two-sided Wilcoxon Signed-Rank Test. These results are based on 3000 Monte Carlo samples from the null distribution: Normal with mean 3.4 and standard deviation 5.4 and the alternative distribution Normal with mean 8.6 and standard deviation equal to 6.3. Power computations are performed with PASS 2020 Power Analysis & Sample Size (https://www.ncss.com/software/pass/). We will include 50 patients in our RCT.

Identification study

The Identification study aims to select eligible patients for the RCT. Based on our previous study, we estimate that 40% of the included ALL patients experience clinically relevant neurobehavioral side effects.[14] Estimating the probability of a 10% dropout rate, a 35% refusal rate and exclusion of 15% based on our exclusion criteria, a total of approximately 150 patients will be included in the Identification study course (Figure 2).

The secondary aims for studies in the Identification cohort are to investigate possible factors associated to the inter-patient variability in dexamethasone-induced neurobehavioral problems, including pharmacokinetics, candidate single nucleotide polymorphisms (SNP) analyses and psychosocial and environmental factors. Patients with a rise of five or more points on the SDQ Total Difficulties score after five days of dexamethasone (T1-T2, Figure 1) will be compared with patients with a rise of four or less points. Dexamethasone kinetics are measured through peak levels (measured 2–3 hours after the first dexamethasone administration on day 1 of the dexamethasone course (T1)) and trough levels (measured on day 6 (T2), after the last dexamethasone dose the previous evening). To identify possible very slow metabolizers, an additional blood sample will be taken on day 8, i.e. two full days after the last dexamethasone dose (T2a). A blood sample to evaluate carrier status of several relevant candidate SNP is taken on T1. Germline DNA will be extracted and candidate SNP analysis of the GR gene (NR3C1), including BclI polymorphism (rs41423247 variant), ER22/23EK polymorphism (rs6189) and N363S/A1220G polymorphism, and the AHSG gene (rs4918 variant) will be included. Psychosocial and environmental factors include parenting stress, measured with the NOSI-K (Nijmeegse Ouderlijke Stress Index),[33] i.e. the adapted and shortened Dutch version of the Parental Stress Index (PSI)[34] and the Distress Thermometer (DT).[35,36]

Several questions about received psychosocial support and education are filled in at T1 and T2. Eating and hunger satiety are measured using an Eating thermometer (a visual analogue scale to indicate hunger).

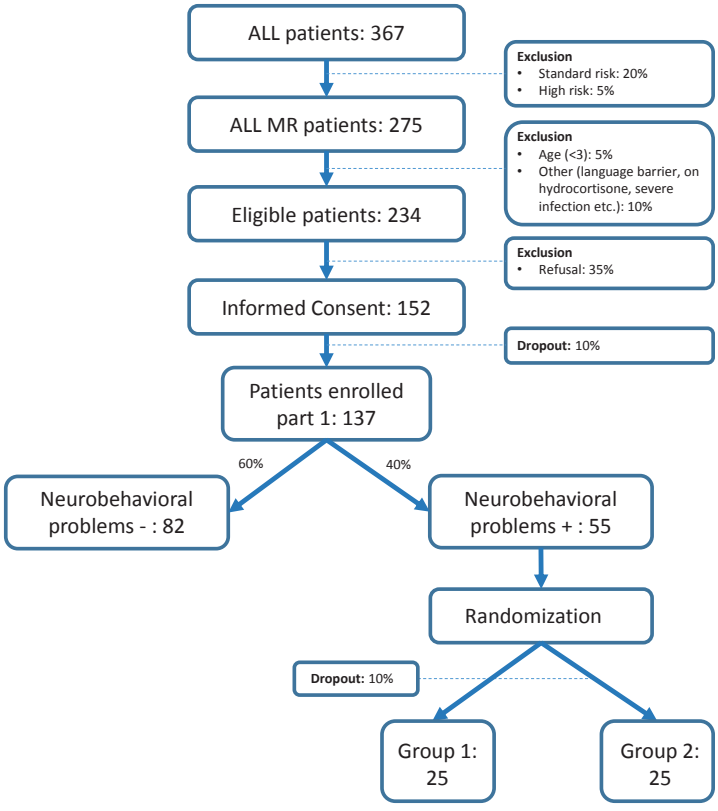


Figure 2. Flow chart of expected number of acute lymphoblastic leukemia (ALL) patients in the DexaDays-2 study. Group 1: starting with two courses hydrocortisone, thereafter cross over to placebo. Group 2: starting with two courses placebo, thereafter cross over to hydrocortisone

Statistical analysis

The effect of treatment (n = 50) is assessed by comparing placebo with hydrocortisone on SDQ Total Difficulties Score (delta scores; subtracting the score on treatment day 1 from the score after treatment day 5) by employing a Paired Student's T-test or Wilcoxon Signed Rank test in case of violation of normality assumption. SDQ sub scores will also be compared between the two groups. The effect of hydrocortisone on sleep and HRQoL (total and sub scores) is evaluated in the same way.

Due to the presence of repeated measures in the design of the RCT a generalized mixed model will be estimated to study the effect of therapy on neurobehavioral outcomes. This model explicitly accounts for the correlations between repeated measurements within each patient. Results from this analysis will provide information about the longitudinal effect of the treatment. A treatment period interaction will be included in the model to investigate the groups effect over time.

To study the associations in the Identification group between potential determinants (genetics, pharmacokinetics and environmental factors) and the occurrence of dexamethasone-induced neurobehavioral problems a binary logistic regression model will be estimated. Odds ratios along with 95% confidence interval will be provided.

Data, monitoring and publication

All data is collected and stored in agreement with good clinical practice (GCP) guidelines. Certified members of the study team collect data on paper case report forms. OpenClinica Enterprise Version 3.13 is used to further collect and manage data. Blood is stored for 15 years. Deblinding takes place at the end of the study, after which the database will be frozen.

All questionnaires are web based and data is collected through a secure website, www.hetklikt.nu, a safe internet environment, which is widely used in pediatric (oncology) care in the Netherlands.[37]

Adverse events are recorded, and all serious adverse events are reported to the competent authority by the investigator without undue delay, according to GCP. Patients can discontinue study participation at all times, without providing a reason for withdrawal. Standard insurance contracts apply in case of any unforeseen harm. Since patients are treated for a short time frame (2x 5 days) and the drug under investigation is well characterized and given in a physiological dose, we do not expect any suspected unsuspected serious adverse reactions.[14]

An independent certified third party (Julius Clinical) monitors the study. All processes including informed consent procedure, data collection and data management are monitored by this party. Monitoring takes place twice per year.

The results of this study will be disclosed unreservedly in the form of scientific publications. Participants are notified of study proceedings through regular newsletters.

When necessary the protocol can be modified or additions can be made. This can be done through amendments, which need approval by the Medical Ethical Committee.

DISCUSSION

This paper describes the DexaDays-2 protocol: a randomized controlled trial set up to replicate the finding that addition of physiological doses of hydrocortisone to standard dexamethasone treatment reduces neurobehavioral side effects in pediatric ALL patients. Our previous study suggests that patients with clinically relevant neurobehavioral problems benefit from treatment with hydrocortisone.[14] Currently no other satisfying options to treat dexamethasone-induced neurobehavioral problems are available.[38] The results of this study may affect the management of future ALL patients with dexamethasone-induced side effects as it may improve HRQoL for those who suffer most from these problems. Our study could also be important for adult patients or children with other conditions who receive dexamethasone and experience the accompanying neurobehavioral side effects.[38,39] Furthermore, by investigating possible risk factors that could influence the inter-patient variability, we might be able to identify patients at risk for dexamethasone-induced neurobehavioral problems at an earlier stage, providing a possible intervention. Besides earlier recognition, the potential identification of risk factors for dexamethasone-induced neurobehavioral problems might lead to new outcomes which could be targeted to deal with these problems. For example, parenting stress or received support could be established as risk factors, providing starting points for non-pharmacological interventions.

Several strong points of this study can be discussed. First, every patient with ALL in the Netherlands can be screened on eligibility for this study, rendering a large and hopefully unbiased population. From this population, we select patients who might benefit from the intervention, following the results of our previous DexaDagen-1 study. Second, the design, a double-blind placebo controlled randomized controlled trial with cross-over, will minimize the risk of bias in our RCT. Third, we measure the effect of hydrocortisone in two subsequent dexamethasone courses. This is an addition to our previous study protocol, by which we want to mimic the normal situation of repetitive dexamethasone courses, and to investigate whether the possible effect of hydrocortisone is lasting.

Some possible study limitations have to be taken into account as well. To begin with, some patients might already use hydrocortisone therapy because patients heard of positive results from participants in our previous DexaDays-1 study. We address this problem by communicating the importance of our current study with all treating pediatric oncologists and asking them to include these patients in the study. Furthermore, because patients are coming to the Princess Máxima Center from the whole country, one extra visit might be a barrier for patients living far away. We try to overcome this problem by offering reimbursement of travel expenses and making it possible to visit a patient at home if the extra visit is the only objection of parents to participate. Another potential limitation is

that mainly patients who experience dexamethasone-induced neurobehavioral problems are motivated to participate in the trial. This could result in too few children without neurobehavioral problems and this may affect the study of possible determinants of dexamethasone-induced side effects. To investigate the presence of possible bias, we ask non-responders to fill in a short non-obligatory questionnaire with questions regarding dexamethasone-induced side effects. This allows for comparison between participants and non-participants. If too few patients are included in the Identification study when we reach 50 patients in the RCT, it may be possible to continue the Identification study to generate a larger group to answer our secondary research questions by amending the protocol.

In conclusion, this study is set up to establish whether hydrocortisone addition to standard dexamethasone treatment is an effective therapy for dexamethasone-induced serious neurobehavioral side effects. With this therapy we aim to improve health related quality of life for ALL patients who suffer from this side effect during their 1.5 year treatment schedule.

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Unraveling dexamethasone-induced neurobehavioral and sleep problems in children with acute lymphoblastic leukemia: which determinants are important?

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ABSTRACT

Purpose

Dexamethasone, the preferred corticosteroid in most treatment protocols for pediatric acute lymphoblastic leukemia (ALL), can induce undesirable side effects. Neurobehavioral and sleep problems are frequently reported, but the inter-patient variability is high. We therefore aimed to identify determinants for parent-reported dexamethasone-induced neurobehavioral and sleep problems in pediatric ALL.

Patients and methods

Our prospective study included medium risk ALL patients and their parents during maintenance treatment. Patients were assessed before and after one five-day dexamethasone course. Primary endpoints were parent-reported dexamethasone-induced neurobehavioral and sleep problems, measured with the Strengths and Difficulties Questionnaire (SDQ) and Sleep Disturbance Scale for Children (SDSC), respectively. Analyzed determinants included patient and parent demographics, disease and treatment characteristics, parenting stress (Parenting Stress Index and Distress Thermometer for parents), dexamethasone pharmacokinetics and genetic variation (candidate single nucleotide polymorphisms *rs41423247* and *rs4918*). Statistically significant determinants identified in univariable logistic regression analyses were incorporated in a multivariable model.

Results

We included 105 patients: median age was 5.4 years (range 3.0-18.8) and 61% were boys. Clinically relevant dexamethasone-induced neurobehavioral and sleep problems were reported by parents in 70 (67%) and 61 (59%) patients respectively. In our multivariable regression models, we identified parenting stress as significant determinant for parent-reported neurobehavioral (odds ratio (OR) 1.16, 95%-confidence interval (95%-CI) 1.07-1.26) and sleep problems (OR 1.06, 95%-CI 1.02-1.10). Furthermore, parents who experienced more stress before start of a dexamethasone course reported more sleep problems in their child (OR 1.16, 95%-CI 1.02-1.32).

Conclusion

We identified parenting stress, and not dexamethasone pharmacokinetics, genetic variation, patient/parent demographics, or disease/treatment characteristics, as a significant determinant for parent-reported dexamethasone-induced neurobehavioral and sleep problems. Parenting stress may be a modifiable target to reduce these problems.

INTRODUCTION

Dexamethasone is currently the preferred corticosteroid in most treatment protocols for pediatric acute lymphoblastic leukemia (ALL).[1-3] However, dexamethasone has various undesirable side effects. Patients and parents often report neurobehavioral problems as harmful side effects, which generally negatively affect quality of life.[4-6] These neurobehavioral problems include mood swings, behavioral changes but also depression or psychosis, and occur in 5 to 75% of patients.[7-10] Sleep problems, such as insomnia and hypersomnia, are also well-established adverse effects of supraphysiological steroid treatment in children, with an estimated prevalence between 19 and 87%.[11-14]

For better understanding of the differences between patients and parents who do and do not report dexamethasone-induced neurobehavioral or sleep problems and to develop targeted interventions, it is important to identify contributing determinants. Our recent systematic review recognized younger patient age as potential determinant for dexamethasone-induced neurobehavioral problems, whereas older patients are at risk of sleep problems.[15] Furthermore, single nucleotide polymorphisms (SNPs) may contribute to inter-individual differences.[16] Two small studies suggested an association between the *Bcl-1* polymorphism (*glucocorticoid receptor (GR)* gene) and depressive symptoms.[17,18] The *rs4918* polymorphism (*Alpha2-HS glycoprotein (AHSG)* gene) was suggested to be associated with impaired sleep during dexamethasone treatment in ALL patients.[19] However, these results remain to be replicated. Furthermore, in adults, a higher steroid dose appears to increase the risk of steroid-induced mental disorders.[20] Younger children have a higher dexamethasone clearance,[21] however, the role of dexamethasone pharmacokinetics in the occurrence of neurobehavioral or sleep problems remains unclear. Other possible determinants such as parenting stress, parental coping or (medical) background have only been suggested in case reports and series.[15] Besides, a large drawback of most studies that investigated prognostic factors for dexamethasone-induced side effects, are their retrospective nature and/or use of unvalidated outcome measurement tools.

Therefore, our current study aimed to identify possible determinants for dexamethasone-induced parent-reported neurobehavioral and sleep problems, in a prospective national cohort of pediatric ALL patients, using validated questionnaires. In addition, we explored parental coping during dexamethasone treatment, since this may be an important and modifiable factor for possible interventions.

METHODS

Study design and participants

This prospective study was pursued in the setting of a randomized controlled trial, of which the design was previously reported.[22] Additional relevant methods are available as Supplement. In brief, patients between 3 and 18 years, treated according to the Dutch Childhood Oncology Group ALL-11 protocol who received dexamethasone during Medium Risk Group maintenance treatment and their parents were asked to participate.

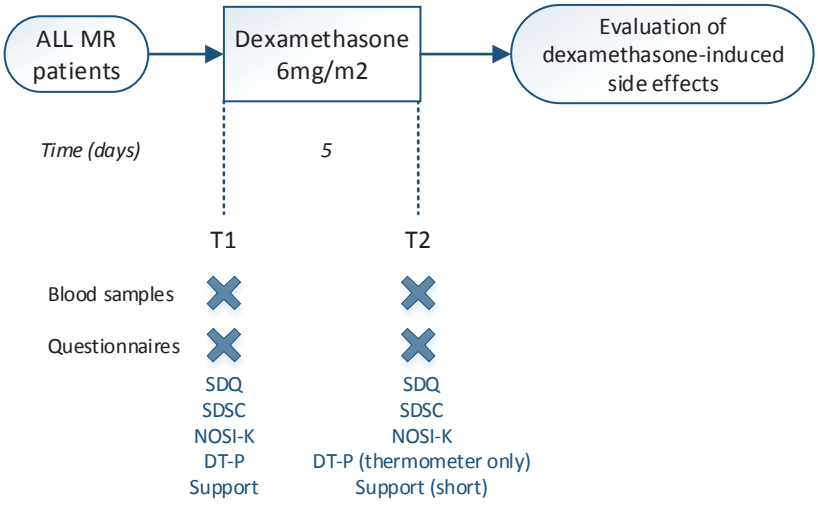

Figure 1. Study design.

Acute lymphoblastic leukemia patients were included during maintenance therapy. Before (T1) and after (T2) five days of dexamethasone treatment peripheral blood samples were collected and validated questionnaires were filled in by parents. At T2 the DT-P consisted of only the thermometer, and the support questionnaire was one additional question.

Abbreviations: ALL: acute lymphoblastic leukemia, DT-P: distress thermometer for parents, MR: medium risk, NOSI-K: Nijmeegse Ouderlijke Stress Index Korte versie (shortened Dutch version of the Parenting Stress Index), SDQ: strengths and difficulties questionnaire, SDSC: sleep disturbance scale for children.

All included patients were assessed on the first day (T1), before the start of a dexamethasone course, and after five full days (T2) of dexamethasone (6 mg/m2/day). Peripheral blood samples were obtained on both days. Parents were asked to complete questionnaires on both days (Figure 1). All parents and/or patients provided written informed consent to participate. The study was approved by the Medical Ethical Committee of Rotterdam (NL62388.078.17).

Outcome measures

Neurobehavioral problems

We used the validated Dutch version of the parent-reported Strengths and Difficulties Questionnaire (SDQ) to assess neurobehavioral problems.[23-27] This questionnaire measures psychological adjustment of children and adolescents using five subscales: emotional symptoms, conduct problems, hyperactivity-inattention, peer problems and prosocial behavior. The Total Difficulties score was calculated by adding the first four subscales; a higher score reflects more problems. Outcome measures were dichotomized: an increase of ≥5 points after five days of dexamethasone was considered as clinically relevant dexamethasone-induced neurobehavioral problems.[11] The SDQ is also available as self-report for children ≥11 years and was therefore offered to these patients.[27]

Sleep problems

Sleep quality was assessed with the parent-reported Sleep Disturbance Scale for Children (SDSC).[28,29] This validated questionnaire yields six subscales: disorders of initiating and maintaining sleep (DIMS), disorders of arousal (DA), sleep-wake transition disorders (SWTD), disorders of excessive somnolence (DES), and sleep hyperhidrosis (SHY). The sum provides a total sleep score; a higher score reflects more problems. An increase of ≥7 points on this total score, after five days of dexamethasone, was considered as clinically relevant sleep problems.[11] The SDSC is not available as self-report.

Determinants

Patient and parent demographics, disease and treatment characteristics

Patient, treatment and disease characteristics taken into account were age, sex, week in maintenance phase, ALL subtype (B-cell ALL or T-cell ALL), concurrent asparaginase use,[21] and central nervous system (CNS) involvement. Both the SDQ Total Difficulties and the SDSC total score at T1 (i.e. pre-existing neurobehavioral or sleep problems) were evaluated as possible determinants. Family characteristics taken into account were number of siblings and parental factors such as age, nationality, highest level of education of both parents, and which parent completed the questionnaires.

Support and parental coping

We developed a short survey regarding received support (see Supplement). Questions concerned whether a child and/or parent received psychological or other support and why. Furthermore, since it is known from literature that coping with dexamethasone pulses in maintenance treatment can be extremely stressful,[4] we asked parents to qualitatively describe how they cope with the dexamethasone treatment: how do they prepare their family and how do they manage during the treatment days. The qualitative responses were collected and pre-defined keywords were highlighted (e.g. 'nothing/normal' or

'resting'). If a response which was not predefined was noted more than five times, this was also highlighted (e.g. 'lax parenting'). The highlighted keywords were totaled. At T2, one question regarding received support during the dexamethasone course was included.

Parenting stress

To measure parenting stress at T1 and T2 we used the NOSI-K (Nijmeegse Ouderlijke Stress Index Korte versie),[30] an adapted version of the PSI (Parenting Stress Index).[31] This validated 25-item questionnaire measures stress experienced within the parenting role. A higher score reflects higher stress levels (range 25-150).

We also used the Distress Thermometer for parents (DT-P).[32] This validated questionnaire consists of three parts: 1) a thermometer (visual analogue scale) ranging from 0 (no distress) to 10 (extreme distress), 2) a total score based on a problem list about everyday problems and 3) additional questions concerning support, lack of understanding, and parental chronic illness. The total questionnaire and thermometer were completed by parents at T1, the thermometer at T2.

Dexamethasone pharmacokinetics

At T1, a peripheral blood sample was obtained approximately two hours after the first dexamethasone dose on day 1 (peak level). The exact time of intake and blood sampling was registered. For the measurement at T2 (trough level), parents were asked to document the exact time of the last dexamethasone administration the evening before. The moment of T2 blood sampling was registered. To calculate area under the curve (AUC) of dexamethasone, we selected patients with both a peak and trough measurement concentration value within 24 hours after dexamethasone administration. We calculated slope and intercept values of the concentration and time after dose (0-24h) plots for each patient using a linear regression method. We then used these values to get the extrapolated intercept on both x-axis and y-axis and calculated the triangle area as the AUC in each patient (Equation 1).

Equation 1: $$AUC = \frac{intercept_x * intercept_y}{2}$$

Candidate SNP assessment

We used a candidate SNP approach, analyzing whether *Bcl1 (rs41423247 G>C, GR gene)* and *rs4918 C>G (AHSG gene*) polymorphisms were associated with dexamethasone-induced neurobehavioral or sleep problems, respectively.[17-19] We analyzed both dominant models (e.g. CC vs. CG+GG: heterozygous carrier status) and recessive models (e.g. CC+CG vs. GG: homozygous carrier status). Details about the methodology are available in the Supplement.

Statistical analysis

Descriptive statistics with either means and standard deviations or medians with interquartile ranges (IQR) were calculated. The difference between SDQ and SDSC scores at T1 versus T2 was tested with a Wilcoxon signed-rank test. The intra-class correlation coefficient (ICC) was calculated to compare SDQ data of parents and children (two-way mixed effects model, single measures, absolute agreement).

Univariable logistic regression models were estimated to explore associations between the potential determinants and either neurobehavioral or sleep problems. Odds ratios (OR) with 95% confidence intervals (95%-CI) were estimated. Significant determinants with a p-value <0.20 and four or more patients in each cell of the contingency table were included in the multivariable model. Furthermore, variables that were clinically relevant based on reported literature (patient age and sex[15,33,34] and maintenance week[35,36]), as well as T1 SDQ score (for neurobehavioral problems) and T1 SDSC score (for sleep problems), were included in the multivariable logistic regression model. Multicollinearity was checked as described before.[37] All analyses were performed using IBM SPSS Statistics version 26.0.

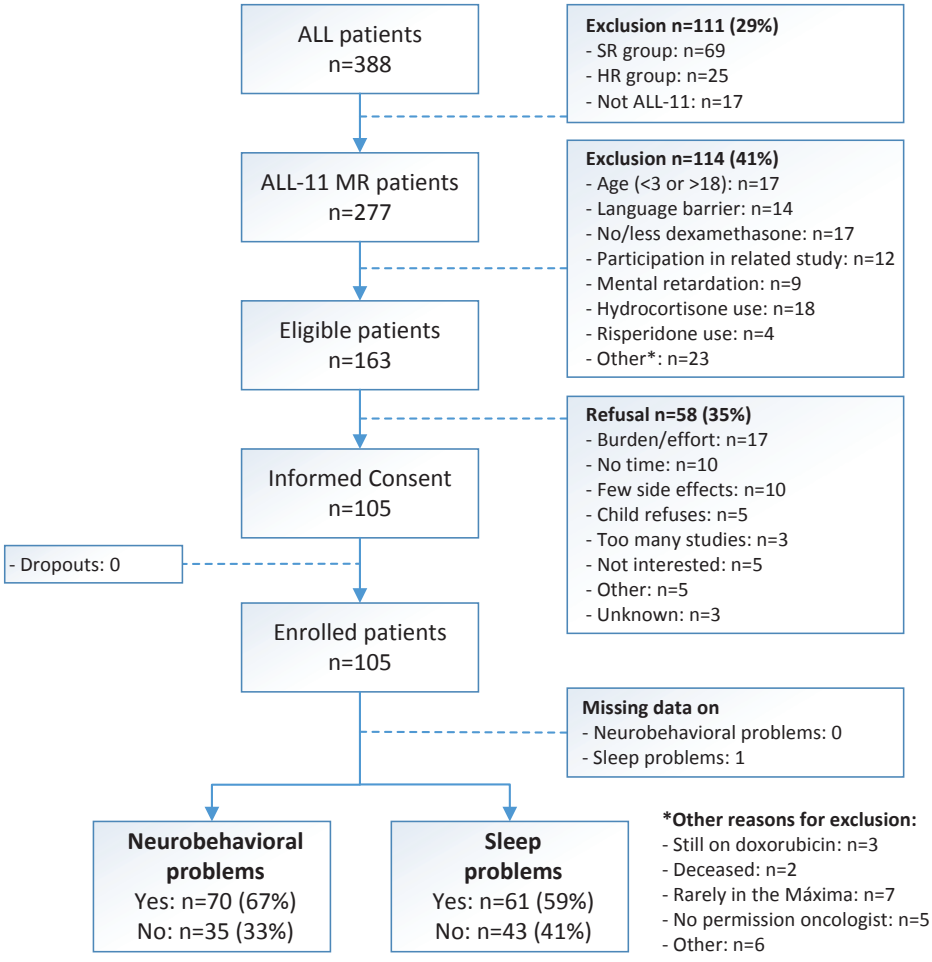

Figure 2. CONSORT diagram

Eligible acute lymphoblastic leukemia patients were approached for inclusion after approval of the treating pediatric oncologist. Reasons for refusal were stated by parents or patients. Patients with a rise of five points on the SDQ or seven points on the SDSC after 5 days of dexamethasone treatment were classified as having clinically relevant dexamethasone-induced neurobehavioral or sleep problems, respectively.

Abbreviations: ALL: acute lymphoblastic leukemia, HRG: high risk group, MR: medium risk, SDQ: Strengths and difficulties questionnaire, SDSC: sleep disturbance scale for children, SRG: standard risk group.

RESULTS

In this nationwide study, patients and their parents were recruited in the Princess Máxima Center for pediatric oncology in the Netherlands between May 17, 2018 and March 27, 2021. Of 163 eligible patients, 105 were included in our study (Figure 2). Non-responders did not differ significantly on baseline characteristics compared to included patients. Median age of the included patients was 5.4 years (range 3.0-18.8) and 61% were boys. All parents completed the SDQ at T1 and T2, the SDSC was missing in one case. The SDQ increased from median 5 points (IQR 3-10) to 16 points (IQR 11-20) ($p<0.001$). The SDSC score increased from median 37 points (IQR 32-46) to 48 points (IQR 38-59) ($p<0.001$) (Figure 3). Clinically relevant dexamethasone-induced neurobehavioral or sleep problems were reported by parents in 70 (67%) and 61 (59%) of the patients respectively. In 53 patients (50%) both problems were reported, whereas in 27 patients (26%) no clinically significant problems occurred (Figure 4). Baseline characteristics did not differ between groups (Table 1).

Results from all questionnaires were low to moderately correlated to each other at T1 (Pearson correlation 0.3-0.7, Supplemental Table 1).

Of the 105 included patients, 19 (18.1%) were ≥11 years and therefore offered the SDQ self-report. Twelve patients (63%) completed the SDQ at both timepoints, four patients (21%) at one timepoint and three patients (16%) did not complete any SDQ. The T1 ICC was 0.40 (95%-CI -0.11-0.72). For T2, the ICC was 0.73 (95%-CI 0.34-0.91). The ICC of the delta SDQ score was 0.30 (95%-CI -0.24-0.72).

Dexamethasone-induced neurobehavioral problems

In univariable regression analyses, child age, SDQ T1 score, maternal age and nationality, paternal age and nationality, NOSI-K and DT-P delta and T2 scores (reflecting parenting stress during a dexamethasone course of the child) were associated with parent-reported clinically relevant dexamethasone-induced neurobehavioral problems (Supplemental Table 2).

Dexamethasone levels (AUC estimated values) were assessed in 86 patients. We did not find an association between dexamethasone AUC and neurobehavioral problems, also after adjusting for concomitant asparaginase use, which may influence dexamethasone pharmacokinetics.[21]

Table 1. Baseline characteristics

		Total group
		N = 105
Age *median (IQR)*		5.4 (4.1-8.9)
Sex *n (%)*	Boy	64 (61)
	Girl	41 (39)
Week maintenance *median (IQR)*		34 (22-43)
Asparaginase during study *n (%)*	No	93 (88.6)
	Yes	12 (11.4)
Type ALL[1] *n (%)*	B-cell ALL	93 (88.6)
	T-cell ALL	11 (10.5)
CNS involvement[2] *n (%)*	No	85 (81)
	Yes	20 (19)

[1] One patient with BPDCN

[2] Patients with CNS involvement (defined as CNS-3 or other CNS manifestations at diagnosis, or TLP+) receive 2 additional intrathecal therapy administrations and are considered as "CNS involvement yes". MRG patients without CNS involvement receive 13 intrathecal administrations, with CNS involvement 15.

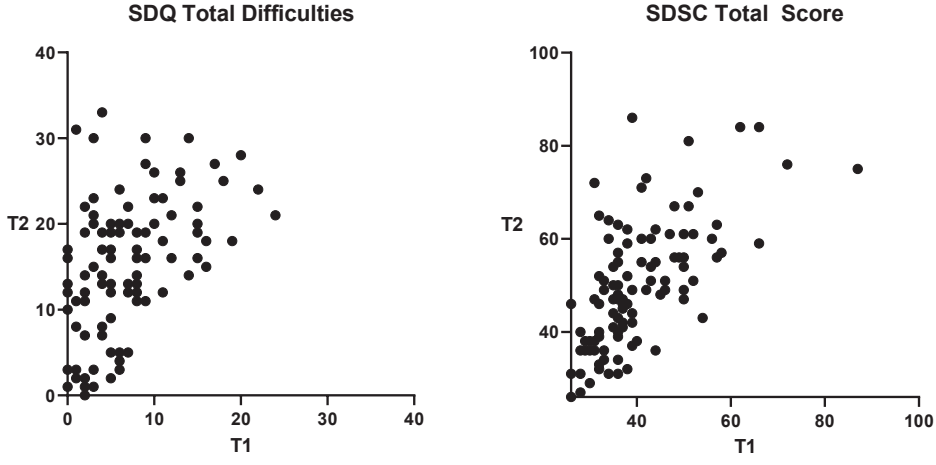

Figure 3. SDQ and SDSC scores at T1 and T2

SDQ (left) and SDSC (right) scores before (T1) and after (T2) a 5-day dexamethasone course. Each dot depicts one patient. Overall, the SDQ and SDSC scores rise between T1 and T2.

Abbreviations: SDQ: strengths and difficulties questionnaire, SDSC: sleep disturbance scale for children

			Outcomes		
	Neurobehavioral problems		Sleep problems		No clinically significant problems
	No	Yes	No	Yes	
	n = 35	*n = 70*	*n = 43*	*n = 61*	*n = 27*
	5.8 (4.3-13.1)	5.3 (4.0-8.4)	5.5 (4.0-9.8)	5.3 (4.1-8.4)	5.4 (4.0-12.1)
	23 (65.7)	41 (58.6)	28 (65.1)	35 (57.4)	17 (63)
	12 (34.3)	29 (41.4)	15 (34.9)	26 (42.6)	10 (37)
	37 (27-43)	33 (21-44)	33 (22-43)	37 (28-48)	34 (25-43)
	32 (91.4)	61 (87.1)	37 (86.0)	56 (91.8)	25 (92.6)
	3 (8.6)	9 (12.9)	6 (14.0)	5 (8.2)	2 (7.4)
	30 (85.7)	63 (90)	39 (90.7)	53 (86.9)	24 (88.9)
	4 (11.4)	7 (10)	3 (7.0)	8 (13.1)	2 (7.4)
	27 (77.1)	58 (82.9)	35 (81.4)	49 (80.3)	20 (74.1)
	8 (22.9)	12 (17.1)	8 (18.6)	12 (19.7)	7 (25.9)

Abbreviations: ALL: acute lymphoblastic leukemia, BPDCN: blastic plasmacytoid dendritic cell neoplasm, CNS: central nervous system, IQR: interquartile range, MRG: medium risk group, TLP: traumatic lumbar puncture.

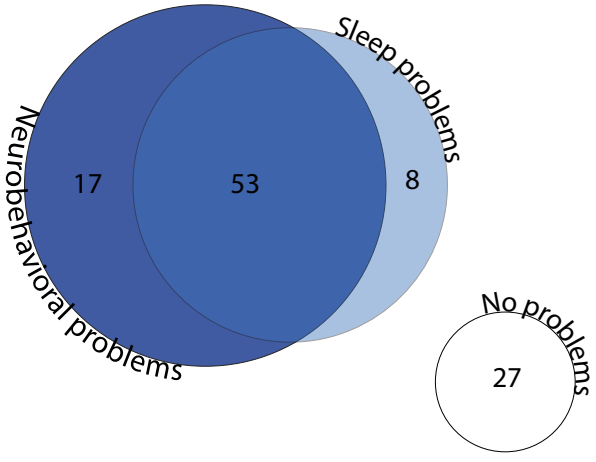

Figure 4. Venn diagram of dexamethasone-induced neurobehavioral and sleep problems

Neurobehavioral problems were reported in 70 patients, sleep problems in 61 patients. In 27 patients, no clinically relevant problems were reported.

Genetic susceptibility data was available for all 105 patients. Neither homozygous nor heterozygous carrier status of the *Bcl-1* polymorphism was associated with neurobehavioral problems in our cohort (Supplemental Table 2).

The multivariable model (Table 2) included patient age and sex, maintenance week, SDQ T1 score, maternal age and nationality and parenting stress (NOSI-K delta score). In multivariable analysis, parenting stress (OR 1.16, 95%-CI 1.07-1.26) remained statistically significantly associated with parent-reported neurobehavioral problems. A one-point increase on the NOSI-K delta score (range -125 to 125) gave 16% higher odds of parent-reported dexamethasone-induced neurobehavioral problems, corrected for the other included variables. SDQ T1 score also remained statistically significant (OR 0.82, 95%-CI 0.68-0.99), indicating that parents of children who had a higher SDQ score at T1, reported less dexamethasone-induced neurobehavioral problems.

Table 2. Multivariable regression models

	Neurobehavioral problems		**Sleep problems**	
	Odds ratio	*95%-confidence interval*	*Odds ratio*	*95%-confidence interval*
Age	1.00	0.80-1.26	1.07	0.93-1.24
Sex	1.26	0.32-5.04	0.89	0.28-2.66
Week maintenance	0.99	0.94-1.04	1.02	0.98-1.06
SDQ / SDSC score T1[1]	**0.82**	**0.68-0.99**	**0.93**	**0.87-1.00**
Parenting stress during dexamethasone[2]	**1.16**	**1.07-1.26**	**1.06**	**1.02-1.10**
Nationality mother	0.62	0.05-7.25	0.54	0.10-3.03
Age mother	0.90	0.78-1.04	-	-
Parental stress T1[3]	-	-	**1.16**	**1.02-1.32**
Psychological support child	-	-	0.24	0.05-1.26

Bold values are statistically significant (p<0.05)

[1] T1 SDQ and SDSC scores were included for neurobehavioral and sleep problems respectively.

[2] Parenting stress during dexamethasone are indicated by the NOSI-K delta score (corrected for the T1 NOSI-K score)

[3] Parental stress is indicated by the DT-P total score measured at T1

Abbreviations: DT-P: Distress Thermometer for parents, NOSI-K: Nijmeegse Ouderlijke Stress Index Korte versie, shortened Dutch version of the Parenting Stress Index, SDSC: Sleep Disturbance Scale for Children, SDQ: Strengths and Difficulties Questionnaire

Dexamethasone-induced sleep problems

The following determinants had a p-value <0.20 in univariable regression analyses for sleep problems: SDSC T1 score, maternal nationality, NOSI-K delta score and DT-P thermometer T2 score (reflecting parenting stress during dexamethasone treatment of the child), DT-P total score (reflecting parental stress before the start of a dexamethasone course), and psychological support of the child (Supplemental Table 3). No significant association was found between other demographics, disease or treatment characteristics, parental support, dexamethasone pharmacokinetics, nor carrier status of *rs4918,* and dexamethasone-induced sleep problems in our cohort (Supplemental Table 3).

The multivariable logistic regression model included age and sex of the child, maintenance week, SDSC T1 score, maternal nationality, parenting stress (delta NOSI-K) and parental stress (DT-P total score) (Table 2). Parenting stress during dexamethasone (delta NOSI-K) remained statistically significantly associated with parent-reported sleep problems of the children (OR 1.06, 95%-CI 1.02-1.10). A one-point rise on the NOSI-K delta score gave 6% higher odds of parent-reported dexamethasone-induced sleep problems, corrected for the other included variables. Furthermore, both SDSC T1 score (OR 0.93, 95%-CI 0.87-1.00) and parental stress at T1 (DT-P total score, OR 1.16, 95%-CI 1.02-1.32) were significantly associated with sleep problems, indicating that parents of children who had a higher SDSC score at T1, reported less dexamethasone-induced sleep problems, whereas parents who experienced more stress before the start of a dexamethasone course reported more sleep problems in their child.

Parental experiences and coping

Ninety-eight parents (93%, 79 mothers and 19 fathers) completed two questions concerning their experiences with dexamethasone and parental coping; multiple responses were possible. Parents' ways of managing during the dexamethasone courses differed from doing nothing extra or acting normal to merely surviving. Phrases like '*it is doom and gloom*' and *´these five days are exhausting*' were mentioned by parents. Interestingly, twenty-six parents spontaneously indicated that they wield lax(er) parenting strategies during dexamethasone treatment.

DISCUSSION

Our prospective national cohort study in 105 pediatric ALL patients identified parenting stress as a significant determinant for both parent-reported dexamethasone-induced neurobehavioral and sleep problems. We did not find an association between dexamethasone pharmacokinetics, genetic variation, patient and parent demographics, or disease and treatment characteristics and behavioral or sleep problems.

Parents with higher levels of parenting stress during a dexamethasone course of their child, reported more dexamethasone-induced neurobehavioral and sleep problems in their children. In addition, parents with more stress before the start of a dexamethasone course, reported more sleep problems. The direction of these associations however, is unclear: parenting stress may lead to disruptive child behavior and perceived sleep problems, vice versa, or the association may be bidirectional. To strengthen our finding, the association between parenting stress and child problems during dexamethasone should be established in an independent cohort. Moderating factors such as other stressful life events could play a role in both parenting stress and child behavioral problems.[38] Previous studies showed that children of parents with higher levels of parenting stress showed more behavioral adjustment problems after ALL diagnosis.[39,40] Also, parental distress and child distress are more strongly linked in a pediatric cancer cohort compared to controls.[38] Targeting parenting stress, in individual cases, may be a useful intervention to decrease behavioral and sleep problems in children. Fedele *et al* showed that a randomly allocated psychosocial intervention focusing on uncertainty, coping, communication, support and problem solving for mothers, reduced internalizing symptoms in children with cancer.[41] Similarly, Williams *et al*. pursued a successful evidence-based parenting intervention for parents with a child in ALL maintenance treatment. Even though this study involved only 12 patients, it showed both an improvement in quantitative difficulties (SDQ) as well as qualitative behavioral improvement (indicated by parents).[42]

Parents of children with a higher SDQ or SDSC score at T1 reported less dexamethasone-induced neurobehavioral and sleep problems, respectively. This could indicate that, when parents already notice more problems in their child, the change during dexamethasone may be less pronounced. Also, if a child has a higher score at T1, a rise of 5 (SDQ) or 7 (SDSC) points may be more difficult to achieve. Still, pre-existing problems and parents' ways of managing these problems are important to take into account, since targeting these problems may be beneficial for both parents and their children.

Even though we evaluated numerous and various determinants, there may still be other mechanisms contributing to behavioral problems. Muriel et al. found family psychiatric history as risk factor of steroid-induced affective symptoms in a cohort of 125 ALL

patients.[43] In the general population, factors such as recent parental unemployment or divorce increased the risk of behavioral or emotional problems in children.[44] In pediatric cancer patients, cumulative exposure to stressful life events was associated with symptoms of depression and anxiety.[38] Screening for family problems and life events, for example with the psychosocial assessment tool (PAT)[45] may therefore be valuable to identify patients and families at risk of (steroid-induced) behavioral problems.

For parent-reported dexamethasone-induced sleep problems, no other determinants besides parenting stress were identified. In the general population and in childhood cancer survivors, female sex is a known risk factor for sleep disturbances.[46,47] We did not find a difference between boys and girls, which may be due to the young age of our cohort, as puberty and hormonal factors seem to play an important role in sex differences of sleep problems.[47] Other factors that could contribute to (dexamethasone-induced) sleep problems, such as sleep hygiene, co-morbidities and pain,[14,48] were unfortunately not available in our cohort.

There was no significant contribution of genetic variation, by exploring carrier status of two most relevant reported candidate SNPs, on dexamethasone-induced neurobehavioral and sleep problems. This may be due to our relatively small cohort for exploring genetic variation. Prior work described the advantage of combining genetic variants to study their effects on brain structure and function, whilst solitary SNPs may not be associated, especially on individual level.[49,50] Studying these combined genetic variants or performing a genome-wide association study in a sufficiently large cohort, could be of value to detect patients with an inborn increased risk for steroid-induced side effects. We did not find an association between behavioral or sleep problems and dexamethasone pharmacokinetics as well; however, we only measured one peak and one trough level, and a more extensive pharmacokinetic(-pharmacodynamic) model, including more timepoints, may give more insight in the differences in dexamethasone clearance and side effects in patients.

Even though parenting strategies were not systematically evaluated and the qualitative responses in our study were meant to gain insight in the ways of managing dexamethasone-induced problems by parents, 25% of the parents spontaneously indicated that they wielded lax(er) parenting strategies during the dexamethasone courses. Interestingly, previous studies showed that parental laxness and inconsistent discipline are associated with increased behavioral and emotional difficulties, as well as parent-reported child sleep problems, in ALL patients.[51-53] Therefore, we anticipate that parenting strategies may be a modifiable target for healthcare providers, thereby improving child behavioral and sleep outcomes. Given the independent value of this factor, we feel that future studies may include systematic evaluation of parenting strategies in order to design successful interventions.

The main strength of our study is the fact that we were able to invite all eligible ALL patients due to our national centralized pediatric cancer care. This rendered a representative study population, and the largest prospective series thus far. We were also able to study a broad range of possible determinants. Limitations are risk of bias in inclusion, as families of patients who experience more dexamethasone-induced side effects may have been more interested to participate. Besides, it may be possible that certain predisposing factors, e.g. differences in dexamethasone kinetics, were not found to be significantly associated with the outcomes due to the relatively small sample size. Additionally, the study was not primarily powered for the multivariable analyses. Our results are based on proxy reports, since most included patients were too young to fill in questionnaires themselves. Only nineteen children were ≥11 years and therefore offered the SDQ self-report. When comparing the delta SDQ of the twelve patients who completed the SDQ on both timepoints with their parents, we found a poor agreement between these scores. It has been described before that parents and children report side effects differently.[54,55]The group of patients who were able to complete a self-report was very small, therefore no conclusions regarding determinants could be made. The use of proxy-reports may also be an explanation for the fact that we only found parental factors to be associated with the reported outcomes.

To conclude, we identified parenting stress during dexamethasone as determinant for parent-reported neurobehavioral and sleep problems, which may be a modifiable target. Future studies including larger cohorts that incorporate other possible risk factors such as coping with stress, (family) psychiatric history, stressful life events and parenting strategies, as well as more extensive genetic evaluation, may be of value.

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SUPPLEMENTAL METHODS

ALL-11 treatment

ALL-11 maintenance treatment contained 28 three weekly treatment cycles. Patients were enrolled in the study after completion of doxorubicin (administered on the first day of the first four treatment cycles). Patients could be included at any cycle thereafter, that is, some patients started directly after cessation of doxorubicin (beginning of maintenance treatment) and some patients started later during maintenance treatment. Dexamethasone 6 mg/m2/day was administered for 5 consecutive days at the beginning of each treatment cycle. Patients also received vincristine once every three weeks, methotrexate once per week and 6-mercaptopurine once per day, in dosages as described in the Dutch Childhood Oncology Group ALL-11 protocol. Depending on randomization, patients also received asparaginase once every three weeks till week 15 or 27 of maintenance treatment.

Determinants

Disease characteristics

CNS involvement was dichotomized into present or absent. CNS involvement was defined as CNS-3 (a non-traumatic lumbar puncture (LP) with >5 white blood cells per microliter in the cerebrospinal fluid with identifiable leukemic cells), intracerebral or meningeal mass, cranial nerve palsy or retinal involvement) or a traumatic LP with leukemic cells (TLP+). Both patients with CNS involvement and a TLP+ receive two additional intrathecal administrations during induction treatment which may potentially increase the risk of neurobehavioral side effects.

Parent characteristics

Information regarding parental factors was extracted from a general questionnaire parents completed upon participation in an online quality of life platform (KLIK).[56] This questionnaire was not available for all included patients.

Parenting stress

We used the NOSI-K (Nijmeegse Ouderlijke Stress Index Korte versie, adapted from the Parenting Stress Index) [30,31] to measure parenting stress. The NOSI-K has been found to have a good internal validity, in the present study the reliability coefficient was 0.92 on T1 and 0.96 on T2.

We also used the Distress Thermometer for parents (DT-P)[32], which is a validated questionnaire and consists of three parts: 1) a thermometer (visual analogue scale) ranging from 0 (no distress) to 10 (extreme distress), 2) a problem list about everyday problems over the past week across six domains (practical, social, emotional, physical, cognitive and parenting problems): the total score adds all domains (higher score reflects more problems), and 3) additional

questions concerning perceived support, lack of understanding, and parental chronic illness. The internal validity of the DT-P ranged from 0.55 (social domain) to 0.91 (total score).

Support and parental coping

This unvalidated short questionnaire contained nine questions, divided in three sections. The first part was about whether a child and/or parent received psychological or other (medical) support and why (e.g. *Has your child received help support from a psychologist in the past month?)*. The second part was about information the parent had received or looked up about dexamethasone (e.g. *Have you ever received information about the possible side effects of dexamethasone in the areas of (multiple answers possible): weight, sleep, behavior, eating, bone problems, risk of infections, other).* In the last part, parents were asked to describe how they usually cope with the dexamethasone treatment period (e.g. *How do you prepare for the days when your child is on dexamethasone?*).

After five days of dexamethasone one question regarding support during this period was asked (*Have you sought extra help for yourself or your child in the past 5 days?*)

Dexamethasone pharmacokinetics

Blood plasma samples were stored at -80°C. Dexamethasone levels were assessed in EDTA-plasma using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Precision of the assay was ensured by using quality control samples in each batch of samples. Between-run precision was 2.4%. The lower limit of the measuring interval (LLMI) was 1.0 nmol/L.

Levels below the lower limit of the measuring interval (LLMI) were set to one half LLMI for the purpose of analyses.

Candidate SNP assessment

Wholeblood was stored at -80°C and shipped on dry ice. DNA was extracted using the ReliaPrep chemistry (Promega) on a Tecan robot. All samples were simultaneously genotyped with the GSA-MD (Multi-disease Finemapping) array version 3. Standard quality control was performed using Plink and zCall.[57,58] Imputations were performed to the 1000 Genomes Phase 3v5 reference panel[59] using SHAPEIT[60] and Minimac4[61]. We used a candidate single nucleotide polymorphism (SNP) approach. In adults, homozygous carriers of the *Bcl-1* polymorphism have an increased risk for major depression.[17] This was also found in a small (n = 49) sample of pediatric ALL patients.[18] In another cohort of pediatric ALL patients, the *rs4918* polymorphism (*Alpha2-HS glycoprotein (AHSG)* gene) was associated with impaired sleep during dexamethasone treatment. We therefore evaluated *Bcl1 (rs41423247)* and *rs4918* (*AHSG gene*) polymorphisms as possible determinants for dexamethasone-induced neurobehavioral or sleep problems respectively. Imputation quality was 0.94 for the *Bcl1 (rs41423247)* SNP and 0.98 for the *rs4918* SNP.

SUPPLEMENTAL TABLES

Supplemental Table 1. Correlations between questionnaires on T1

		SDSC T1	DT-P Thermometer T1	DT-P total T1	NOSI-K score T1
SDQ T1	**Pearson Correlation**	0.61	0.32	0.50	0.54
	Sig. (2-tailed)	<0.0001	0.001	<0.0001	<0.0001
	N	105	100	100	105
SDSC T1	**Pearson Correlation**	-	0.37	0.45	0.45
	Sig. (2-tailed)	-	<0.0001	<0.0001	<0.0001
	N	-	100	100	105
NOSI-K T1	**Pearson Correlation**	-	0.39	0.62	-
	Sig. (2-tailed)	-	<0.0001	<0.0001	-
	N	-	100	100	-

Abbreviations: SDQ: strengths and difficulties questionnaire, SDSC: sleep disturbance scale for children, NOSI-K: Nijmeegse ouderlijke stress index korte versie (shortened Dutch version of the Parenting Stress Index), DT-P: distress thermometer for parents. DT-P total adds the practical, social, emotional, physical, cognitive and parenting domains.

Very high correlation	0.9-1.0
High correlation	0.7-0.9
Moderate correlation	0.5-0.7
Low correlation	0.3-0.5
Negligible correlation	0-0.3

Supplemental Table 2. Univariable logistic regression analyses dexamethasone-induced neurobehavioral problems

		No dexamethasone-induced neurobehavioral problems (ΔSDQ<5)
		Median (IQR) or n (%)
Patient demographics		*n = 35*
Age, years		5.8 (4.3-13.1)
Sex	Boy	23 (65.7)
	Girl	12 (34.3)
T1 SDQ Total Difficulties score		6 (2-14)
T1 SDSC Total score		37 (32-46)
Disease and treatment characteristics		*n = 35*
Week maintenance		37 (27-43)
Asparaginase during study	No	32 (91.4)
	Yes	3 (8.6)
Type ALL[1]	B-cell ALL	30 (85.7)
	T-cell ALL	4 (11.4)
CNS involvement[2]	No	27 (77.1)
	Yes	8 (22.9)
Family characteristics		n = 32
Age mother		38 (36-44)
Age father		42 (38-46)
Who completed questionnaires	Mother	25 (71.4)
	Father	10 (28.6)
Nationality mother[3]	Dutch	24 (75)
	Other	8 (25)
Nationality father	Dutch	24 (77.4)
	Other	7 (22.6)
Level of education mother	Low/middle	11 (35.5)
	High	20 (64.5)
Level of education father	Low/middle	15 (50.0)
	High	15 (50.0)
Number of siblings		1 (1-2)
NOSI-K		*n = 32*
NOSI-K T1		37 (28-52)
NOSI-K T2		41 (31-49)
NOSI-K delta (T2-T1)[4]		0.5 (-3-6)

	Dexamethasone-induced neurobehavioral problems (ΔSDQ≥5)		
	Median (IQR) or n (%)	*OR*	*95%-CI*
n = 70			
5.3 (4.0-8.4)		**0.90**	**0.81-0.99**
41 (58.6)			
29 (41.4)		1.36	0.58-3.16
5 (3-9)		**0.95**	**0.88-1.02**
37 (32-45)		1.00	0.96-1.03
n = 70			
34 (21-44)		0.99	0.96-1.02
61 (87.1)			
9 (12.9)		1.57	0.40-6.22
63 (90)			
7 (10)		0.83	0.23-3.07
58 (82.9)			
12 (17.1)		0.70	0.26-1.91
n = 64			
37 (33-41)		**0.96**	**0.90-1.02**
39 (35-46)		**0.94**	**0.89-1.01**
61 (87.1)			
9 (12.9)		0.37	0.13-1.02
59 (93.7)			
4 (6.3)		**0.20**	**0.06-0.74**
56 (88.9)			
7 (11.1)		**0.43**	**0.14-1.36**
29 (48.4)			
31 (51.7)		0.59	0.24-1.44
31 (54.4)			
26 (45.6)		0.84	0.35-2.03
1 (1-2)		1.12	0.77-1.61
n = 70			
44 (33-59)		1.02	1.00-1.05
69 (53-88)		**1.06**	**1.03-1.08**
21 (10-35)		**1.11**	**1.06-1.16**

Supplemental Table 2. Continued

		No dexamethasone-induced neurobehavioral problems (ΔSDQ<5)
		Median (IQR) or n (%)
DT-P		*n = 34*
Thermometer T1		4 (0-7)
Thermometer T2		5.5 (2-7)
Thermometer delta (T2-T1)[4]		0 (0-2)
Total score		6 (1-12)
Received support[5]	No	4 (11.8)
	Yes	30 (88.2)
Perceived lack of understanding[6]	No	33 (97.1)
	Yes	1 (2.9)
Parental chronic illness	No	27 (79.4)
	Yes	7 (20.6)
Support questionnaire		*n = 34*
Psychological support child	No	29 (85.3)
	Yes	5 (14.7)
Psychological support parent(s)[7]	No	31 (91.2)
	Yes	3 (8.8)
Social work support for parent(s)	No	29 (85.3)
	Yes	5 (14.7)
Information regarding side effects	None	2 (5.9)
	Only behavioral	3 (8.8)
	Only somatic	0
	Both	29 (85.3)
Information sufficient[8]	No	4 (11.8)
	Yes	30 (88.2)
Extra support on T2[9]	No	31 (96.9)
	Yes	1 (3.1)
Dexamethasone pharmacokinetics		n = 31
Dexamethasone AUC *mg*h/L*		0.185 (0.128-0.320)
Candidate SNP Bcl-1 *rs41423247*		n = 35
Additive model (alternate allele dosage G>C)		0.881 (0.049-0.998)
Dominant risk allele	CC	16 (45.7)
	CG+GG	19 (54.3)

	Dexamethasone-induced neurobehavioral problems (ΔSDQ≥5)		
	Median (IQR) or n (%)	*OR*	*95%-CI*
n = 66			
	4.5 (2-7)	1.10	0.95-1.27
	7 (6-8)	**1.51**	**1.23-1.86**
	2 (1-4)	**1.77**	**1.33-2.34**
	8 (4-13)	1.04	0.97-1.11
	10 (15.2)		
	56 (84.8)	0.75	0.22-2.58
	54 (81.8)		
	12 (18.2)	7.33	0.91-59.02
	53 (80.3)		
	13 (19.7)	0.95	0.34-2.65
n = 64			
	55 (85.9)		
	9 (14.1)	0.95	0.29-3.10
	52 (81.3)		
	12 (18.8)	2.39	0.62-9.12
	53 (82.8)		
	11 (17.2)	1.20	0.38-3.80
	2 (3.1)		
	9 (14.1)	-	-
	0	-	-
	53 (82.8)	-	-
	13 (20.3)	1.91	0.57-6.40
	51 (79.7)		
	55 (83.3)	0.16	0.02-1.31
	11 (16.7)		
n = 55			
	0.180 (0.147-0.264)	1.14	0.05-25.55
n = 70			
	0.963 (0.034-1.006)	1.13	0.57-2.23
	28 (40.0)		
	42 (60.0)	1.26	0.56-2.87

Supplemental Table 2. Continued

		No dexamethasone-induced neurobehavioral problems (ΔSDQ<5)
		Median (IQR) or n (%)
Recessive risk allele	CC+CG	30 (85.7)
	GG	5 (14.3)

Numbers are depicted as median (interquartile range) or number (%). Bold values are regarded as statistically significant with a p-value <0.20 and were subsequently included in multivariable analyses. Bold underlined values are statistically significant with a p-value <0.05.

[1] One patient with BPDCN was excluded from this analysis

[2] Patients with CNS involvement (defined as CNS-3 or other CNS manifestations at diagnosis, or TLP+) receive 2 additional intrathecal therapy administrations and are considered as "CNS involvement yes". MRG patients without CNS involvement receive 13 intrathecal administrations, with CNS involvement 15.

[3] Fisher's Exact test: p=0.019

[4] Corrected for score on T1 (correction for two repeated measurements)

[5] Fisher's Exact test (2-sided): p=0.767

	Dexamethasone-induced neurobehavioral problems (ΔSDQ≥5)		
	Median (IQR) or n (%)	*OR*	*95%-CI*
	62 (88.6)		
	8 (11.4)	0.77	0.23-2.57

[6] Fisher's Exact test (2-sided): p=0.055

[7] Fisher's Exact test (2-sided): p=0.248

[8] Fisher's Exact test (2-sided): p=0.403

[9] Fisher's Exact test (2-sided): p=0.096

Abbreviations: SDQ: strengths and difficulties questionnaire, SDSC: sleep disturbance scale for children, OR: odds ratio, 95%-CI: 95%-confidence interval, ALL: acute lymphoblastic leukemia, CNS: central nervous system, NOSI-K: Nijmeegse ouderlijke stress index korte versie (shortened Dutch version of the Parenting Stress Index), DT-P: distress thermometer for parents, AUC: area under the curve, SNP: single nucleotide polymorphism, BPDCN: Blastic plasmacytoid dendritic cell neoplasm, TLP: traumatic lumbar puncture, MRG: medium risk group. IQR: interquartile range

Supplemental Table 3. Univariable logistic regression analyses dexamethasone-induced sleep problems

		No dexamethasone-induced sleep problems (ΔSDSC<7)
		Median (IQR) or n (%)
Patient demographics		*n = 43*
Age		5.5 (4.0-9.8)
Sex	Boy	28 (65.1)
	Girl	15 (34.9)
T1 SDSC Total score		38 (33-50)
T1 SDQ Total Difficulties score		7 (2-13)
Disease and treatment characteristics		*n = 43*
Week maintenance		33 (22-43)
Asparaginase during study	No	37 (86.0)
	Yes	6 (14.0)
Type ALL[1]	B-cell ALL	39 (90.7)
	T-cell ALL	3 (7.0)
CNS involvement[2]	No	35 (81.4)
	Yes	8 (18.6)
Family characteristics		*n = 34*
Age mother		38 (34-46)
Age father		42 (36-47)
Who completed questionnaires	Mother	32 (74.4)
	Father	11 (25.6)
Nationality mother	Dutch	27 (79.4)
	Other	7 (20.6)
Nationality father	Dutch	26 (78.8)
	Other	7 (21.2)
Level of education mother	Low/middle	14 (42.4)
	High	19 (57.6)
Level of education father	Low/middle	15 (45.5)
	High	18 (54.5)
Number of siblings		1 (1-2)
NOSI-K		*n = 42*
NOSI-K T1		43 (32-56)
NOSI-K T2		46 (36-70)
NOSI-K delta (T2-T1)[3]		1 (-2-19)

	Dexamethasone-induced sleep problems (ΔSDSC≥7)		
	Median (IQR) or n (%)	*OR*	*95%-CI*
	n = 61		
	5.3 (4.1-8.4)	0.96	0.87-1.05
	35 (57.4)		
	26 (42.6)	1.39	0.62-3.11
	36 (32-43)	**0.97**	**0.93-1.01**
	5 (3-9)	0.93	0.86-1.00
	n = 61		
	37 (28-48)	1.01	0.99-1.04
	56 (91.8)		
	5 (8.2)	0.55	0.16-1.94
	53 (86.9)		
	8 (13.1)	1.96	0.49-7.88
	49 (80.3)		
	12 (19.7)	1.07	0.40-2.90
	n = 60		
	38 (33-41)	0.97	0.91-1.03
	40 (34-46)	0.97	0.91-1.03
	53 (86.9)		
	8 (13.1)	0.44	0.16-1.21
	55 (91.7)		
	5 (8.3)	**0.35**	**0.10-1.21**
	53 (88.3)		
	7 (11.7)	0.49	0.16-1.55
	25 (43.9)		
	32 (56.1)	0.94	0.40-2.24
	30 (56.6)		
	23 (43.4)	0.64	0.27-1.53
	1 (1-2)	0.85	0.61-1.18
	n = 60		
	42 (32-63)	1.01	0.99-1.03
	69 (50-91)	**1.03**	**1.01-1.05**
	21 (9-35)	**1.05**	**1.02-1.08**

Supplemental Table 3. Continued

		No dexamethasone-induced sleep problems (ΔSDSC<7)
		Median (IQR) or n (%)
DT-P		*n = 41*
Thermometer T1		3 (1-7)
Thermometer T2		6 (4-7)
Thermometer delta (T2-T1)[3]		1 (0-3)
Total score		6 (1-13)
Received support	No	5 (12.2)
	Yes	36 (87.8)
Perceived lack of understanding	No	35 (85.4)
	Yes	6 (14.6)
Parental chronic illness	No	33 (80.5)
	Yes	8 (19.5)
Support questionnaire		*n = 40*
Psychological support child	No	32 (80.0)
	Yes	8 (20.0)
Psychological support parent(s)	No	34 (85.0)
	Yes	6 (15.0)
Support social work for parent(s)	No	33 (82.5)
	Yes	7 (17.5)
Information regarding side effects	None	1 (2.5)
	Only behavioral	3 (7.5)
	Only somatic	0
	Both	36 (90.0)
Information sufficient	No	5 (12.5)
	Yes	35 (87.5)
Extra support on T2[4]	No	39 (95.1)
	Yes	2 (4.9)
Dexamethasone pharmacokinetics		n = 32
Dexamethasone AUC *mg*h/L*		0.18 (0.13-0.28)

	Dexamethasone-induced sleep problems (ΔSDSC≥7)		
	Median (IQR) or n (%)	*OR*	*95%-CI*
n = 58			
	5 (2-7)	0.12	0.97-1.30
	7 (6-8)	**1.20**	**1.01-1.41**
	2 (0-4)	1.18	0.96-1.44
	8 (4-13)	**1.05**	**0.98-1.11**
	49 (84.5)		
	9 (15.5)	0.76	0.23-2.45
	51 (87.9)		
	7 (12.1)	0.80	0.25-2.59
	46 (79.3)		
	12 (20.7)	1.08	0.40-2.93
n = 57			
	51 (89.5)		
	6 (40.5)	**0.47**	**0.15-1.48**
	48 (84.2)		
	9 (15.8)	1.06	0.35-3.27
	48 (84.2)		
	9 (15.8)	0.88	0.30-2.61
	3 (5.3)	-	-
	9 (15.8)	-	-
	0	-	-
	45 (78.9)	-	-
	12 (21.1)	1.87	0.60-5.80
	45 (78.9)		
	47 (82.5)	0.24	0.05-1.17
	10 (17.5)		
n = 53			
	0.18 (0.15-0.28)	1.19	0.05-27.45

Supplemental Table 3. Continued

		No dexamethasone-induced sleep problems (ΔSDSC<7) *Median (IQR) or n (%)*
Candidate SNP AHSG *rs4918*		n = 43
Additive model (Alternate allele dosage C>G)		0.980 (0.960-1.932)
Dominant risk allele	GG	5 (11.6)
	GC+CC	38 (88.4)
Recessive risk allele	GG+GC	29 (67.4)
	CC	14 (32.6)

Numbers are depicted as median (interquartile range) or number (%). Bold values are regarded as statistically significant with a p-value <0.20 and were subsequently included in multivariable analyses. Bold underlined values are statistically significant with a p-value <0.05.

[1] One patient with BPDCN was excluded from this analysis

[2] Patients with CNS involvement (defined as CNS-3 or other CNS manifestations at diagnosis, or TLP+) receive 2 additional intrathecal therapy administrations and are considered as "CNS involvement yes". MRG patients without CNS involvement receive 13 intrathecal administrations, with CNS involvement 15.

	Dexamethasone-induced sleep problems (ΔSDSC≥7)		
	Median (IQR) or n (%)	*OR*	*95%-CI*
n = 61			
0.990 (0.958-1.953)	1.03	0.57-1.86	
10 (16.4)			
51 (83.6)	0.70	0.22-2.23	
38 (62.3)			
23 (37.7)	1.25	0.55-2.85	

[3] Corrected for score on T1 (correction for two repeated measurements)

[4] Fisher's exact test: p=0.069

Abbreviations: SDQ: Strengths and difficulties questionnaire, SDSC: sleep disturbance scale for children, OR: odds ratio, 95%-CI: 95%-confidence interval, ALL: acute lymphoblastic leukemia, CNS: central nervous system, NOSI-K: Nijmeegse ouderlijke stress index korte versie (shortened Dutch version of the Parenting Stress Index), DT-P: distress thermometer for parents, AUC: area under the curve, SNP: single nucleotide polymorphism, BPDCN: Blastic plasmacytoid dendritic cell neoplasm, TLP: traumatic lumbar puncture, MRG: medium risk group.


5

Leptin increase during dexamethasone and its association with hunger and fat, in pediatric acute lymphoblastic leukemia

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Under Review

ABSTRACT

Background & Aims

Children with acute lymphoblastic leukemia (ALL) receive high doses dexamethasone during treatment, which induce acute side effects. The aims of the current study were to determine the influence of a five-day dexamethasone course on changes in leptin, fat mass, BMI, hunger, sleep and fatigue and to explore associations between these changes.

Methods

Pediatric ALL patients were included during maintenance treatment. Data was collected before (T1) and after (T2) a five-day dexamethasone course (6mg/m^2/day). BMI, fat mass (bioelectrical impedance analysis) and leptin were assessed on both timepoints, as well as parent-reported questionnaires regarding hunger, fatigue and sleep problems. Changes between T1 and T2 were assessed using paired tests. Correlation coefficients were calculated to assess associations between these changes (Delta scores: T2-T1). Univariable regression models were estimated to study associations between covariates and elevated leptin.

Results

We included 105 children with median age 5.4 years (range 3.0-18.8). Leptin and fat mass, as well as hunger scores, fatigue and sleep deteriorated after five days of dexamethasone ($p<0.001$), in contrast to BMI ($p=0.12$). No correlations between delta leptin and delta fat mass, BMI, hunger, fatigue or sleep were found. Elevated leptin on T1 was associated with older age (odds ratio (OR) 1.51, 95%-confidence interval (95%-CI) 1.28-1.77), higher fat mass (OR 1.19, 95%-CI 1.07-1.33) and earlier maintenance week (OR 0.96, 95%-CI 0.92-0.99).

Conclusions

Five days of high dose dexamethasone treatment lead to direct and significant changes in leptin, hunger scores and fat mass, which may suggest a dexamethasone-induced state of acute leptin resistance. Since children with ALL are at increased risk for metabolic adverse events, understanding underlying mechanisms is important, and leptin resistance might play a role.

INTRODUCTION

Since survival rates of pediatric acute lymphoblastic leukemia (ALL) have increased to over 90% in high-income countries, more attention is being paid to acute and late toxicities.[1] These toxicities are due to the disease itself, but also to the intensity and type of treatment. Dexamethasone is an important component of ALL treatment, but is notorious for its numerous side effects.[2,3] Dyslipidemia and adiposity are well-known side effects of dexamethasone, as well as increased fatigue and sleep problems.[4-6] Additionally, increased appetite and consequent unhealthy eating behavior are reported acute side effects of dexamethasone treatment.[7-9] Previous pediatric ALL studies showed that merely four or five days of glucocorticoid treatment increased blood pressure as well as fasting glucose and lipid levels, and significantly induced insulin resistance.[6,10] This illustrates that the high dose glucocorticoid pulses, which are frequently administered in ALL treatment, trigger significant metabolic changes, which in turn may precede long-term metabolic side effects with its attendant health consequences.[11]

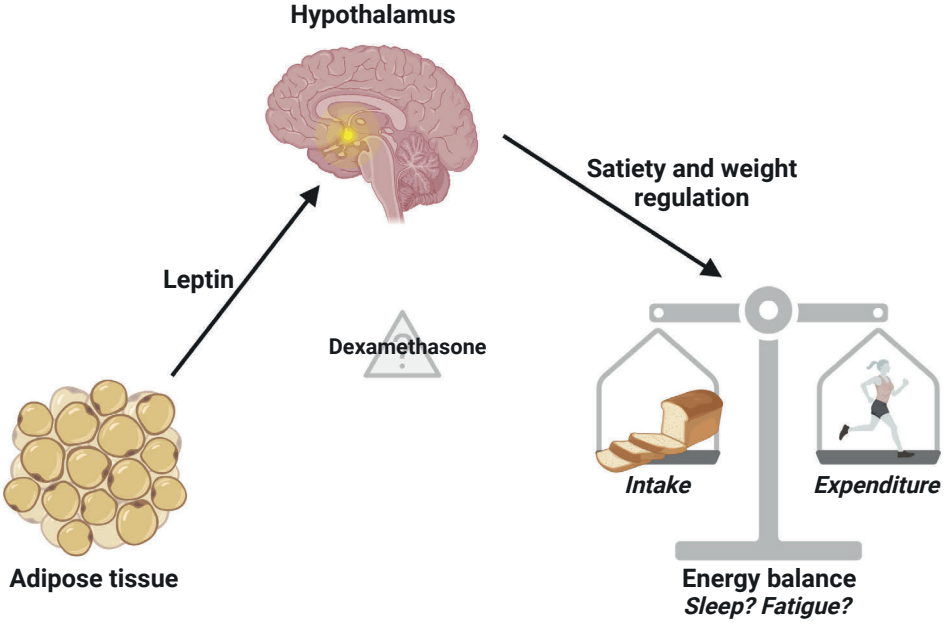

Figure 1. Regulation of energy balance through leptin pathway

Leptin is produced by adipose tissue and exerts its effect on both intake and energy expenditure through the hypothalamus. Low levels of leptin induce a physiological response including feeling of hunger and decreases energy expenditure. High leptin levels reduce food intake and increases energy expenditure. The exact effect of dexamethasone and sleep and fatigue is unknown. *Created with BioRender.com*

In physiological conditions, regulation of food intake and weight homeostasis is regulated by leptin (Figure 1).[12-14] Leptin is an adipokine that is mainly produced by adipose tissue, and circulating leptin concentrations are highly correlated with the amount of fat mass. It is known that in obese individuals hyperleptinemia occurs without an adequate response that reduces these high levels of leptin, suggesting a state of leptin resistance.[14] A previous study in ALL patients showed that leptin levels increased almost twofold after four days of dexamethasone,[8] similar to what was shown in healthy adults after two days of dexamethasone.[15,16] It may be possible that the short-term side effects of dexamethasone are mediated through (partial) leptin resistance. Furthermore, sleep deprivation is known to decrease leptin levels and increase hunger and appetite,[17] and leptin is associated with cancer-related fatigue in adults.[18] However, the associations between dexamethasone-induced side effects and leptin remain unclear.

Therefore, the aims of the current study were to determine the influence of a five-day dexamethasone course on changes in leptin, as well as fat mass, hunger, sleep and fatigue and to assess correlations between these changes. Furthermore, we aimed to explore contributing factors to high leptin levels before and during a dexamethasone course.

MATERIALS AND METHODS

This study was conducted within the framework of the DexaDays-2 study: a national randomized clinical trial on dexamethasone-induced neurobehavioral problems in ALL patients at the Princess Máxima Center for Pediatric Oncology in the Netherlands, between 2019 and 2021. The design of this trial has been published previously.[19,20] This study was approved by the Medical Ethical Committee of Rotterdam (NL62388.078.17) and was performed in compliance with the ethical standards of the Princess Máxima Center as well as with the Declaration of Helsinki. All parents and/or patients provided written informed consent to participate.

Patients and treatment

Patients between 3 and 18 years treated according to the Dutch Childhood Oncology Group (DCOG) ALL-11 protocol were eligible during maintenance treatment phase, after cessation of doxorubicin, as previously described.[19] Dexamethasone (6 mg/m^2/day) was administered for five consecutive days at the start of each three-weekly cycle. Data and venous blood samples were collected on the first day of a five-day dexamethasone course (T1) and on the morning after the same course (i.e. after five full days of dexamethasone treatment) (T2). Weight (kg) and height (cm) were measured at these timepoints and body mass index (BMI) was calculated. Parents completed several questionnaires at T1 and T2 (Figure 2).

Measurements

Hunger scores

Parents were asked to indicate how hungry their child was at T1 and T2 by completing an 11-point Likert-type hunger scale (Eating Thermometer), where possible together with the child. Four different hunger scores were generated, with 0 indicating not hungry at all, and 10 indicating the hungriest possible. These four scores specified the average, most, least and fasting hunger score, with a recall over the past 24 hours. Such Likert-type hunger scales have not been validated, but have been used previously to assess feeling of hunger in adults and children.[21,22]

Fatigue and sleep

Parents completed the validated Pediatric Quality of Life Inventory (PedsQL) – Multidimensional Fatigue Scale (MFS) to assess fatigue. Parental versions for four different age groups were used: 3-4, 5-7, 8-12 and 13-18 years. The total scores were compared to Dutch reference values to generate standardized deviation scores (SDS).[23]

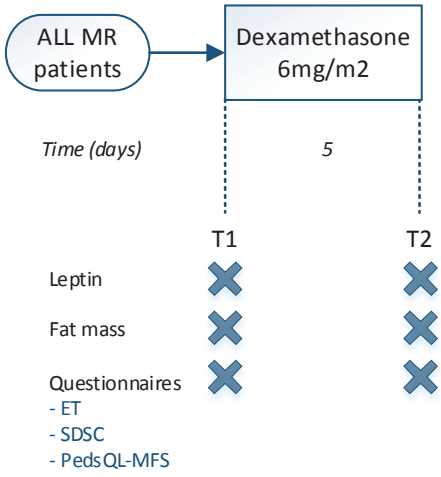

Figure 2. Study Design

Acute lymphoblastic leukemia patients were included during maintenance therapy. Assessments took place before (T1) and after (T2) a five-day dexamethasone course. Leptin and fat mass were measured and parents completed three questionnaires on both timepoints.

Abbreviations: ALL: acute lymphoblastic leukemia, MR: medium risk, ET: eating thermometer (hunger scores), SDSC: sleep disturbance scale for children, PedsQL-MFS: Pediatric Quality of Life Inventory – Multidimensional Fatigue Scale.

We used the parent-reported validated Sleep Disturbance Scale for Children (SDSC) to assess sleep. The SDSC contains 26 items which combined generate a total sleep score: a higher score represents more sleep problems.[24] Furthermore we used the first item of the SDSC to explore whether children slept more/the same or less during a dexamethasone course. This question asks parents to indicate how many hours their child slept on average per night the last week: 9-11 hours, 8-9 hours, 7-8 hours, 5-7 hours or less than 5 hours.

Fat mass

Total body fat mass (kg) was estimated using a multi-frequency segmental bioelectrical impedance analyzer (BIA) (Tanita MC-780, Tanita Corporation, Tokyo, Japan). Unadjusted values were reported since no normative values for fat mass are available for Dutch children under the age of five.

Leptin

Serum, from peripheral blood samples obtained on T1 and T2, was stored at -80°C and leptin levels were assessed all together after study closure to avoid variability in laboratory conditions. Leptin was quantified by ELISA (Mediagnost E07, Mediagnost, Tübingen, Germany) in an ISO15189 accredited laboratory. Kit controls were within range for all measurements.

Since leptin values are highly variable between patients and are known to depend on sex, body mass index (BMI) and puberty stage, they are presented as SDS using previously described normative values taking these factors into account.[25] Since we did not document the puberty stage of our cohort, for this study, puberty stage was approximated per patient using the median age of reaching the stages of secondary sex characteristics in the general Dutch population, using reference values of the 1997 Dutch Growth Study.[26]

Statistical analyses

Patient characteristics and measurement results were reported as mean along with standard deviation (SD) or median with interquartile range (IQR) depending on the distribution of the variables. Delta values were calculated by subtracting T1 values from T2 values.

The changes in leptin SDS, fat mass, hunger scores, fatigue and sleep after five days of dexamethasone (T2 versus T1) were assessed using paired tests: a Paired T-test in case of normally distributed measures or a Wilcoxon Signed Rank test in case of skewed distribution.

To explore correlations between delta leptin and delta fat mass, hunger scores, fatigue and sleep, Spearman correlation coefficients were estimated together with the 95%-confidence intervals (95%-CI). A correlation between 0.0 and 0.3 is negligible, between 0.3 and 0.5 low, between 0.5 and 0.7 moderate, between 0.7 and 0.9 high and >0.9 very high.[27] To explore possible contributing factors (patient demographics and treatment characteristics) for a high leptin value on T1, univariable logistic regression models were estimated: a cutoff of SDS >1,5 was used to define high leptin values at T1. Furthermore, linear regression models were estimated to explore potential influencing factors for change in leptin levels after five days of dexamethasone (delta leptin), with correction for T1 leptin values. All analyses were performed using IBM SPSS Statistics version 26.0.

RESULTS

During the inclusion period, 163 medium risk ALL patients were eligible, of which 105 gave informed consent and therefore were enrolled in our study (Figure 3). Median age of the included patients was 5.3 years (range 3.0-18.8 years) and 61% were boys. The mean week of maintenance treatment phase in which patients were enrolled was week 35 (±14 weeks) (Table 1).

For each measurement there were missing values (Figure 3). The baseline characteristics of the patients who completed measurements and the patients with missing values were similar, except for the fat mass measurement: bioelectrical impedance analysis was obtained in less boys than girls: 19/64 boys (30%) refused this measurement, as opposed to 3/41 girls (7%).

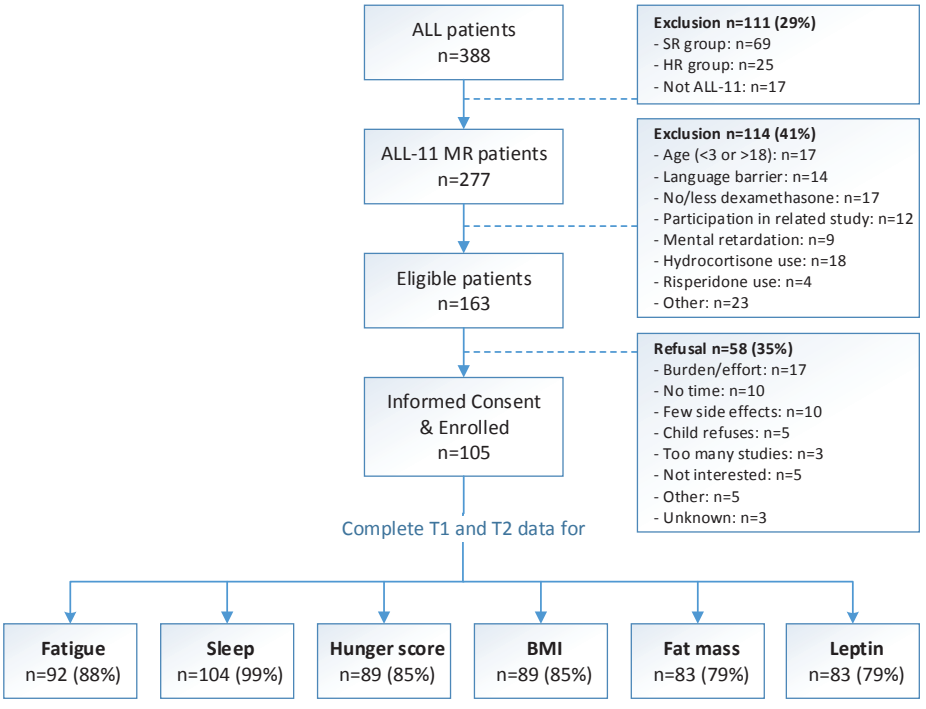

Figure 3. Flow diagram

After screening on in- and exclusion criteria, 163 eligible patients were asked to participate, of which 105 were enrolled in the study. Complete data for both timepoints (i.e. measurement before the start of a five-day dexamethasone course (T1) and after this course (T2) is depicted.

Abbreviations: ALL: acute lymphoblastic leukemia, SR: standard risk, HR: high risk, MR: medium risk

Table 1. Baseline characteristics

Age (years)	
Median (range)	5.3 (3.0 ; 18.8)
Sex n (%)	
Boy	64 (61)
Girl	41 (39)
Type ALL n (%)	
B-cell	93 (89)
T-cell	11 (10)
BPDCN	1 (1)
CNS involvement n (%)	
Yes	20 (19)
No	85 (81)
Maintenance week	
Mean (SD)	35 (14)

Abbreviations: n: number, ALL: acute lymphoblastic leukemia, BPDCN: blastic plasmacytoid dendritic cell neoplasm, CNS: central nervous system, SD: standard deviation

Changes after five days of dexamethasone

At T1, before the start of a five-day dexamethasone course, mean leptin SDS was -0.09 (±2.1), which increased to 1.8 (±1.5) ($p<0.001$) at T2 (Table 2, Figure 4A, Supplemental Figure 1). Fat mass increased significantly as well, from 5.1kg (IQR 3.8 to 8.5) at T1 to 5.6kg (IQR 4.3 to 9.6) at T2 ($p<0.001$) (Figure 4B), whereas BMI remained stable (17.3 kg/m^2 (IQR 16.3 to 19.1) at T1 to 17.7 kg/m^2 (IQR 16.5 to 19.0) at T2 ($p=0.112$) (Figure 4C).

The median hunger scores at T1 were 5 (IQR 3 to 6) for average hunger, 6 (IQR 5 to 7) for most hunger, 2 (IQR 0 to 4) for least hunger and 5 (IQR 2 to 6) for fasting hunger. All hunger scores had increased significantly ($p<0.001$) at T2 to 7 (IQR 6 to 8), 8 (IQR 7 to 10), 4 (IQR 2 to 6) and 7 (IQR 5 to 9), respectively (Figure 4D).

Median fatigue SDS was -0.5 (IQR -2.2 to 0.5) at T1, which decreased to -3.5 (IQR -4.6 to -2.0) at T2, indicating a significant increase in fatigue ($p<0.001$) (Figure 4E). The SDSC Total sleep score increased from 37 (IQR 32 to 46) at T1 to 48 (38 to 59) at T2 ($p<0.001$) (Figure 4F), indicating significantly more sleep problems. Sleep duration, based on the first question of the SDSC, decreased in 42 (40%) of patients, whereas in 62 (60%) sleep duration stayed the same or increased at T2.

Table 2. Measurements at two timepoints along with delta scores

	n	T1	T2	Delta	p-value
Leptin SDS	83				
mean (SD)		-0.1 (2.1)	1.8 (1.5)	1.9 (1.5)	*<0.001*
Fat mass (kg)	83				
median (IQR)		5.1 (3.8 ; 8.5)	5.6 (4.3 ; 9.6)	0.7 (0.3 ; 1.1)	*<0.001*
BMI (kg/m2)	89				
median (IQR)		17.5 (16.3 ; 19.4)	17.7 (16.5 ; 19.0)	0.1 (-0.2 ; 0.3)	0.112
Hunger score	89				
median (IQR)					
- Average		5 (3 ; 6)	7 (6 ; 8)	2 (1 ; 4)	*<0.001*
- Most		6 (5 ; 7)	8 (7 ; 10)	2 (1 ; 3)	*<0.001*
- Least		2 (0 ; 4)	4 (2 ; 6)	1 (0 ; 3)	*<0.001*
- Fasting (morning)		5 (2 ; 6)	7 (5 ; 9)	2 (1 ; 5)	*<0.001*
Fatigue PedsQL SDS	92				
median (IQR)		-0.5 (-2.2 ; 0.5)	-3.5 (-4.6 ; -2.0)	-2.3 (-3.4 ; -0.5)	*<0.001*
Sleep time (SDSC) *n (%)*	104				
- 9-11 hours		80 (77)	53 (51)	≥sleep	*<0.001*
- 8-9 hours		18 (17)	19 (18)	62 (60)	
- 7-8 hours		2 (2)	13 (13)		
- 5-7 hours		4 (4)	13 (13)	<sleep	
- <5 hours		0	6 (6)	42 (40)	
Sleep Score (SDSC)	104				
median (IQR)		37 (32 ; 46)	48 (38 ; 59)	8 (3 ; 16)	*<0.001*

≥sleep or <sleep is based on the first question of the SDSC.

Abbreviations: SDS: standardized deviation score, SD: standard deviation, n: number, IQR: interquartile range, PedsQL: Pediatric Quality of Life Inventory, SDSC: sleep disturbance scale for children.

Correlations between leptin changes and other side effects

No significant correlations between delta leptin SDS and changes after five days of dexamethasone in fat mass or the different hunger scores were found (Table 3, Supplemental Figure 2). Furthermore, there was no correlation between delta leptin SDS and delta fatigue and sleep problems (SDSC total score) (Table 3).

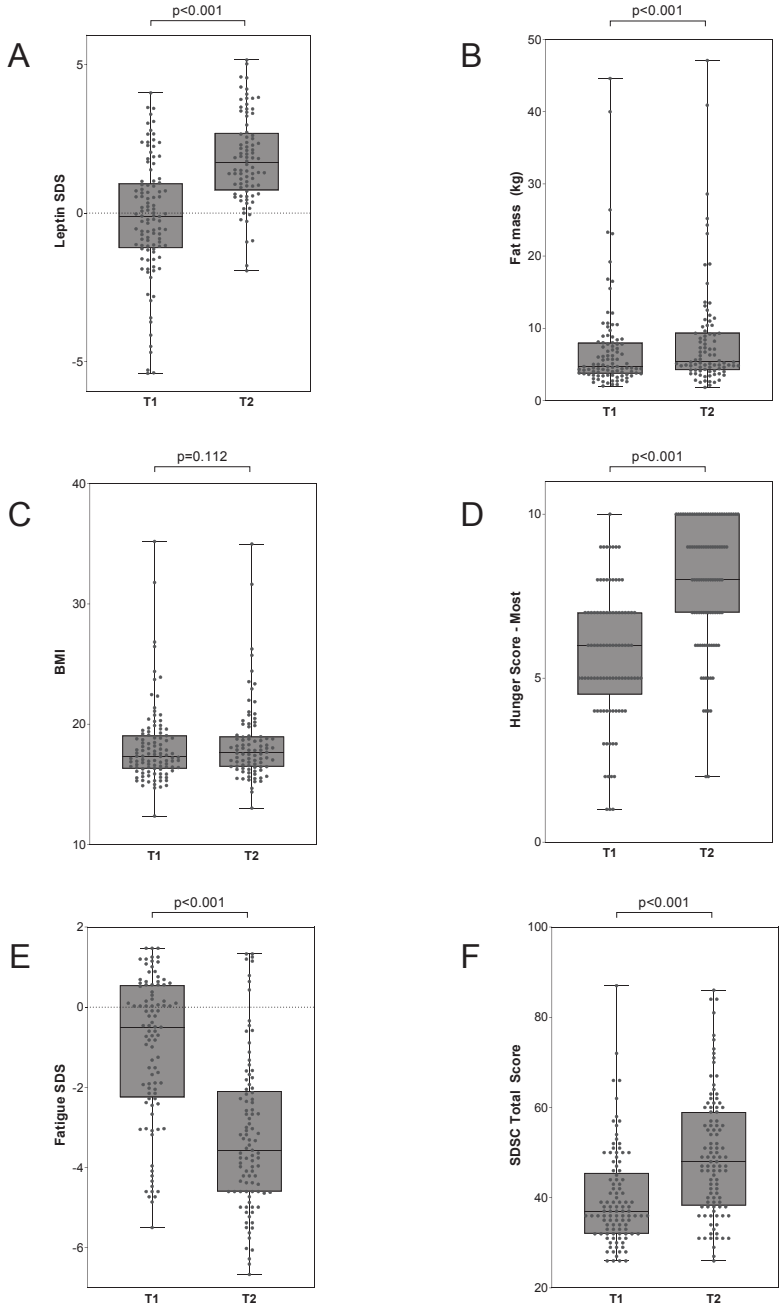

Figure 4. Boxplots before (T1) and after (T2) a five-day dexamethasone course

Boxplots visualize measurements before (T1) and after (T2) a five-day dexamethasone course.

Abbreviations: BMI: body mass index, SDS: standardized deviation score, SDSC: sleep disturbance scale for children.

Table 3. Spearman correlation coefficients for delta leptin values and delta fatigue, sleep, hunger score and fat mass.

		Delta Leptin SDS		
			95%-Confidence Interval	
	n	r_s	Lower bound	Upper bound
Delta fat mass (kg)	78	-0.18	-0.38	0.05
Delta BMI	83	0.13	-0.09	0.33
Delta Hunger score	71			
Average		0.18	-0.06	0.40
Most		0.05	-0.19	0.28
Least		0.08	-0.16	0.31
Fasting		0.04	-0.20	0.27
Delta Fatigue SDS	73	0.04	-0.20	0.26
Delta Sleep SDSC Total score	82	-0.14	-0.35	0.08

Abbreviations: SDS: standardized deviation score, SDSC: sleep disturbance scale for children.

Potential influencing factors for high leptin

To explore which patient and treatment factors may contribute to a high leptin level (SDS>1.5) on T1, we estimated univariable logistic regression models (Table 4). An older age increased the odds of a high leptin level at T1 with 1.51 per year (95%-CI 1.28 to 1.77). A higher fat mass at T1 increased the odds with 1.19 per kg (95%-CI 1.07 to 1.33). Earlier weeks of maintenance treatment (i.e. how far along a patient was in his/her treatment) revealed higher leptin values: every week further in maintenance gave a 0.96 lower odds (95%-CI 0.92 to 0.99) of high leptin. Fatigue, sleep problems and hunger scores at T1 were not associated with a high leptin at T1.

Linear regression models were estimated to study the effect of possible explanatory variables on the change in leptin SDS during a dexamethasone course, with a correction for T1 leptin values (Table 5). Age at measurement was associated with the change in leptin: one year increase, increased delta leptin SDS with 0.08 (95%-CI 0.02 to 0.15). Week of maintenance was also negatively associated with delta leptin values: -0.02 (95%-CI -0.04 to -0.01). Whether a child received asparaginase during the study was also associated with the increase in leptin: if a child received asparaginase, delta leptin SDS was 1.09 higher (95%-CI 0.39 to 1.79). Of note, only 11 children received asparaginase during the study measurements.

Table 4. Descriptive statistics for patients demographics, disease and treatment characteristic; odds ratio (OR) along with 95% confidence interval (95%-CI) estimated from a univariable logistic regression model for high leptin at T1

		Normal leptin levels (<1.5 SD)	**High leptin levels (≥1.5 SD)**		
		Median (IQR) or n (%)	Median (IQR) or n (%)	OR	95%-CI
Patient demographics		n = 83	n = 21		
Age, years		4.8 (4.0-6.5)	12.1 (9.5-15.8)	*1.51*	*1.28-1.77*
Sex	Boy	48 (58)	15 (71)		
	Girl	35 (42)	6 (29)	0.55	0.19-1.56
Fatigue T1, SDS		-0.6 (-2.3-0.6)	-0.1 (-0.9-0.2)	1.26	0.90-1.76
SDSC total score T1		36 (32-45)	39 (35-45)	1.00	0.96-1.05
Hunger scores T1					
Average		5 (3-5)	5 (4-7)	1.27	0.92-1.74
Most		6 (5-7)	6 (4-7)	1.00	0.76-1.30
Least		2 (0-4)	3 (0-4)	0.99	0.74-1.32
Fasting		5 (2-6)	4 (2-5)	0.93	0.74-1.16
Fat mass T1, kg		4.4 (3.5-5.9)	9.6 (8.2-16.7)	*1.19*	*1.07-1.33*
Disease and treatment characteristics					
Week maintenance		37 (25-49)	27 (19-37)	*0.96*	*0.92-0.99*
Asparaginase during study	No	74 (89)	19 (91)		
	Yes	9 (11)	2 (9)	0.87	0.17-4.34
CNS involvement[1]	No	69 (83)	15 (71)		
	Yes	14 (17)	6 (29)	1.97	0.65-5.97

Numbers are depicted ad median (interquartile range) or number (%). Italicized values are statistically significant (p-value <0.05).

[1] Patients with CNS involvement defined as CNS-3 or other CNS manifestations at diagnosis, or TLP+) receive 2 additional intrathecal therapy administrations and are considered as "CNS-involvement yes". MRG patients without CNS involvement receive 13 intrathecal administrations, with CNS involvement 15.

Abbreviations: SDS: standardized deviation score, SDSC: sleep disturbance scale for children, CNS: central nervous system, SD: standard deviation, IQR: interquartile range

Table 5. Estimated regression coefficients (β) along with 95%-confidence interval (CI) from a multivariable linear regression models for delta leptin (corrected for leptin T1)

Patient characteristics		**Leptin SDS delta**		
		β	95%-Confidence Interval	
			Lower bound	Upper bound
Age, years		0.08	0.02	0.15
Leptin T1		-0.60	-0.73	-0.47
Intercept		1.18		
Sex	Boy			
	Girl	-0.32	-0.78	0.14
Leptin T1		-0.49	-0.60	-0.38
Intercept		1.96		
Fat mass T1		0.01	-0.03	0.40
Leptin T1		-0.48	-0.60	-0.35
Intercept		1.76		
Fatigue SDS T1		0.07	-0.05	0.20
Leptin T1		-0.53	-0.65	-0.41
Intercept		1.82		
SDSC total scoreT1		-0.01	-0.03	0.01
Leptin T1		-0.50	-0.61	-0.39
Intercept		2.25		
Disease and treatment characteristics		β	95%-Confidence Interval	
			Lower bound	Upper bound
Week maintenance		-0.02	-0.04	-0.01
Leptin T1		-0.50	-0.60	-0.40
Intercept		2.65		
Asparaginase	No			
	Yes	1.09	0.39	1.79
Leptin T1		-0.47	-0.58	-0.37
Intercept		1.71		
CNS involvement	No			
	Yes	0.29	-0.29	0.86
Leptin T1		-0.49	-0.60	-0.38
Intercept		1.77		

Abbreviations: SDS: standardized deviation score, SE: standard error, 95%-CI: 95%-confidence interval, SDSC: sleep disturbance scale for children.

DISCUSSION

In this national cohort of children with acute lymphoblastic leukemia, we showed that leptin SDS increased from -0.09 (±2.1) to 1.8 (±1.5) after merely five days of high dose dexamethasone. Fat mass, hunger scores, fatigue and sleep problems increased significantly as well, whereas BMI remained stable. No correlations between delta leptin and delta fat mass, hunger scores, fatigue or sleep problems were found.

Our results confirm results from previous studies in ALL patients which also established an increase in leptin during glucocorticoid treatment.[8,28-34] However, none of these studies adjusted the leptin values for BMI, sex or age. The current study showed that adjusted leptin values increased considerably after five days of dexamethasone. The feeling of hunger, measured with four different hunger scores, also increased significantly during these days. Under physiological circumstances, an increase in leptin is accompanied by reduced feeling of hunger.[12-14] In obese patients, elevated leptin levels also do not exert their usual anorexigenic effect, which may imply leptin resistance.[35] The combination of increased leptin levels and feeling of hunger in our cohort, may also suggests a state of acute leptin resistance. However, the interaction between dexamethasone and food intake is regulated through more complex processes than leptin alone.[36] Still, since patients with ALL frequently receive high doses glucocorticoids for at least 1,5 year during their treatment, it is possible that the elevated leptin levels may precede certain long-term side effects in survivors, such as obesity.[37-39] A study to longitudinally evaluate leptin and other appetite-regulating hormones, in combination with anthropometric measurements, feeling of hunger and caloric intake may be of value to shed more light on possible leptin resistance during treatment. Furthermore, interventions designed to mediate the risk of metabolic adverse events should begin timely, to diminish late toxicities.

Even though leptin is mainly produced by adipocytes and is considered as a marker of fat accumulation,[33] we did not find a correlation between the rise in leptin SDS and rise in fat mass. This may be due to the fact that bioelectrical impedance analysis tends to underestimate fat mass and is sensitive to changes in fluid balance.[40] We measured an average increase of 0.5kg in fat mass in five days, which may also reflect increased fluid retention. Ideally, a dual-energy X-ray absorptiometry (DXA) scan would be used to analyze body composition,[41] however, DXA use is limited in children due to logistic issues, the radiation burden and need for sedation in very young children. Besides the most appropriate measurement tool, the question arises whether adipocyte hyperplasia or hypertrophy occurs. Due to the fast increase in fat mass, the latter seems more plausible. Hypertrophic adipocytes seem to secrete less

leptin than normal adipocytes.[42] Therefore rise in leptin could be a result of leptin resistance and not fat accumulation. It would be interesting to evaluate fat mass and leptin longitudinally and in a standardized way during multiple dexamethasone courses to gain better understanding in the interplay between both.

Despite the fact that we observed a significant rise in leptin levels and hunger scores after five days of dexamethasone, we could not establish the expected association between both. This may be due to the fact that parents reported the feeling of hunger for their child and it was not always possible to ask the children to participate in these questions because of their young age. Validated questionnaires that measure feeling of hunger or eating behavior are scarce, not available in every language and often only parent-reported or as self-report commence from the age of seven or higher.[8,43] Thus, measuring the feeling of hunger in young children remains challenging and this may have influenced our results. Still, previous research showed a dexamethasone-induced increase in food intake, including increased total protein, fat, saturated fat, carbohydrate, as well as sodium intake, after four days of dexamethasone treatment.[8] This undesirable increase in quantitative and qualitative food intake may be a direct effect of dexamethasone, independent of leptin signaling.

Parents reported that fatigue and sleep problems increased during the dexamethasone course, as was previously reported by us and others.[5,44-47] In the general population, sleep deprivation is known to decrease leptin levels and to increase hunger and appetite.[17] In our cohort, we did not find an association between the change in leptin values and sleep problems, nor between changes in sleep problems and hunger scores. Interestingly, previous studies showed that, when measuring sleep objectively with actigraphy, sleep duration increased during dexamethasone administration.[45,46] Furthermore, one study in healthy children (n = 37) showed that increased sleep duration was associated with decreased leptin values.[48] In our cohort, 60% of the parents indicated that their child slept the same or more during dexamethasone, but this was not associated with the change in leptin values. However, we based our results on a single item of the SDSC questionnaire, which is a meagre substitute for true sleep duration. There are no studies in children linking fatigue and leptin. Leptin has been linked to pathological inflammatory fatigue in adults, possibly through the release of proinflammatory cytokines.[49-52] Dexamethasone suppresses inflammatory responses and may therefore moderate the association between fatigue and leptin in our cohort. In addition, it is conceivable that in children with ALL, other factors such as chemotherapy, immobilization and hospital visits may influence sleep, fatigue and leptin values independently, influencing possible associations between the changes that occur during dexamethasone.

At T1 (before start of the dexamethasone course), we observed a large variation of leptin SDS (range -5.4 to +4.1). We explored possible contributing factors for this variation, and found that a higher age and higher fat mass were associated with increased leptin level at T1, even for leptin values adjusted for age, sex, pubertal stage and BMI. The cause is not known, but could indicate an increased risk of leptin resistance in children during ALL treatment. Possible contributors to this phenomenon could be concomitant asparaginase treatment, which was associated with delta leptin in our study. Asparaginase is known to influence dexamethasone pharmacokinetics as well as to cause hypertriglyceridemia and may therefore mediate the association between higher dexamethasone and increased leptin.[53,54] Also, we found that children who were further in their maintenance treatment, had lower leptin levels. This is surprising, since children further in maintenance have had a higher cumulative dose of administered dexamethasone. For some dexamethasone-induced side effects, such as osteonecrosis, a higher (cumulative) dose is associated with more physical problems.[55] Moreover, lipid accumulation in hepatocytes is associated with higher cumulative doses of (endogenous) glucocorticoids.[56] The reversed phenomenon in our study may be due to a longer time since asparaginase, which is administered in the beginning of maintenance only. Additionally, physical activity increases in the course of treatment and exercise may assert a protective role on metabolic adverse events and leptin resistance.[57] Longitudinal studies that include physical activity in combination with leptin and body composition are needed to get more insight in the effect of multiple dexamethasone courses on these outcomes.

The current study is the first, and largest, to evaluate leptin SDS in ALL patients, before and after a dexamethasone course. Furthermore, we were able to study leptin SDS in combination with feeling of hunger, as well as fat mass and sleep and fatigue, which has not been evaluated previously. Some limitations may be worth mentioning. The reference cohort for the leptin SDS values was based on children from the age of 5.8 years. Our study also included younger children, which may have influenced the SDS values. However, the standardized values are calculated based on Tanner stage and BMI, which will not differ greatly for younger children. Additional analyses excluding children <5.8 years did not show dissimilar results. Furthermore, the relatively small number of patients prohibited larger multivariable analyses to investigate associations between leptin and other measurements.

To conclude, standardized leptin levels increase significantly after merely five days of dexamethasone, as well as fat mass, hunger scores, fatigue and sleep problems. Our findings suggest a dexamethasone-induced state of acute leptin resistance. Since children with ALL are at increased risk for metabolic adverse events, it is important to understand the underlying mechanisms, and leptin resistance might play a role.

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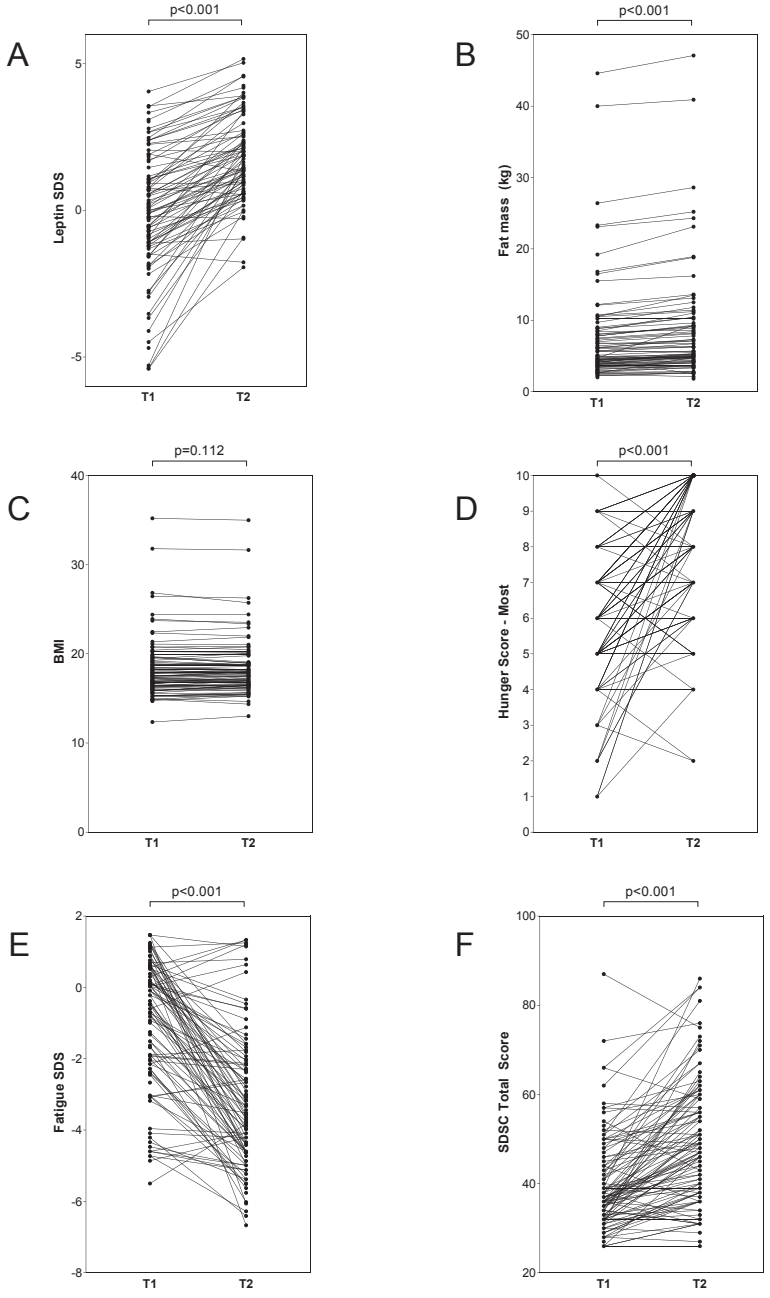
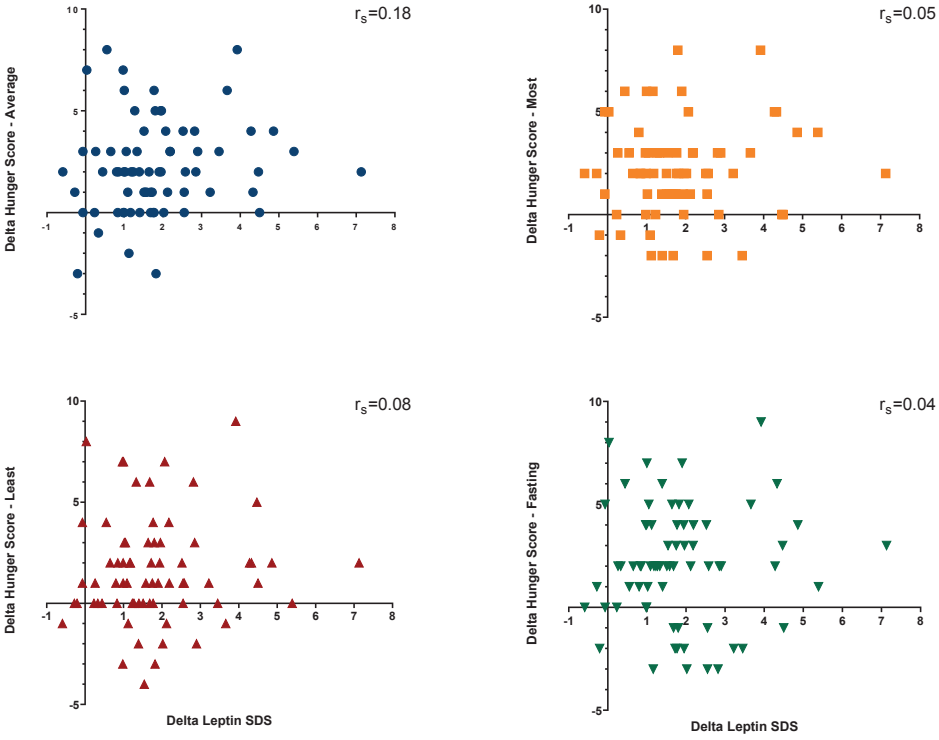
SUPPLEMENTAL FIGURES



Supplemental figure 1. Individual changes before (T1) and after (T2) five days of dexamethasone

Each dot and line represent an individual patient, measured before (T1) and after (T2) a five-day dexamethasone course.



Supplemental Figure 2. Delta leptin and delta hunger score correlations

Spearman correlation coefficients between delta leptin SDS values (x-axes) and the delta of the four different hunger scores (y-axes) is depicted. No significant correlations were found.

Abbreviations: SDS: standardized deviation score

Hydrocortisone to reduce dexamethasone-induced neurobehavioral side-effects in children with acute lymphoblastic leukemia

Results of a double-blind, randomized controlled trial with cross-over design

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ABSTRACT

Background

Dexamethasone is a cornerstone of pediatric acute lymphoblastic leukemia (ALL) treatment, although it can induce serious side-effects. Our previous study suggests that children who suffer most from neurobehavioral side-effects might benefit from physiological hydrocortisone in addition to dexamethasone treatment. This study aimed to validate this finding.

Methods

Our phase three, double-blind, randomized controlled trial with cross-over design included ALL patients (3–18 years) during medium-risk maintenance therapy in a national tertiary hospital between 17th May 2018 and 5th August 2020. A baseline measurement before and after a 5-day dexamethasone course was performed, whereafter 52 patients with clinically relevant neurobehavioral problems were randomized to receive an intervention during four subsequent dexamethasone courses. The intervention consisted of two courses hydrocortisone (physiological dose 10 mg/m2/d in circadian rhythm), followed by two courses placebo, or vice versa. Neurobehavioral problems were assessed before and after each course using the parent-reported Strengths and Difficulties Questionnaire (SDQ) as primary end-point. Secondary end-points were sleep problems, health-related quality of life (HRQoL), hunger feeling, and parental stress, measured with questionnaires and actigraphy. A generalized mixed model was estimated to study the intervention effect.

Results

The median age was 5.5 years (range 3.0–18.8) and 61.5% were boys. The SDQ filled in by 51 primary caregivers showed no difference between hydrocortisone and placebo in reducing dexamethasone-induced neurobehavioral problems (estimated effect -2.05 (95% confidence interval (CI) -6.00–1.90). Also, no benefit from hydrocortisone compared to placebo was found for reducing sleep problems, hunger, parental stress or improving HRQoL.

Conclusions

Hydrocortisone, when compared to placebo, had no additional effect in reducing clinically relevant dexamethasone-induced neurobehavioral problems. Therefore, hydrocortisone is not advised as standard of care for children with ALL who experience dexamethasone-induced neurobehavioral problems.

INTRODUCTION

The introduction of dexamethasone for the treatment of pediatric acute lymphoblastic leukemia (ALL) significantly contributed to the current overall 5-year survival rate of more than 90%.[1] However, dexamethasone may cause severe adverse effects, of which emotional or behavioral disturbances and sleep problems are experienced as detrimental with respect to health-related quality of life (HRQoL) by both patients and parents.[2,3] Currently, in most pediatric ALL treatment protocols, dexamethasone is administered in monthly 5-day courses, during at least one and a half year of maintenance treatment, thereby significantly impacting well-being of child and family for a substantial amount of time. Children and parents can be supported through psychological interventions; however, no effective treatment to overcome dexamethasone-induced neurobehavioral problems exists to date.[4,5]

The pathophysiology of dexamethasone-induced neurobehavioral problems is complex. Previous studies emphasized that both the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) in the brain play an important role in the regulation of mood, behavior, and sleep.[6,7] The MR and GR are activated by binding of endo- and exogenous glucocorticoids. Dexamethasone has a high affinity for the GR, but in contrast to other glucocorticoids, binds the MR to a minimal extent.[8] Simultaneously, the endogenous production of cortisol, which has a high affinity for the MR, is suppressed due to the supra-physiological dose of dexamethasone.[9] Dexamethasone treatment may therefore lead to a relatively insufficient activation of the MR, and this can lead, as shown in preclinical studies in MR knockout mice, to increased anxiety behaviour.[7] In adults with major depression, treatment with MR antagonists was associated with impaired cognitive function and sleep.[7] Our hypothesis was that the relatively underactivated MR contributes to the dexamethasone-induced neurobehavioral side-effects observed in ALL patients.[6,7,10]

Based on this hypothesis, we previously performed a double-blind, randomized placebo-controlled trial (RCT) in which we investigated whether neurobehavioral side-effects could be ameliorated by adding physiological dosages of hydrocortisone, to activate the MR in the brain.[10] The intervention suggested a beneficial effect of hydrocortisone, however only for the subset of children who suffered most from dexamethasone-induced neurobehavioral side-effects.[10] Since the results of this study were based on a relatively small number of patients with clinically relevant side-effects, we aimed to validate this finding in a larger targeted patient cohort. The current study therefore aimed to validate that hydrocortisone decreases dexamethasone-induced neurobehavioral problems in an independent cohort of children with ALL who suffer from these problems. Our secondary aims were to examine whether adding hydrocortisone could reduce dexamethasone-induced sleep problems and feeling of hunger, and improve patient HRQoL and parental stress.[11]

METHODS

Study design

This phase three, double-blind, placebo-controlled, RCT with cross-over design, the DexaDays-2 study, was conducted in the Princess Máxima Center for pediatric oncology in the Netherlands (national tertiary hospital). The study was approved by the Medical Ethical Committee of Rotterdam (NL62388.078.17) and was included in the Netherlands Trial Register (NTR6695/NL6507).[12] Detailed methods have been published previously[11] and an additional relevant method section is available as Supplement.

Participants

Medium Risk Group (MRG) ALL patients, aged 3–18 years, treated according to the Dutch Childhood Oncology Group ALL-11 protocol who received dexamethasone during maintenance treatment were eligible. All included parents and/or patients gave written informed consent to participate in the study. Patients were assessed before and after one dexamethasone course, whereafter patients with an increase of ≥5 points (clinically relevant dexamethasone-induced problems)[10,13] on the parent-reported Strengths and Difficulties Questionnaire (SDQ) were eligible for the RCT (Figure 1).

Intervention

The intervention consisted of oral physiological dosage of liquid hydrocortisone: 10 mg/m^2/day in a circadian rhythm; 5 mg/m^2 in the morning directly after awakening, 3 mg/m^2 in the afternoon and 2 mg/m^2 in the evening. Hydrocortisone was administered for five consecutive days, in addition to dexamethasone. Placebo was administered similarly and had the same appearance and taste as hydrocortisone. Patients were randomized using the method of a prefixed randomization list, prepared by the pharmacy, to receive two courses hydrocortisone followed by two courses placebo, or vice versa (Figure 1). The administration of study medication was blinded for physicians, parents, patients and research personnel.

At the close-out visit, parents were asked whether they thought their child had started with hydrocortisone or with placebo during the RCT.

Outcomes

The primary outcome was measured at all timepoints (T1-T10). Secondary outcomes were measured on T1/T2, T3/T4 and T7/T8, except for health-related quality of life and objective sleep through actigraphy, which were measured at T3/T4 and T7/T8 only, to minimize patient burden (Figure 1).

Primary outcome

Neurobehavioral problems

To answer our primary aim, we used the Dutch version of the parent-reported SDQ.[13-17] This 25-item questionnaire assesses psychological adjustment of children and youths and provides five subscales: emotional symptoms, conduct problems, hyperactivity and inattention, peer relationship problems, and prosocial behavior. The Total difficulties score is the sum of the first four subscale scores (i.e. without prosocial behavior), a higher score reflects more problems.

Secondary outcomes

Sleep problems

Children wore a wrist-worn actigraph (ActiGraph wGT3X-BT, Pensacola, FL, USA) for seven consecutive days twice: once during hydrocortisone and once during placebo (Figure 1). The parent kept an additional sleep diary. To assess subjective sleep quality and sleep disturbances, we used the Sleep Disturbance Scale for Children (SDSC).[18] This questionnaire contains 26 items and yields six subscales and a Total sleep score: a higher score reflects more problems.

Hunger score

To measure dexamethasone-induced feeling of hunger, we used an Eating Thermometer (ET): a visual analogue scale to indicate hunger.[19,20] Four different thermometers were administered: to indicate average, least and worst hunger the past 24 hours, and fasting feeling of hunger. The scale ranged from 0 (no hunger at all) to 10 (terrible hunger).

Health-related quality of life

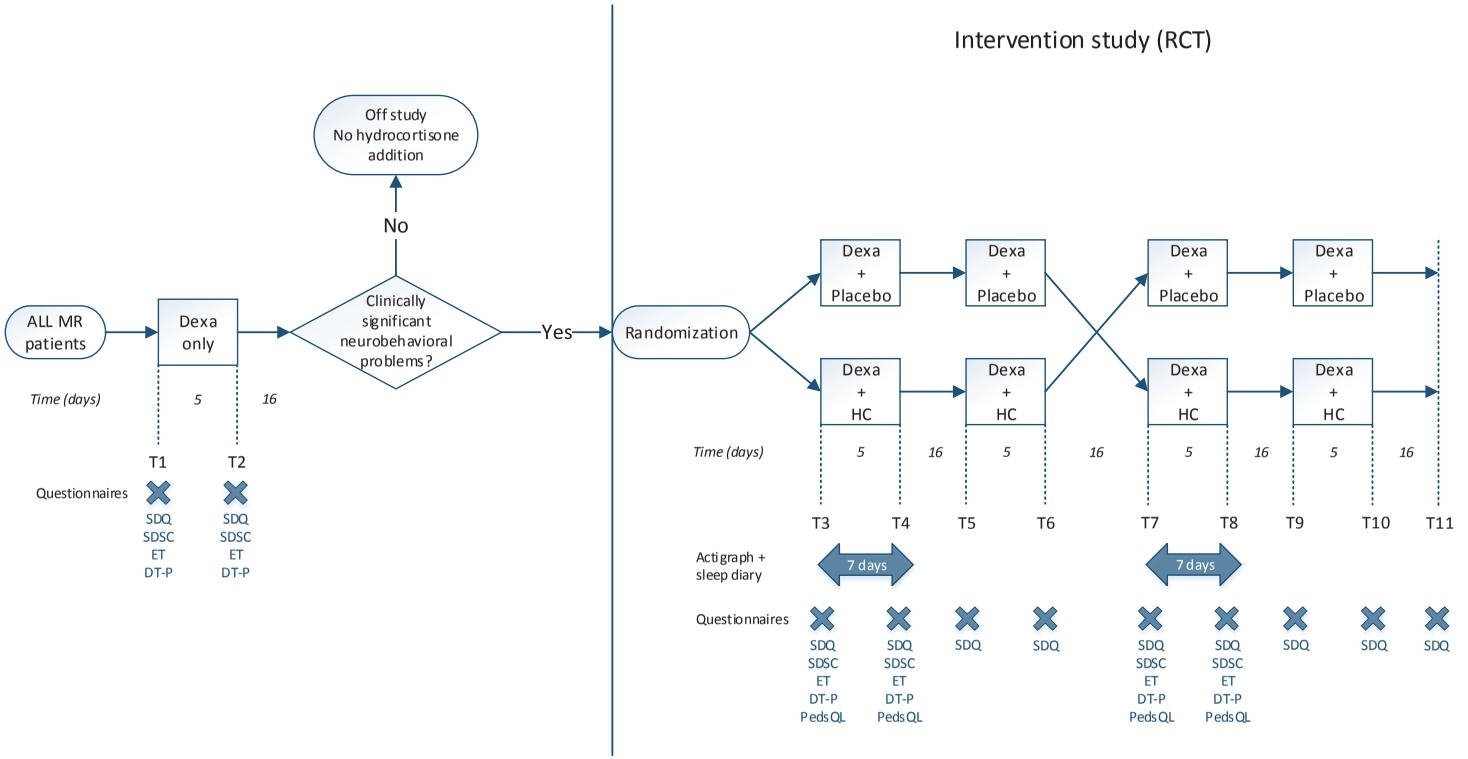
The Pediatric Quality of Life Inventory (PedsQL), a 21- (for toddlers) or 23-item questionnaire, was used to assess HRQoL.[21] A higher score reflects a better HRQoL in the child.

Parental distress

We used the Distress Thermometer for parents (DT-P) to assess parental distress.[22] Parents were asked to rate their overall distress from 0 (no distress) to 10 (extreme distress).

Adverse events

All adverse events, defined as any change in condition between the very first dose and 16 days after the last dose of study medication, were recorded consistent with the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 5.0.[23]


Intervention study (RCT)
ALL MR patients
Dexa only
Clinically significant neurobehavioral problems?
No
Off study
No hydrocortisone addition
Yes
Randomization
Time (days)
5
16
T1
T2
Questionnaires
SDQ
SDSC
ET
DT-P
Dexa + Placebo
Dexa + HC
T3
T4
T5
T6
T7
T8
T9
T10
T11
Actigraph + sleep diary
7 days
DT-P PedsQL

◂ **Figure 1.** DexaDays-2 Study Design

Eligible ALL patients were first enrolled to identify clinically relevant dexamethasone-induced neurobehavioral problems. Parents filled in several questionnaires before (T1) and after (T2) a 5 day 'dexamethasone only' treatment. If patients showed ≥5 points increase on the SDQ Total difficulties score, they were included in the RCT and subsequently randomized to start with either placebo or hydrocortisone. After two courses cross-over took place. Before and after each treatment block, parents filled in several questionnaires (T3-T10). During the first course of each treatment (hydrocortisone and placebo), patients also wore an actigraph to measure sleep objectively. T11 was used as a close-out visit.

Abbreviations: ALL: acute lymphoblastic leukemia, dexa: dexamethasone, DT-P: distress thermometer for parents, ET: eating thermometer, HC: hydrocortisone, MR: medium risk, PedsQL: pediatric quality of life questionnaire, RCT: randomized placebo-controlled trial SDSC: sleep disturbance scale for children, SDQ: Strengths and Difficulties Questionnaire

Statistical analysis

Descriptive statistics for baseline characteristics with either means and standard deviations or medians with interquartile ranges, depending on distribution, were calculated. Comparison of baseline characteristics between included patients and not included patients was done with χ^2 test or Mann-Whitney U test in case of violation of normality assumption.

First, the data was analyzed for carry-over effect or period effect (i.e. the order of treatment), using a paired-samples T-test or Mann-Whitney U test. To assess the effect of hydrocortisone on neurobehavioral problems we calculated delta SDQ scores by subtracting the SDQ score at the start of a dexamethasone course, from the SDQ score after five days of dexamethasone (e.g. T6-T5 or T4-T3, Figure 1). These delta scores were compared using the Wilcoxon signed-rank test, as was described in our study design.[11]

Furthermore, due to the presence of repeated measures, a generalized mixed model was estimated to study the effect of hydrocortisone. Included covariates were age, sex, start group (hydrocortisone/placebo), week of maintenance treatment, concomitant asparaginase treatment (yes/no),[24] and whether mother or father completed the questionnaire.[25] An interaction term between intervention and time was also included. To assess the effect of hydrocortisone compared to placebo, we estimated a mixed model for timepoints T3 to T10. The toeplitz covariance matrix structure was used in the model since the within subjects' correlation gets weaker for times further apart.

Subscores and secondary outcomes were analyzed in a similar way as described above. A decrease on the SDQ Total Difficulties score of 5 points (1 standard deviation (SD) of the norm) was considered clinically significant. A p-value <0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics version 26.0.

RESULTS

Of 256 newly diagnosed ALL patients (17th May 2018 till 5th August 2020), 123 patients were eligible, of whom 79 gave informed consent to participate. The most common reported reason for refraining from participation was the burden and time-consuming nature of the study (38%). Of the 79 included patients, 52 (66%) experienced clinically relevant dexamethasone-induced side-effects and were therefore eligible for the RCT and subsequently randomized to start with hydrocortisone (n = 26) or placebo (n = 26) (Figure 2).

Median age at the start of the RCT was 5.5 years (range 3.0–18.8) and 61.5% were boys. The randomized subgroups (hydrocortisone or placebo first) did not differ significantly with respect to baseline characteristics and baseline questionnaire measurements. The total group (n = 79), patients who refused to participate (n = 44), as well as the included patients who were not eligible for the RCT (n = 27), were not statistically different with regard to baseline characteristics either (Supplemental Table 1). There was no carry-over effect (p = 0.49), nor a period effect (p = 0.77) in our study, based on the primary outcome.

Primary outcome: neurobehavioral problems

The median increase in SDQ Total Difficulty score (delta SDQ) during 'dexamethasone only' was 12 points (interquartile range (IQR) 8–15). During hydrocortisone courses the median delta SDQ was 5 points (IQR 2–9) and during placebo courses 6 points (IQR 3–9) (Table 1). There was no statistically significant difference between hydrocortisone and placebo in reducing dexamethasone-induced neurobehavioral problems (p = 0.33). The mixed model analysis showed the same trend: estimated effect hydrocortisone compared to placebo −2.05 (95% CI −6.00 to 1.90) (Figure 3, Table 1, Supplemental Table 2).

None of the covariates included in the model were associated with the primary outcome. The findings were consistent in the analyses of the SDQ subscores, however with smaller estimated effects (Table 1, Supplemental Table 2).

At the end of the study period for each individual child, parents indicated whether they thought their child had started with placebo or hydrocortisone. Of 52 parents, 24 (46%) were correct, 24 (46%) were not, and four parents (8%) were unsure.

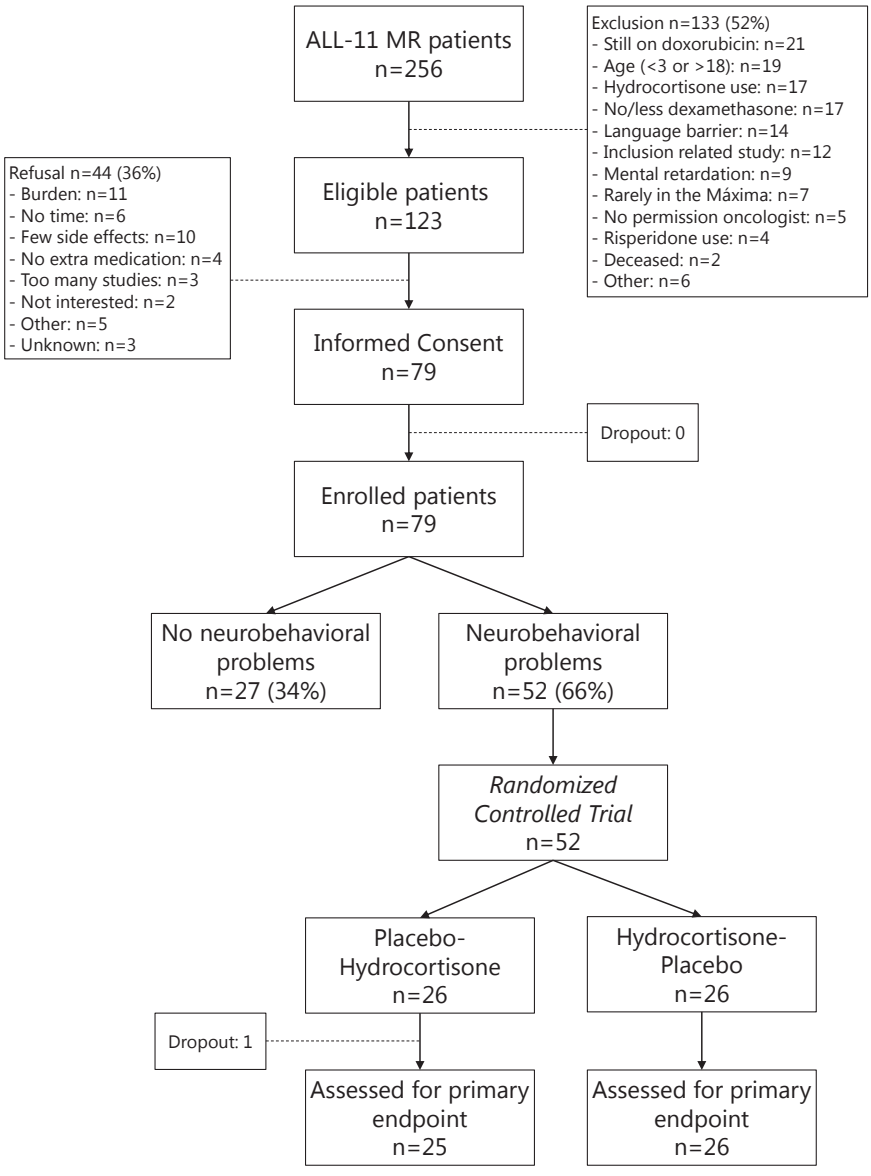


Figure 2. CONSORT flow diagram

ALL patients were screened on our in- and exclusion criteria and after approval of the treating pediatric oncologist approached for inclusion. Reasons for refusal are what parents or patients themselves reported. After enrollment, patients were measured during a 'dexamethasone only' course. Patients with clinically significant neurobehavioral problems were subsequently included in the randomized controlled trial. Due to one dropout 26 children who started with hydrocortisone and 25 children who started with placebo were assessed for our primary endpoint.

Abbreviations: ALL: acute lymphoblastic leukemia, MR: medium risk

Table 1. Difference in neurobehavioral side effects measured with the SDQ

n = 51 median (IQR)	**Randomized Controlled Trial**			
	Δ score dexa only	*Average Δ 2 courses hydrocortisone*	*Average Δ 2 courses placebo*	**Hydrocortisone vs placebo** estimated effect (95%CI)
Total Difficulties	12.0 (8.0;15.0)	5.0 (2.0;9.0)	5.8 (3.0;9.0)	-2.05 (-6.00;1.90)
Emotional problems	4.0 (3.0;6.0)	1.5 (0.5;3.0)	2.0 (1.5;3.5)	-0.94 (-2.49;0.60)
Conduct problems	2.0 (1.0;3.0)	1.0 (0.5;2.0)	1.0 (0.0;2.0)	-0.32 (-1.54;0.89)
Hyperactivity	4.0 (2.0;5.0)	1.5 (0.0;3.0)	2.0 (1.0;4.0)	-1.64 (-3.29;0.01)
Peer problems	2.0 (1.0;3.0)	1.0 (0.0;2.0)	0.5 (0.0;1.5)	0.88 (-0.18;1.93)
Prosocial	-4.0 (-5.0;-2.0)	-2.0 (-3.5;-0.5)	-2.0 (-3.0;-1.0)	-0.37 (-1.85;1.10)

Delta scores are calculated for the SDQ Total difficulties score and all subscales by subtracting day 1 (start dexamethasone course) from day 5 (end of dexamethasone course) scores. Hydrocortisone and placebo were added during two subsequent courses, for these courses the average delta score was calculated. The estimated effect is corrected for age, sex, start group (hydrocortisone/placebo), week of maintenance treatment, concomitant asparaginase treatment (yes/no), whether mother or father completed the questionnaire and an interaction term between intervention and time. Italicized values are statistically significant.
Abbreviations: 95%CI: 95% confidence interval, dexa: dexamethasone, IQR: interquartile range, SDQ: strengths and difficulties questionnaire

Secondary outcomes

In total, 75 actigraphy weeks of 39 children were available for analysis. The main reason for missing data was the child refusing to wear the Actigraph (n = 11). No statistically significant difference in any sleep outcome between hydrocortisone and placebo was observed (Supplemental Table 3).

The median delta SDSC Total score (n = 42) was 11 points (IQR 6–18) in 'dexamethasone only' course, and 4 (IQR 1–10) and 3 (IQR 1–8) in the hydrocortisone and placebo courses, respectively. There was no significant difference between hydrocortisone and placebo (Figure 4a, Supplemental Table 4). Results did suggest that parents reported less sleep problems if their child was further in maintenance treatment (Supplemental Table 5).

The median delta most extreme hunger score (n = 38) was 2 points during 'dexamethasone only' (IQR 2–4), 2 points during hydrocortisone (IQR 1–4) and 2 points during placebo (IQR 1–3). Results showed that hydrocortisone led to an increased average and fasting hunger score compared to placebo (Figure 4b, Supplemental Tables 4 and 6).

The median delta PedsQL score (n = 41) was −14 points (IQR −24 to −4) during hydrocortisone and −15 points (−26 to −7) during placebo, a difference which was not statistically significant (Figure 4c, Supplemental Tables 4 and 7).

The delta distress thermometer score (n = 40) was 2 (IQR 1–4) during 'dexamethasone only' and the hydrocortisone and placebo courses, no difference between hydrocortisone and placebo in reducing parental distress was found (Figure 4d, Supplemental Tables 4 and 8).

Adverse Events

All adverse events (AEs) are depicted in Supplemental Table 9. Overall, adverse events were usually minor (grade 1 or 2) and equally divided between hydrocortisone and placebo periods. Most serious adverse events (SAEs) were scored as being related to leukemia treatment (Supplemental Table 10). However, one patient left the study during the third study course due to abnormal behavior (CTCAE grade 2). The mother described that her daughter became angry, delusional and associative after starting study medication. Therefore, after 2.5 days, her study medication was discontinued, her behavior normalized, and deblinding took place for this patient. The study medication was hydrocortisone, and the episode was reported as an SAE, possibly related to the study medication.

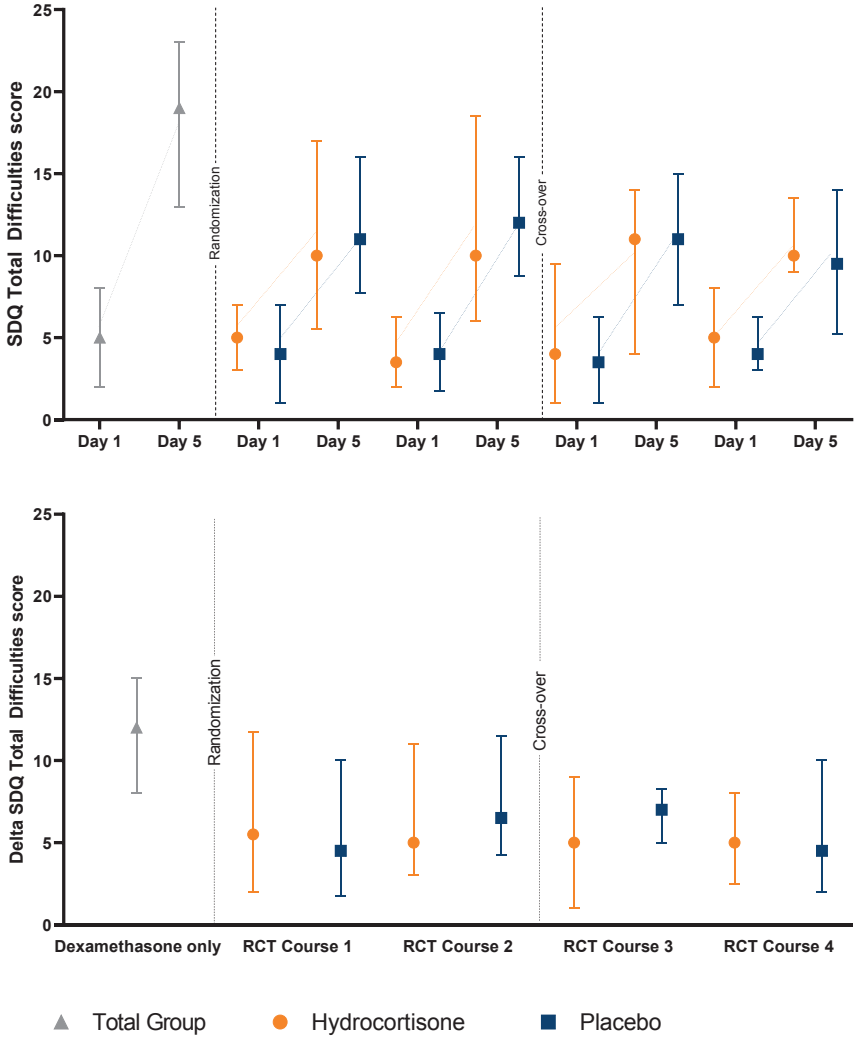


Figure 3. Effect of hydrocortisone and placebo on dexamethasone-induced neurobehavioral problems

A) SDQ Total difficulties scores (median with IQR) on day one (start dexamethasone) and day five (stop dexamethasone). Grey triangles represent the total group (n = 51) during the 'dexamethasone only' course. During the RCT, patients who receive hydrocortisone or placebo (n = 25 or n = 26) are indicated with orange circles or blue squares, respectively.

B) Delta SDQ Total difficulties score (median with IQR) of the total group (n = 51) is indicated in a grey triangle. After randomization patients who receive hydrocortisone or placebo (n = 25 or n = 26) are indicated with an orange circle or a blue square, respectively.

Abbreviations: IQR: interquartile range, SDQ: strengths and difficulties questionnaire, RCT: randomized clinical trial

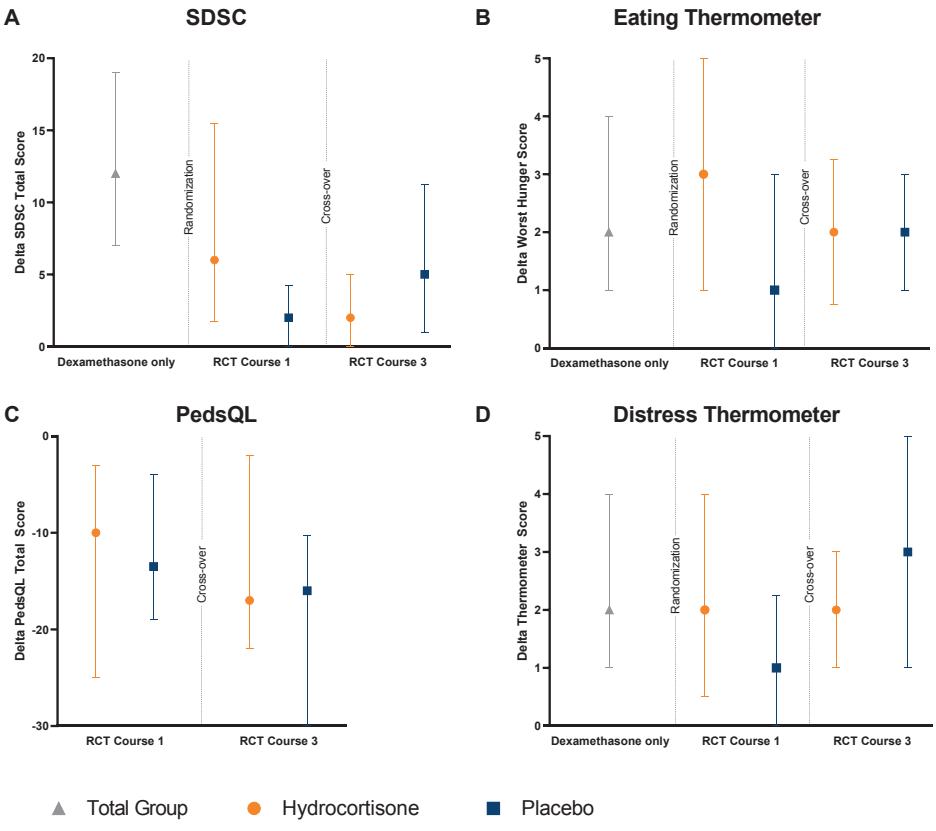


Figure 4. Effect of hydrocortisone and placebo on (dexamethasone-induced) sleep problems, feeling hungry, quality of life and parental distress

The total group (n = 51) during a 'dexamethasone only' course is indicated with a grey triangle. After randomization patients who receive hydrocortisone are indicated with an orange circle, and patients who receive placebo are indicated with a blue square (n = 26 or n = 25).

(A) Delta SDSC Total score (median with IQR). (B) Delta Worst Hunger score (median with IQR). (C) Delta PedsQL total score (median with IQR). The PedsQL was only measured during the RCT. (D) Delta Thermometer score: Parental distress measured with the Distress thermometer for parents (median with IQR).

Abbreviations: IQR: interquartile range, SDSC: sleep disturbance scale for children, PedsQL: pediatric quality of life (questionnaire), RCT: randomized clinical trial.

DISCUSSION

Our study showed that hydrocortisone, when compared to placebo, had no additional effect in reducing clinically relevant dexamethasone-induced neurobehavioral problems in children with ALL. Similarly, hydrocortisone was not better in reducing dexamethasone-induced sleep problems, feeling of hunger, parental distress or improving quality of life as compared to placebo.

The finding that, when compared to placebo, hydrocortisone did not significantly reduce dexamethasone-induced neurobehavioral problems was surprising, since our previous RCT suggested a beneficial effect of hydrocortisone.[10] Several choices in the current study design may have contributed to this different outcome. First, we selected patients with a rise of ≥5 points on the SDQ during a 'dexamethasone only' course, whereas the previous study did a post-hoc analysis on selected patients with a rise of ≥5 points during a placebo course. The results of the previous study may have been based on regression to the mean, rather than an effect of hydrocortisone. Second, we increased the inclusion age to 18 years, compared to 16 in the former study. This may have influenced our results, since older children may have a lower risk of behavioral problems.[26] Nevertheless, only three patients older than 16 were included in our study. A third difference was the presence of two courses hydrocortisone and placebo instead of one, by which we aimed to mimic the repetitive dexamethasone courses with an often changing burden of side-effects. We accounted for the presence of repeated measurements by using a generalized mixed model to estimate the effect of hydrocortisone on the outcomes. This is a different analysis than the previously published study where the Wilcoxon-signed-rank test was used.

Due to our nationalized pediatric cancer care, more extensive information about side-effects of dexamethasone and experiences from other patients and parents may have influenced our results, illustrated by the fact that 66% of the included patients experienced clinically significant side-effects, in contrast to 35% in our previous study.[10] Previous negative experiences, worrisome information, mistaken beliefs and negative expectations induced by verbal suggestions are known to increase or even cause side-effects, and are described as *nocebo*-effects.[27-29] This nocebo-effect (by proxy)[30] of dexamethasone may have played an important role in our findings. In children, nocebo-effects can be severe and often anticipatory.[31] Behavioral and anticipatory adjustment of both child and family, may give rise to intensified behavioral changes. Hence, despite the fact that informing parents and children regarding side-effects is standard of care, overextended information may provoke non-intended adverse effects.

The secondary outcomes of this study were sleep, quality of life, and hunger feeling. Hydrocortisone did not reduce parental distress or improve sleep problems or quality of life of patients. Additionally, the average hunger score and fasting hunger score increased during hydrocortisone compared to placebo. The effect of glucocorticoids on hunger is not completely unraveled, and our findings may be explained by the fact that glucocorticoids act differently on appetite than on other side-effects, for example by altering excretion of appetite-regulating hormones, such as leptin.[32] Besides a different mechanism, a bias in reporting the hunger score could play a role since this proved to be difficult for parents, resulting in fewer patients to evaluate.

An interesting observation in our data is that the delta scores of the first, 'dexamethasone only' course are remarkably higher than the subsequent delta scores in the RCT (figures 3 and 4, tables 1 and 4). This may be caused by regression to the mean, however other explanations may be possible. The decrease in side-effects during the RCT may be attributed to a placebo (by proxy) effect.[29,30,33] Expectancies, which are an important learning mechanism and may steer placebo-effects,[34] may have played a role in our study, since both parents and children were informed about the potential positive effect of hydrocortisone. Furthermore, a participation effect or classical conditioning may have occurred: by adding an oral suspension to standard treatment, patients can be triggered to show physiological responses to additional medication.[29,35-37] However, since we did not include a third observational arm with treatment as usual, a direct comparison between the intervention with hydrocortisone or placebo and no intervention (natural course) cannot be made.

Clinical implications and future directions

The question remains, should we use hydrocortisone in clinical practice? Our study suggests that hydrocortisone has the same effect as placebo on the outcome. Therefore, hydrocortisone is not advised as standard of care for children with ALL who experience dexamethasone-induced neurobehavioural problems. The current study was not designed including a third 'treatment as usual' arm, therefore we cannot show that both hydrocortisone and placebo improve side-effects compared to a non-intervention setting. Based on the observations in our study, it would be interesting to explore the possibilities of nocebo- and placebo-effects in the respective prevention and treatment of dexamethasone-induced side-effects. A recent expert consensus paper regarding placebo- and nocebo-effects in adults stresses the importance of making optimal use of placebo-effects to achieve better treatment outcomes.[38] Studying the effect of hydrocortisone and open-label placebo, which has been proven effective in children with functional abdominal pain or attention deficit hyperactivity disorder (ADHD), would be very interesting.[37,39] Besides further research on the placebo-effect, we propose to create awareness about possible nocebo-effects of dexamethasone in clinical practice.

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SUPPLEMENTAL METHODS

Acute lymphoblastic leukemia treatment

Only patients with medium risk acute lymphoblastic leukemia (ALL) were eligible for our study since standard risk and high risk patients do not receive regular courses of dexamethasone. The ALL-11 medium risk group maintenance phase in which our research was conducted contains 28 three week treatment cycles. Patients could start with the study after cessation of doxorubicin, which is given on the first day of the first four treatment cycles. Patients could be included in the study at any given time, that is, some patients started directly after cessation of doxorubicin (beginning of maintenance treatment) and some patients started later or at the end of maintenance treatment.

Dexamethasone 6 mg/m2/day was given during 5 consecutive days at the beginning of each treatment cycle. Patients also received vincristine once every three weeks, methotrexate once per week and 6-mercaptopurine once per day. A subgroup of patients also received asparaginase once every three weeks.

Sample size calculation

A sample size of 23 pairs with a correlation equal to 0 achieves 79% power to detect a difference of -5,2 between the null hypothesis mean difference of 0 and the actual mean difference of -5,2 at the 5% significance level (alpha) using a two-sided Wilcoxon Signed-Rank Test. These results were based on 3000 Monte Carlo samples from the null distribution: Normal with mean 3.4 and standard deviation 5.4 and the alternative distribution Normal with mean 8.6 and standard deviation equal to 6.3. Power computations were performed with PASS 2020 Power Analysis & Sample Size (https://www.ncss.com/software/pass/). We included 52 patients in our RCT and therefore met the aim of including 50 individual patients.

Objective sleep measurement with ActiGraph

Patients (sometimes through their parents) were instructed to wear the ActiGraph non-stop for seven consecutive days twice (during hydrocortisone and placebo). The ActiGraph could only be removed for showering or swimming. The parent kept an additional sleep-diary to document bedtimes, time of awakening, and removal periods. Actigraphy data were processed using ActiLife version 6.13.3. The Sadeh algorithm was used to generate sleep outcomes.[40] Incomplete or invalid data were removed from further analyses and only wearing periods with 4 or more valid nights were assessed.[41,42]

Using the Sadeh algorithm, several sleep outcomes were generated: total sleep time (TST: number of minutes asleep during the time spent in bed); time in bed (TiB: total number of minutes spent in bed); sleep efficiency (SE: the ratio between TST and TiB), sleep onset latency (SOL: number of minutes between bedtime and first minute scored as sleep); and wake after sleep onset (WASO: number of minutes awake after onset of sleep).

Parent reported questionnaires

Parents were asked to fill in several questionnaires on different timepoints. All questionnaires were web based and data was collected through a secure website, www.hetklikt.nu, a safe internet environment which is widely used in pediatric (oncology) care in the Netherlands.

Parents were instructed to provide information regarding the previous five days: that is, with or without dexamethasone and/or study medication.

Strengths and difficulties questionnaire (SDQ)

The parent-reported SDQ contains 25 items and is divided in 5 subscales: emotional symptoms, conduct problems, hyperactivity-inattention, peer problems and prosocial behavior. A Total difficulties score can be calculated by adding up the first four subscales (i.e. excluding prosocial behavior). Clinical cut-off scores are age dependent, and an increase of one SD above norm values is considered clinically significant and lies between 4 and 6 points.[13] The primary caregiver was asked to complete the SDQ on all eleven testing moments.

Sleep Disturbance Scale for Children (SDSC)

The parent-reported SDSC contains 26 items and yields 6 subscales which cover the most common sleep problems in childhood and adolescence: disorders of initiating and maintaining sleep (DIMS), disorders of arousal (DA), sleep-wake transition disorders (SWTD), disorders of excessive somnolence (DES) and sleep hyperhidrosis (SHY). The total sleep score is the sum of the 26 items with a range of 26-130.

The primary caregiver was asked to complete the SDSC before and after the dexamethasone course and before and after one hydrocortisone and placebo course.

Eating Thermometer (ET)

To measure a hunger score we used the ET questionnaire, which consisted of 4 different thermometers. Parents were asked to fill (together with their child when possible) in how much hunger their child experienced on average the past 24 hours, when waking up in the morning (fasting score) and to indicate the least and worst hunger feeling the past day on a visual analogue scale (range 0-10).

The ET was completed before and after a dexamethasone, hydrocortisone and placebo course (figure 1).

Pediatric Quality of Life Inventory (PedsQL)

Health-related quality of life (HRQoL) was measured with the PedsQL, a 21 or 23-item questionnaire which encompasses 4 scales: physical functioning, emotional functioning, social functioning and school functioning. Items are reverse-scored and linearly transformed to a 0-100 scale. A higher score therefore reflects a better HRQoL. The PedsQL was completed before and after one course of hydrocortisone and placebo.

Distress thermometer (DT-P)

Parental distress was measured with the Distress thermometer for parents: a visual analogue scale to indicate parental distress. before and after their child received dexamethasone, hydrocortisone and placebo (figure 1).

SUPPLEMENTAL TABLES

Supplemental Table 1. Description of baseline characteristics.

		Refusal n = 44	Total group n = 79
Age at start study		6.0 (4.1;8.7)	6.0 (4.3;9.3)
Sex	Male	24 (54.5)	50 (63.3)
	Female	30 (45.5)	29 (36.7)
Week of maintenance phase		33 (23;46)	37 (28;49)
ALL subtype	B-ALL	39 (88.6)	68 (86.1)
	T-ALL	5 (11.4)	10 (12.7)
	BPDCN	0	1 (1.3)
CNS-status at diagnosis	CNS-1	23 (52.3)	32 (40.5)
	CNS-2	15 (34.1)	27 (34.2)
	CNS-3	1 (2.3)	5 (6.3)
	TLP+	4 (9.1)	12 (15.2)
	Undetermined	1 (2.3)	3 (3.8)

Numbers are depicted as median (interquartile range) or number (%).

Abbreviations: ALL: acute lymphoblastic leukemia, BPDCN: blastic plasmacytoid dendritic cell neoplasm, CNS: central nervous system, TLP: traumatic lumbar puncture, RCT: randomized controlled trial

	Dexamethasone course only n = 27	RCT n = 52	Start hydrocortisone n = 26	Start placebo n = 26
	7.6 (4.4;13.1)	5.5 (4.1;8.4)	5.8 (4.1;9.1)	5.4 (4.1;8.0)
	18 (66.7)	32 (61.5)	19 (73.1)	13 (50.0)
	9 (33.3)	20 (38.5)	7 (26.9)	13 (50.0)
	37 (28;49)	37 (25;49)	34 (25;44)	42 (27;53)
	22 (81.5)	46 (88.5)	25 (96.2)	21 (80.8)
	4 (14.8)	6 (11.5)	1 (3.8)	5 (19.2)
	1 (3.7)			
	11 (40.7)	21 (40.4)	10 (38.5)	11 (42.3)
	9 (33.3)	18 (34.6)	12 (46.2)	6 (23.1)
	3 (11.1)	2 (3.8)	1 (3.8)	1 (3.8)
	4 (14.8)	8 (15.4)	2 (7.7)	6 (23.1)
	0	3 (5.8)	1 (3.8)	2 (7.7)

Supplemental Table 2. Repeated measurement of ΔSDQ in the RCT (T3-T10)

	Total Difficulties		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-2.05	-6.00	1.90
Start group			
- Placebo			
- Hydrocortisone	0.68	-1.67	3.04
Week of maintenance treatment	-0.04	-0.13	0.06
Interaction intervention and week of maintenance treatment			
- Placebo	0.03	-0.06	0.12
- Hydrocortisone			
Sex	-0.64	-3.02	1.75
Asparaginase	0.66	-3.63	4.95
Age at registration	-0.16	-0.50	0.18
Parent who completed questionnaire	-0.24	-2.16	1.68
	Emotional Problems		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-0.94	-2.49	0.60
Start group			
- Placebo			
- Hydrocortisone	0.79	-0.18	1.75
Week of maintenance treatment	0.003	-0.04	0.04
Interaction intervention and week of maintenance treatment			
- Placebo	0.01	-0.02	0.05
- Hydrocortisone			
Sex	0.30	-0.67	1.28
Asparaginase	0.21	-1.54	1.95
Age at registration	-0.05	-0.18	0.09
Parent who completed questionnaire	-0.02	-0.79	0.75

Supplemental Table 2. Continued

	Conduct problems		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-0.32	-1.54	0.89
Start group			
- Placebo			
- Hydrocortisone	-0.11	-0.72	0.49
Week of maintenance treatment	-0.02	-0.04	0.01
Interaction intervention and week of maintenance treatment			
- Placebo	0.01	-0.02	0.04
- Hydrocortisone			
Sex	-0.43	-1.04	0.18
Asparaginase	0.41	-0.69	1.52
Age at registration	-0.05	-0.14	0.04
Parent who completed questionnaire	-0.07	-0.61	0.47
	Hyperactivity		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-1.64	-3.29	0.01
Start group			
- Placebo			
- Hydrocortisone	-0.21	-1.22	0.80
Week of maintenance treatment	-0.01	-0.05	0.03
Interaction intervention and week of maintenance treatment			
- Placebo	0.02	-0.02	0.06
- Hydrocortisone			
Sex	-0.52	-1.54	0.51
Asparaginase	0.62	-1.21	2.45
Age at registration	-0.01	-0.16	0.13
Parent who completed questionnaire	0.04	-0.77	0.85

Supplemental Table 2. Continued

	Peer problems		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	0.88	-0.18	1.93
Start group			
- Placebo			
- Hydrocortisone	0.21	-0.45	0.88
Week of maintenance treatment	-0.01	-0.04	0.01
Interaction intervention and week of maintenance treatment			
- Placebo	-0.02	-0.04	0.01
- Hydrocortisone			
Sex	-0.01	-0.68	0.67
Asparaginase	-0.59	-1.79	0.62
Age at registration	-0.05	-0.15	0.04
Parent who completed questionnaire	-0.23	-0.75	0.30
	Prosocial		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-0.37	-1.85	1.10
Start group			
- Placebo			
- Hydrocortisone	0.31	-0.59	1.21
Week of maintenance treatment	0.03	-0.01	0.06
Interaction intervention and week of maintenance treatment			
- Placebo	0.01	-0.03	0.04
- Hydrocortisone			
Sex	0.55	-0.36	1.46
Asparaginase	0.27	-1.36	1.90
Age at registration	0.07	-0.06	0.20
Parent who completed questionnaire	0.61	-0.12	1.33

Supplemental table 3. Descriptive statistics of sleep outcomes measured with Actigraph

	Hydrocortisone *Mean (SD)*	**Placebo** *Mean (SD)*	**Estimated effect** (95%CI)
Sleep Onset Latency (SOL) *Min*	22.7 (11.4)	21.7 (13.4)	1.60 (-1.39;4.59)
Sleep Efficiency (SE) *TST/TiB*	76.6 (5.0)	76.9 (5.5)	-0.15 (-1.63;1.34)
Time in Bed (TiB) *Min*	669.3 (43.4)	656.8 (46.0)	10.23 (-5.00;25.45)
Total Sleep Time (TST) *Min*	512.0 (41.4)	504.2 (42.1)	6.88 (-7.26;21.01)
Wake After Sleep Onset (WASO) *Min*	134.6 (34.2)	130.9 (39.7)	1.62 (-9.35;12.59)
Number of Awakenings (NA) *N*	30.0 (5.1)	28.2 (5.6)	1.62 (-0.29;3.54)

Estimated effects are corrected for age, sex, start group (hydrocortisone/placebo), week of maintenance treatment, concomitant asparaginase treatment (yes/no).
Abbreviations: 95%CI: 95% confidence interval, min: minutes, SD: standard deviation.

Supplemental table 4. Results other parent-reported questionnaires

		Δ dexa only course
SDSC n = 42	Total Score	11.0 (5.8;18.0)
	DIMS	4.0 (0.0;7.3)
	Sleep breathing disorder	0.0 (0.0;1.0)
	Arousal	(0.0;0.0)
	SWTD	1.0 (0.0;3.3)
	DES	4.5 (1.8;6.3)
	Hyperhidrosis	0.5 (0.0;2.0)
Eating thermometer n = 38	Average	2 (0;5)
	Most	2 (1.8;4.3)
	Least	1 (0;3.3)
	Fasting	3.5 (2;5)
PedsQL n = 41	Total Score	
	Physical functioning	
	Emotional functioning	
	Social functioning	
	School functioning	
	Psychosocial functioning	
DT-P n = 40	Thermometer score	2 (1;4)

Results are reported as median (IQR). Estimated effects are corrected for age, sex, start group (hydrocortisone/placebo), week of maintenance treatment, concomitant asparaginase treatment (yes/no). Bold values are statistically significant.

Randomized controlled trial		
Δ hydrocortison course	*Δ placebo course*	**HC vs placebo** estimated effect (95%CI)
3.5 (0.8;10.0)	3.0 (1.0;8.0)	-1.96 (-9.27;5.35)
1.5 (0.0;6.0)	2.0 (-0.3;4.3)	0.12 (-4.20;4.44)
0.0 (0.0;0.0)	0.0 (0.0;0.0)	0.20 (-0.53;0.93)
0.0 (0.0;0.0)	0.0 (0.0;0.0)	0.38 (-0.32;1.08)
0.0 (-1.0;1.3)	0.0 (0.0;1.0)	-0.93 (-3.33;1.48)
2.0 (0.0;4.5)	2.0 (0.0;4.0)	-1.23 (-4.38;1.93)
0.0 (0.0;1.0)	0.0 (-1.0;0.0)	-0.45 (-1.67;0.77)
2 (0.8;4)	2 (0;4)	**2.52 (0.23;4.81)**
2 (1;4)	2 (0.75;3)	1.25 (-1.10;3.60)
0.5 (0;2.3)	2 (0;3)	0.63 (-1.87;3.14)
2 (0;4)	2 (0;3)	**2.74 (0.07;5.41)**
-14.1 (-23.8/-4.2)	-15.2 (-25.6/-7.1)	3.25 (-13.69;20.20)
-12.5 (-29.7/-3.1)	-18.8 (-31.3/-9.4)	14.28 (-6.77;35.34)
-20.0 (-27.5/-5.0)	-15.0 (-27.5/-5.0)	-0.10 (-22.22;22.02)
-10.0 (-20.0/-2.5)	-15.0 (-20.0/0.0)	0.86 (-16.31;18.03)
-8.3 (-22.5/0.0)	-5.0 (-30.0/0.0)	-11.08 (-39.29;17.12)
-11.5 (-23.2/-3.9)	-11.5 (-23.3/-3.3)	-3.13 (-21.53;15.28)
2 (1;4)	2 (1;4)	0.62 (-2.23;3.46)

Abbreviations: DES: disorders of excessive somnolence, dexa: dexamethasone, DIMS: disorders of initiating and maintaining sleep, DT-P: distress thermometer for parents, HC: hydrocortisone, PedsQL: pediatric quality of life questionnaire, SDSC: sleep disturbance scale for children, SWTD: sleep wake transition disorders

Supplemental table 5. Repeated measurement of ΔSDSC in the RCT (T3-T10)

	Total Score		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-1.96	-9.27	5.35
Start group			
- Placebo			
- Hydrocortisone	**5.22**	**1.58**	**8.87**
Week of maintenance treatment	**-0.20**	**-0.36**	**-0.04**
Interaction intervention and week of maintenance treatment			
- Placebo	0.09	-0.08	0.26
- Hydrocortisone			
Sex	0.73	-2.96	4.41
Asparaginase	-0.68	-7.50	6.13
Age at registration	-0.23	-0.74	0.29
Parent who completed questionnaire	-2.83	-5.86	0.19
	Disorders of initiating and maintaining sleep		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	0.12	-4.20	4.44
Start group			
- Placebo			
- Hydrocortisone	**2.66**	**0.56**	**4.75**
Week of maintenance treatment	-0.08	-0.17	0.01
Interaction intervention and week of maintenance treatment			
- Placebo	0.02	-0.08	0.12
- Hydrocortisone			
Sex	0.46	-1.66	2.57
Asparaginase	1.78	-2.14	5.70
Age at registration	-0.01	-0.30	0.29
Parent who completed questionnaire	-1.31	-3.06	0.45

Supplemental table 5. Continued

	Sleep breathing disorder		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	0.20	-0.53	0.93
Start group			
- Placebo			
- Hydrocortisone	0.31	-0.02	0.64
Week of maintenance treatment	0.003	-0.01	0.02
Interaction intervention and week of maintenance treatment			
- Placebo	-0.003	-0.02	0.01
- Hydrocortisone			
Sex	-0.02	-0.35	0.31
Asparaginase	0.23	-0.38	0.85
Age at registration	-0.01	-0.06	0.04
Parent who completed questionnaire	0.06	-0.22	0.34
	Disorders of arousal		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	0.38	-0.32	1.08
Start group			
- Placebo			
- Hydrocortisone	0.11	-0.18	0.40
Week of maintenance treatment	0.008	-0.005	0.22
Interaction intervention and week of maintenance treatment			
- Placebo	-0.004	-0.02	0.01
- Hydrocortisone			
Sex	0.15	-0.14	0.44
Asparaginase	-0.01	-0.56	0.54
Age at registration	0.01	-0.03	0.05
Parent who completed questionnaire	0.03	-0.23	0.28

Supplemental table 5. Continued

	Sleep wake transition disorders		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-0.93	-3.33	1.48
Start group			
- Placebo			
- Hydrocortisone	0.47	-0.41	1.35
Week of maintenance treatment	**-0.05**	**-0.10**	**-0.01**
Interaction intervention and week of maintenance treatment			
- Placebo	0.02	-0.03	0.08
- Hydrocortisone			
Sex	-0.02	-0.90	0.86
Asparaginase	-1.18	-2.85	0.50
Age at registration	-0.07	-0.19	0.06
Parent who completed questionnaire	-0.10	-0.91	0.72
	Disorders of excessive somnolence		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-1.23	-4.38	1.93
Start group			
- Placebo			
- Hydrocortisone	**1.41**	**0.09**	**2.73**
Week of maintenance treatment	-0.06	-0.12	0.0005
Interaction intervention and week of maintenance treatment			
- Placebo	0.03	-0.04	0.11
- Hydrocortisone			
Sex	0.36	-0.97	1.69
Asparaginase	-1.85	-4.34	0.65
Age at registration	-0.13	-0.32	0.06
Parent who completed questionnaire	-0.78	-1.95	0.38

Supplemental table 5. Continued

	Sleep hyperhidrosis		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-0.45	-1.67	0.77
Start group			
- Placebo			
- Hydrocortisone	0.24	-0.37	0.85
Week of maintenance treatment	-0.02	-0.05	0.002
Interaction intervention and week of maintenance treatment			
- Placebo	0.02	-0.01	0.05
- Hydrocortisone			
Sex	-0.18	-0.79	0.44
Asparaginase	0.16	-0.97	1.30
Age at registration	-0.02	-0.11	0.06
Parent who completed questionnaire	-0.46	-0.96	0.05

Supplemental table 6. Repeated measurement of ΔEating Thermometer in the RCT (T3-T10)

	Average hunger feeling		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	**2.52**	**0.23**	**4.81**
Start group			
- Placebo			
- Hydrocortisone	-0.04	-1.26	1.17
Week of maintenance treatment	0.0002	-0.05	0.05
Interaction intervention and week of maintenance treatment			
- Placebo	-0.05	-0.10	0.004
- Hydrocortisone			
Sex	-1.11	-2.32	0.09
Asparaginase	-1.36	-3.58	0.86
Age at registration	-0.13	-0.32	0.06
Parent who completed questionnaire	-0.46	-1.52	0.59
	Most extreme hunger feeling		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	1.25	-1.10	3.60
Start group			
- Placebo			
- Hydrocortisone	0.54	-0.54	1.62
Week of maintenance treatment	-0.004	-0.05	0.04
Interaction intervention and week of maintenance treatment			
- Placebo	-0.02	-0.07	0.04
- Hydrocortisone			
Sex	-0.64	-1.71	0.44
Asparaginase	-0.14	-2.14	1.85
Age at registration	-0.13	-0.30	0.04
Parent who completed questionnaire	**-1.04**	**-2.02**	**-0.06**

Supplemental table 6. Continued

	Least extreme hunger feeling		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	0.63	-1.87	3.14
Start group			
- Placebo			
- Hydrocortisone	0.26	-0.89	1.41
Week of maintenance treatment	0.003	-0.05	0.05
Interaction intervention and week of maintenance treatment			
- Placebo	-0.03	-0.08	0.03
- Hydrocortisone			
Sex	0.08	-1.06	1.22
Asparaginase	-0.53	-2.65	1.59
Age at registration	**-0.21**	**-0.39**	**-0.29**
Parent who completed questionnaire	-0.61	-1.65	0.44
	Hunger feeling in the morning (fasting)		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	**2.74**	**0.07**	**5.41**
Start group			
- Placebo			
- Hydrocortisone	-0.42	-1.93	1.09
Week of maintenance treatment	0.02	-0.04	0.08
Interaction intervention and week of maintenance treatment			
- Placebo	-0.06	-0.12	0.005
- Hydrocortisone			
Sex	-1.18	-2.68	0.33
Asparaginase	0.13	-2.62	2.89
Age at registration	-0.05	-0.29	0.19
Parent who completed questionnaire	-0.09	-1.38	1.20

Supplemental table 7. Repeated measurement of Δ Pediatric Quality of Life questionnaire (PedsQL) in the RCT (T3-T10)

	Total Score		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	3.25	-13.69	20.20
Start group			
- Placebo			
- Hydrocortisone	-2.80	-9.30	3.70
Week of maintenance treatment	0.14	-0.16	0.45
Interaction intervention and week of maintenance treatment			
- Placebo	-0.06	-0.44	0.33
- Hydrocortisone			
Sex	-1.62	-8.11	4.88
Asparaginase	4.54	-7.68	16.76
Age at registration	0.10	-0.93	1.12
Parent who completed questionnaire	2.75	-3.08	8.58
	Physical functioning		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	14.28	-6.77	35.34
Start group			
- Placebo			
- Hydrocortisone	-3.08	-12.86	6.71
Week of maintenance treatment	0.17	-0.26	0.59
Interaction intervention and week of maintenance treatment			
- Placebo	-0.23	-0.71	0.25
- Hydrocortisone			
Sex	-0.84	-10.69	9.00
Asparaginase	3.16	-15.07	21.39
Age at registration	-0.75	-0.81	2.31
Parent who completed questionnaire	6.25	-2.02	14.52

Supplemental table 7. Continued

	Emotional functioning		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-0.10	-22.22	22.02
Start group			
- Placebo			
- Hydrocortisone	-6.04	-14.51	2.43
Week of maintenance treatment	0.23	-0.16	0.63
Interaction intervention and week of maintenance treatment			
- Placebo	-0.09	-0.60	0.41
- Hydrocortisone			
Sex	-4.90	-13.36	3.56
Asparaginase	-1.48	-17.40	14.44
Age at registration	-0.13	-1.46	1.21
Parent who completed questionnaire	5.62	-1.97	13.22
	Social functioning		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	0.86	-16.31	18.03
Start group			
- Placebo			
- Hydrocortisone	-1.75	-9.79	6.29
Week of maintenance treatment	0.07	-0.29	0.42
Interaction intervention and week of maintenance treatment			
- Placebo	-0.03	-0.42	0.36
- Hydrocortisone			
Sex	2.39	-5.70	10.47
Asparaginase	1.77	-13.21	16.74
Age at registration	-0.35	-1.63	0.93
Parent who completed questionnaire	3.13	-3.66	9.91

Supplemental table 7. Continued

	School functioning		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-11.08	-39.29	17.12
Start group			
- Placebo			
- Hydrocortisone	-0.10	-10.36	10.17
Week of maintenance treatment	0.005	-0.59	0.50
Interaction intervention and week of maintenance treatment			
- Placebo	0.32	-0.33	0.96
- Hydrocortisone			
Sex	-4.24	-14.44	5.97
Asparaginase	17.70	-1.67	37.06
Age at registration	-0.54	-2.14	1.06
Parent who completed questionnaire	-6.22	-15.63	3.18
	Psychosocial functioning		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	-3.13	-21.53	15.28
Start group			
- Placebo			
- Hydrocortisone	-2.60	-9.55	4.35
Week of maintenance treatment	0.13	-0.20	0.46
Interaction intervention and week of maintenance treatment			
- Placebo	0.05	-0.38	0.47
- Hydrocortisone			
Sex	-2.12	-9.06	4.81
Asparaginase	5.81	-7.26	18.89
Age at registration	-0.29	-1.38	0.81
Parent who completed questionnaire	1.02	-5.24	7.28

Supplemental table 8. Repeated measurement of Δ Distress Thermometer for parents in the RCT (T3-T10)

	Thermometer Score		
	Estimated effect	95% CI	
		Lower bound	Upper bound
Intervention			
- Placebo			
- Hydrocortisone	0.62	-2.23	3.46
Start group			
- Placebo			
- Hydrocortisone	0.76	-0.47	1.98
Week of maintenance treatment	-0.03	-0.09	0.02
Interaction intervention and week of maintenance treatment			
- Placebo	-0.01	-0.08	0.05
- Hydrocortisone			
Sex	0.77	-0.46	2.00
Asparaginase	-1.14	-3.43	1.16
Age at registration	-0.06	-0.25	0.14
Parent who completed questionnaire	0.03	-1.03	1.10

Supplemental table 9. Adverse Events

Structurally measured AE's					
		Day 5 course 1		**Day 5 course 2**	
		Hydrocortisone	Placebo	Hydrocortisone	Placebo
Pain	grade 0	31	34	29	33
	grade 1	21	16	16	16
	grade 2	0	2	4	2
	grade 4	0	0	0	1
	NP	0	0	3	0
Fatigue	grade 0	37	28	29	32
	grade 1	14	22	19	17
	grade 2	0	2	1	3
	NP	1	0	3	0
Abdominal pain	grade 0	41	43	41	42
	grade 1	11	8	8	10
	NP	0	1	3	0
Constipation	grade 0	43	44	45	43
	grade 1	9	8	3	9
	grade 2	0	0	1	0
	NP	0	0	3	0
Agitation	grade 0	44	45	43	45
	grade 1	7	5	4	6
	grade 2	0	1	0	0
	NP	1	1	5	1
Nausea	grade 0	48	47	46	46
	grade 1	4	5	3	6
	NP	0	0	3	0
Headache	grade 0	49	47	45	47
	grade 1	3	5	4	4
	NP	0	0	3	1
Fever	grade 0	46	51	47	47
	grade 1	2	0	0	3
	grade 2	0	0	1	0
	NP	4	1	4	2
Anorexia	grade 0	45	46	44	44
	grade 1	0	2	2	2
	NP	7	4	6	6
Vomiting	grade 0	51	51	48	51
	grade 1	1	1	1	1
	NP	0	0	3	0
Rash	grade 0	45	46	44	47
	grade 1	1	1	1	1
	NP	6	5	7	4

Supplemental table 9. Continued

Diarrhea	grade 0	52	49	47	51
	grade 1	0	2	2	0
	NP	0	0	3	1
Anxiety	grade 0	50	51	44	51
	grade 1	0	0	3	0
	NP	2	1	5	1
Allergic reaction	grade 0	37	39	40	39
	grade 1	0	1	0	0
	NP	15	12	12	13
Occasionally measured or mentioned AE's					
		Day 5 course 1		**Day 5 course 2**	
		Hydrocortisone	Placebo	Hydrocortisone	Placebo
Pruritus	grade 1	6	3	4	7
	NP	46	49	48	45
Anemia	grade 0	2	1	3	1
	grade 1	2	2	1	2
	NP	48	49	48	49
Cough	grade 1	0	3	0	3
	NP	52	49	52	49
Flushing	grade 1	1	0	1	1
	grade 2	0	0	1	0
	NP	51	52	50	51
ALAT elevated	grade 0	0	0	0	0
	grade 1	0	0	0	0
	grade 2	0	1	0	1
	grade 3	1	0	0	0
	NP	51	51	52	51
ASAT elevated	grade 1	0	0	0	1
	grade 2	0	1	0	0
	grade 3	1	0	0	0
	NP	51	51	52	51
Cushingoid	grade 0	0	0	0	1
	grade 1	1	1	0	1
	NP	51	51	52	50
Hypertension	grade 0	1	2	0	1
	grade 2	0	1	0	0
	grade 3	0	0	1	0
	NP	51	49	51	51

Supplemental table 9. Continued

Allopecia	grade 0	1	2	1	1
	grade 1	0	0	0	1
	grade 2	0	1	0	0
	NP	51	49	51	50
Weight gain	grade 0	3	5	0	0
	grade 1	0	0	1	1
	NP	49	47	51	51
Mucositis oral	grade 0	28	25	27	24
	grade 1	0	0	0	1
	NP	24	27	25	27
Ankle pain	grade 1	0	1	0	0
	NP	52	51	52	52
Arthritis	grade 1	0	0	1	0
	NP	52	52	51	52
Back pain	grade 1	0	0	0	1
	NP	52	52	52	51
Muscle cramp	grade 1	1	0	0	0
	NP	51	52	52	52
Otitis media	grade 2	0	0	0	1
	NP	52	52	52	51
Urinary frequency	grade 1	1	0	0	0
	NP	51	52	52	52
Vertigo	grade 1	0	1	0	0
	NP	52	51	52	52

Abbreviations NP:not performed

Supplemental table 10. Serious Adverse Events (SAE)

CTCAE term	Grade	Type SAE	Timing	Related to study medication
Fever	2	(Prolongation of) hospitalization	Between course 2 and course 3	Not related
Psychosis *	2	Other medically important condition	During course 3 (hydrocortisone)	Possibly related
Febrile neutropenia	3	(Prolongation of) hospitalization	Between course 3 and course 4	Not related
Febrile neutropenia	3	(Prolongation of) hospitalization	Between course 1 and course 2	Not related
Device related infection	3	(Prolongation of) hospitalization	Between course 2 and course 3	Not related
Fever	1	(Prolongation of) hospitalization	Between course 1 and course 2	Unlikely related
Arthritis	2	(Prolongation of) hospitalization	During course 4 (hydrocortisone)	Unlikely related
Fever	1	(Prolongation of) hospitalization	During course 2 (placebo)	Not related

All SAE resolved without sequelae.

* Due to lack of a more appropriate CTCAE term, psychosis was reported for one patient. However, she displayed psychosis-like symptoms, and a diagnosis of psychosis was never made by a psychiatrist.

Abbreviations: CTCAE: common terminology criteria for adverse events

The role of the mineralocorticoid receptor in steroid-induced cytotoxicity in pediatric acute lymphoblastic leukemia

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ABSTRACT

Synthetic glucocorticoids such as dexamethasone and prednisone are cornerstone drugs in the treatment of pediatric acute lymphoblastic leukemia (ALL) because of their cytotoxic effect on leukemic cells. The effect of these steroids is mediated through activation of the glucocorticoid receptor (encoded by the *NR3C1* gene). Besides the glucocorticoid receptor, leukemic cells are known to express the mineralocorticoid receptor (encoded by the *NR3C2* gene). Currently, the role of the mineralocorticoid receptor in steroid-induced cytotoxicity is unclear. Furthermore, hydrocortisone, the synthetic equivalent of the natural occurring hormone cortisol, has never been considered as a potential cytotoxic steroid. In this preclinical study, we show that hydrocortisone can induce the expression of steroid-regulated genes through both steroid receptors, and effectively induces cell death in Reh cell lines that by doxycycline-induction express NR3C1 or NR3C2. Moreover, dexamethasone induces cell death in NR3C2-expressing Reh cells that lack an endogenous functional *NR3C1* receptor gene. These results highlight that the mineralocorticoid receptor is a potent receptor to induce leukemic cell death after activation by steroid treatment, and that hydrocortisone treatment can induce cell death in leukemic cells. In PDX and patient samples, the role of the mineralocorticoid receptor in steroid-induced cytotoxicity seems less pronounced, possibly due to the (relative) low expression of *NR3C2* in ALL patients.

INTRODUCTION

Glucocorticoids, also denoted as steroids, were among the first drug classes used in the treatment of childhood acute lymphoblastic leukemia (ALL) and are still regarded as cornerstone drugs in ALL therapy.[1-3] Different synthetic glucocorticoids, with different molecular aspects and varying properties, are used.[1] Dexamethasone is currently the preferred glucocorticoid in most ALL treatment protocols since its use is linked to less relapses and a higher event-free survival compared to prednisone.[4]

Two receptors exist which can bind glucocorticoids: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR), both nuclear receptors encoded by the *NR3C1* and *NR3C2* gene respectively. The cytotoxic effect of glucocorticoids seems to be mainly exerted through the GR, and previous steroid cytotoxicity studies in childhood ALL mainly focused on prednisone and dexamethasone in relation to the expression levels of and mutations in the *NR3C1* gene.[1,5,6] Clinical steroid resistance as well as *in vitro* steroid resistance, which have been shown to be a poor prognostic factor for the survival of ALL, are related to *NR3C1* aberrations.[6,7] Mutations in the *NR3C2* gene are less frequently studied, maybe due to the fact that MR expression on leukemic cells is relatively low.[8] The role of the MR in steroid cytotoxicity therefore has remained unclear.

Synthetic glucocorticoids differ in their ability to bind and activate the GR and MR. Prednisone has a high affinity for both the GR and MR, whereas dexamethasone does not bind the MR and has a high potency to activate the GR.[9] Hydrocortisone, which is identical to the natural occurring hormone cortisol, can bind both receptors, but has a higher affinity for the MR.[9,10] Interestingly, hydrocortisone seems to potentiate the cytotoxic effect of both prednisolone and dexamethasone in steroid sensitive ALL cell lines.[8] Moreover, when hydrocortisone was given as a single drug, it appeared to be as potent as dexamethasone or prednisolone in cytotoxicity assays.[8] Hydrocortisone has never been considered as a potential cytotoxic steroid in ALL treatment protocols. Since hydrocortisone has far fewer side effects compared to dexamethasone or prednisone, and may even ameliorate certain neurobehavioral side effects of dexamethasone treatment,[11] it would be of interest to investigate the cytotoxic effect of hydrocortisone, compared to prednisone and dexamethasone.

The purpose of the present study was to establish the role of the MR in steroid-induced cytotoxicity in patients with ALL. Furthermore, we evaluated in vitro antileukemic activity of prednisolone, dexamethasone and hydrocortisone in cell lines that selectively can be induced to express the GR or the MR.

METHODS

Cell lines

Generation and culturing

The Reh cell line, which lacks expression of a functional glucocorticoid and mineralocorticoid receptor, was used to generate two inducible cell line models with either a GR (*NR3C1*) or MR (*NR3C2*) construct. Gateway multisite recombination (Invitrogen) was used for gateway cloning of lentiviral expression vectors as previously described.[7] The entry vectors used for Reh^{NR3C1} or Reh^{NR3C2} cells were (1) *attL1/attR5*-flanked doxycycline-inducible promotor (third generation; Clontech), (2) *attL5/attL4*-NR3C1 or *attL5/attL4*-NR3C2 complementary DNA sequence, (3) *attR4/attR3*-flanked DDK-tag followed by a stop codon, Woodchuck hepatitis virus Posttranscriptional Regulatory Element (WPRE) sequence, and a constitutive spleen focus forming virus promotor, and (4) tetracycline (doxycycline)-induced transcriptional activator protein-Thosea asigna virus 2A-truncated Nerve Growth Factor Receptor reporter. Single cells were plated to acquire cell lines with significant inducibility of the constructs. After exposure to doxycycline (0,5µg/ml) for 16 hours, the inducibility of the NR3C1- or NR3C2-constructs was assessed through flow cytometry following intracellular DDK staining. For both cell lines, two clones were selected (named $Reh^{NR3C1\text{-}A}$, $Reh^{NR3C1\text{-}B}$, $Reh^{NR3C2\text{-}A}$ and $Reh^{NR3C2\text{-}B}$). Both clone A cell lines had the best inducibility and were therefore primarily used in our main experiments. Cells were cultured in RPMI 1640 medium (Gibco) supplemented with 1x Glutamax, 2% fetal calf serum (FCS) and 1% penicillin-streptomycin-fungizone solution (PFS).

Cytotoxicity assays

Prednisolone, dexamethasone and hydrocortisone were plated in concentrations ranging from 500µM to $8.192*10^{-8}$ µM. Both Reh^{NR3C1} and Reh^{NR3C2} cell lines were incubated with doxycycline 0,5µg/ml for approximately 16 hours (overnight). Doxycycline-induced and non-induced cells were plated in a concentration of $0.80*10^{6}$ cells/mL and subsequently exposed to steroid treatment. After four days, viability was measured by methylthiazolyldiphenyl-tetrazolium bromide (MTT, Sigma Aldrich).

Western blotting

Reh^{NR3C1} and Reh^{NR3C2} cells were incubated with doxycycline 0,5µg/ml for approximately 16 hours. Reh^{NR3C1} cells were treated with 0.8µM prednisolone, 0.16µM dexamethasone or 0.032µM hydrocortisone and Reh^{NR3C2} cells with 0.032µM prednisolone, 0.16µM dexamethasone or 0.0028µM hydrocortisone. Protein extraction and subsequent blotting procedure on Reh cells was performed as described previously.[12] Primary antibodies used for western blotting were NR3C1 (3660S, Cell signaling), DDK (DYKDDDK Tag, Rabbit mAb, 2368S, Cell Signaling), BIM (#ab32158, Abcam) and β-actin (#ab6276, Abcam).

Real-time quantitative polymerase chain reaction (RTQ-PCR)

RNA was isolated using TRIzol reagent (Thermo Fisher Scientific). Real-time quantitative polymerase chain reaction (RTQ-PCR) was performed as previously described.[12] Expression levels were calculated relative to the expression of the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) household gene. For normalized expression levels, the expression of non-doxycycline induced cells in the absence of steroid treatment was set to one. Primers used were *GAPDH* Fw primer 5'-GTCGGAGTCAACGGATT-3', *GAPDH* Rev primer 5'-AAGCTTCCCGTTCTCAG-3', *NR3C1* Fw primer 5'-TGTTTTGCTCCTGATCTGA-3', *NR3C1* Rev primer 5'-TCGGGGAATTCAATACTCA-3', *NR3C2* Fw primer 5'-GAGCTGGCAGAGGTTCTA-3', *NR3C2* Rev primer 5'-CTGGTCGCTGATGATCTC-3', *BIM* Fw primer: 5'-CGCCCAGAGATATGGAT-3', *BIM* Rev primer: 5'-CGCAAAGAACCTGTCAAT-3', *GILZ* Fw primer 5'-TGGCCATAGACAACAAGAT-3', *GILZ* Rev primer 5'-TTGCCAGGGTCTTCAA-3', *FKBP5* Fw primer 5'-GAATGGTGAGGAAACGC-3', *FKPB5* Rev primer 5'-ATGCCTCCATCTTCAAATAA-3'.

Antagonist approach

To be able to distinguish between the cytotoxic effect of the GR and MR in primary patient samples, we assessed three MR antagonists (spironolactone, eplerenone and RU-28318). First, we performed a cytotoxicity (MTT) assay with fixed prednisolone concentrations (0.5879 μM for the NRC31 clone, 0.0245 μM for the NR3C2 clone) and increasing concentrations of antagonists. After establishing three effective antagonist doses, we performed an MTT assay with these doses and ascending prednisolone concentrations. Thereafter the best antagonist and optimal dose was determined and subsequently added in our MTT assays with different steroids in ascending concentrations. RU-28318 was the most effective MR antagonist and therefore used in the subsequent experiments (4 μM).

Patient and PDX samples

As proof of concept, we used one ALL patient-derived xenograft model (PDX) as well as two primary patient samples and treated cells with different steroid concentrations in combination with RU-28318. Since cells with an ETV6-RUNX1 gene fusion have a relatively high expression of both GR and MR,[8] the used samples all harbored this gene fusion. Viability readout was performed by amino staining using FACS (viability staining: LIVE/DEAD Fixable Far Red Dead Cell Stain Kit, for 633 or 635 nm excitation (Cytoflex_S, Beckman Coulter)). Amino staining was performed in accordance with manufacture guidelines. Patient cells were first fixated using human telomerase reverse transcriptase mesenchymal stem cells (hTERT MSCs). Because of loss of cells after fixation, the percentage of live cells was used in the final viability calculations. Area under the curve (AUC) values were calculated to compare the in vitro cytotoxicity of prednisolone, dexamethasone and hydrocortisone in combination with RU-28318 (Prism software Version 9.3.0 from GraphPad).

Patient data and outcomes

GR and MR expression levels of 278 ALL patients were available and plotted based on ALL subtype. For these samples, mRNA sequencing (RNA-seq) was performed as previously described.[13] In brief, total RNA was isolated using the AllPrep DNA/RNA/Protein Mini Kit (QIAGEN) according to standard protocol on the QiaCube (Qiagen). RNA-seq libraries were generated with 300ng RNA using the KAPA RNA HyperPrep Kit with RiboErase (Roche) and subsequently sequenced on a NovaSeq 6000 system (2x150 bp) (Illumina). The raw sequencing reads were aligned using STAR (version 2.7.0f) to GRCh38 and gencode version 29.[13] Finally, expression counts were determined at gene level using Subread Counts.[14]

Outcome data regarding survival and relapse was available on 131 of the 278 patients. This comprised a subgroup of patients treated according to the Dutch Childhood Oncology Group ALL-11 protocol. Patients and/or patients' parents or legal guardians provided informed consent to use clinical data and leftover diagnostic material for research, compliant with the biobanking procedure of the Princess Máxima Center (MEC-2016-739, Netherlands Trial Register (NTR) NL7744) and the Declaration of Helsinki.

Statistical analyses

The association between MR and GR expression levels and prednisone response on day 8 was estimated with a multivariable logistic regression model. MR and GR expression levels were categorized as low or high, with the median as cut-off value, as described before.[15] We included National Cancer Institute (NCI) risk category (standard risk: <10 years *and* white blood cell count at diagnosis <50x10^9/L, high risk: other)[16] and sex as covariates.

Event free survival (EFS), defined as time since diagnosis to the occurrence of induction failure, relapse, secondary malignancy or death in complete remission as event of interest, was estimated with Kaplan Meier's methodology.[17] Median follow up was estimated by using reverse Kaplan Meiers.[18] To study the effect of prognostic factors on EFS, Cox proportional hazard models were estimated. First, univariable analyses were performed which included patient, disease and treatment characteristics. Statistically significant variables were entered with MR or GR expression levels in a multivariable Cox model. Landmark analysis (with land mark points day 33 or 79) was employed to study the effect of minimal residual disease (MRD) on survival outcomes.[19] Differences with a p-value <0.05 were considered statistically significant. All analyses were performed with IBM SPSS Statistics version 26.0.

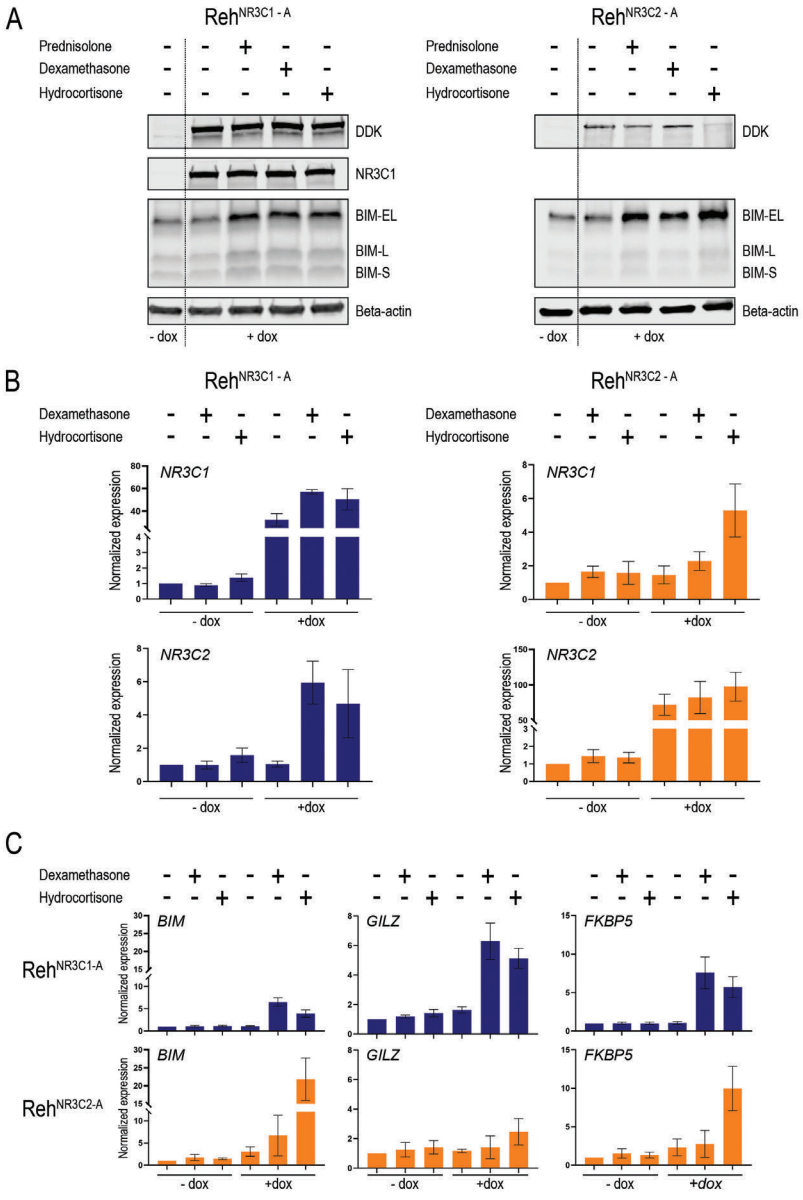


Figure 1. Hydrocortisone can induce expression of NR3C1 and NR3C2 via both the GR and MR.

(A), Western blot analysis of DDK, NR3C1 and BIM in Reh cell lines that were transfected with either doxycycline-inducible DDK-tagged NR3C1 or NR3C2 constructs, after treatment with prednisone, dexamethasone or hydrocortisone, as indicated. (B), Transcriptional steroid response of Reh cell lines transfected with doxycycline-inducible NR3C1 or NR3C2 constructs. After doxycycline induction, cells were treated with 0.16μM dexamethasone or 0.032μM (RehNR3C1) or 0.0028μM (RehNR3C2) hydrocortisone. Expression of NR3C1 (upper panels) and NR3C2 (lower panels) was measured in both cell lines, as well as (C), expression of GR and MR target genes BIM, GILZ and FKBP5.

RESULTS

To investigate the cytotoxic effects of dexamethasone, prednisolone and hydrocortisone via the GR or the MR in leukemic cells, we generated bulk-transduced Reh cells with a doxycycline-inducible DDK-tagged *NR3C1* or *NR3C2* construct, respectively. Doxycycline exposure induced the expression of DDK-tagged *NR3C1* (referred to as Reh^{NR3C1}) or DDK-tagged *NR3C2* (referred to as Reh^{NR3C2}) in Reh parental cells that lack a functional GR and MR (Figure 1A, Supplemental Figures 1A-B).[20] In both cell lines, the DDK signal became apparent after induction with doxycycline, and steroid treatment further enhanced this signal and correspondingly showed strong induction of BIM.

Next, we studied the expression of *NR3C1* and *NR3C2* in both cell line models upon treatment with dexamethasone (known to specifically bind the GR) or hydrocortisone (known to specifically bind the MR). As expected, exposure to doxycycline induced the expression of *NR3C1* in the Reh^{NR3C1} cell line (Figure 1B, left panels). Treatment with dexamethasone and hydrocortisone significantly enhanced the expression of the *NR3C1* gene in these cells. Interestingly, both dexamethasone and hydrocortisone treatment also induced the expression of the endogenous *NR3C2* gene, signifying *NR3C2* as a target gene of *NR3C1*. Of note, this induced expression of *NR3C2* was significantly lower than the induced expression of *NR3C1* in Reh^{NR3C1} cells.

In the Reh^{NR3C2} cell line, doxycycline exposure induced the expression of *NR3C2*, which was enhanced by hydrocortisone and to a lesser extent by dexamethasone treatment (Figure 1B, right panels). Moreover, and in contrast to Reh^{NR3C1} cells, only hydrocortisone treatment was able to induce the expression of *NR3C1* in Reh^{NR3C2} cells. To further study the differences in GR and MR activation by hydrocortisone and dexamethasone, we further explored steroid-induced expression of known *NR3C1* transcriptional target genes. In line with the induced expression of *NR3C1* or *NR3C2*, the expression of *BIM*, *GILZ* and *FKBP5* was strongly induced by hydrocortisone and dexamethasone in Reh^{NR3C1} cells (Figure 1C, upper panels). In contrast, strong transcriptional upregulation of these genes was only achieved by hydrocortisone treatment in Reh^{NR3C2} cells (Figure 1C, lower panels), confirming the low potency of dexamethasone to activate the MR. Combined, these data show that hydrocortisone can induce the expression of steroid-regulated genes via both the GR and the MR, while dexamethasone predominantly induces transcription via the GR.

The strong induction of BIM by hydrocortisone treatment in Reh^{NR3C2} cells (Figure 1A, C) is of specific interest since BIM mediates steroid-induced apoptosis of lymphoid cells.[21] The induction of BIM by dexamethasone, prednisolone and hydrocortisone in Reh^{NR3C2} cells indicates that the MR may play a role in steroid-induced cytotoxicity

of leukemic cells. We therefore examined the cytotoxic effects of steroid treatment in *NR3C1*- or *NR3C2*- expressing Reh cells. In the absence of doxycycline, Reh^{NR3C1} and Reh^{NR3C2} cells were completely refractory to treatment with dexamethasone, prednisolone or hydrocortisone (Figure 2A, Supplemental Figure 2A). Interestingly, doxycycline-induced *NR3C1* or *NR3C2* expression sensitized Reh cells for all three steroids. Moreover, hydrocortisone appeared to be the most potent cytotoxic steroid in both Reh^{NR3C1} and Reh^{NR3C2} cells. Furthermore, the cytotoxic effect induced by dexamethasone was comparable in cells expressing the GR or the MR. The notable difference in hydrocortisone sensitivity between Reh^{NR3C2} and Reh^{NR3C1} cells to induce cell death is consistent with the superior induction of *BIM* by hydrocortisone in Reh^{NR3C2} cells (Figure 1C). Combined, these results show that hydrocortisone can induce significant steroid-induced cell death in leukemic cells, either by activation of the MR or the GR. Interestingly, but in contrast to our RTQ-PCR data, dexamethasone induces significant steroid-induced cell death in Reh^{NR3C2} cells, albeit at a slightly higher concentration than in Reh^{NR3C1} cells.

To verify the role of the MR in steroid-induced cell death, we treated Reh^{NR3C1} and Reh^{NR3C2} cells with RU28318, a specific MR-antagonist.[22] In the absence of steroid treatment, RU28318 was minimally cytotoxic for Reh^{NR3C2} cells (Supplemental Figure 2B). As expected, the treatment with RU28318 did not affect steroid sensitivity of doxycycline-induced Reh^{NR3C1} cells upon treatment with prednisolone, dexamethasone or hydrocortisone. This further confirms the absence of endogenous functional *NR3C2* expression in these cells (Figure 2B). RU28318 treatment in Reh^{NR3C2} cells completely inhibited the cytotoxic potential of the MR following steroid treatment. Similar results with MR antagonists Eplerenone and Spironolactone were observed (Supplemental Figure 3A and B).

Our results in Reh^{NR3C1} and Reh^{NR3C2} cell lines highlighted a potential role for the MR in steroid-induced cell death in ALL cells. To study the potential clinical relevance of these observations, we determined the relative expression of *NR3C1* and *NR3C2* in 279 primary ALL patient samples. Overall, the relative expression of *NR3C1* was higher than *NR3C2* (Figure 3A). After dissecting the cohort according to molecular ALL characteristics, we observed that the expression of *NR3C2* in T-ALL patients was relatively low. Of the B-cell precursor acute lymphoblastic leukemia patients, those with an ETV6-RUNX1 fusion gene harbored the highest (relative) expression of *NR3C2*, as described before.[8] This is highly interesting, considering the favorable outcome of this specific leukemic subgroup.[23] As a proof of concept, we treated one PDX sample and two patient samples with an ETV6-RUNX1 fusion gene with prednisolone, dexamethasone and hydrocortisone in the presence or absence of RU28318 to measure

the potential contribution of the MR in steroid-induced cytotoxicity. In these steroid sensitive samples, we saw a modest decrease in steroid sensitivity after treatment with RU28318, which was most pronounced in the PDX model albeit not significant (Figure 3B). However, no effects of RU28318 could be seen on expression of glucocorticoid target genes in these steroid treated samples (Supplemental Figure 4A).

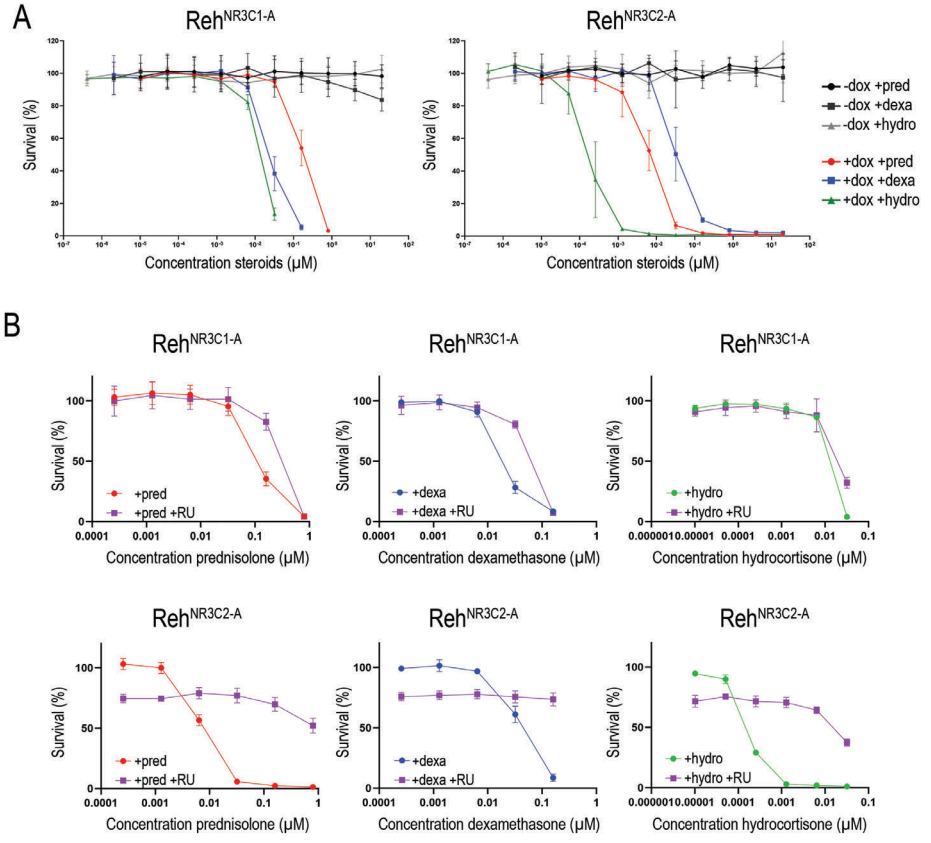


Figure 2. Hydrocortisone most potent steroid in NR3C1 and NR3C2 overexpressing cells

(A), Cell toxicity screening of RehNR3C1 (left) and RehNR3C2 (right) cells with (color) and without (gray-scales) doxycycline induction and after treatment with prednisolone, dexamethasone or hydrocortisone. Steroid sensitivity was determined with an MTT-assay. Data represents biological triplicates, with standard deviations. (B), Cell toxicity screening of doxycycline-induced RehNR3C1 (upper panels) and RehNR3C2 (lower panels) with and without 4μM RU28318 (MR antagonist) treatment in combination with prednisolone, dexamethasone or hydrocortisone. RU28318 treatment in RehNR3C2 cells reversed the acquired steroid sensitivity.

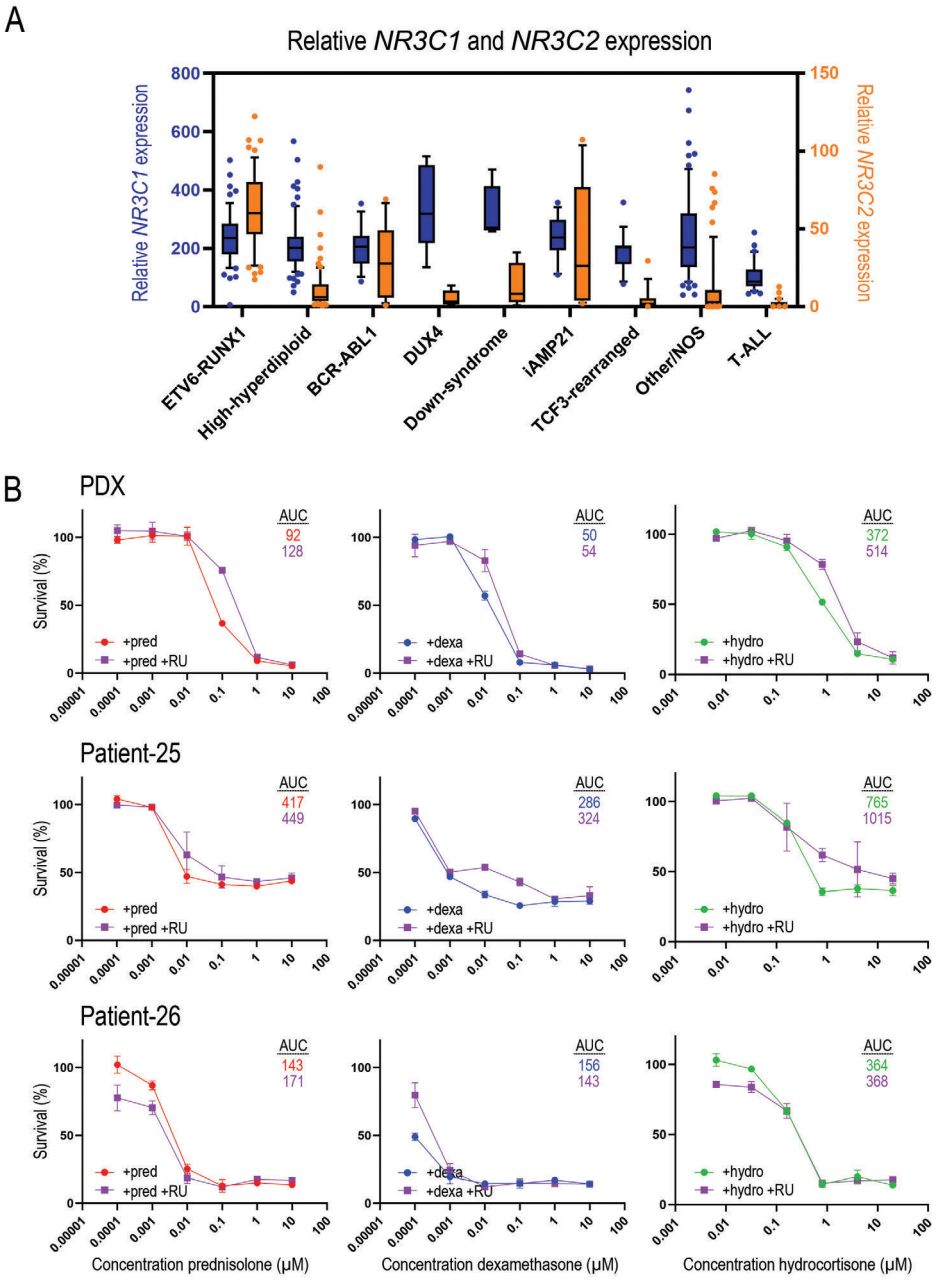
Finally, we measured NR3C1 and NR3C2 mRNA expression in a cohort of 131 ALL patients with different ALL subtypes, including 28 ETV6-RUNX1 patients (Supplemental Table 1) and studied the association on early clinical response to seven days of prednisone and event-free survival (EFS). Median *NR3C1* expression in leukemic blasts (199.6 counts per million (CPM), range 41.9 to 567.1 CPM) was significantly higher than median *NR3C2* expression (5.4 CPM, range 0.2 to 122.3 CPM).

Only fourteen patients (11%) had a poor prednisone response, prohibiting both a univariable and multivariable regression analysis in this cohort to study a possible association between GR or MR expression and prednisone response (Supplemental Tables 1 and 2). One induction failure (0.8%), 22 relapses (16.8%) and two secondary malignancies (1.5%) were observed. In a univariable Cox regression, we did not find a significant association between high *NR3C1* expression (hazard ratio (HR) 0.96, 95%-confidence interval (95%-CI) 0.40-2.30), nor high *NR3C2* expression (HR 0.57, 95%-CI 0.24-1.33), and any event (Supplemental Table 4), suggesting that high expression of *NR3C1* or *NR3C2* does not predict for favorable outcome in our patient cohort. Kaplan-Meijer's analysis also did not show an association between EFS and high or low *NR3C1* or *NR3C2* expression (Supplemental Figure 5).

Together, these findings indicate that, even though a pronounced contribution of the MR exists in our models, the role of the MR in steroid-induced cytotoxicity is limited in ALL patients.

Figure 3. NR3C2 expression in patients is relatively low

(A), Relative expression of NR3C1 (blue) and NR3C2 (orange) in 279 primary ALL patient samples, dissected according to genetic background. (B), Cell toxicity screening of two primary patient samples and one PDX sample, all harboring the ETV6-RUNX1 fusion gene. Toxicity screening was performed using amino staining and data represents technical duplicates with standard deviations. Samples were treated with prednisolone, dexamethasone or hydrocortisone, in presence or absence of 4μM RU28318 (MR antagonist). ►


A
Relative NR3C1 and NR3C2 expression
Relative NR3C1 expression
Relative NR3C2 expression
800
600
400
200
0
150
100
50
0
ETV6-RUNX1
High-hyperdiploid
BCR-ABL1
DUX4
Down-syndrome
iAMP21
TCF3-rearranged
Other/NOS
T-ALL
B
PDX
Patient-25
Patient-26
Survival (%)
AUC
92
128
50
54
372
514
417
449
286
324
765
1015
143
171
156
143
364
368
+pred
+pred +RU
+dexa
+dexa +RU
+hydro
+hydro +RU
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Concentration prednisolone (μM)
Concentration dexamethasone (μM)
Concentration hydrocortisone (μM)

DISCUSSION

In the current study, we found that hydrocortisone is a potent steroid to induce steroid-induced cell death and that this steroid-induced cell death can be achieved solely by activation of the mineralocorticoid receptor (MR). By selectively inducing either MR (*NR3C2* gene) or glucocorticoid receptor (GR, *NR3C1* gene) expression in MR/GR-naive Reh cells, we observed that hydrocortisone induces the expression of *NR3C1* via both the GR and MR, and that hydrocortisone can induce significant cell death in these cells. In contrast, dexamethasone mainly induced *NR3C1* transcription via the GR, but was not able to induce *NR3C2* or other downstream target genes including *BIM*, *GILZ* or *FKBP5* via the MR, despite the fact that it could induce cell death in *NR3C2*-overexpressing Reh cells that lack a functional NR3C1 receptor. These results highlight that the MR is potentially capable of inducing leukemic cell death after activation by steroid treatment.

The finding that hydrocortisone is the most potent steroid in both our *in vitro* NR3C1 and NR3C2 models is interesting, since the glucocorticoid activity of hydrocortisone is reported to be inferior compared to other steroids. Conventionally, dexamethasone is reported to be the most potent steroid, with high glucocorticoid, but no mineralocorticoid activity.[24] However, this is based on anti-inflammatory and Na^+-retaining potency of the different steroids. Several in vitro studies in primary ALL samples with different cellular backgrounds showed that dexamethasone had higher antileukemic activity compared to either prednisone or hydrocortisone than the traditionally used equivalent doses.[9,25-27] In contrast to this previous research, hydrocortisone seems more efficient in inducing steroid-induced cell death than prednisolone and dexamethasone in both our Reh^{NR3C1} and Reh^{NR3C2} cell line models. This may be related to the high expression of *NR3C1* and *NR3C2* in our models compared to PDX or patient samples. Indeed, in the primary patient and PDX samples, hydrocortisone appeared less cytotoxic compared to dexamethasone or prednisone and the expression of *NR3C2* was minor compared to the induced expression levels in our Reh^{NR3C2} model (Supplemental Figure 4B). The inter-patient variability in steroid sensitivity is high, with cytotoxicity values sometimes varying more than 1000-fold among patient samples.[25,26] It therefore cannot be excluded that certain patients respond better to other steroids than the traditionally used dexamethasone or prednisone. Moreover, our Reh cell line model underscores the potential of hydrocortisone to induce steroid-induced cell death in leukemic cells.

It is known that ALL patients with a t(12;21)(p13;q22) lesion, which leads to the ETV6-RUNX1 fusion gene, also known as TEL/AML1, have an excellent prognosis.[23,28-30] Interestingly, this pre-B ALL subgroup has a relatively high expression of both *NR3C1* and *NR3C2* (Figure 3A). It is therefore tempting to speculate that the MR contributes to the overall

steroid response in those patients. We attempted to distinguish between the contribution of the GR and MR on steroid-induced cytotoxicity in this specific genetic subgroup, by treating two primary ALL patient samples and one PDX model with different steroids in combination with the MR antagonist RU28318 at a concentration that was minimally cytotoxic by itself but could complete block steroid-induced cell death via the MR. In these samples, we saw a minimal shift in our cell toxicity curves towards resistance as well as minimal reduced expression levels of GR/MR target genes after addition of RU28318, although not significant. This indicates a minimal and subtle involvement of the MR in steroid-induced death in ETV6-RUNX1 rearranged pre-B ALL patients. An explanation for the difference between these patient and PDX samples and our experimental setting may be a lower expression or lower transcriptional activity of *NR3C2* compared to *NR3C1* in patient leukemic cells and the presence of other more dominant (genetic and/or cellular) factors in these patients. Due to the lack of a functional antibody recognizing NR3C2, we were unable to test this at the protein level. Moreover, no genes have been identified to be specifically regulated by either *NR3C1* or *NR3C2*, prohibiting more specific transcriptional analysis.[9,31] Therefore, the contribution of the MR in steroid-induced cytotoxicity in our patient samples remains unclear.

In vivo sensitivity to glucocorticoids is an important prognostic factor in the treatment of ALL. In our ALL patient cohort, we did not find an association between basal *NR3C1* or *NR3C2* mRNA expression levels and event free survival or poor steroid response, as was described before for *NR3C1* expression.[7] This may be partially explained by other important underlying mechanisms of steroid resistance in pediatric ALL, such as loss of IKZF1 function, epigenetic silencing of the *BIM* locus, IL7-induced signaling or IL7R signaling mutations.[32-34] Furthermore, the median follow up of our cohort was only 26 months, therefore only early events could be analyzed. Since many relapses occur three years after therapy, our results concerning a possible association between *NR3C1* and *NR3C2* expression levels to event free survival are limited, especially in this relatively small cohort. It is conceivable however, that other crucial processes play a more dominant role in relapse, such as chemotherapy induced mutations.[35]

In conclusion, in experimental models, the mineralocorticoid receptor (*NR3C2*) potently induces steroid-induced cell death and hydrocortisone is a potent steroid to initiate this process. However, the contribution of MR-regulated steroid-induced toxicity appears to be minimal or subtle in leukemic patient samples, and the clinical relevance of *NR3C2* expression or functionality for patients with acute lymphoblastic leukemia remains to be elucidated.

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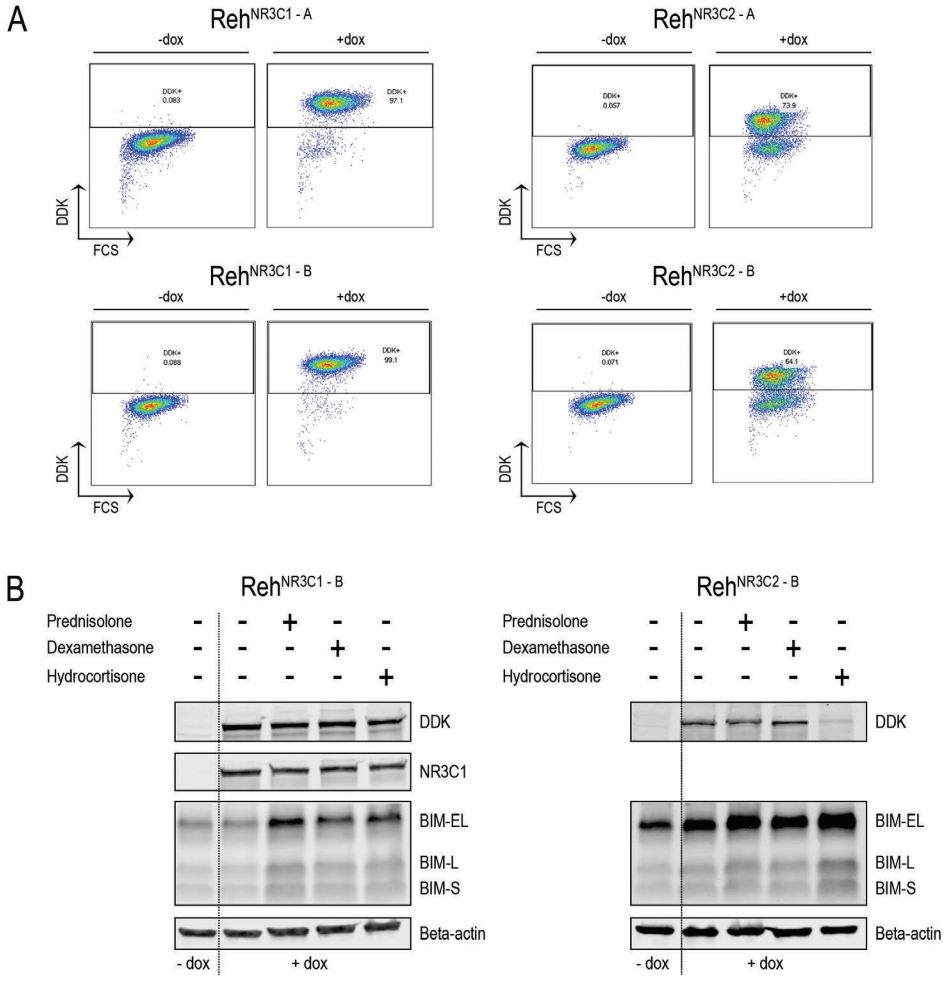
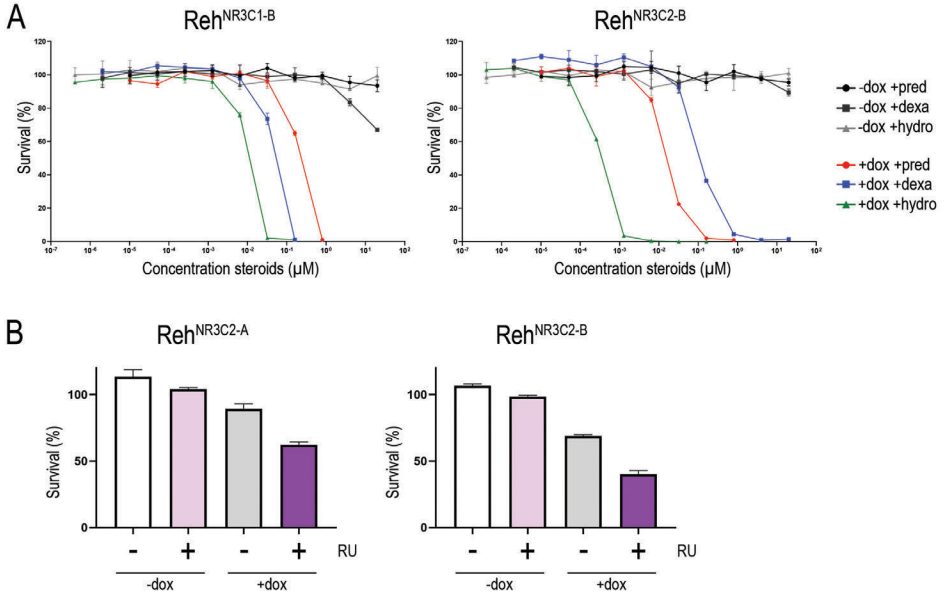
35. Li B, Brady SW, Ma X, et al: Therapy-induced mutations drive the genomic landscape of relapsed acute lymphoblastic leukemia. Blood 135:41-55, 2020

SUPPLEMENTAL FIGURES



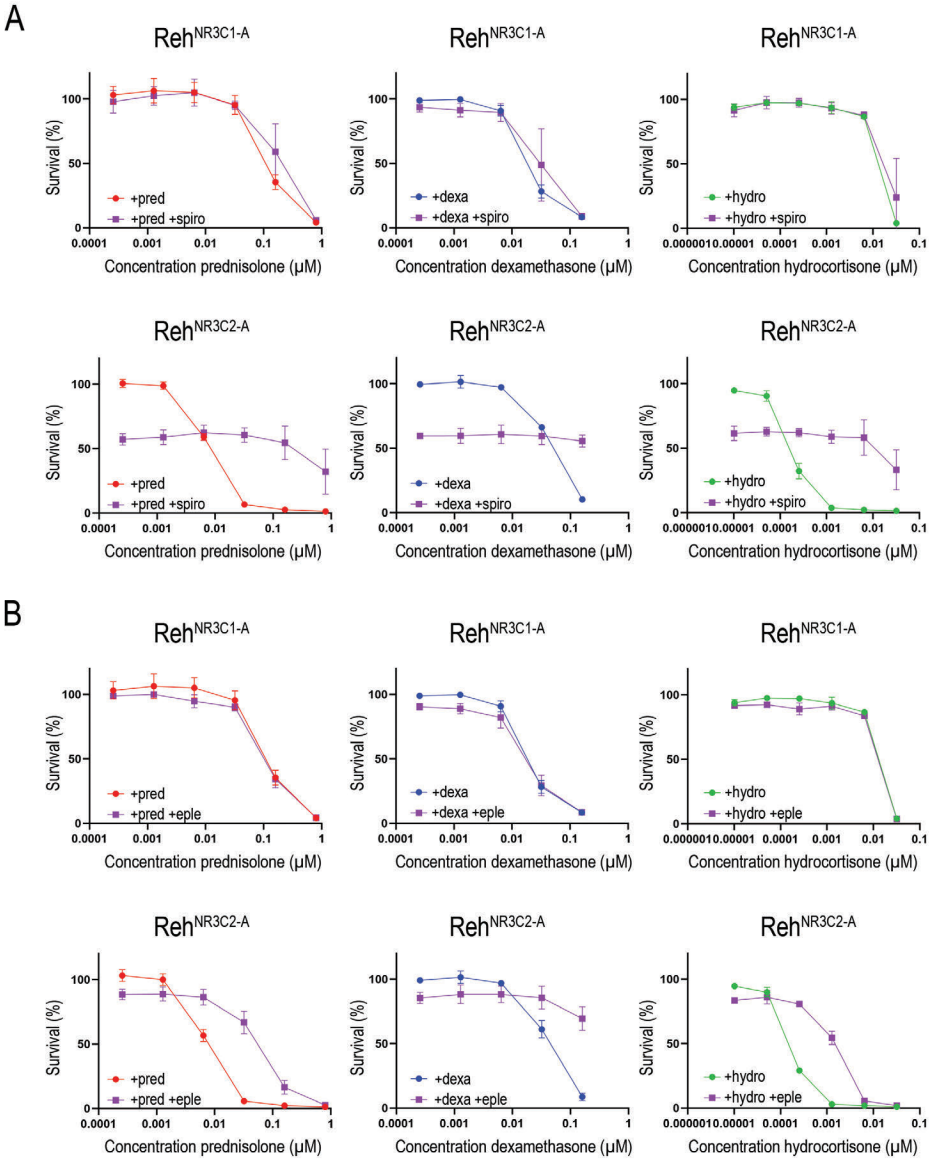
Supplemental Figure 1.

(A), Reh cells were transduced with a doxycycline-inducible *NR3C1* or *NR3C2* construct. The inducibility of the NR3C1- or NR3C2- constructs was measured through flow cytometry following intracellular DDK staining. Doxycycline exposure induced the expression of DDK-tagged *NR3C1* (referred to as Reh^{NR3C1}) or *NR3C2* (referred to as Reh^{NR3C2}) in Reh single cells that intrinsically lack *NR3C1* and *NR3C2* expression. (B), Western blot analysis of our secondary Reh cell lines (Reh$^{NR3C1\text{-}B}$ and Reh$^{NR3C2\text{-}B}$) that were transfected with either doxycycline-inducible DDK-tagged *NR3C1* or *NR3C2* constructs, after treatment with prednisone, dexamethasone or hydrocortisone, as indicated.



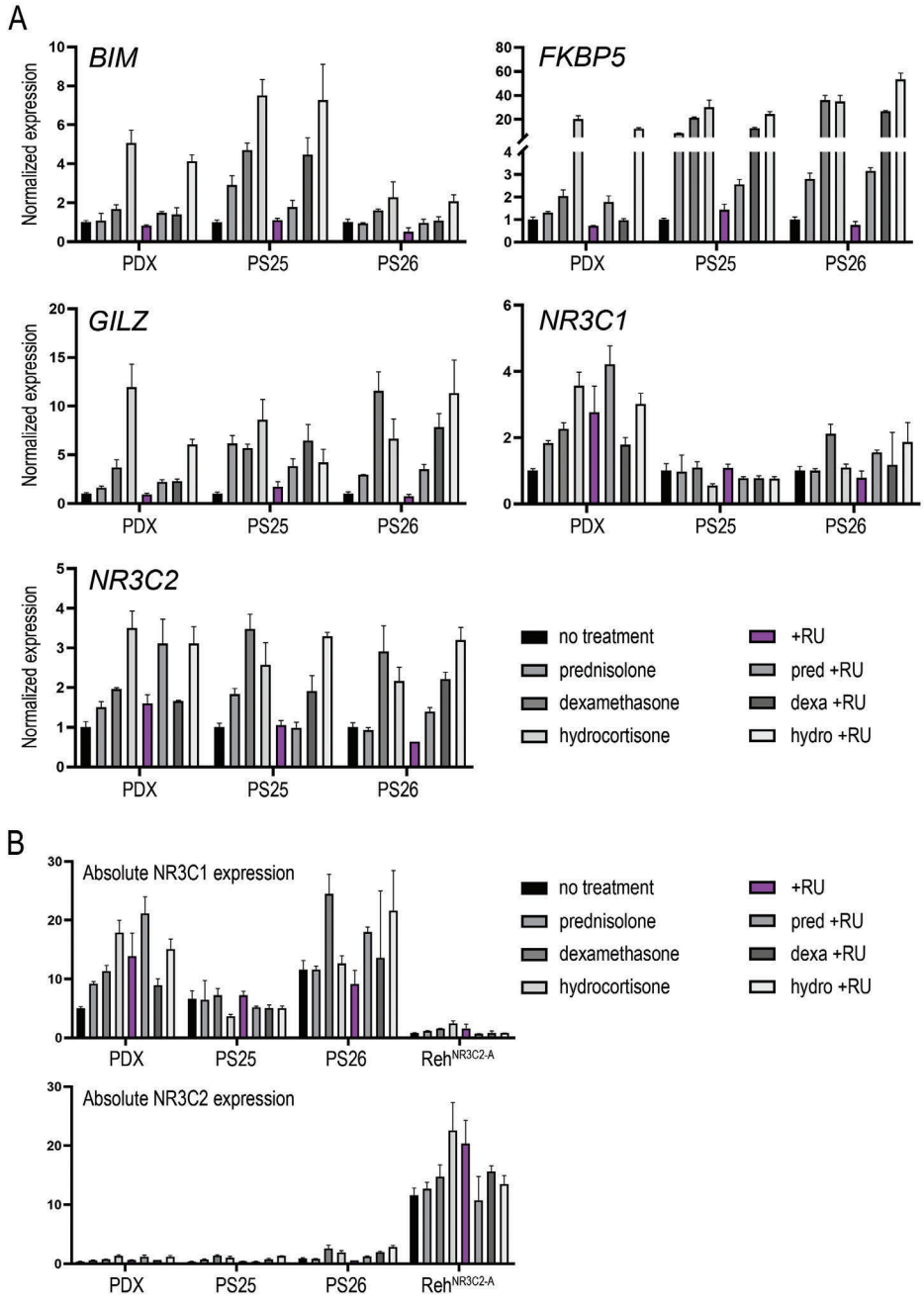
Supplemental Figure 2.

(A), Cell toxicity screening of our Reh$^{NR3C1-B}$ and Reh$^{NR3C2-B}$ cell lines with (color) and without (gray-scales) doxycycline induction and after treatment with prednisolone, dexamethasone or hydrocortisone. These secondary cell lines showed the same pattern of steroid sensitivity as our primary cell lines. (B), Cell toxicity screening of RU28318 monotherapy. In the presence of doxycycline, 4μM RU28318 was slightly cytotoxic for Reh^{NR3C2} cells.



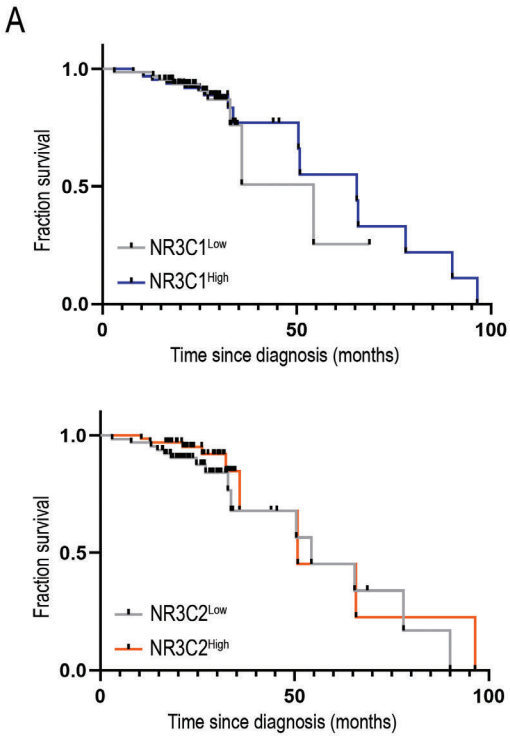
Supplemental Figure 3.

(A), Cell toxicity screening of doxycycline-induced Reh^{NR3C1} (upper panels) and Reh^{NR3C2} (lower panels) with and without 20μM spironolactone (MR antagonist) treatment in combination with prednisolone, dexamethasone or hydrocortisone. Spironolactone treatment in Reh^{NR3C2} cells reversed the acquired steroid sensitivity. (B), Cell toxicity screening of doxycycline-induced Reh^{NR3C1} (upper panels) and Reh^{NR3C2} (lower panels) with and without 4μM eplerenone (MR antagonist) treatment in combination with prednisolone, dexamethasone or hydrocortisone. Eplerenone treatment in Reh^{NR3C2} cells reversed the acquired steroid sensitivity.


A
BIM
FKBP5
GILZ
NR3C1
NR3C2
Normalized expression
PDX
PS25
PS26
no treatment
prednisolone
dexamethasone
hydrocortisone
+RU
pred +RU
dexa +RU
hydro +RU
B
Absolute NR3C1 expression
Absolute NR3C2 expression
PDX
PS25
PS26
Reh NR3C2-A

◂ Supplemental Figure 4.

Transcriptional steroid response of two primary patient samples (PS25 and PS26) and one PDX sample, all harboring ETV6-RUNX1 fusion gene. Cells were treated with 0.05μM prednisolone, 0.05μM dexamethasone or 2μM hydrocortisone, with or without addition of 4μM RU28318. Expression of *NR3C1, NR3C2, BIM, GILZ and FKBP5* was measured. (B) Transcriptional steroid response of two primary patient samples (PS25 and PS26), one PDX sample and doxycycline-induced Reh^{NR3C2}. Patient and PDX cells were treated with 0.05μM prednisolone, 0.05μM dexamethasone or 2μM hydrocortisone, Reh^{NR3C2} cells were treated with 0.032μM prednisolone, 0.16μM dexamethasone or 0.0028μM hydrocortisone, all in presence or absence of MR antagonist RU28318. Absolute *NR3C1* (upper panel) and *NR3C2* (lower panel) expression was measured.



Supplemental Figure 5.

Event free survival for the 131 ALL patients with either high or low *NR3C1* or *NR3C2* expression.

SUPPLEMENTAL TABLES

Supplemental Table 1. Patients' characteristics for prednisone response

		Poor prednisone response n = 14	Good prednisone response n = 114
Sex n (%)	Girl	5 (35.7)	44 (38.6)
	Boy	9 (64.3)	70 (61.4)
NCI risk group n (%)	Standard risk	4 (28.6)	66 (57.9)
	High risk	9 (64.3)	48 (42.1)
NR3C1 n (%)	Low	13 (92.9)	49 (43.0)
	High	1 (7.1)	65 (57.0)
NR3C2 n (%)	Low	11 (78.6)	52 (45.6)
	High	3 (21.4)	62 (54.4)

NCI-risk category: standard risk= <10 years and white cell count at diagnosis <50x109/L, high risk= other

Abbreviations: n: number, NCI: National Cancer Institute

Supplemental Table 2. Estimated odds ratio (OR) with 95%-CI for prednisone response

		Poor prednisone response	
		OR (MVA)	95%-CI
Sex n (%)	Girl		
	Boy	0.922	0.262-3.244
NCI risk group n (%)	Standard risk		
	High risk	3.221	0.893-11.622
NR3C1 n (%)	Low		
	High	0.060	0.007-0.481
		Poor prednisone response	
		OR (MVA)	95%-CI
Sex n (%)	Girl		
	Boy	0.998	0.298-3.340
NCI risk group n (%)	Standard risk		
	High risk	3.257	0.943-11.245
NR3C2 n (%)	Low		
	High	0.240	0.062-0.923

Odds ratio's (OR) for either GR (*NR3C1)* or MR (*NR3C2)* on prednisone response are depicted, corrected for sex and NCI risk group (multivariable regression analysis). Due to low number of patients with poor prednisone response, interpretation of the OR is not possible. NCI-risk category: standard risk= <10 years and white cell count at diagnosis <50x109/L, high risk= other

Abbreviations: n: number, CI: confidence interval, NCI: National Cancer Institute, OR: odds ratio, MVA: multivariable analysis

Supplemental Table 3. Patients' characteristics for survival analyses

Patients	Number	131
	Median follow up (95%-CI) *months*	26.1 (23.8-28.4) *Reverse Kaplan Meier*
Events	Induction failure n (%)	1 (0.8)
	Relapse n (%)	22 (16.8)
	Secondary malignancy n (%)	2 (1.5)
	Death n (%)	8 (6.1) *NB: all second events*
		n (%)
Age at diagnosis *years*	Median (range)	5 (1-18)
	1-4	63 (48.1)
	5-9	24 (18.3)
	10-14	24 (18.3)
	15-18	20 (15.3)
Sex	Girl	50 (38.2)
	Boy	81 (61.8)
ALL subclass	B-cell ALL	114 (87)
	T-cell ALL	17 (13)
Cytogenetic subtype	ETV6-RUNX1	28 (21.4)
	High hyperdiploid	35 (26.7)
	BCR-ABL	3 (2.3)
	DUX4	3 (2.3)
	Down syndrome	3 (2.3)
	iAMP21	8 (6.1)
	TCF3 rearranged	5 (3.8)
	T-cell ALL	17 (13)
	Other/NOS	29 (22.1)
CNS involvement	No	122 (93.1)
	Yes	4 (3.1)
	Unknown	5 (3.8)
Leucocytes at diagnosis	Median (range)	11.2 (0.8-705)
	<10	59 (45.0)
	10-25	29 (22.1)
	25-50	14 (10.7)
	50-100	15 (11.5)
	>100	14 (10.7)

Supplemental Table 3. Continued

NCI risk group	NCI standard risk	71 (54.2)
	NCI high risk	60 (45.8)
Prednisone response day 8	Good	114 (87)
	Poor	14 (10.7)
	Unknown	3 (2.3)
Risk group stratification	SR	24 (18.3)
	MR	95 (72.5)
	HR	12 (9.2)
MRD day 33	Negative	30 (22.9)
	Positive	97 (74)
	Not done	4 (3.1)
MRD day 79	Negative	80 (61.1)
	Positive	45 (34.4)
	Not done	6 (4.6)
GR (NR3C1) expression	Median (range)	199.6 (41.9-567.1)
	Low	65 (49.6)
	High	66 (50.4)
MR (NR3C2) expression	Median (range)	5.4 (0.2-122.3)
	Low	65 (49.6)
	High	66 (50.4)

Abbreviations: n: number, ALL: acute lymphoblastic leukemia, CNS: central nervous system, NCI: National Cancer Institute, SR: standard risk, MR: medium risk, HR: high risk, MRD: minimal residual disease, GR: glucocorticoid receptor, MR: mineralocorticoid receptor

Supplemental Table 4. Estimated hazard ratio with 95%-CI for any first event

		Hazard ratio	**95%-CI**	
			Lower bound	**Upper bound**
Age at diagnosis *years*	1-4			
	5-9	0.38	0.11	1.28
	10-14	0.93	0.33	2.63
	15-18	0.63	0.14	2.85
Sex	Girl			
	Boy	0.86	0.37	1.99
ALL subclass	B-cell ALL			
	T-cell ALL	2.17	0.72	6.53

Supplemental Table 4. Continued

		Hazard ratio	95%-CI	
			Lower bound	Upper bound
Cytogenetic subtype[1]	ETV6-RUNX1			
	Other	2.45	0.57	10.51
CNS involvement[2]	No			
	Yes	7.73	1.70	35.15
	Unknown	0.62	0.14	2.81
Leucocytes at diagnosis	<10			
	10-25	1.38	0.52	3.66
	25-50	0.73	0.15	3.58
	50-100	1.51	0.32	7.20
	>100	1.91	0.51	7.17
NCI risk group	NCI standard risk			
	NCI high risk	1.33	0.58	3.03
Prednisone response day 8[3]	Good			
	Poor	2.34	0.66	8.25
Risk group stratification[4]	SR			
	MR	2.10	0.59	7.54
	HR	10.33	2.19	48.71
MRD day 33[5]	Negative			
	Positive	2.59	0.74	9.06
MRD day 79[6]	Negative			
	Positive	4.22	1.57	11.28
GR (NR3C1) expression	Low			
	High	0.96	0.40	2.30
MR (NR3C2) expression	Low			
	High	0.57	0.24	1.33

Hazard ratio is depicted for the first occurring event. NCI-risk category: standard risk= <10 years and white cell count at diagnosis <50x109/L, high risk= other.

[1] Groups too small: only 2 events in ETV6-RUNX1 group

[2] Groups too small: only 2 events in group with CNS involvement

[3] Landmark analysis, starting at day 8: 1 event (induction failure) lost

[4] Groups too small: only 3 events in the standard risk group

[5] Landmark analysis, starting at day 33: 1 event (induction failure) lost

[6] Landmark analysis, starting at day 79: 1 event (induction failure) lost

Abbreviations: CI: confidence interval, ALL: acute lymphoblastic leukemia, CNS: central nervous system, NCI: National Cancer Institute, SR: standard risk, MR: medium risk, HR: high risk, MRD: minimal residual disease, GR: glucocorticoid receptor, MR: mineralocorticoid receptor

General discussion
and
Future perspectives

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

The aims of this thesis were threefold. First, we aimed to increase current knowledge on the prevalence and determinants of dexamethasone-induced side effects in children with acute lymphoblastic leukemia (ALL). Second, we aimed to validate the finding that hydrocortisone addition to dexamethasone treatment leads to a significant reduction of clinically relevant dexamethasone-induced neurobehavioral and sleep problems. Third, we aimed to describe the role of the mineralocorticoid receptor in steroid-induced cytotoxicity. The findings described in this thesis are relevant for the identification, possible prevention and treatment of the burdensome side effects of dexamethasone in children with ALL, as discussed in this chapter. Table 1 provides an overview of studies and the main findings of this thesis.

Prevalence and measurement of dexamethasone-induced side effects

Neurobehavioral problems

This thesis showed that 67% of ALL patients who received dexamethasone during maintenance treatment experienced clinically relevant dexamethasone-induced neurobehavioral problems *(Chapter 4)*. Previous prospective studies in children with ALL which used validated measurement tools, reported adverse psychological reactions between 38% and 86%.[1-5] This wide range may partly be due to the differences in outcome measurement tools. We consider the used strengths and difficulties questionnaire (SDQ) very valuable since it is validated in children from 3 to 18 years.[6] However, this 25-item questionnaire is not fitting for the evaluation of other more specific adverse psychological reactions, such as depressive symptoms or psychosis, which occur more often in older children.[7,8] A validated questionnaire which evaluates both behavioral problems in young children and (e.g.) depressive symptoms in older children is currently not available. A promising future option would be to use a patient-reported outcome measures information system (PROMIS) which measures patient-reported specific outcomes with highly accurate, precise and short measures.[9,10] Combining short forms (4-items each) for anxiety, depression and anger, as recently specified by the Outcome-Based Healthcare Program Working Group Generic PROMs,[11] could provide more insight in the different psychological reactions which occur during dexamethasone treatment. Preferably, these measurements should be self-reported when possible, since children are known to report less (severe) side effects than their parents.[12]

Table 1. Overview of the main findings in this thesis

Chapter	Short title	Purpose	Sample
2	Risk factors – a review	To provide an overview of literature on risk factors for steroid-induced adverse psychological reactions and sleep problems.	n = 24 included articles 19 described adverse psychological reactions, 7 sleep problems, and 2 both
3	DexaDagen-2 study design	To describe the design and methods used in the DexaDagen-2 study.	-
4	Determinants for dexamethasone-induced side-effects	To identify determinants for parent-reported dexamethasone-induced neurobehavioral and sleep problems.	n = 105 pediatric ALL patients and their parents
5	Leptin increase	To determine the influence of a five-day dexamethasone course on changes in leptin, fat mass, hunger, sleep and fatigue and to explore the role of leptin in these changes.	n = 105 pediatric ALL patients

	Measurements	Main findings
	-	- Quality of evidence was very low. - Younger patients seem at risk for behavior problems. - Older patients may be at risk for sleep problems. - Type/dose of steroid may be related to sleep problems. - No studies describing parental stress or medical history were identified. - Limited studies on genetic susceptibility which remain to be replicated.
	-	- Prospective Identification study: designed to select patients for the RCT and to identify risk factors for steroid-induced neurobehavioral and sleep problems. - Double-blind placebo-controlled RCT with a cross-over design: designed to compare hydrocortisone with placebo as intervention for dexamethasone-induced problems.
	Outcomes - Behavior: SDQ questionnaire - Sleep: SDSC questionnaire *Determinants* - Patient/parent demographics - Treatment characteristics - Parenting stress: NOSI-K - Parental stress: DT-P - Dexamethasone pharmacokinetics - Genetic variation: candidate SNP	- Parents reported clinically relevant neurobehavioral side effects in 70 (67%) and sleep problems in 61 (59%) patients. - Parenting stress was a significant determinant for neurobehavioral and sleep problems in their children. - Parents who experienced more stress before start of a dexamethasone course reported more sleep problems in their child. - Dexamethasone pharmacokinetics, genetic variation, patient/parent demographics, or disease/treatment characteristics were not associated with the outcomes.
	- Leptin SDS - Fat mass (bioelectrical impedance analysis) - BMI - Hunger: four VAS scores - Fatigue: PedsQL-MFS questionnaire - Sleep: SDSC questionnaire	- Leptin and fat mass, as well as hunger scores, fatigue and sleep deteriorated significantly after five days of dexamethasone ($p<0.001$) - No significant correlations between delta leptin and delta hunger, fatigue, sleep or BMI were found. - This suggests a dexamethasone-induced state of acute leptin resistance. - Elevated leptin SDS was associated with older age, higher fat mass and earlier maintenance week.

Table 1. Continued

Chapter	Short title	Purpose	Sample
6	Hydrocortisone as an intervention	To validate the finding that hydrocortisone addition to dexamethasone treatment reduces clinically relevant dexamethasone-induced neurobehavioral problems	n = 52 pediatric ALL patients with clinically relevant dexamethasone-induced neurobehavioral problems
7	The role of MR in steroid cytotoxicity	To evaluate the role of the mineralocorticoid receptor (MR) in steroid-induced cytotyoxicity	- Doxycycline-inducible Reh cell lines (with MR *or* GR) - 1 ALL PDX model, 2 primary ALL patient samples (all ETV6-RUNX1) - n = 278 pediatric ALL patients

Abbreviations: ALL: acute lymphoblastic leukemia, BMI: body mass index, DT-P: distress thermometer for parents, GR: glucocorticoid receptor, MR: mineralocorticoid receptor, NOSI-K: Nijmeegse Ouderlijke Stress Index Korte versie, adapted from the Parenting Stress Index, PedsQL(-MFS): Pediatric Quality of Life Pediatric Quality of Life Inventory (– Multidimensional Fatigue Scale),

	Measurements	Main findings
	Intervention Oral hydrocortisone (10mg/m^2/day in circadian rhythm) compared to placebo *Outcomes* - Behavior: SDQ questionnaire - Sleep: actigraphy and SDSC questionnaire - Hunger: four VAS scores - Quality of Life: PedsQL questionnaire - Parental stress: DT-P	- Compared to placebo, hydrocortisone had no additional beneficial effect in reducing clinically relevant dexamethasone-induced neurobehavioral problems. - Hydrocortisone did not improve sleep, quality of life or parental stress. - Hydrocortisone led to an increased average and fasting hunger score compared to placebo. - Placebo and nocebo effects may play an important role in behavioral side effects.
	Cell lines - Cytotoxicity of dexamethasone, prednisolone and hydrocortisone - Transcriptional activity *PDX / patient samples* - Cytotoxicity of dexamethasone, prednisolone and hydrocortisone, combined with RU28318 (MR antagonist) *ALL patients* - MR and GR expression levels - Prednisone response - Outcome (survival / events)	- Hydrocortisone can induce the expression of steroid-regulated genes via both the GR and the MR. - Dexamethasone predominantly induces transcription via the GR. - Hydrocortisone can induce significant steroid-induced cell death by activation of the MR or the GR. - Dexamethasone induces significant steroid-induced cell death via the GR, but also via the MR. - The MR is potentially capable of inducing leukemic cell death after activation by steroid treatment. - Relative expression of the GR is higher than the MR in ALL patients - The role of the MR in steroid-induced cytotoxicity is limited in ALL patients

PDX: patient derived xenograft, RCT: randomized clinical trial, SDS: standardized deviation score, SDSC: sleep disturbance scale for children, SDQ: strengths and difficulties questionnaire, SNP: single nucleotide polymorphism, VAS: visual analogue scale

Sleep problems

Clinically relevant sleep problems during dexamethasone treatment were reported by parents in 59% of our patients *(Chapter 4)*. Prospectively measured sleep problems during glucocorticoid treatment were previously reported between 24% and 97% of patients with ALL.[1,4,13,14] Subjective measurement of sleep in children remains challenging, especially across a wide age range, due to the limited psychometric validation of sleep questionnaires.[15,16] A promising future subjective measurement tool is a (proxy-report) short PROMIS form that measures sleep disturbances, which is currently being validated in Dutch.

Objective measurement of sleep problems, using actigraphy, is widely used to assess sleep-wake patterns in children.[15] We used actigraphy in our RCT (*Chapter 6*) and found that the data was usable for 36/52 patients (69%). Especially younger children refused to wear the actigraph. Besides, even though actigraphy is excellent to detect sleep-wake pattern deviations, it is not able to identify all sleep problems, e.g. excessive somnolence, which are represented in questionnaires such as the SDSC. We therefore anticipate that combining both subjective (questionnaire) and objective (actigraphy) sleep measures would provide the most optimal insight in different sleep problems in children treated with dexamethasone.

Metabolic side effects

Besides neurobehavioral and sleep problems, patients report physical side effects of dexamethasone. This thesis revealed that standardized leptin values, as well as fat mass and hunger scores, increased tremendously after five days of dexamethasone treatment (*Chapter 5*). We did not find a correlation between these changes, which may reflect a dexamethasone-induced state of acute leptin resistance. This contributes to understanding the underlying mechanisms of metabolic adverse events in children with ALL, which in turn are risk factors for sequential cardiovascular diseases and subsequent morbidity and mortality after treatment.[17]

Appetite signaling and (behavioral) eating are complex processes and disruption in one or more of the pathways of satiety and weight regulation may lead to metabolic dysregulation and/or obesity. High dosages of glucocorticoids such as dexamethasone may cause or mediate such disruptions.[18] We established that high dose dexamethasone treatment is associated with dysregulation of one of the players of the hormonal satiety pathway, i.e. leptin, and the feeling of hunger. Dexamethasone is known to upregulate leptin expression and release, but also leptin receptors.[19-21] It is conceivable that the increase in leptin during glucocorticoid treatment has another role than solely appetite control, but to truly determine the regulating role of leptin signaling deficits during or after dexamethasone

treatment, other assessments, e.g. new quantitative biomarkers, are needed.[18] Furthermore, to design clinically relevant interventions, investigating other contributing factors for the strongly increased appetite during dexamethasone treatment is of value. Subsequent steps towards elucidating the mechanisms of (short-term) metabolic changes induced by dexamethasone include the measurement of more appetite-regulating hormones with either orexigenic or anorexigenic effects,[22-27] in combination with extensive exploration and registration of feeling of hunger, eating behavior and caloric intake through a dietary diary. Measurement of body composition may be considered to be more accurately performed using an air-displacement plethysmograph or a dual energy X-ray absorptiometry (DXA) scan.[28,29] These assessments could be performed longitudinally throughout the different treatment phases, since metabolic changes worsen over time.[30] Thereafter, targeted interventions, e.g. physical activity programs, may be started to prevent or overcome worsening of metabolic side effects of glucocorticoid treatment.

Identification of patients at risk of dexamethasone-induced side effects

Neurobehavioral problems

Our systematic review of literature suggested younger age as a possible risk factor for behavioral problems (*Chapter 2*),[4] but this was not confirmed in our prospective study (*Chapter 4)*. Parenting stress was the only factor significantly associated with dexamethasone-induced neurobehavioral problems in our national cohort. It is unknown whether parents experienced more stress due to the perceived problems in their child, or vice versa, or whether the association may be bidirectional. A recent study in 7208 healthy children and their primary caregiver supports the bidirectionality of the association between parenting stress and child behavioral problems.[31] Regardless, parenting stress may be a modifiable target to influence child problems.[32,33] Hence, we propose that future studies should consider parenting stress interventions to explore whether they can improve or prevent dexamethasone-induced neurobehavioral problems in children with ALL.

Other possible determinants which influence the inter-patient variability in the development of dexamethasone-induced neurobehavioral problems are worth mentioning. Even though we did not find an association between the candidate *Bcl1* single nucleotide polymorphism (SNP) and behavioral problems, still the interpatient variation in behavioral side effects suggests genetic susceptibility. Large scale patient cohorts and replication studies are needed to identify genetic susceptibility and to develop polygenic risk scores.[34,35] Furthermore, other factors such as parental coping, family and medical history, as reported in case series and retrospective studies, are conceivably important possible contributing factors for steroid-induced behavioral problems, but they have not been assessed prospectively.[36-42] Screening new patients and their families for psychosocial risk at diagnosis is currently part of standard of care in the Princess Máxima Center, using the Psychosocial Assessment Tool (PAT).[43]

This risk profile is indicative of the distress levels families are likely to experience during the treatment of their child,[44] and may therefore prove useful to identify parents and patients who will benefit from interventions or more support early during treatment, to eventually improve child behavior. This may be a first step towards improved knowledge, identification and future intervention for children who are at risk of dexamethasone-induced neurobehavioral problems. Ultimately, the development of a risk prediction model, taking into account both child and parental factors, can lead towards targeted identification of children at risk of dexamethasone-induced neurobehavioral problems, and subsequent selection of a group that may benefit from interventions.

Sleep problems

When reviewing existing literature on steroid-induced sleep problems (*Chapter 2)*, older age, higher steroid dose and the use of dexamethasone (as opposed to prednisone) were associated with increased sleep problems during glucocorticoids. In contrast, in our cohort, parenting and parental stress were the only significant determinants for sleep problems (*Chapter 4*). The association between child sleep problems and parenting stress has been shown in healthy children, as well as in children with psychological problems.[45-49] The bidirectional association was previously proposed in a transactional model which also included other aspects such as infant and environmental factors, which may influence child sleep.[50,51] Another factor which is associated with poor child's sleep is poor parental sleep, as was shown previously in children with ALL.[52] We did not evaluate parental sleep in our cohort, but since poor sleep is also associated with (parenting) stress,[53] it is feasible that parental sleep has influenced both parenting stress and the reporting of child sleep in our cohort. Still, positive effects on child sleep may be expected when targeting parenting stress, as was previously suggested in children with behavioral sleep problems.[54] It would be of value to measure parenting stress, parental and child sleep problems throughout the treatment of ALL to detect changes and to be able to timely intervene in one or more of these domains. In our center, sleep is assessed in clinical practice through the KLIK PROM portal and awareness during consultation with the treating physician is strongly advocated.[55]

Interestingly, there appear to be different associations between genetic and environmental factors and objective versus subjective sleep measurements.[56] The fact that we did not find a genetic (*rs4918* SNP) or pharmacokinetic risk factor for dexamethasone-induced sleep problems may be due to our use of subjective, parent-reported outcome measures. This endorses the recommendation to use both actigraphy combined with parent-reported outcomes, and combine this knowledge with previously described SNPs and other risk factors, such as parenting stress, to gain more insight in the mechanisms behind and risk factors of steroid-induced sleep problems.

An intervention to reduce dexamethasone-induced side effects

Our previous clinical trial suggested that children who suffer most from dexamethasone-induced neurobehavioral and sleep problems may benefit from physiological hydrocortisone addition to dexamethasone treatment.[5] However, our randomized controlled trial did not establish a beneficial effect of hydrocortisone when compared to placebo (*Chapter 6*). Of note, both trials did not compare the intervention with a treatment as usual arm, prohibiting a direct comparison between the intervention with hydrocortisone or placebo and the natural course of side effects.

Neurobehavioral problems

Both hydrocortisone and placebo seemed to diminish dexamethasone-induced neurobehavioral problems, a finding which may be contributed to a placebo-effect. This does not completely exclude the hypothesis that hydrocortisone in itself has a beneficial effect through restored activation of the MR. However, if this is the case, the effect is not stronger than a placebo-effect. The equivalent dose of the physiological dose of hydrocortisone (10mg/m^2) is 0.375mg/m^2 dexamethasone,[57] a fraction of the 6mg/m^2 dexamethasone children receive every day during their ALL treatment. It is conceivable, that the high dose of dexamethasone results in upregulation of both the GR and MR, and that the relatively low dose of hydrocortisone is not enough to completely saturate the MR. It has been reported that high dexamethasone doses can activate the MR in the brain, although with lower potency,[58] thereby generating competition with the low hydrocortisone dose, and minimizing the potential effect of hydrocortisone addition. For better understanding of the binding of physiological quantities of hydrocortisone in the brain during high dose dexamethasone treatment, future research may use (radioactively) labelled hydrocortisone in animal models.[59]

Sleep problems

Both objective (actigraphy) and subjective sleep outcomes did not improve after hydrocortisone (or placebo) addition. This lack of efficacy could be due to a different pathophysiology of sleep problems than GR:MR imbalance. A previous study in healthy adult volunteers showed that both dexamethasone and hydrocortisone decrease rapid-eye-movement (REM) sleep, whereas slow-wave sleep (SWS) increased during hydrocortisone but decreased during dexamethasone.[60] Interestingly, when adding hydrocortisone to dexamethasone treatment, both REM and SWS seemed to improve in comparison with the dexamethasone only condition, albeit not significantly.[60] Hence, it would be interesting to use polysomnography to measure sleep in children with ALL under dexamethasone treatment with or without hydrocortisone addition, to further elucidate the effect of hydrocortisone on sleep.

Hunger feeling

We hypothesized that hydrocortisone addition would diminish dexamethasone-induced feeling of hunger. In contrast, we found that hydrocortisone increased fasting and average hunger scores when compared with placebo. This is in line with a recent randomized, double-blind study with cross-over design in 16 healthy adult volunteers, which showed an increase in fasting hunger after overnight administration of stress doses hydrocortisone, compared to saline.[61] This study showed decreased perfusion in emotion regions of the brain, as well as in reward and executive control regions, supporting the role of hydrocortisone in regulating (increasing) appetite. A study in mice investigated the effect of corticosterone, the natural occurring glucocorticoid in rodents, add-on therapy during dexamethasone treatment to prevent dexamethasone-induced metabolic side effects.[62] Conversely, corticosterone aggravated dexamethasone-induced hyperinsulinemia, hyperglycemia, and glucose intolerance.[62] Hence, future studies on the treatment of dexamethasone-induced hunger and metabolic side effects should consider other options than hydrocortisone addition , e.g. physical activity interventions, to increase energy expenditure.

Health related quality of life

All the dexamethasone-induced problems described in this thesis have impact on health related quality of life (HRQoL) during ALL treatment.[3,63-65] We did not find a difference between hydrocortisone and placebo in improving HRQoL of the child, as reported by parents. Unfortunately, we did not measure HRQoL during a course without hydrocortisone or placebo addition (i.e. a 'dexamethasone only' course), therefore we were not able to ascertain whether a placebo-effect played a role in improving HRQoL. We measured parent-reported HRQoL with the general pediatric quality of life questionnaire, which does not account for treatment-specific problems during dexamethasone treatment. Another questionnaire, the Quality of life Evaluation in patients receiving Steroids (QuESt) tool, examines changes attributable to corticosteroids.[66] This tool is a self-report questionnaire for children ≥8 years and even though further validity and reliability testing is still required, it is a promising tool to measure treatment-specific HRQoL in children receiving dexamethasone.

Placebo-effect

Based on the results of our previous study,[5] we considered the design of our current study (randomized, double blind, placebo-controlled, with cross-over), as most optimal to measure differences between hydrocortisone and placebo. However, we did not anticipate that a third, treatment as usual arm, would have been beneficial to irrefutably prove a placebo-effect, which eventually occurred in our cohort. Still, our findings in children with clinically relevant dexamethasone-induced neurobehavioral problems suggest a strong placebo-effect which influences both patient and family. The mechanisms of placebo effects are not extensively

studied in children, but in adults complex neurobiological reactions, molecular events and neural network changes have been described to be involved.[67] For example, placebo analgesia has been shown to be associated with the release of endogenous opioids.[68,69] It is conceivable, that both hydrocortisone and placebo induce a similar neurobiological effect, independent of the MR binding effect. Such a neurobiological effect of both hydrocortisone and placebo may also have been mediated by expectancies of parents and children, which are an important learning mechanism and often steer placebo-effects.[70] Not all children were aware of the possible beneficial effect due to their young age, and since all our assessments were proxy-reported, a placebo-effect by proxy could have occurred: parents who expect their child to improve during the study would experience such an improvement.[71,72] Classical conditioning may have played a role as well: by adding an oral suspension to standard treatment, patients can be triggered to show physiological responses to additional medication.[67,73,74] Another mechanism, which probably increased the placebo-effect, is the participation effect: through attention and (frequent) contact during participation in a clinical study, a general positive effect can be obtained.[75] In our study, the research physician contacted all parents at extra time points, after each dexamethasone course. Parents were asked about adverse events, but they could also talk about encountered problems during the dexamethasone course. Even though no improvement in parental distress was observed, asking parents every dexamethasone course about their child's behavior, may have made them more perceptible to their child. This can cause adjustment of parental behavior towards the child, which in turn can yield children to behave differently, as previously reported in children with autism.[76]

Nocebo-effect

Substantially more patients (67%) experienced clinically relevant dexamethasone-induced neurobehavioral and sleep problems than in our previous DexaDays-1 study (33%).[5] The side effects of dexamethasone are widely known and parents and patients are currently informed extensively about the severity of potential problems, even before they exist. Previous negative experiences, worrisome information, mistaken beliefs and negative expectations induced by verbal suggestions are known to increase or even cause side effects, and are described as *nocebo*-effects.[67,77,78] It is plausible that this nocebo-effect (by proxy)[71] of dexamethasone played an important role in our findings and even contributed to the pronounced observed placebo-effect. The increased emphasis on providing comprehensive, regular and extensive information about the side effects of dexamethasone, driven by a genuine desire to prioritize psychological well-being, may have inadvertently led to a nocebo-effect experienced by patients and parents at our recently established national pediatric cancer center. This unintended consequence could be attributed to heightened awareness and concerns surrounding potential negative effects, impacting their perceptions and experiences.

In children, nocebo-effects can be severe and often anticipatory. For example, children with juvenile idiopathic arthritis who receive methotrexate and experience nausea as side effect, can become nauseous even before they start their next dose methotrexate upon entering the hospital.[79] During ALL maintenance treatment, children usually know when the 'DexaDays' will start again: this fact and the behavioral and anticipatory adjustment of the family members, may give rise to intensified behavioral changes. Furthermore, all patients described in this thesis already had received at least twelve dexamethasone courses before they were included. The experiences from patients and parents in the preceding dexamethasone courses could have influenced the perception of the measured dexamethasone-induced problems. Therefore, it would be interesting to measure dexamethasone-induced problems during the first encounter with dexamethasone, to explore whether a negative first experience contributes to dexamethasone-induced problems further in maintenance, especially since dexamethasone is currently used in induction of the ALLTogether1 protocol, similar to the previously applied DCOG ALL-9 protocol.

Steroid receptor function in steroid-induced cytotoxicity

While previously studying the influence of hydrocortisone on dexamethasone-induced cytotoxicity, for safety purposes, we found that hydrocortisone potentiated the cytotoxic effect of both prednisolone and dexamethasone in steroid sensitive ALL cell lines.[80] In this thesis, we describe that hydrocortisone is an extremely potent steroid to induce steroid-induced cell death (*Chapter 7*). This steroid-induced cell death can be achieved solely by activation of the mineralocorticoid receptor (MR). Furthermore, dexamethasone was able to induce cell death through activation of the MR, highlighting that the MR is potentially capable of inducing leukemic cell death after activation by steroid treatment.

The fact that hydrocortisone is a potent anti-leukemic steroid is of interest. Since several studies showed better overall and event free survival and less central nervous system relapses using dexamethasone compared to prednisone, dexamethasone became the preferred steroid during maintenance therapy in most treatment protocols.[81-84] It is still debated whether these advantages of dexamethasone are due to a higher equivalent cytotoxic dose than the generally presumed factor seven, or due to higher CNS penetration.[85] The downside of dexamethasone compared to prednisone is an overall increased toxicity.[86,87] Apart from intrathecal therapy, hydrocortisone has not been used in the treatment of ALL, possibly due to the shorter biological half-life, consequential logistic dosing challenges, and higher mineralocorticoid activity.[57] However, hydrocortisone seems to penetrate the cerebrospinal fluid, and has a favorable toxicity profile.[88] Although our PDX model and patient samples did not confirm that hydrocortisone was more cytotoxic than dexamethasone or prednisone, it was at least equal, and it may therefore still be of

value as an additional steroid to induce cell death, or as an alternative therapy for those patients who do not tolerate dexamethasone or prednisone due to extreme side effects. However, the short half-life (1-3 hours) would require frequent administration, or modified-release prescriptions could be used. Still, this should first be explored in clinical trials.

Our study was the first to compare the cytotoxic activity of dexamethasone, prednisolone and hydrocortisone in equivalent concentrations (500 micromolar (μM) to $8.19*10^{-8}$ μM) in ALL cell lines with either a functional GR *or* MR, thereby comparing both receptors in their abilities to induce cell death. Previous studies in ALL cell lines only looked into cytotoxic activity of different steroids without distinguishing between GR and MR activity, and most studies or trials so far, did not consider hydrocortisone as a potent steroid.[89-92] We found that dexamethasone is a potent steroid to induce cell death through MR activation, however, a somewhat similar experiment in CV-1 cells (monkey kidney cells) transfected with human GR or MR, showed that dexamethasone had a high glucocorticoid potency, but only minor mineralocorticoid potency.[89] This indicates that the MR and GR may exert different mechanisms in different cell types. A recent case report using brain tissue from an eight-year-old patient who died from a brain tumor whilst using high-dose dexamethasone suggests that dexamethasone is able to bind the MR sufficiently to induce partial nuclear translocation.[58] Nevertheless, it is unknown whether this binding actually regulates gene transcription and subsequent effects. This needs further investigation in neural cell lines or, preferably, in brain tissue from more glucocorticoid-treated, resistant and sensitive subjects.

Future directions: implementation of our findings

Based on the findings described in this thesis, the most important question which arises is: *Can we use the intervention with hydrocortisone or placebo in clinical care to reduce dexamethasone-induced neurobehavioral problems?* In a recent expert consensus paper regarding placebo- and nocebo-effects, the importance of making optimal use of placebo-effects to achieve better treatment outcomes is strongly advocated in adults.[93] Studies evaluating the use of open-label placebo showed its effect in children with functional abdominal pain or ADHD.[75,94-96] To our knowledge, the use of open-label placebo has not been evaluated so far in the pediatric oncology setting. Besides open-label placebo, hydrocortisone could also be considered as an effective intervention, bearing in mind that it might increase feeling of hunger. As we realized that introducing open-label placebo in children with cancer is a novel strategy, we discussed this clinical implementation extensively with different stakeholders, including several feedback loop conversations (Figure 1).

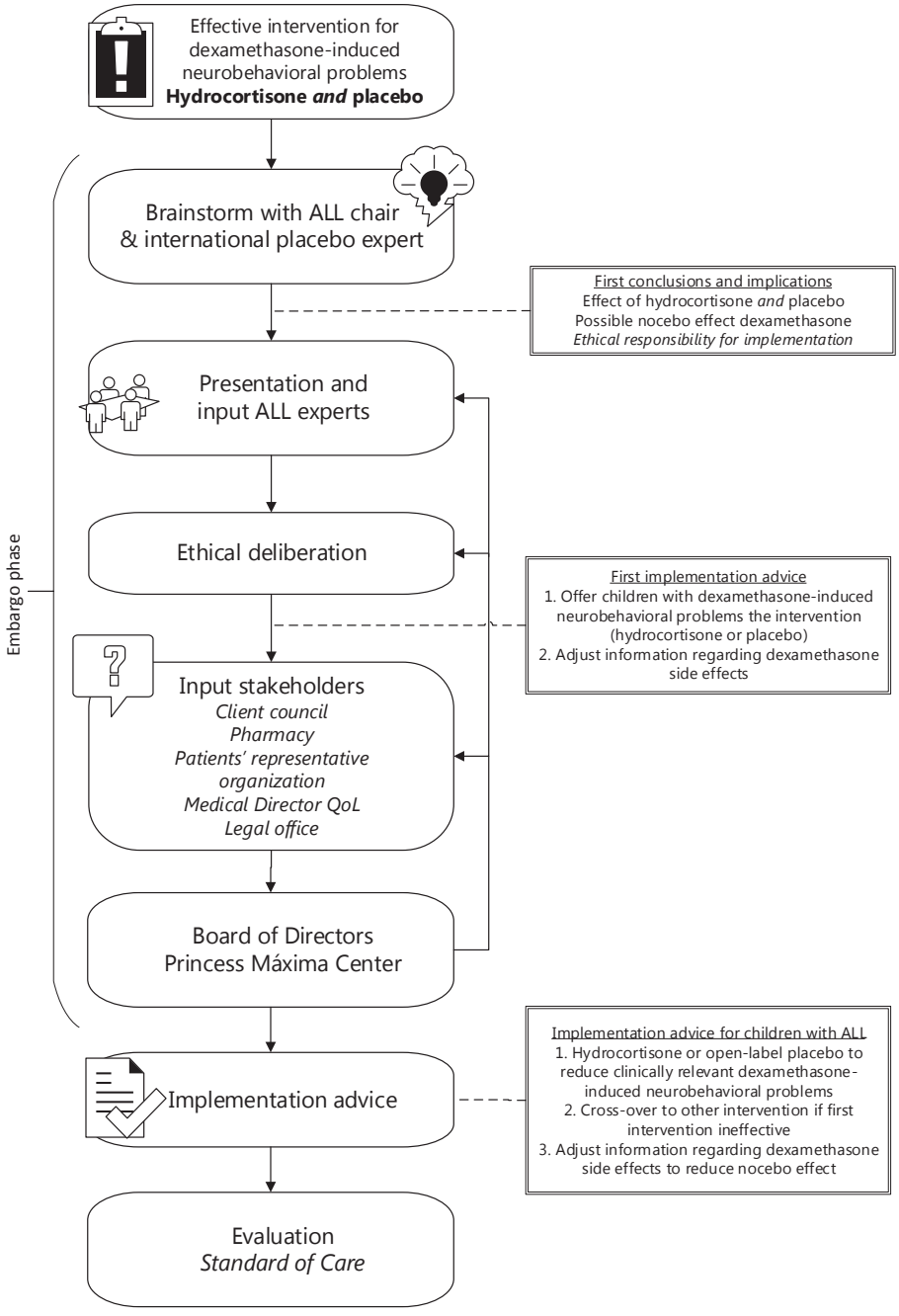


Figure 1. Flow-chart illustrating our advisory and implementation process.

The results were kept under embargo until a final decision regarding implementation was reached.

Abbreviations: ALL: acute lymphoblastic leukemia, QoL: quality of life

Taking all advice into account, we decided to propose the innovative strategy to offer parents the choice between open-label placebo or hydrocortisone in a shared decision making setting whenever children (or parents) report clinically relevant dexamethasone-induced neurobehavioral problems. Both expectancies and believes of parents/patients and healthcare providers will play a role in the choice between hydrocortisone or placebo. The pros and cons of both interventions are depicted in Table 2.

Table 2. Pros and cons of hydrocortisone and open-label placebo use

Hydrocortisone		**Open-label placebo**	
Pros	*Cons*	*Pros*	*Cons*
Expectations of parents and patients	Side effects?	No side effects	Hard(er) to explain
Believes of parents and patients	Still a medicine	No extra active medication	Open-label placebo ≠ study placebo
Expectations of the healthcare provider	Increased feeling of hunger	Believes of parents and patients	Skepticism of healthcare providers
Easy to explain			

This novel intervention started in May 2023 in the Princess Maxima Center, and standard of care evaluation will show whether the effect of the choice of hydrocortisone or open-label placebo is comparable to the effect described in this thesis. Besides the implementation of offering a choice between the interventions as standard of care, we attempt to downscale the nocebo effect of dexamethasone, by creating awareness, adjusting dexamethasone information, and training healthcare providers to discuss dexamethasone-induced side effects in a uniformly prepared, concise, less repeated and more neutral way.

In conclusion

The research and implications for clinical care described in this thesis are an essential step towards beating dexamethasone-induced side effects. We do not expect all the side effects of dexamethasone to suddenly disappear, but if the edges are taken off, a step towards enhanced quality of life for both patients and their families is achieved during the 2 years of ALL treatment. In addition to the implementation steps which we already undertook, we identified several gaps of knowledge that may be addressed either in future research and/or in clinical practice (summarized in Table 3). These include directions for measurement of dexamethasone-induced side effects, identification of patients at risk and treatment and evaluation options. The ultimate goal is to improve quality of life for children with ALL during dexamethasone treatment and thereafter.

Table 3. Gaps of knowledge and future directives

Domain	Directions for future research and clinical practice
Neurobehavioral problems	Outcome measurement - Measure neurobehavioral problems systematically in children receiving dexamethasone, include the first administration phase - Use specific PROMIS items when available - Use self-report questionnaires when possible - Include screening for depressive symptoms in older children Identification of patients at risk - Genetic susceptibility: identification of genetic variants - Polygenic risk score development - Include parental coping and family and medical history - Use the PAT to screen for psychosocial risk before the start of dexamethasone - Development of a prediction model to identify patients at risk Interventions - Implementation of hydrocortisone and open-label placebo for those children who suffer most - Evaluation of this implementation: both the process and effect of (offering) the intervention - Targeting of parenting stress to possibly reduce child (dexamethasone related) problems
Sleep problems	Outcome measurement - Subjective sleep measurement: development of PROMIS item - Objectively measure sleep problems with actigraphy, preferably on multiple time points during dexamethasone treatment - Combine subjective and objective sleep measurement - Measurement of sleep with polysomnography during glucocorticoid treatment Identification of patients at risk - Combining measurements of child sleep, parental sleep and child and environmental factors - Development of a risk prediction model Intervention - Targeting of parenting and parental stress to possibly improve child sleep

Table 3. Continued

Domain	Directions for future research and clinical practice
Metabolic changes and hunger	Outcome measurement - New quantitative biomarkers which identify leptin signaling deficits - Measurement of more appetite-regulating hormones before and after high dose dexamethasone - Further exploration of feeling of hunger, eating behavior and caloric intake through a dietary diary - Measurement of body composition using air-displacement plethysmography or DXA scan Identification of patients at risk - Longitudinal cohort including all known risk factors and incorporating expenditure as well as intake Intervention - Development of targeted (physical activity) interventions
(Pre-)clinical studies	- Activation of the MR upon dexamethasone treatment in neural cell lines or brain tissue - Gene transcription (based on MR activation) in the brain of glucocorticoid treated patients - Labelled hydrocortisone addition in animal models to study uptake in the brain under dexamethasone treatment - Effectivity of hydrocortisone in comparison with dexamethasone or prednisone in the treatment of ALL

Abbreviations: ALL: acute lymphoblastic leukemia, DXA: dual energy X-ray absorptiometry, MR: mineralocorticoid receptor, PAT: psychosocial assessment tool, PROMIS: Patient-Reported Outcomes Measurement Information System

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English summary

ENGLISH SUMMARY

Glucocorticoids, such as dexamethasone and prednisone, are indispensable components in the treatment of childhood acute lymphoblastic leukemia (ALL). Dexamethasone is currently the preferred glucocorticoid in most treatment protocols and is administered during maintenance therapy for five days every 3-4 weeks, during 1,5 year of ALL treatment. Besides the positive anti-leukemic effect, dexamethasone can induce various undesirable side effects. Patients and parents often report neurobehavioral and sleep problems as harmful side effects, which generally negatively affect quality of life. Increased feeling of hunger, dyslipidemia and adiposity are also well-known side effects of dexamethasone. The inter-patient variability in all these side effects is high and no clear risk factors are known. The first aim of this thesis was to increase current knowledge on the prevalence and determinants of dexamethasone-induced side effects in children with ALL.

We first performed a systematic literature review to identify previously described risk factors for steroid-induced neurobehavioral and sleep problems in children with ALL (**Chapter 2**). Overall, the quality of evidence was very low. Available literature suggested that type or dose of steroid is not related to neurobehavioral problems, but might be to sleep problems. Younger patients seem at risk for behavioral problems, whereas older patients are at risk for sleep problems. No studies describing parental stress or medical history were identified, and genetic susceptibility associations remain to be replicated.

We furthermore performed a prospective cohort study in 105 children (3.0-18.8 years) with ALL (**Chapters 3 and 4**). Clinically relevant dexamethasone-induced neurobehavioral and sleep problems were reported by parents in 70 (67%) and 61 (59%) patients respectively. We identified parenting stress, and not dexamethasone pharmacokinetics, genetic variation, patient and parent demographics, or disease and treatment characteristics, as a significant determinant for parent-reported dexamethasone-induced neurobehavioral and sleep problems. Parenting stress may be a modifiable target to reduce these problems in the future.

We also found that merely five days of dexamethasone lead to direct and significant increase in leptin (a fat hormone that regulates satiety), hunger scores and fat mass (**Chapter 5**). We found no correlations between these measurements, which may suggest a dexamethasone-induced state of acute leptin resistance. Since children with ALL are at increased risk for metabolic adverse events, understanding underlying mechanisms is important, and leptin resistance might play a role.

Even though the side effects of dexamethasone are well-known and negatively impact quality of life during ALL treatment, currently no pharmacological treatment to overcome dexamethasone-induced side effects exists. Glucocorticoids can bind to two receptor types: the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Dexamethasone has a high affinity for the GR, but no affinity for the MR. Furthermore, dexamethasone suppresses the endogenous production of cortisol, which has a high affinity for the MR. We previously hypothesized that the neurobehavioral side effects of dexamethasone are due to cortisol depletion of the MR, caused by dexamethasone, which might be overcome by hydrocortisone (the synthetic equivalent of cortisol) addition. Our previous randomized controlled trial (RCT) investigated the effect of physiological hydrocortisone addition to dexamethasone treatment. In those patients who suffered most, hydrocortisone diminished neurobehavioral and sleep problems, but these results required further validation before implementation in clinical practice.

The second aim of this thesis was therefore to validate the finding that hydrocortisone addition to dexamethasone treatment leads to a significant reduction of clinically relevant dexamethasone-induced neurobehavioral and sleep problems. We performed a double-blind RCT with cross-over design in 52 children with ALL who suffered from clinically relevant neurobehavioral problems (**Chapters 3 and 6**). We found no difference between hydrocortisone and placebo in reducing dexamethasone-induced neurobehavioral problems. However, the neurobehavioral problems decreased equally during both hydrocortisone and placebo treatment, suggesting a placebo-effect which influences both patient and family. This placebo-effect may be used in clinical practice to alleviate some of the burden of dexamethasone-induced neurobehavioral problems.

Finally, we aimed to describe the role of the MR in steroid-induced cytotoxicity and to evaluate the cytotoxic effect of hydrocortisone (**Chapter 7**). In a preclinical study, we showed that hydrocortisone can induce the expression of steroid-regulated genes through both GR and MR, and effectively induces cell death in Reh cell lines that by doxycycline-induction express the GR or MR. Moreover, dexamethasone induces cell death in MR-expressing Reh cells that lack an endogenous functional GR gene. These results highlight that the MR is a potent receptor to induce leukemic cell death after activation by steroid treatment, and that hydrocortisone treatment can induce cell death in leukemic cells. In patient samples, the role of the mineralocorticoid receptor in steroid-induced cytotoxicity seems less pronounced, possibly due to the (relative) low expression of MR in ALL patients. Still, hydrocortisone may be considered as a potential anti-leukemic agent, especially for those patients who suffer from severe dexamethasone-induced side effects.

The research and implications for clinical care described in this thesis are an essential step towards beating dexamethasone-induced side effects. We implemented the innovative strategy to offer parents and patients the choice between open-label placebo or hydrocortisone in a shared decision making setting whenever clinically relevant dexamethasone-induced neurobehavioral problems occur. Furthermore, we attempt to downscale the nocebo-effect of dexamethasone, by creating awareness, adjusting dexamethasone information, and training healthcare providers to discuss dexamethasone-induced side effects in a uniformly prepared, concise, less repeated and more neutral way.

We do not expect all the side effects of dexamethasone to suddenly disappear, but if the edges are taken off, a step towards enhanced quality of life for both patients and their families is achieved during the 2 years of ALL treatment.

Nederlandse samenvatting

NEDERLANDSE SAMENVATTING

Glucocorticoïden, zoals dexamethason en prednison, zijn onmisbare componenten in de behandeling van acute lymfatische leukemie (ALL) bij kinderen. Dexamethason is momenteel het glucocorticoïd waaraan de voorkeur wordt gegeven in de meeste behandelingsprotocollen. Gedurende de onderhoudsfase van de ALL behandeling krijgen kinderen elke 3-4 weken vijf dagen dexamethason, gedurende 1,5 jaar. Naast het positieve antileukemische effect kan dexamethason verschillende ongewenste bijwerkingen veroorzaken. Patiënten en ouders melden vaak gedrag- en slaapproblemen als vervelende bijwerkingen, die over het algemeen de kwaliteit van leven negatief beïnvloeden. Een verhoogd hongergevoel, dyslipidemie en adipositas zijn ook bekende bijwerkingen van dexamethason. De variabiliteit tussen patiënten in het voorkomen van al deze bijwerkingen is hoog en er zijn geen duidelijke risicofactoren bekend. Het eerste doel van dit proefschrift was het vergroten van de huidige kennis over de prevalentie en determinanten van dexamethason-geïnduceerde bijwerkingen bij kinderen met ALL.

We voerden eerst een systematisch literatuuronderzoek uit om eerder beschreven risicofactoren voor steroïd-geïnduceerde gedrag- en slaapproblemen bij kinderen met ALL te identificeren (**Hoofdstuk 2**). Over het algemeen was de kwaliteit van de geïdentificeerde artikelen zeer laag. Het beschikbare bewijs suggereerde dat het type of de dosis steroïden geen verband houdt met gedragsproblemen, maar mogelijk wel met slaapproblemen. Jongere patiënten lijken risico te lopen op gedragsproblemen, terwijl oudere patiënten risico lopen op slaapproblemen. Er waren geen onderzoeken die ouderlijke stress of medische voorgeschiedenis beschreven, en studies naar genetische susceptibiliteit moeten nog worden gerepliceerd.

Verder voerden we een prospectieve cohortstudie uit bij 105 kinderen (3,0-18,8 jaar) met ALL (**Hoofdstukken 3 en 4**). Klinisch relevante door dexamethason veroorzaakte gedrag- en slaapproblemen werden door de ouders gemeld bij respectievelijk 70 (67%) en 61 (59%) patiënten. We identificeerden opvoed stress bij ouders, en niet dexamethason farmacokinetiek, genetische variatie, demografische gegevens van patiënten en ouders, of ziekte- en behandelingsdeterminanten, als een significante risicofactor voor door ouders gerapporteerde dexamethason-geïnduceerde gedrag- en slaapproblemen. Opvoed stress bij ouders kan wellicht een aanknopingspunt zijn om door dexamethason geïnduceerde gedragsproblemen in de toekomst te verminderen.

We ontdekten ook dat slechts vijf dagen dexamethason leidde tot directe en significante stijging in leptine (het vethormoon dat de eetlust regelt), hongerscores en vetmassa (**Hoofdstuk 5**). We vonden geen correlaties tussen deze metingen, wat kan wijzen op

een door dexamethason veroorzaakte toestand van acute leptine resistentie. Aangezien kinderen met ALL een verhoogd risico lopen op metabole toxiciteit, is het belangrijk om de onderliggende mechanismen te begrijpen, en leptine resistentie zou hierbij een rol kunnen spelen.

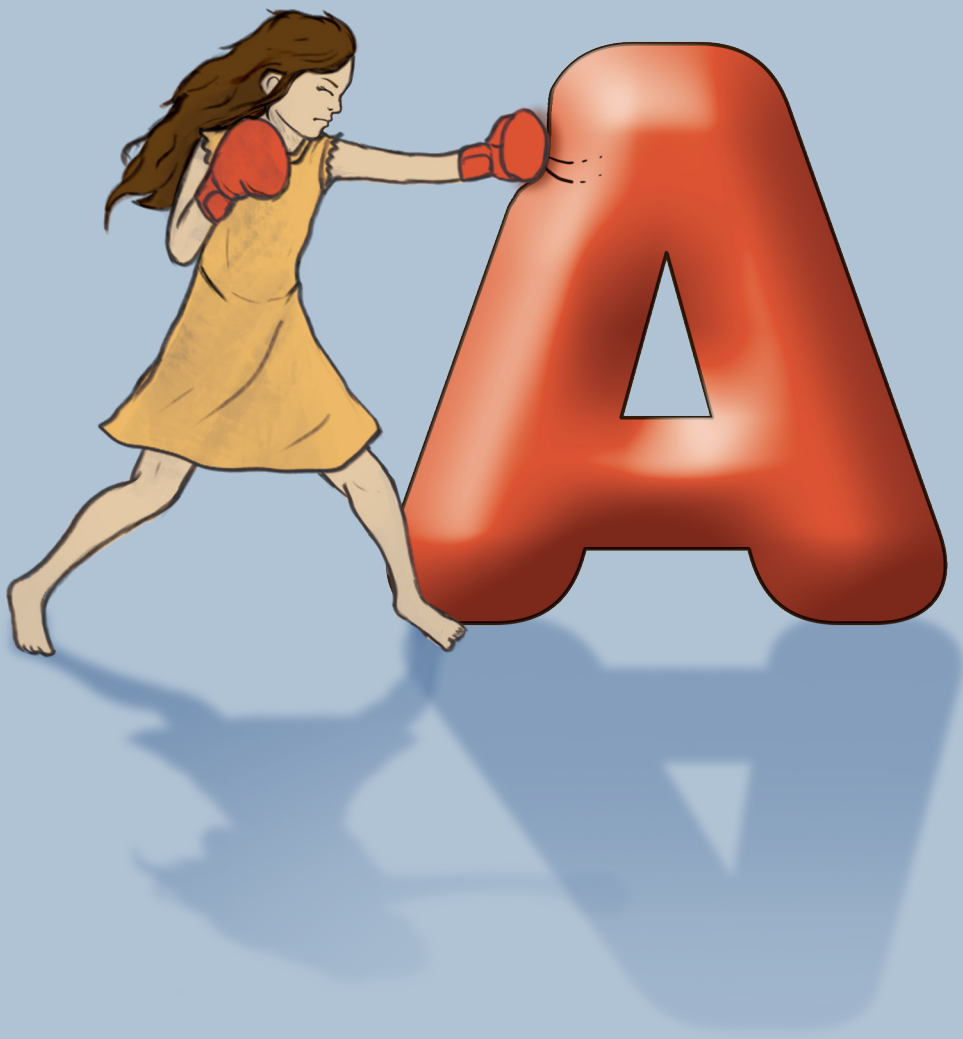
Hoewel de bijwerkingen van dexamethason bekend zijn en een negatieve invloed hebben op de kwaliteit van leven tijdens de behandeling van ALL, bestaat er momenteel geen medicamenteuze behandeling om de door dexamethason veroorzaakte bijwerkingen te ondervangen. Glucocorticoïden kunnen zich binden aan twee receptortypen: de glucocorticoïdreceptor (GR) en de mineralocorticoïdreceptor (MR). Dexamethason heeft een hoge affiniteit voor de GR, maar weinig affiniteit voor de MR. Bovendien onderdrukt dexamethason de endogene productie van cortisol, dat een hoge affiniteit heeft voor de MR. We stelden eerder de hypothese dat de gedragsmatige bijwerkingen van dexamethason te wijten zijn aan cortisoldepletie van de MR, veroorzaakt door dexamethason. Dit zou kunnen worden verholpen door hydrocortison (het synthetische equivalent van cortisol) te suppleren. Onze eerdere gerandomiseerde gecontroleerde trial (RCT) onderzocht het effect van fysiologische toevoeging van hydrocortison aan dexamethason behandeling. Bij de patiënten die er het meeste last van hadden, verminderde hydrocortison de gedrag- en slaapproblemen, maar deze resultaten moesten worden gevalideerd voordat ze in de klinische praktijk konden worden toegepast.

Het tweede doel van dit proefschrift was daarom het valideren van de bevinding dat toevoeging van hydrocortison aan dexamethason behandeling leidt tot een significante vermindering van klinisch relevante dexamethason-geïnduceerde gedrag- en slaapproblemen. We voerden een dubbelblinde RCT met cross-over uit bij 52 kinderen met ALL die last hadden van klinisch relevante gedragsproblemen (**Hoofdstukken 3 en 6**). We vonden geen verschil tussen hydrocortison en placebo in het verminderen van dexamethason-geïnduceerde gedragsproblemen. De gedragsproblemen namen echter in gelijke mate af tijdens zowel de behandeling met hydrocortison als placebo, wat duidt op een placebo-effect dat zowel de patiënt als de familie beïnvloedt. Dit placebo-effect kan in de klinische praktijk worden gebruikt om een deel van de dexamethason-geïnduceerde gedragsproblemen te verlichten.

Tot slot wilden we de rol van de MR in steroïd-geïnduceerde cytotoxiciteit beschrijven en het cytotoxische effect van hydrocortison evalueren (**Hoofdstuk 7**). In een preklinische studie toonden we aan dat hydrocortison de expressie van steroïd-gereguleerde genen kan induceren via zowel GR als MR, en effectief celdood induceert in Reh cellijnen die door doxycycline-inductie de GR of MR tot expressie brengen. Bovendien induceert dexamethason celdood in MR-uitende Reh-cellen die geen endogeen functioneel GR-gen

hebben. Deze resultaten benadrukken dat de MR een krachtige receptor is om leukemische celdood te induceren na activering door steroïdenbehandeling, en dat hydrocortison celdood kan induceren in leukemische cellen. In patiënt samples lijkt de rol van de MR in steroïd-geïnduceerde cytotoxiciteit minder uitgesproken, mogelijk door de (relatief) lage expressie van MR bij ALL-patiënten. Toch kan hydrocortison worden beschouwd als een potentieel anti-leukemisch middel, vooral voor patiënten die last hebben van ernstige door dexamethason veroorzaakte bijwerkingen.

Het onderzoek en de implicaties voor de klinische zorg die in dit proefschrift worden beschreven, zijn een essentiële stap op weg naar het overwinnen van door dexamethason veroorzaakte bijwerkingen. We implementeerden de innovatieve strategie om ouders en patiënten de keuze te bieden tussen open-label placebo of hydrocortison wanneer klinisch relevante dexamethason-geïnduceerde gedragsproblemen optreden. Verder proberen we het nocebo-effect van dexamethason te verminderen door bewustwording te creëren, de informatie over dexamethason aan te passen en zorgverleners te trainen om dexamethason-geïnduceerde bijwerkingen op een uniform voorbereide, beknopte, minder herhaalde en meer neutrale manier te bespreken. We verwachten niet dat alle bijwerkingen van dexamethason plotseling zullen verdwijnen, maar als de scherpe kantjes eraf worden gehaald, wordt er een stap gezet in de richting van een verbeterde kwaliteit van leven voor zowel patiënten als hun families gedurende de 2 jaar dat de ALL behandeling duurt.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About the author

ABOUT THE AUTHOR

Annelienke van Hulst was born on February 14th 1990 in Amersfoort, The Netherlands. She attended secondary school at the Johan van Oldenbarnevelt Gymnasium, from which she obtained her VWO gymnasium diploma in 2008. Her medical training at Utrecht University commenced that same year. After obtaining her medical degree in 2014, Annelienke gained a few years of hands-on clinical experience. She first worked as a resident (ANIOS) in pediatrics at the Gelre hospital in Apeldoorn, after which she continued as resident in adult intensive care at the Gelderse Vallei hospital in Ede. In 2017 she started her PhD project at the Princess Maxima Center for Pediatric Oncology (Utrecht) under the supervision of prof. dr. M.M. van den Heuvel-Eibrink (pediatric oncology), prof. dr. M.A. Grootenhuis (pediatric psycho-oncology) and prof. dr. E.L.T. van den Akker (pediatric endocrinology), which resulted in this thesis. Since the beginning of 2022, she was responsible for the implementation of the main results of the research described in this thesis.

As of September 2023, Annelienke started her training to become a general practitioner at the UMC Utrecht. She lives in Leusden with her husband and two children and enjoys boxing in her spare time.


P

List of publications

THIS THESIS

van Hulst AM*, Peersmann SHM*, van den Akker ELT, Schoonmade LJ, van den Heuvel-Eibrink MM, Grootenhuis MA, van Litsenburg RRL. *Shared first authorship. Risk factors for steroid-induced adverse psychological reactions and sleep problems in pediatric acute lymphoblastic leukemia: A systematic review. *Psychooncology. 2021 Jul;30(7):1009-1028.*

van Hulst AM, Verwaaijen EJ, Fiocco M, Pluijm SMF, Grootenhuis MA, Pieters R, van den Akker ELT, van den Heuvel-Eibrink MM. Study protocol: DexaDays-2, hydrocortisone for treatment of dexamethasone-induced neurobehavioral side effects in pediatric leukemia patients: a double-blind placebo controlled randomized intervention study with cross-over design. *BMC Pediatr. 2021 Sep;21(1):427-435.*

van Hulst AM, Grootenhuis MA, Verwaaijen EJ, van Litsenburg RRL, Li L, van Zelst BD, Broer L, Pluijm SMF, Pieters R, Fiocco M, van den Akker ELT#, van den Heuvel-Eibrink MM#. #Shared last authorship. Unraveling dexamethasone-induced neurobehavioral and sleep problems in children with acute lymphoblastic leukemia: which determinants are important? *JCO Precis Oncol. 2023 Jun;7.*

van Hulst AM, Verwaaijen EJ, van den Berg SAA, van Litsenburg RRL, Grootenhuis MA, Fiocco M, Neggers SJCMM, van den Heuvel-Eibrink MM, van den Akker ELT. Leptin increase during dexamethasone and its association with hunger and fat, in pediatric acute lymphoblastic leukemia. *Under Review.*

van Hulst AM, van den Akker ELT, Verwaaijen EJ, Fiocco M, Rensen N, van Litsenburg RRL, Pluijm SMF, Zwaan CM, van Santen HM, Pieters R, Evers AWM, Grootenhuis MA§, van den Heuvel-Eibrink MM§. §Shared last authorship. Hydrocortisone to reduce dexamethasone-induced neurobehavioral side-effects in children with acute lymphoblastic leukemia – Results of a double-blind, randomized controlled trial with cross-over design. *Eur J Cancer. 2023 Apr;187:124-133.*

van Hulst AM¤, van der Zwet JCG¤, Buijs-Gladdines JGCAM, Smits WK, Fiocco M, Pieters R, van Leeuwen FN, van den Heuvel-Eibrink MM, van den Akker ELT, Meijerink JPP. ¤Shared first authorship. The role of the mineralocorticoid receptor in steroid-induced cytotoxicity in pediatric acute lymphoblastic leukemia. *Under Review.*

OTHER PUBLICATIONS

van Hulst AM, Grootenhuis MA, van den Akker ELT, van den Heuvel-Eibrink MM. Response to letter re: Hydrocortisone to reduce dexamethasone-induced neurobehavioral side-effects in children with acute lymphoblastic leukaemia - results of a double-blind, randomised controlled trial with cross-over design. *Eur J Cancer. 2023;191:112980.*

Verwaaijen EJ, van Hulst AM, Fiocco M, Hartman A, Grootenhuis MA, Pluijm SMF, Pieters R, van den Akker ELT, van den Heuvel-Eibrink MM. Dexamethasone-Induced Sarcopenia and Physical Frailty in Children With Acute Lymphoblastic Leukemia: Protocol for a Prospective Cohort Study. *JMIR Research Protocols, 2022 Apr 11;11(4):e33517.*

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Verwaaijen EJ, Corbijn DM, van Hulst AM, Neggers SJCMM, Boot AM, van den Heuvel-Eibrink MM, Hartman A, Pluijm SMF. Frailty in long-term Dutch adult survivors of childhood Acute Myeloid Leukemia, Neuroblastoma and Wilms Tumor. *JCSM, 2021 Jan;6(1):3-10.*

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P

PhD Portfolio

PHD PORTFOLIO

Name:	Annelienke Michelle van Hulst
PhD period:	June 2017 – March 2023
Research School:	Clinical and Translation Oncology (Utrecht University)
Department:	Pediatric Oncology (Princess Máxima Center for Pediatric Oncology)
Promotores:	Prof. dr. Marry M. van den Heuvel-Eibrink
	Prof. dr. Martha A. Grootenhuis
	Prof. dr. Erica L.T. van den Akker

PhD training	**Year**
Courses	
Behandeleffecten verbeteren via communicatie – IVM (online)	2022
SNP Course XVIII: SNPs and Human diseases – MolMed, Erasmus MC (online)	2020
Writing a Scientific Paper – GSLS, UU (online)	2020
The Art of Presenting Science – GSLS, UU (online)	2020
Practical Biostatistics – Amsterdam UMC (online)	2020
Castor EDC database study building and management – Castor (online)	2020
Basic Human Genetics Course: Genetics for Dummies – MolMed, Erasmus MC (online)	2018
Musculoskeletale Echografie Histologie/Fysiologie – Sonoskills, Rotterdam	2018
Introductory Course – CTO, UU	2018
Achieving your goals and performing more successfully in your PhD – GSLS, UU	2017
Research Data Management – RDM Support, UU	2017
Basiscursus Regelgeving en Organisatie voor Klinisch Onderzoekers (BROK)	2017

PhD training	**Year**
Seminars and Workshops	
Clinical and Translation Oncology PhD Retreat	2019, 2020, 2022
PhD Retreat Van den Heuvel-Eibrink Group	2019
Research Retreat Princess Máxima Center	2018, 2021
GSLS PhD Day	2018
Weekly PhD Meetings Van den Heuvel-Eibrink Group	2017 – 2023
Weekly PhD Meetings Grootenhuis Group	2018 – 2023
Conferences	
4th Congress of the International Society for Paediatric Oncology (SIOP) Europe Valencia, Spain. *Oral presentation*	2023
64th American Society of Hematology (ASH) Annual Meeting Online. *Poster presentation*	2022
54th Congress of SIOP Barcelona, Spain. *Poster discussion*	2022
63rd ASH Annual Meeting Online. *Attended*	2021
53rd Congress of SIOP Online. *Attended*	2021
52nd Congress of SIOP Online. *Poster discussion*	2020
51st Congress of SIOP Lyon, France. *Poster presentation*	2019
Teaching activities	
Supervising a master student (UU, Pharmacy)	2018


D

Dankwoord

DANKWOORD

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