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Incidence of adrenal insufficiency in paediatric, oncologic patients with fever during chemotherapy

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1 Introduction

1.1 Adrenal Insufficiency in children

Adrenal insufficiency (AI) can be classified as primary or central depending on the underlying aetiology. Primary adrenal insufficiency (PAI) results from disease intrinsic to the adrenal cortex. This results in insufficient production of steroid hormones, mineralocorticoids and adrenal hormones. [1] Impaired production of adrenocorticotropic hormone (ACTH) from the pituitary gland can lead to a hypofunction of the adrenal cortex and cause central adrenal insufficiency (CAI). [2]

1.1.1 Primary Adrenal Insufficiency (PAI)

PAI, also known as Addison's disease, is estimated to affect 90-140 per one million people and results from disease intrinsic to the adrenal cortex. [2-5] When Thomas Addison described this disease in 1855, bilateral adrenal destruction by tuberculosis was its most common cause. [6] The most causes of PAI in children have an inherited, monogenic origin and can be divided according to their pathogenesis into impaired steroidogenesis, adrenal hypoplasia, resistance to ACTH action and adrenal destruction. [7] A recent study in Canada evaluated the aetiologies of primary AI in their paediatric population over twenty years. 72% of the children were diagnosed with congenital adrenal hyperplasia, 13% had autoimmune AI, and the remaining 15% had either adrenoleukodystrophy, other syndromes or unexplained AI. [8] Clinical symptoms of children with PAI vary depending on the type and severity of hormonal class (glucocorticoids, mineralocorticoids, adrenal androgens) affected. In general, children affected present with nonspecific symptoms of fatigue, nausea and vomiting. Signs of glucocorticoid deficiency include weakness, failure to thrive, increased insulin sensitivity, fasting hypoglycaemia, and morning headache. Increased production of pro-opiomelanocortin (POMC) due to deficient cortisol secretion leads to hyperpigmentation of areas exposed to sunlight and is also salient in areas not typically exposed to sun (palmar creases, axillae, tongue, palate, gingival borders). It is one of the most remarkable sign of PAI in children and was the initial presenting sign in 94% of 95 children presenting with PAI in a Turkish cohort. [7] Hypotension, dizziness, muscle weakness, salt-craving, weight loss, dehydration and electrolyte abnormalities are symptoms caused by urinary sodium loss and would indicate mineralocorticoid deficiency. Androgen deficiency in both sexes presents with delayed adrenarche, lack of pubic/axillary hair and dry skin.

An adrenal crisis (AC) is an acute, life-threatening episode of adrenal insufficiency (AI) and can be triggered by fever, severe infection, surgery or trauma. It presents with signs of acute cardiovascular decompensation such as hypotension, tachycardia and hypovolemia as well as altered mental status. [8, 9]

1.1.2 Central adrenal insufficiency (CAI)

Central adrenal insufficiency (CAI) is most often caused by the abrupt discontinuation of glucocorticoid therapy and is therefore frequently observed in children treated for leukaemia or lymphoma. Genetic causes for CAI are rare and include mutations in genes that play a role in the differentiation of pituitary POMC cells. [10, 11] In patients with congenital malformations of the brain, CAI is usually associated with other pituitary hormone deficiencies. [12] It has an estimated prevalence of 150-280 per one million people. [12-15] The suppressing effect of glucocorticoids on the adrenal function can be enhanced by the use of CYP3A4 inhibitors or antifungal drugs that interfere with glucocorticoid synthesis. CAI can occur with all forms of steroid therapy, including local application. [16-18] There are no absolute values established that can predict adrenal suppression, however adrenal atrophy and subsequent CAI should be suspected in all patients who have undergone prolonged steroid therapy (> two weeks). Infants and children have similar symptoms compared to children with primary adrenal insufficiency, solely salt craving and hyperpigmentation are not observed.

1.1.3 Critical illness related corticosteroid insufficiency (CIRCI)

The HPA (Hypothalamic-pituitary-adrenal) axis plays a crucial role in metabolic and immunological pathways during severe stress by modulating serum glucose level, suppressing the inflammatory response and regulating the blood pressure. The prevalence of adrenal insufficiency in critical ill children varies from 4 to 77% depending on the population and criteria used to define it. [19-23] Critical illness related corticosteroid insufficiency (CIRCI) describes an inadequate corticosteroid activity for the severity of the disease that results in an strong proinflammatory response. [24] This can be the consequence of either a decrease in adrenal steroid production or a resistance in the tissue and target organs to glucocorticoids. CRH, ACTH and hitherto cortisol secretion is physiologically stimulated by stress, hypoglycaemia, hypotension, tissue damage, hypoxia or cytokine secretion. CRH then again stimulates the secretion of catecholamine as well as arginine vasopressin,

the latter leading to water retention and vasoconstriction. [25] The exact pathomechanism in CIRCI in children is not clear but pituitary ischemia or necrosis in sepsis could be causal for a decrease in pituitary hormone secretion. [26] Another explanation could be the competition of proinflammatory cytokines with ACTH at its receptors. [27] In a prospective study of 72 children with meningococcal sepsis admitted to ICU, the non-survivors had significantly lower cortisol and higher ACTH as well as interleukin-6 levels than the survivors. [28]

1.2 HPA (Hypothalamic-pituitary-adrenal) axis suppression during treatment for paediatric cancer

Adrenal insufficiency represents a possible life-threatening adverse effect of chemotherapy and its incidence is not well known in oncologic children. HPA axis suppression may result from glucocorticoid treatment in leukaemia and lymphoma patients but may also occur due to other chemotherapeutic regimens for solid tumours.

1.2.1 HPA axis suppression after treatment with glucocorticoid therapy for childhood acute lymphoblastic leukaemia (ALL)

Corticosteroids are an important element in the therapeutic regimen of childhood leukaemia and lymphoma since they induce apoptosis of lymphoblastic cells. [29] Depending on the diagnosis and treatment protocol, patients receive dexamethasone (DXM), methylprednisolone (MPN) or prednisolone (PDN) over a certain timeframe with or without tapering. High-dose glucocorticosteroid may suppress the secretion of corticotrophin-releasing hormone (CRH) and of adrenocorticotropic hormone (ACTH) by the hypothalamus or pituitary gland, respectively. The resulting atrophy of the adrenal cortex leads to an inadequate cortisol production. [30] Indeed, abrupt cessation of glucocorticoid therapy is the most common cause of secondary adrenal insufficiency. [2] The HPA axis plays an important role in the stress response and defence against infection. Cortisol has direct anti-inflammatory effects and inhibits cytokines that would enhance the inflammatory cascade. [31, 32] In turn, HPA axis suppression and hence reduced adrenal cortisol production, remains a cause of morbidity and mortality in children. [2]

Several observational studies and two randomized controlled (RCT) studies have evaluated the HPA axis of children with acute lymphoblastic leukaemia after

glucocorticoid treatment. [34-42] These studies are very heterogeneous but have demonstrated that nearly all children present with adrenal insufficiency (AI) during the first days after cessation of steroid treatment and most of them recover within seven weeks. However, some children have a persisting AI lasting up to 34 weeks. Investigators used either early morning plasma cortisol level or the (low dose) ACTH stimulation tests. The two RCT studies found no difference between PDN and DXM treatment with regard to occurrence and duration of AI. [36, 37] The observational study of Salem et al. revealed that children receiving PDN recovered earlier than those receiving DXM. [41] In four studies the glucocorticoid was tapered over nine days. However, due to the heterogeneity of these studies, it was not possible to draw a final conclusion about the effect of steroid tapering on the incidence adrenal insufficiency. [34, 37, 39, 40]

Two of the studies evaluated the influence of infections or stress on the presence of AI. The study of Kuperman et al. reported 35 episodes of infection (defined by hospitalization due to fever) and could not observe a correlation between the presence of infection and/or stress and the response to the low dose ACTH test. [36] Salem et al. demonstrated in his cohort study, that for both DXM and PDN groups, longer duration of AI was associated with an increased occurrence of infection. [41] The use of antifungal therapy is frequently necessary in children treated for ALL. Fluconazole therapy was evaluated as a risk factor for persistence of AI in two cohort studies, especially with doses above 10 mg/kg/d. [39, 41]

1.2.2 HPA axis suppression after treatment with chemotherapy/immunotherapy

We did not find any published data on HPA axis suppression regarding chemotherapy used in childhood cancer. We explicitly browsed current literature for adrenal insufficiency or HPA axis suppression related to vincristine, doxorubicin, methotrexate, asparaginase, etoposidphosphate, cisplatin, dacarbazine, actinomycin D, ifosfamide, cytarabine, mitoxantrone, amsacrine, fludarabine, cladribine. We therefore think it is important to assess the adrenal function in paediatric cancer patients that receive chemotherapy without corticosteroids.

Immune checkpoint inhibitors that enhance the effector T cell response to tumour cells have led to autoimmune related toxicities including endocrine, gastrointestinal and dermatologic toxicities. Hypophysitis, thyroiditis and adrenalitis have been described. [43] However, no patient in our study received the above-mentioned therapy.

Mitotane, a drug that is used to treat adrenocortical carcinoma, can cause adrenal necrosis and interfere with steroid metabolism. [44] We therefore excluded patients treated with mitotane from our study.

1.2.3 HPA axis suppression after radiotherapy

The effect of cranial irradiation on the HPA axis is well known. Many studies demonstrated negative effects on growth, pubertal development, thyroid function and adrenal function. [45-47] The incidence of growth hormone deficiency is estimated to be up to 80% after cranial irradiation with > 30 Gy. [48] In a Danish cohort of 73 children treated with cranial/craniospinal irradiation, 19% had adrenal insufficiency diagnosed by either standard dose short Synacthen test (SDSST) or insulin tolerance test (ITT). [49] In another cohort of 310 childhood cancer survivors, 18% had adrenal insufficiency and 95% of these patients had received cranial irradiation. [50] Hence, we excluded patients with a history of radiotherapy or a tumour involving the hypothalamus/pituitary gland from our study.

1.2.4 HPA axis suppression after adrenalectomy

We did not exclude patients after unilateral adrenalectomy for neuroblastoma or nephroblastoma treatment since it is known that the other adrenal gland is able to compensate the steroid synthesis. In a prospective study that evaluated the adrenal function of 103 survivors after neuroblastoma and nephroblastoma treatment, no adrenal insufficiency was observed. 39 patients in this cohort received unilateral adrenalectomy since the tumour was located in or close to the adrenal gland. [51]

1.3 Diagnosis of adrenal insufficiency in children

Primary and central AI both present with low morning serum cortisol concentrations. A morning serum cortisol level of 3 µg/dl is suggestive, a level ≥ 15 µg/dl eliminates it. [52] Primary AI is further indicated by an elevated plasma ACTH (>50 pg/ml) level, low levels would be associated with CAI. Relatively low aldosterone level despite high levels of renin as well as hyponatremia and/or hypokalaemia can confirm mineralocorticoid deficiency in PAI and are not expected in CAI. [1] If the results of morning cortisol and ACTH are not definitive, a dynamic stimulation test can be performed. In patients with PAI the cortisol level would fail to rise with stimulation.

1.3.1 Static test of HPA axis

Testing for serum or plasma cortisol concentration should be performed in the early morning. Cortisol is secreted according to a diurnal pattern, regulated by corticotropin (ACTH) released from the pituitary gland that is, in turn, regulated by corticotropin-releasing hormone (CRH) from the hypothalamus. Cortisol secretion of the adrenal gland reduces CRH release via a negative feedback loop. The normal cortisol peak is around 8 am, thus hypocortisolism is suspected if the cortisol level is abnormal. Cortisol levels taken at other times during the day are hard to asses since lower cortisol values could be normal during this time of the day. However, cortisol levels should rise in response to stress such as fever, hypoglycaemia, pain, trauma or anxiety and diurnal variation is usually lost. [53] Elevated levels of circulating cytokines stimulate the HPA axis leading to higher secretion of CRH and ACTH and a reduction in negative feedback from cortisol. [54] Thus, high cortisol level during stress makes adrenal insufficiency very unlikely. It is recommended to use plasma total cortisol rather than plasma free cortisol to diagnose adrenal insufficiency. [55] More than 90% of circulating cortisol is bound to corticosteroid-binding globulin (CBG) and albumin; 5 – 8% is free, unbound and therefore active cortisol. Moreover, in critical illness the circulation of acute phase proteins rises and albumin as well as CBG decreases thereby increasing the amount of free cortisol. It had been feared that the use of total cortisol level in hypoproteinaemia would lead to the underestimation of active cortisol. However, recent studies demonstrated that CBG and albumin level did not differ significantly between survivor and nonsurvivor in septic shock and, more importantly, that serum total cortisol levels were strongly correlated with free cortisol levels measured in blood or saliva in children with septic shock. [21, 56, 57] Taken together, serum total cortisol proves to be a reliable parameter in the evaluation of adrenal functions in paediatric patients. We decided to take random cortisol levels as a first step in this explorative study since the patients presented with fever and fever should be an adequate stimulator of the adrenal gland. We decided against additional dynamic tests with repeated blood draws to reduce the risk of infection and to prevent a delay in antibiotic therapy.

1.3.2 Dynamic test of HPA axis

Until recently, the gold-standard to confirm the suspicion of central AI by mimicking severe stress was the hypoglycaemia inducing insulin tolerance test (ITT). Therefore 0.1 U/kg of intravenous regular insulin was applied followed by measurement of

serum cortisol at baseline and 30, 60, 90 and 120 minutes later with an expected cortisol response ≥ 20 µg/dl (550 nmol/L). Due to the increased risk of hypoglycaemic seizure and severe hypokalaemia, many paediatric centres are no longer using this test. [58, 59]

For the evaluation of central disorders of the HPA axis, the corticotropin analog stimulation test is indicated. [60] Chronic ACTH deficiency causes secondary adrenal atrophy and thus a reduced cortisol response. Therefore, this test is able to detect long existing ACTH insufficiency, but does not identify moderate or recent ACTH deficiency that did not yet lead to adrenocortical atrophy. For the standard dose short Synacthen test (SDSST), 250 µg of Synacthen is applied and cortisol levels are measured at baseline, after 30 and 60 minutes after injection. A peak cortisol value < 18 µg/dl suggests CAI, a cortisol value > 30 µg/dl rules it out in children. [12, 52] The low dose corticotropin test (LDSST) has been developed due to false negative results of the standard dose test in patients with clinical signs of CAI. 1 µg of ACTH is able to elicit the same cortisol reaction 20 minutes after injection compared to the standard dose but is more sensitive to detect central AI and to monitor the adrenal function after cessation of oral steroids. (cortisol < 18 µg/dl suggests central AI, cortisol > 22 µg/dl rules out central AI) [52, 61]

1.3.3 Diagnosis of CIRCI (Critical illness related corticosteroid insufficiency)

The diagnosis criteria of CIRCI are not well defined. However, haemodynamic instability, vasopressor dependency and occurrence of hypoglycemia are typical signs of CIRCI. [53]

A multicenter study in Canada including 381 critical ill children used the LDSST with an increment of 9 µg/dl from baseline cortisol to diagnose adrenal insufficiency and found a prevalence of 30% in their cohort. Adrenal insufficiency based on these criteria was also associated with increased fluid and catecholamine requirements and, interestingly, higher baseline cortisol levels. Thus, in many children with AI the baseline cortisol was above 18 µg/dl, but their adrenal glands were not able to mount an adequate response to exogenous ACTH stimulation. [21] The 2017 published guidelines for the diagnosis and management of CIRCI in critical ill adult and paediatric patients suggested to use either an increment of 9 µg/dl after the SDSST or a random plasma cortisol of < 10 µg/dl. [55] Interestingly, the surviving Sepsis Campaign Guidelines published in 2016, advise against using the SDSST or random plasma cortisol level in septic shock in order to make the decision wether to treat a

patient with hydrocortisone or not. They recommend the hydrocortisone treatment only in a septic shock that is vasopressor and fluid resistant; stating that random cortisol levels are not helpful since they may over- or underestimate the actual cortisol level. [62, 63]

1.4 Therapy of adrenal insufficiency

1.4.1 Physiologic replacement doses of glucocorticoid

The treatment goal in primary adrenal and secondary insufficiency is the prevention of adrenal crisis, to achieve normal growth and to avoid excess glucocorticoid replacement. Androgen excess has to be reduced in patients with congenital adrenal hyperplasia.

Hydrocortisone (HC) is the therapy favoured in children, since prednisolone or dexamethasone might suppress growth. Since the replacement therapy has the aim to mimic the physiological circadian rhythm, a thrice daily regime with the highest HC dose in the morning and the last dose four to six hours before bedtime, should be pursued. [64] The dose ranges between 7 and 12 mg/m_2/day. [1] Patients have to be monitored closely regarding growth, weight gain and general well-being. Patients with secondary adrenal insufficiency might need lower doses.

Mineralocorticoid replacement with fludrocortisone is required in patients with primary adrenal insufficiency.

1.4.2 Stress dosing

Physiologic stress requires additional doses of glucocorticoids in patients with primary or central adrenal insufficiency in order to avoid complications of adrenal crisis. In illness, depending on the severity of infection, the usual HC dose has to be increased up to four times the normal replacement. [65] A common recommendation is a dose of 30 to 50 mg/m_2 HC per day. If children are unable to tolerate oral therapy in this situation, intramuscular injection is necessary. Major surgeries or sepsis often requires doses up to 100 mg/m_2 HC per day intravenously. [2]

1.4.3 Therapy in CIRCI (Critical illness related corticosteroid insufficiency)

The role of steroids in septic shock is still controversial since studies in critical ill patients generated inconsistent data. [55] Previous studies in adults with septic shock demonstrated no decrease in mortality even though hydrocortisone accelerated the reversal of shock. It has been recommended to consider HC treatment in patients

with septic shock that respond poorly to fluid resuscitation and vasopressor agents. [66] Clinical trials that examine the role of steroid therapy in critical ill children are scarce. However, according to the American college of critical care medicine, hydrocortisone should be given in children that are at risk for adrenal insufficiency and fail to respond to vasopressor therapy. Previous steroid exposure, congenital adrenal hyperplasia, purpura fulminans or hypothalamic/pituitary abnormalities are defined as risk factors in this context. [67]

2 Hypothesis

The primary aim of this prospective, explorative study was to evaluate the incidence of adrenal insufficiency in children treated with chemotherapy under stress (fever). We hypothesized that paediatric oncologic patients do not secrete enough cortisol to come up with the stress of an infection irrespective of steroids given as part of the treatment protocol. Adrenal insufficiency was hereby defined by a random cortisol level < 18 µg/dl (= 497 nmol/l). We further wanted to assess whether there is a difference in the occurrence rate between patients receiving glucocorticosteroid in their treatment and those who are steroid naive.

3 Materials and Methods

3.1 Study design

We prospectively examined the random cortisol, corticotropin (ACTH) and renin level of 75 children (age 1 months – 18 years) treated with chemotherapy and presenting with fever between August 2015 and April 2018 at the Dr. von Hauner' Children's Hospital in Munich. The patients were all treated with chemotherapy due to an oncologic disease and had no previously known history of adrenal- or hypopituitary insufficiency. Every patient was included only once in this study.

We decided to take random cortisol levels as a first step, since the patients were under stress. The blood had to be taken from a central venous line in immunosuppressed children under chemotherapy. We decided against additional dynamic tests with repeated blood draws in this study, in order to reduce the risk of infection and to prevent a delay in antibiotic therapy. Fever was defined as body temperature greater or equal 38.5°C once or greater 38.0°C twice in 24 hours, respectively. Renin levels were measured to get an estimation of the mineral corticosteroid function of the adrenal gland. Age below 1 month, previous cranial irradiation, preknown adrenal- or hypopituitary insufficiency, no central venous line and/or a missing informed consent led to the exclusion of the study.

Age at inclusion, gender, height, weight, diagnosis, time of initial diagnosis and treatment protocol was collected for all patients included in this study. We further collected data on the corticosteroid doses, derivative and time distance to last intake in the patient group who received steroids in their chemotherapy. Laboratory and clinical values of C-reactive protein (CrP) at submission to the hospital and the following two days, blood count, albumin, sodium, potassium, blood cultures, blood pressure, heart rate and antifungal prophylaxis were collected.

3.2 Ethics committee

Prior to conducting the study, a vote by the ethics committee of the Ludwig-Maximilans-University was made to assess ethical and legal issues. The commission gave its consent. All parents and or custodians as well as the patients were informed about the content, risks and benefits of the study at least 24 hours before the blood was taken. The patients were only included if they or their parent/custodian respectively consented to this study. Patients and parents/custodians each received a copy of the informed consent.

3.3 Sample size estimation

Since there was no published data on the incidence of adrenal insufficiency in paediatric oncologic patients with chemotherapy who present with fever, a reasonable sample size planning was not possible. This study should therefore be able to estimate its range. In order to allow expressing the validity of the forthcoming exploratory study, the confidence interval according to Clopper-Pearson was determined. The sample size should be set so that the worst-case confidence interval has a maximum width of 0.2. This most unfavourable case would arrive with an occurrence probability of 50%. Let X be the proportion of cases of adrenal insufficiency. Making sure that the confidence interval has a maximum width of 0.2 under all possible occurrence rates, n=104 patients seemed required:

X =	**95% confidence interval**	**(Clopper-Pearson)**
0	0.0000	0.0348
0.096 (=10/104)	0.0471	0.1697
0.202 (=21/104)	0.1296	0.2919
0.298 (31/104)	0.2123	0.3957
0.404 (=42/104)	0.3087	0.5046
0.5 (=52/104)	0.4003	0.5997

The duration of the study was initially estimated at two years. The inclusion of patients took longer than expected. Therefore, we decided to perform an early evaluation after 2 years and 9 months. At this time, 75 patients were included and the incidence of adrenal insufficiency was 77% [Mean 0.77, 95% CI based on single sample t test: 0.6764 – 0.8703] with a confidence interval < 0.2. Thus, our initial condition on the tolerated uncertainty for estimating the incidence of adrenal insufficiency was fulfilled. Accordingly, we reconsidered our recruiting strategy and decided to stop the trial early for benefit.

3.4 Sample handling/analysis

If the parents/custodians gave their informed consent to the study and the patients presented with fever as defined above, three blood samples à 1.2 ml (3.6 ml) were taken via central venous catheter (Hickman line/port) in the same procedure as the routine diagnostic by the respective doctor in charge. The blood samples were immediately taken to the central laboratory and handled as well as stored according to the standard procedure. Every sample received an ongoing study-sample number.

The samples were analysed in badges and the treating doctor did not receive the results.

3.4.1 Cortisol

Serum cortisol concentration was carried out from serum on the Roche cobas® e601, a electrochemiluminescence immunoassay. The standard values indicated a range of 6 – 20 µg/dl.

3.4.2 Adrenocorticotropic hormone (ACTH)

The determination of ACTH was carried out from EDTA plasma on the Roche Cobas® e601, an electrochemiluminescence immunoassay. The standard values indicated a range of 10 – 50 pg/ml.

3.4.3 Renin

Direct Renin was measured in EDTA plasma using DiaSorin, Liason® Analyzer. The standard values indicated a range of 1.7 – 23.9 ng/l.

3.5 Statistical Analysis

The program Microsoft Office Excel 2016 (Microsoft, Redmond, USA) was used for general recording and evaluation of the data. The statistical evaluation of the data was performed with Prism version 7.0 (GraphPad, La Jolla, Ca).

Since no normal distribution could be assumed for the data, continuous data were expressed in median and range and compared by the Wilcoxon-Mann-Whitney test or ANOVA one-way analysis. The relationship between two two-level categorical variables was tested with the Fisher's exact test. The relationship between categorical variables with more levels was tested with the Kruskal-Wallis test. In total, 20 tests for differences between subgroups of patients were applied. Bonferroni correction was applied to adjust for multiple testing. Consequently, p values $< 0.05/20 = 0.0025$ were considered statistically significant.

The following analyses were done:

Primary analyses:

I. distribution of adrenal insufficiency (cortisol < 18 µg/dl) for all patients *(Fig. 4a)*

Secondary analyses:

I. Incidence of adrenal insufficiency (cortisol < 18 µg/dl)
    a. between corticosteroid and naïve group (Fisher's exact test) *(Fig. 5a)*
    b. for corticosteroid group
        i. between different corticosteroid derivatives (5 groups) (Kruskal Wallis test) *(Fig. 6a)*
        ii. between distances to last steroid intake (3 groups) (Kruskal Wallis test) *(Fig. 7a)*
        iii. between distance of ≤14 days and > 14 days (Fisher's exact test) *(Fig. 8a)*
        iv. between distance of ≤7 days and > 7 days (Fisher's exact test) *(Fig. 8c)*
    c. between all patients and patients without posaconazole prophylaxis (Fisher's exact test) *(Fig. 10c)*
II. Cortisol level differences
    a. between corticosteroid and naïve group (Wilcoxon-Mann-Whitney test) *(Fig. 8b)*
    b. for corticosteroid group
        i. between different corticosteroid derivatives (5 groups) (ordinary one-way ANOVA analysis) *(Fig. 6b)*
        ii. between distances to last intake (3 groups) (ordinary one-way ANOVA analysis) *(Fig. 7b)*
        iii. between distance of ≤14 days and > 14 days (Wilcoxon-Mann-Whitney test) *(Fig. 8b)*
        iv. between distance of ≤7 days and > 7 days (Wilcoxon-Mann-Whitney test) *(Fig. 8d)*
III. Correlation analysis
    a. between cortisol level and distance to last steroid intake (spearman correlation) *(Fig. 7c)*

b. between ACTH level and distance to last steroid intake (spearman correlation) *(Fig. 12d)*

IV. Incidence of adrenal insufficiency (cortisol < 10 µg/dl) between corticosteroid and naïve group (Fisher's exact test) *(Fig.9a)*

V. Incidence of high ACTH level (>50 pg/ml)
   a. Between patients with cortisol <18 µg/dl and patients with cortisol ≥ 18 µg/dl in all patients (Fisher's exact test) *(Fig. 11a)*
   b. Between patients with cortisol <18 µg/dl and patients with cortisol ≥ 18 µg/dl in patients with cortisone (Fisher's exact test) *(Fig. 11b)*
   c. Between patients with cortisol <18 µg/dl and patients with cortisol ≥ 18 µg/dl in patients without cortisone (Fisher's exact test) *(Fig. 11c)*

VI. ACTH level differences
   a. between corticosteroid and naïve group (Wilcoxon-Mann-Whitney test) *(Fig. 12a)*
   b. between patients with cortisol <18 µg/dl and patients with cortisol ≥ 18 µg/dl in patients without cortisone (Wilcoxon-Mann-Whitney test) *(Fig. 12b)*
   c. between patients with cortisol <18 µg/dl and patients with cortisol ≥ 18 µg/dl in patients with cortisone (Wilcoxon-Mann-Whitney test) *(Fig. 12c)*

VII. Incidence of high Renin (>23.9 ng/l) between patients with cortisol <18 µg/dl and patients with cortisol ≥ 18 µg/dl in all patients (Fisher's exact test) *(Fig. 13a)*

VIII. Renin level differences between patients with cortisol <18 µg/dl and patients with cortisol ≥ 18 µg/dl in all patients (Wilcoxon-Mann-Whitney test) *(Fig. 13b)*

IX. Distribution of adrenal insufficiency for patients with posaconazole prophylaxis *(Fig. 10a)*

4 Results

4.1 Patient characteristics

75 patients were included in this study from August 2014 until April 2018. 47 of these patients received corticosteroids in their treatment protocol and 28 patients were cortisol naïve at time of inclusion. The diseases included in our study matched the usual distribution of childhood cancer with the exception of the low rate of brain tumours since cranial irradiation was an exclusion criteria. *(see Fig. 1)* [68]

The median age was four years [range 0.5 – 17 years, interquartile range 2 – 12.5 years] in the corticosteroid group and five years [range 1.0 – 17 years, interquartile range 2 – 10 years] in the corticosteroid-naïve group. 58.7% of the patients were male, 41.3% were female. There were no differences in baseline characteristics between patients receiving corticosteroids in their treatment protocol and those who were not. *(see Tab. 1)*

We did not assess HPA axis function before glucocorticoid therapy. We analysed albumin levels of the included patients since hypalbuminaemia could increase the amount of circulating, free cortisol. Only two of the 75 patients had an albumin level < 2.5 g/dl (2.6%) at the time of blood examination.

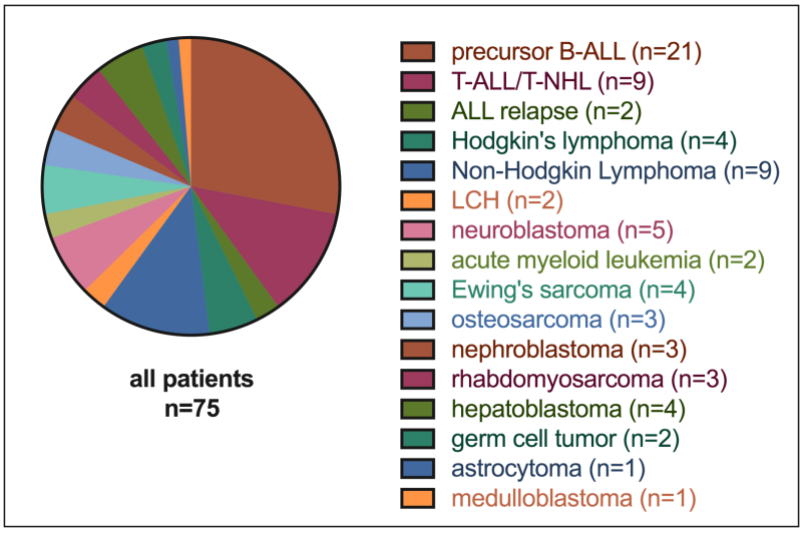

Figure 1: Diseases of all patients included in this study (n=75)

Characteristic	Treatment with corticosteroids n = 47	Treatment without corticosteroids n=28	Odds Ratio (95% CI) With Reference = Treatment without corticosteroids
Age – yr			
Median	4	5	
Interquatile range	2 – 12.50	2 – 10	
Range	0.5 – 17	1 – 17	
Gender			
Male sex – no. (%)	29 (61.7)	15 (53.6)	1.40 (0.45-2.98)
Female sex – no. (%)	18 (38.3)	13 (46.4)	
Clinical Presentation			
Infect Focus – no. (%)	10 (21.3)	13 (46.4)	0.31 (0.22-1.67)
Arterial Hypotension – no. (%)	2 (4.3)	2 (7.1)	0.58 (0.10-5.93)
Laboratory Diagnostic			
Hyponatremia/Hyperkalaemia	8 (17.0)	3 (10.7)	1.71 (0.31-5.22)
Neutropenia (<500 cells/µl) – no. (%)	30 (63.8)	20 (71.4)	0.71 (0.31-2.37)
Negative CrP at presentation (≤ 0.5 mg/dl) – no. (%)	18 (38.3)	6 (21.4)	2.28 (0.49 – 4.20)
CrP at presentation (> 0.5 mg/dl) – no. (%)	29 (61.7)	22 (78.6)	0.44 (0.24-2.05)
Germ proof in blood culture – no. (%)	1 (2.1)	1 (3.6)	0.59 (0.05-13.21)
Albumin level < 2.5 g/dl	2 (4.3)	0 (0)	

Table 1: Characteristics of patients included in this study (n = 75)

4.1.1 Corticosteroid group

32 (68.1%) of the 47 patients in the corticosteroid group were treated due to a precursor B- or T- cell acute lymphoblastic leukaemia (ALL), four (8.5%) due to Hodgkin lymphoma, nine (19.1%) patients had a Non-Hodgkin lymphoma (mature B cell-, anaplastic large cell- or T-lymphoblastic lymphoma) and two patients (4.3%) were treated due a systemic Langerhans cell histiocytosis (LCH). *(see Tab. 2., Fig. 2)* The respective treatment protocol, protocol phase and corticosteroid derivative is described in table 3.

Ten (21.3%) patients in the corticosteroid group had an infectious focus, only two patients presented with arterial hypotension. 30 (63.8%) patients were in cell aplasia after chemotherapy with absolute neutrophil counts (ANC) below 500/µl. Only one

patient had a germ proof in the blood culture taken at admission. Regarding inflammatory values, 29 (61.7%) patients presented with an elevated C-reactive protein (CrP) [median 1,3 mg/dl, range 0,52 – 20,5] at admission. *(see Tab.1)*

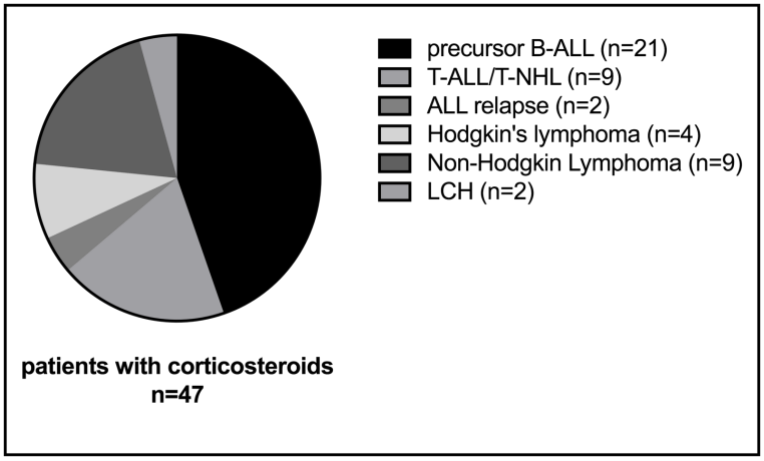

Figure 2: Diseases of patients included in this study with corticosteroids in their treatment protocol

Characteristic of patients with corticosteroid intake (n=47) **Disease – No. (%)**	
Acute lymphoblastic leukaemia (ALL)	
Precursor B-ALL	21 (44.7)
T-ALL/T-NHL	9 (19.1)
ALL relapse	2 (4.3)
Hodgkin's lymphoma	4 (8.5)
Mature B cell lymphoma	6 (12.8)
Anaplastic large cell lymphoma	2 (4.3)
T-lymphoblastic lymphoma	1 (2.1)
Langerhans cell histiocytosis (LCH)	2 (4.3)

Table 2: Diseases of patients included in this study with corticosteroids in their treatment protocol (n=47)

Disease	age	study	Protocol phase	Dose + duration	Tapering	Patients treated
precursor B-ALL + T-ALL	≥ 1 – ≤ 18 years	COALL 08-09	Prophase and Induction	MPN 60 mg/m$_2$ orally for 28d	No	n=22
			Reinduction	DXM 10 mg/m$_2$ orally for 14d (1/2 cycles depending on risk group)	No	n=5
			HR1	DXM 20 mg/m$_2$ orally for 5d	No	n=1
Precursor B-ALL + T-ALL	≥ 1 – ≤ 18 years	AEIOP BFM ALL	Prephase, IA, IA', AI-CPM	PDN 60 mg/m$_2$ orally for 21d	Yes	n=1
			IA-Dexa, IIA, II Asp+	DXM 10 mg/m2 orally for 21d	Yes	
			HR-1', HR-2', HR3'	DXM 20 mg/m$_2$ orally for 5d	No	
			III	DXM 10 mg/m$_2$ orally for 14d	Yes	
Precursor-B-ALL	≤ 1 year	ALL-Interfant	Induction	PDN 60 mg/m$_2$ orally d1-d7, DXM 6 mg/m$_2$ d8-28	Yes	n=1
			OCTADAD	DXM 6 mg/m$_2$ orally for 15 days	Yes	
ALL relapse	≤ 18 years	ALL-REZ-BFM	Prephase	DXM 6 mg/m2 orally for 5d	No	
			F1/F2/R1/R2	DXM 20 mg/m2 orally for 5d	No	n=2
			II-IDA	DXM 6mg/m2 orally for 14d	Yes	
Mature B-NHL/B-AL	< 18 years	NHL-BFM-Registry 2012	Prephase	DXM 5 mg/m$_2$ d1+d2, 10 mg/m$_2$ d3-d5	No	
			A_4, AA_{24}, B_4, BB_{24}	DXM 10mg/m$_2$ orally for 5	No	n=3
			AAZ1, AAZ2, BBZ1, BBZ2	DXM 20 mg/m$_2$ orally for 6 days	No	n=3
Lymphoblastic Lymphoma	< 18 years	NHL-BFM Registry 2012	Prephase/Induction	PDN 60 mg/m$_2$ orally for 7+28 days	Yes	
			Reinduction Protocol II	DXM 10 mg/m$_2$ orally for 21 days	Yes	n=1
ALCL	< 18 years	NHL-BFM Registry 2012	Prephase	DXM 5 mg/m$_2$ d1+d2, 10 mg/m$_2$ d3-d5	No	
			AM, BM	DXM 10mg/m2 orally for 5	No	n=2
Hodgkin's lymphoma	< 18 years	Euronet-PHL	OEPA	PDN 60 mg/m$_2$ orally for 15 days	No	n=1
			COPDAC, DECOPDAC	PDN 40 mg/m$_2$ orally for 15 days	No	n=3

LCH	< 18 years	LCH-III	Initial treatment course 1,2	PDN 40 mg/m$_2$ orally for 28 days	Yes	n=1
			Continuation treatment	PDN 40 mg/m$_2$ orally for 5 days	No	n=1

Table 3: Overview of leukemia, lymphoma and LCH study protocols

4.1.2 Corticosteroid naive group

This group consisted of 28 patients treated for various solid tumours (neuroblastoma, Ewing's sarcoma, osteosarcoma, nephroblastoma, rhabdomyosarcoma, hepatoblastoma, germ cell tumour, astrocytoma and medulloblastoma) and acute myeloid leukaemia. (s*ee Tab. 4, Fig. 3)* We included only medulloblastoma and astrocytoma patients that did not have cranial irradiation yet, since cranial irradiation was an exclusion criteria of this study. All neuroblastoma patients had a unilateral adrenalectomy in the course of their therapy but only two of them had the tumour resection before the blood for the study was taken. The clinical presentation and laboratory signs in this group were quite similar to the corticosteroid group. 13 (46.4%) patients presented with an infectious focus and two (7.1%) patients presented with arterial hypotension at admission. Comparable to the corticosteroid group, 20 (71.4%) patients had ANC counts below 500/µl and 22 (78.6%) an elevated CRP value at the first day [median 2.43 mg/dl, range 0,79 – 9,9].

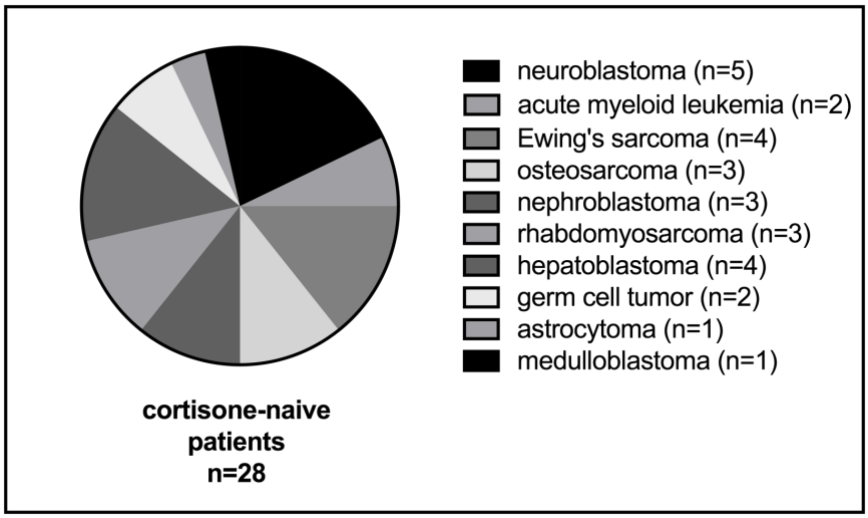

Figure 3: Diseases of patients included in this study without corticosteroids in their treatment protocol

Characteristic of patients without corticosteroid intake (n=28) Disease – No. (%)	
Neuroblastoma	5 (18)
Acute myeloid leukaemia	2 (7)
Ewing's sarcoma	4 (14)
Osteosarcoma	3 (11)
Nephroblastoma	3 (11)
Rhabdomyosarcoma	3 (11)
Hepatoblastoma	4 (14)
Germ cell tumour	2 (7)
Astrocytoma	1 (3.5)
Medulloblastoma	1 (3.5)

Table 4: Diseases of patients included in this study without corticosteroids in their treatment protocol (n=28)

4.2 Incidence of adrenal insufficiency

This prospective, single-centre study evaluated the incidence of adrenal insufficiency in 75 children treated chemotherapy and admitted with fever in our paediatric oncologic centre. Every patient included met the inclusion criteria. Of the 75 paediatric oncologic patients treated with chemotherapy and admitted with fever (as defined above), 58 patients [77.3%; 95% CI, 67.6 – 87.0%] had a random cortisol level < 18 µg/dl which had been defined as adrenal insufficiency in our study [median 9.7 µg/dl, range 0.2 – 39.6]. *(see Fig. 4)*

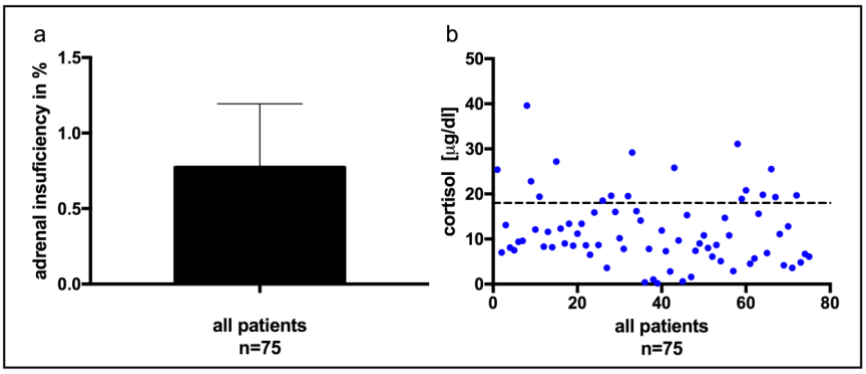

Figure 4:
a) Incidence of adrenal insufficiency (defined by cortisol < 18 µg/dl) in paediatric, oncologic patients with fever [n=75, mean=0.773, SD=0.42, 95% CI 0.68 – 0.87]
b) Absolute values of cortisol in all patients (n=75), dotted line at 18 µg/dl. [median= 9.7 µg/dl, range 0.2 – 39.6]

4.2.1 Incidence of adrenal insufficiency in children treated without corticosteroids

28 (37.3%) patients in our cohort were treated for solid tumours or acute myeloid leukaemia and did not receive corticosteroids in their respective treatment protocol. We observed that 21 patients [75.0%; 95% CI, 57.9 – 92.1%] had cortisol values below 18 µg/dl under stress. The median cortisol level was 11.4 µg/dl [range 3.6 – 39.6].

There was no significant difference between the incidence of adrenal insufficiency (defined by cortisol < 18 µg/dl) in children without and with corticosteroids. [p=0.77, Fisher's exact test] Again, when comparing the absolute cortisol values between children receiving corticosteroids in their treatment and the steroid naïve patients, there was no significant difference. [p=0.1145, Wilcoxon-Mann-Whitney test]

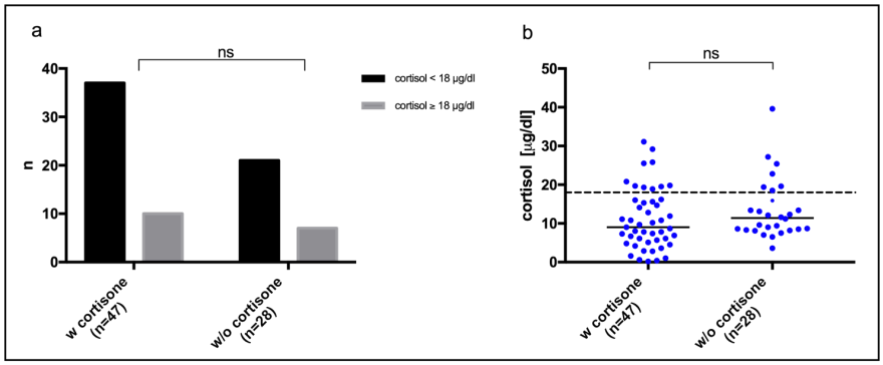

Figure 5:
a) No significant difference in incidence of adrenal insufficiency between patients with corticosteroids [78.7% (n=37) < 18 µg/dl; 21.3% (n=10) ≥ 18 µg/dl] and patients without corticosteroids [75.0% (n=21) < 18 µg/dl; 25.0% (n=7) ≥ 18 µg/dl] in their treatment based on Fisher's exact test [p=0.7789]
b) Absolute values of cortisol, dotted line at 18 µg/dl; no signficant difference between patients with corticosteroids [median = 9 µg/dl, range 0.2 – 31.1] and patients without corticosteroids [median = 11.4 µg/dl, range 3.6 – 39.6] in their treatment based on Wilcoxon-Mann-Whitney test [p=0.1145, U=513.5]

4.2.2 Incidence of adrenal insufficiency in children treated with corticosteroids

47 (62.6%) patients in our cohort received corticosteroids in their treatment protocol. These children were treated due to ALL, Hodgkin's lymphoma, Non-Hodgkin Lymphoma or systemic Langerhans cell histiocytosis (LCH) and either received dexamethasone, methylprednisolone or prednisolone in their protocol. Seven children were treated with methylprednisolone as well as dexamethasone. *(see Tab. 3*)

37 [78.7%; 95% CI, 66,6 – 90.9%] of 47 patients showed an inadequate cortisol response under stress as defined by a cortisol < 18 µg/dl. The median cortisol level was 9 µg/dl [range 0.2 – 31.1].

We further analysed the incidence of an impaired stress response with respect to the different corticosteroid derivatives taken. All patients who had taken dexamethasone (n=11), compared to 85.7% after prednisolone (n=7), 85.7% after methylprednisolone as well as dexamethasone (n=7) and 63.6% after methylprednisolone (n=22) intake had random cortisol levels < 18 µg/dl. *(see Fig. 6)* The Kruskal-Wallis test was conducted to examine the difference in the incidence of adrenal insufficiency according to the different corticosteroid derivatives taken. No significant differences (chi-square=6.236, p=0.1007) were found among the four therapies. *(see Fig. 6)*

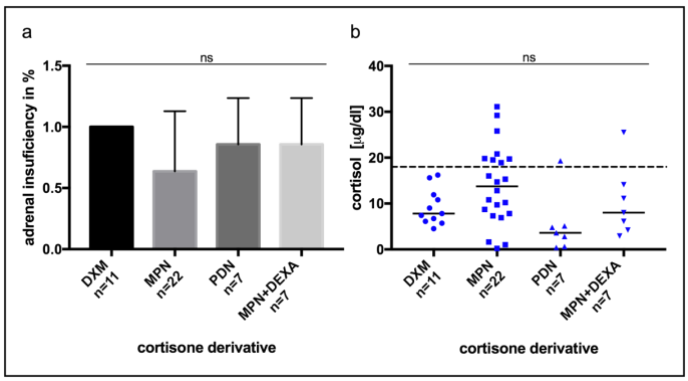

Figure 6:
a) Incidence of adrenal insufficiency (defined by cortisol < 18 µg/dl) varies between the corticosteroid derivatives [dexamethasone 100%, methylprednisolone 63.6%, prednisolone 85.7%, methylprednislone and dexamethasone 85.7%], Kruskal-Wallis test shows no significant difference between the four groups [p=0.1007, chi-square = 6.236]
b) Absolute values of cortisol, dotted line at 18 µg/dl. Dexamethasone: median = 7.8 µg/dl, range 4.5 – 16.2; methylprednisolone: median = 13.75 µg/dl, range 0.2 – 31.1; prednisolone: median = 3.6 µg/dl, range 0.4 – 19.3; methylprednisolone and dexamethasone: median = 8 µg/dl, range 2.9 – 25.5 µg/dl. Ordinary one-way ANOVA analysis shows no significant difference between the absolute cortisol values of the different corticosteroid derivatives [p=0.0465, F(3,43)=2.885; adjusted level of significance p=0.0025 after Bonferroni correction, see chapter 3.5]

We also analysed the cortisol level with regard to the distance to the last cortisone intake and divided the results into three groups: distance of less than or equal 7 days, 8 – 14 days and more than 14 days to the last corticosteroid intake. ANOVA one way analysis showed a highly significant difference of the absolute cortisol values between the three groups [p=0.0005, F(2,44)=1.667]. Further multiple comparison analysis revealed a significant difference between cortisol values that were taken less than or equal to seven days after the last corticosteroid intake and cortisol values taken after a time gap of at least 14 days [p=0.0006, 95% CI based on tukey's multiple comparisons test -14.93 – -3.734]. In a correlation analysis, the distance from the last cortisone intake correlated positively with the cortisol value. [r=0.6165, p=<0.0001, 95% CI based on spearman correlation test 0.3928 – 0.7713]

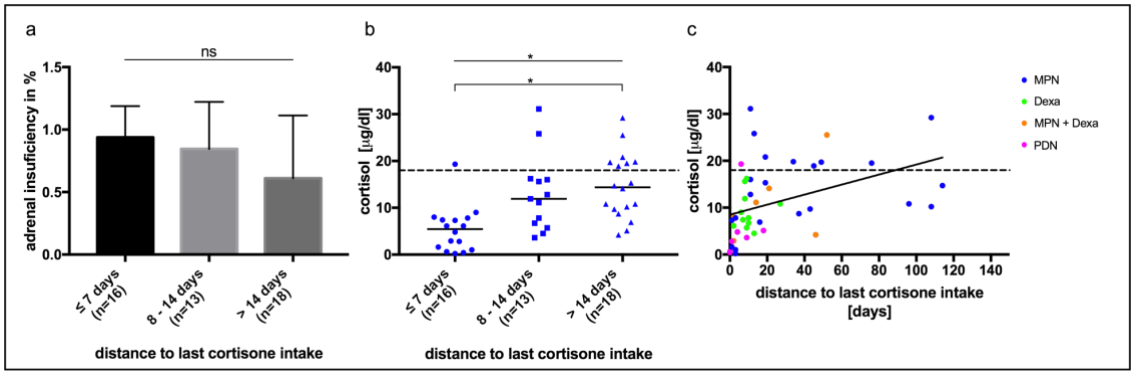

Figure 7: Incidence of adrenal insufficiency (defined by cortisol < 18 µg/dl) with regard to the distance to the last cortisone intake.
a) Incidence decreases with more distance to the last cortisone intake [≤ 7 days 93.8%, 8-14 days 84.6%, >14 days 61.1%] Kruskal-Wallis test shows no signficant difference between the three groups [p=0.0597, chi-square = 5.637]
b) Absolute values of cortisol, dotted line at 18 µg/dl; ≤ 7 days: median = 5.45 µg/dl, range 0.2 – 19.3; 8 – 14 days: median = 11.9 µg/dl, range 3.6 – 31.3; > 14 days: median = 14.4 µg/dl, range 4.2 – 29.2. Difference between the three groups is significantly different [ordinary one-way ANOVA analysis, p=0.0005, F(2,44) = 1.677], multiple comparisons test shows signficant difference between difference between "≤ 7 days" and "> 14 days" [p=0.0006, 95% CI based on tukey's multiple comparisons test -14.93 - -3.734]
c) absolute cortisol values plotted against the distance from the last cortisone intake and marked according to the corticosteroid derivative, blue = methylprednisolone, green = dexamethasone, orange = methylprednisolone + dexamethasone, pink = prednisolone. Values correlate signficantly. [spearman correlation, r = 0.6165, p= <0.0001, 95% CI = 0.3928 – 0.7713]

In a second step, we analysed the results once with a cut-off of seven days and once with a cut-off of 14 days distance to the last cortisone intake. With a cut-off of 14 days, we found no significant difference, neither in terms of incidence, nor in terms of absolute values. Compared to these results, the cut-off of seven days revealed no significant difference regarding the incidence, but a significant difference between the absolute values of cortisol between patients with less than or equal seven days or more than seven days distance to the last cortisone intake. [p=<0.0001, U=74.5] *(see Fig. 8)*

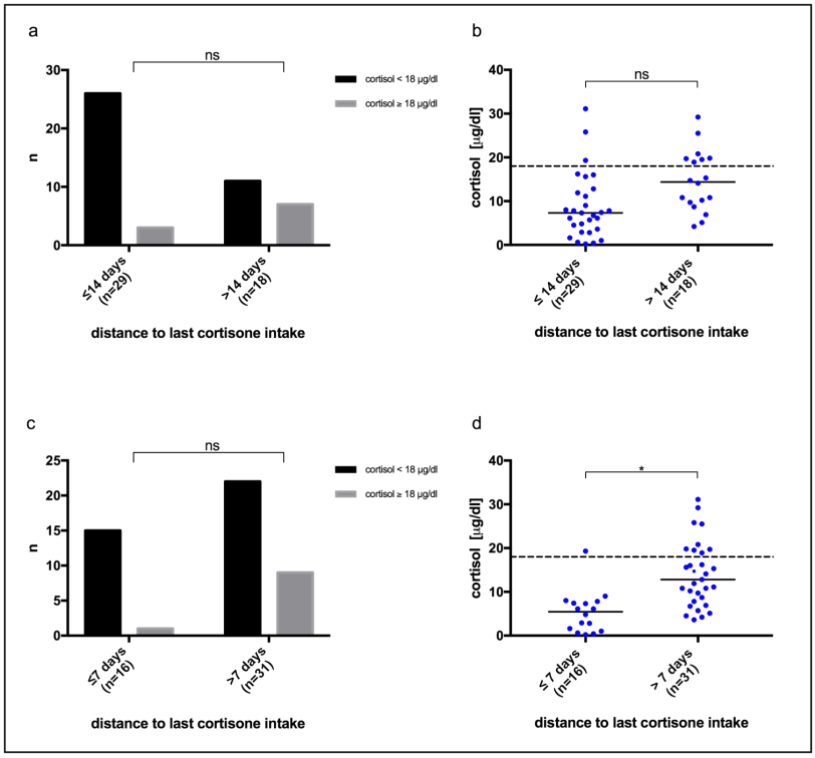

Figure 8: Incidence of adrenal insufficiency (defined by cortisol < 18 µg/dl) with regard to the distance to the last cortisone intake
a) No significant difference in incidence of adrenal insufficiency between distance ≤ 14 days [89.7% < 18 µg/dl; 10.3% ≥ 18 µg/dl] versus >14 days [61.1% < 18 µg/dl; 38.9% ≥ 18 µg/dl] by Fisher's exact test [p=0.0300; adjusted level of significance p=0.0025 after Bonferroni correction, see chapter 3.5]
b) Absolute values of cortisol, dotted line at 18 µg/dl; no signficant difference between distance ≤ 14 days [median=7.3 µg/dl, range 0.2 – 31.1] versus >14 days by Wilcoxon-Mann-Whitney test [median=14.4 µg/dl, range 4.2 – 29.2] [p=0.0044, U=133; adjusted level of significance p=0.0025 after Bonferroni correction, see chapter 3.5]
c) No significant difference in incidence of adrenal insufficiency between distance ≤ 7 days [93.8% < 18 µg/dl; 6.2% ≥ 18 µg/dl] versus >7days [71% < 18 µg/dl; 29% ≥ 18 µg/dl] by Fisher's exact test [p=0.1307]
d) Absolute values of cortisol, dotted line at 18 µg/dl; signficant difference between distance ≤ 7 days [median=5.45 µg/dl, range 0.2 – 19.3] versus >7 days by Wilcoxon-Mann-Whitney test [median=12.8 µg/dl, range=3.6 – 31.1] [p=<0.0001, U=74.5]

Finally, we analysed the results with respect to the corticosteroid derivative as well as the distance to the last cortisone intake. Looking at the dexamethasone group, it became noteworthy that only three of the eleven patients had a distance of less than eight days to the last cortisone intake despite the high incidence of adrenal insufficiency in this group. The methylprednisolone group had the lowest rate of adrenal insufficiency compared to the other groups. This might be linked to the fact that 13 patients of the 22 patients had a distance of at least 14 days to the last cortisone intake. The high incidence of adrenal insufficiency in the prednisolone group might be associated with the high rate of patients that had less than 8 days distance to the last corticosteroid intake. Since only four of 47 patients in our cohort

tapered the corticosteroid according to their protocol, we were not able to analyse this as risk factor.

Corticosteroid derivative	n (%) adrenal insufficiency [cortisol < 18 µg/dl]	n (% of AI) distance < 8 days	n (% of AI) distance 8 – 14 days	n (% of AI) distance > 14 days
Dexamethasone (n=11)	11 of 11 (100)	3 of 3 (100)	7 of 7 (100)	1 of 1 (100)
Methylprednisolone (n=22)	14 of 22 (63.3)	5 of 5 (100)	2 of 4 (50)	7 of 13 (53.8)
Prednisolone (n=7)	6 of 7 (85.7)	4 of 5 (80)	1 of 1 (100)	1 of 1 (100)
Methylprednisolone + Dexamethasone (n=6)	6 of 7 (85.7)	3 of 3 (100)	1 of 1 (100)	2 of 3 (66.7%)

Table 5: Incidence of adrenal insufficiency (defined by cortisol < 18 µg/dl) with regard to distance to the last corticosteroid intake.

4.2.3 Incidence of adrenal insufficiency with a cortisol set point of 10 µg/dl

As stated above, the current CIRCI guidelines recommend a random cortisol below 10 µg/dl as cut-off for adrenal insufficiency in critically ill children. Knowing that our patient population differs, we also analysed our patient cohort with the reduced cortisol set point.

Under application of this limit, 38 patients [50.7%; 95% CI, 39,1 – 62.3%] had an adrenal insufficiency in the entire cohort. Regarding our two patient groups, 25 [53.2%; 95% CI, 38.4 – 68%] of 47 patients with corticosteroids in their therapy and 13 [46.4%; 95 CI, 26.7 – 66.1%] of 28 without, had a random cortisol < 10 µg/dl. Again, there was no significant difference regarding the incidence of adrenal insufficiency between the two groups. [p=0.6371, Fisher's exact test]

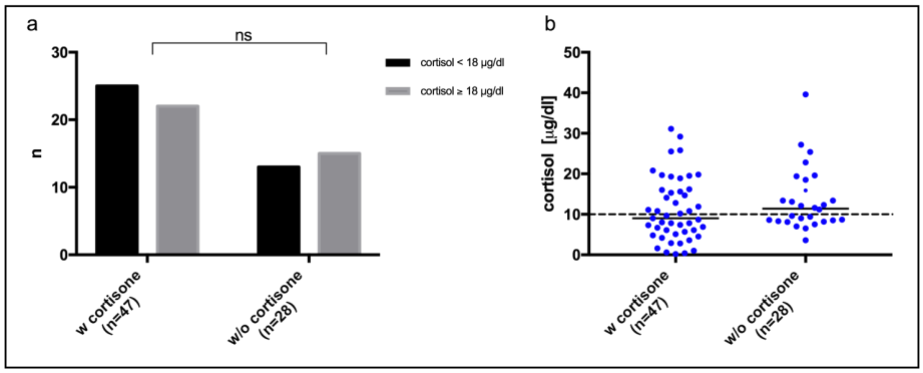

Figure 9:
a) No significant difference in incidence of adrenal insufficiency between patients with corticosteroids [53.2% < 18 µg/dl; 46.8% ≥ 18 µg/dl] and patients without corticosteroids [46.4% < 18 µg/dl; 53.6% ≥ 18 µg/dl] in their treatment by Fisher's exact test. [p=0.6371]

b) Absolute values of cortisol, line at 10 µg/dl. Patients with corticosteroids [median = 9 µg/dl, range 0.2 – 31.1] and patients without corticosteroids [median = 11.4 µg/dl, range 3.6 – 39.6] in their treatment.

4.2.4 Incidence of adrenal insufficiency under antifungal prophylaxis with posaconazole

Seven (9.3%) of 75 children in our cohort took posaconazole antifungal prophylaxis during their therapy due to long neutropenic phases. Three of them had corticosteroids in their treatment protocol, four of them had not. As described before, the influence of fluconazole on the adrenal gland is known, especially with doses above 10 mg/kg/day. However, there is no data on posaconazole and adrenal insufficiency in children. In our cohort, all seven patients had cortisol values < 18 µg/dl (100%). After exclusion of these patients from our cohort, the incidence of adrenal insufficiency was only slightly decreased (75% versus 77.3%). Therefore, we did not exclude these patients from our study.

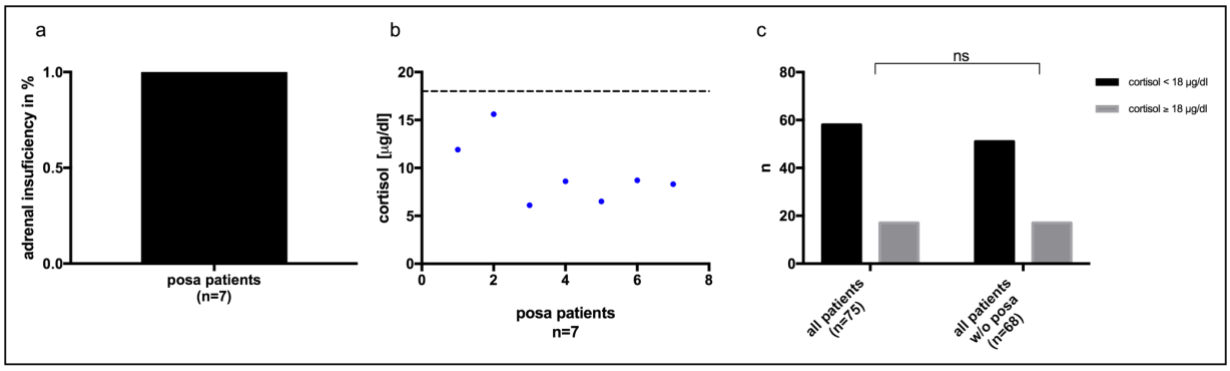

Figure 10:
a) Incidence of adrenal insufficiency (defined by cortisol < 18 µg/dl) in all patients with posaconazole prophylaxis is 100%.
b) Absolute values of cortisol in all posaconazole patients (n=7), dotted line at 18 µg/dl. [median = 8.6 µg/dl, range 6.1 – 15.6]
c) No significant difference in incidence of adrenal insufficiency between all patients [77.3% < 18 µg/dl; 22.7% ≥ 18 µg/dl] and patients with posaconazole prophylaxis [75% < 18 µg/dl; 25% ≥ 18 µg/dl] in their treatment based on Fisher's exact test [p=0.8446]

4.3 Pituitary stimulation of the adrenal gland by ACTH

We analysed ACTH level in every patient included in this study to evaluate the pituitary stimulation of the adrenal gland. Overall, only two of 58 patients had high ACTH level [> 50 pg/ml] in response to stress despite a cortisol < 18 µg/dl under stress. *(see Tab. 6)* Compared with this, five of 17 patients with cortisol levels ≥ 18 µg/dl had high ACTH levels. We observed a similar behaviour of the values in our subgroups with and without corticosteroids in their treatment protocol. (see Fig. 11) Six patients had an adequate cortisol response under stress despite low ACTH levels.

	Cortisol	
ACTH	**< 18 µg/dl (n=58)**	**≥18 µg/dl (n=17)**
Low [< 10 pg/ml]	25	6
Normal [10 – 50 pg/ml]	31	6
High [>50 pg/ml]	2	5

Table 6: Cortisol and ACTH levels of all patients (n=75)

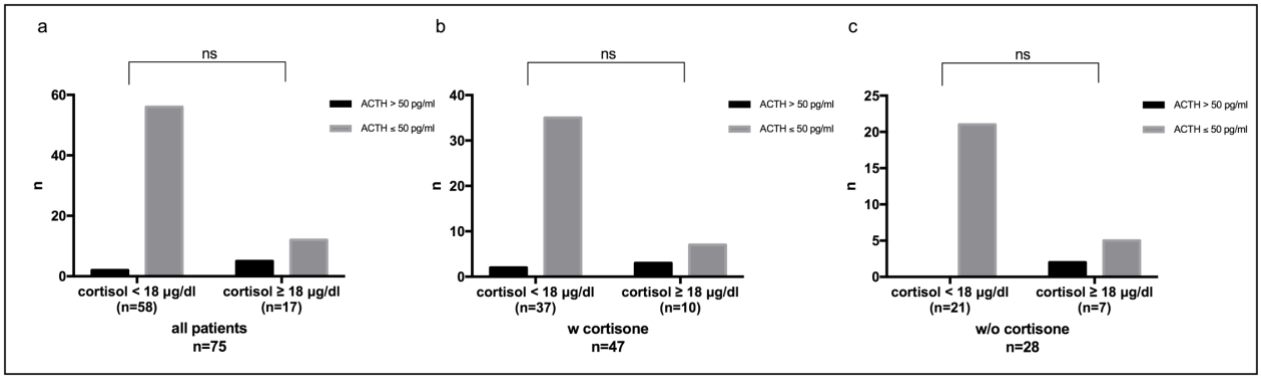

Figure 11: Incidence of a high ACTH level [>50 pg/ml]
a) All patients: Incidence is not significant different between patients with cortisol < 18 µg/dl [3.5% ACTH >50 pg/ml, 94.5% ACTH ≤ 50 pg/ml] and patients with cortisol ≥ 18 µg/dl [29.4% ACTH >50 pg/ml, 70.6% ACTH ≤ 50 pg/ml] in response to stress based on Fisher's exact test [p=0.0055]
b) Patients with cortisone in their treatment protocol: Incidence is not significant different between patients with cortisol < 18 µg/dl [5.4% ACTH >50 pg/ml, 94.6% ACTH ≤ 50 pg/ml] and patients with cortisol ≥ 18 µg/dl [30% ACTH >50 pg/ml, 70% ACTH ≤ 50 pg/ml] based on Fisher's exact test [p=0.0573]
c) Patients without cortisone in their treatment protocol: Incidence is not significant different between patients with cortisol < 18 µg/dl [0% ACTH >50 pg/ml, 100% ACTH ≤ 50 pg/ml] and patients with cortisol ≥ 18 µg/dl [28.6% ACTH >50 pg/ml, 71.4% ACTH ≤ 50 pg/ml] based on Fisher's exact test [p=0.0556]

Regarding the patients with cortisone in their treatment protocol who had cortisol level < 18 µg/dl, 35 of 37 patients had low or normal ACTH values and only two patients had high ACTH level [median = 10 pg/ml, range 3.4 – 601]. *(see Tab.7)* In the patient cohort without cortisone in their treatment, 7 of 21 patients with cortisol < 18 µg/dl had low ACTH level, 14 had normal ACTH level and no patient had high ACTH level [median = 14.7 pg/ml, range 2.7 – 32.7].

Adrenal insufficiency defined by	n corticosteroid group (n=47)	n naïve group (n=28)
Random cortisol < 18 µg/dl	37	21
Random cortisol < 18 µg/dl and low ACTH [<10 g/dl]	18	7
Random cortisol < 18 µg/dl and normal ACTH [10-50 pg/ml]	17	14
Random cortisol < 18 µg/dl and high ACTH [> 50 g/dl]	2	0

Table 7: Cortisol and ACTH values in patients with corticosteroids in their therapy (n=47) and corticosteroid naïve patients (n=28)

We found no significant difference regarding the absolute values of ACTH between patients with corticosteroids in their treatment protocol and those without. [p=0.0830, U=499.5, Wilcoxon-Mann-Whitney test]. Similarly, we observed no significant difference between patients with cortisol <18 µg/dl and patients with cortisol ≥ 18 µg/dl in both patient cohorts, respectively. The ACTH values did not correlate with the distance from the last cortisone intake. [r = -0.0452, p= 0.7629, based on spearman correlation test] *(see Fig.12)*

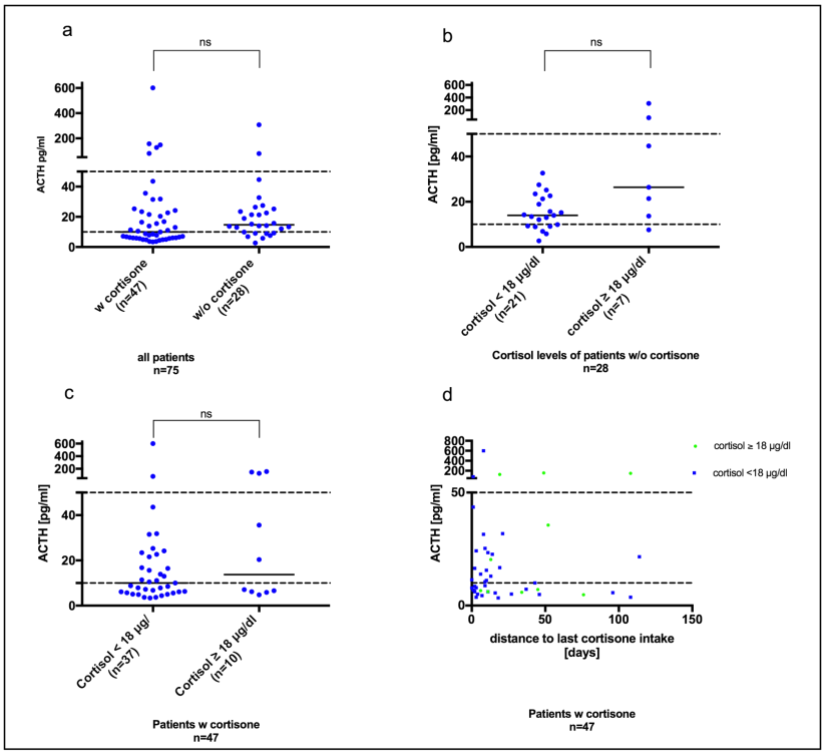

Figure 12: Absolute values of ACTH [pg/ml], dotted line at 10 and 50 pg/ml
a) No significant difference between absolute values of ACTH [pg/ml] in patients with [median = 10 pg/ml, range 3.4 – 601] and without corticosteroids [median = 14.7 pg/ml, range 32.7 – 308] in their treatment protocol based on Wilcoxon-Mann-Whitney test. [p=0.0830, U=499.5]
b) Absolute values of ACTH [pg/ml] in patients without corticosteroids in their treatment protocol: No significant difference between patients with cortisol < 18 µg/dl [median = 14 pg/ml, range 2.7 – 32.7] and cortisol ≥ 18 µg/dl [median = 26.4 pg/ml, range 7.6 – 308] based on Wilcoxon-Mann-Whitney

test. [p=0.0497, U=36, adjusted level of significance p=0.0025 after Bonferroni correction, see chapter 3.5]
c) Absolute values of ACTH [pg/ml] in patients with corticosteroids in their treatment protocol. No significant difference between patients with cortisol < 18 µg/dl [median = 10 pg/ml, range 3.4 – 601] and patients with cortisol cortisol ≥ 18 µg/dl [median = 13.75 pg/ml, range 4.8 – 157] based on Wilcoxon-Mann-Whitney test. [p=0.5185, U=152]
d) Absolute ACTH values [pg/ml] plotted against the distance from the last cortisone intake and marked according to the cortisol value, blue = cortisol < 18 µg/dl, green = cortisol ≥ 18 µg/dl. The ACTH values did not correlate with the distance from the last cortisone intake. [r = -0.0452, p= 0.7629, based on spearman correlation test]

4.4 Renin secretion in paediatric oncologic children under stress (fever)

We measured the renin concentration in every patient included in this study. 50 of 75 (66.7%; 95% CI, 55.8 – 77.6%) patients had high renin levels [>23.9 ng/l] under stress. *(see Tab.8)* Only one patient had a renin concentration below 1.7 ng/l, 24 patients had a renin level within the normal range [1.7 – 23.9 ng/l]. In patients that presented with a cortisol <18 µg/dl, 42 of 58 (72.4%; 95% CI, 60.6 – 84.3%) patients had a high renin level versus 8 of 17 (47.1%; 95% CI, 20.6 – 73.5%) patients in the cohort that had adequate cortisol level under stress. However, the incidence of high renin level [> 23.9 ng/l] was not significant different between the latter groups. [p=0.0780, Fisher's exact test] *(see Fig. 13)* Eleven of 75 patients in our cohort presented with low sodium levels [<135 mmol/l] and seven of these eleven patients had a high renin level at admission. Two of 75 patients had arterial hypotension at the presentation with fever, one of these two patients had high renin levels.

	Cortisol	
Renin	**< 18 µg/dl (n=58)**	**≥18 µg/dl (n=17)**
Low [< 1.7 ng/l]	1	0
Normal [1.7 – 23.9 ng/l]	15	9
High [>23.9 ng/l]	42	8

Table 8: Renin and cortisol level of all patients.

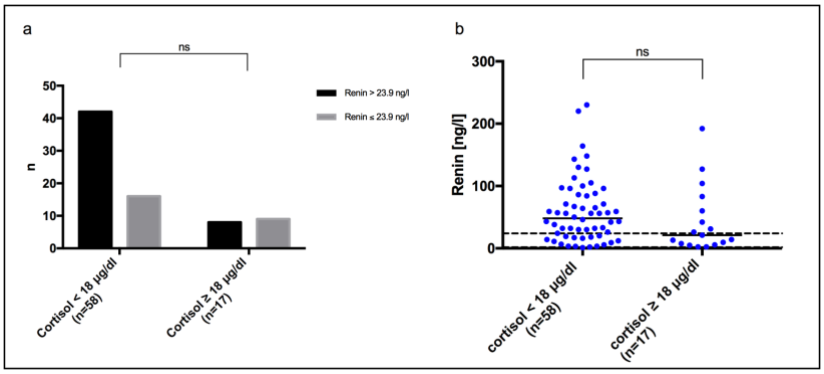

Figure 13:
a) incidence of high renin [>23.9 ng/l] was not significantly different between patients with cortisol < 18 µg/dl [72.4% > 23.9 ng/l, 27.6% ≤ 23.9 ng/l] and patients with cortisol ≥ 18 µg/dl [47.1% > 23.9 ng/l, 52.9% ≤ 23.9 ng/l] in response to stress based on Fisher's exact test. [p=0.0780]
b) Absolute renin levels in patients with cortisol < 18 µg/dl [median = 48 ng/l, range 1 – 230] and patients with cortisol ≥ 18 µg/dl [median = 21 ng/l, range 2.3 - 192], dotted line at 1.7 ng/l and 23.9 ng/l. There was no significant difference between of the absolute renin values between the two groups based on Wilcoxon's test [p=0.1036, U=364]

5 Discussion

This explorative study aimed to assess the incidence of adrenal insufficiency in children treated with chemotherapy under stress. Stress was hereby defined as fever with a body temperature ≥ 38.5°C once or > 38.0°C twice in 24 hours.

Overall, we prospectively analysed random cortisol, corticotropin (ACTH) and renin levels in 75 paediatric patients (age 6 months to 17 years) treated with chemotherapy due to oncologic diseases at the Dr. von Hauner Children's Hospital from 2015 to 2018. These children were either admitted to the clinic due to fever or they developed fever during an inpatient stay. Exclusion criteria were cranial irradiation, age below one month, previously known adrenal- or hypopituitary insufficiency, no central venous line and/or a missing informed consent. Overall, 77.3% (95% CI, 67.6 – 87.0%) of the patients showed an inadequate stress response as defined by our criteria (cortisol < 18 µg/dl). There was no significant difference between children receiving corticosteroids and those who did not (p=0.7789). Regarding the corticosteroid group, cortisol levels correlated positively with the distance from the last cortisone intake (p=<0.0001). We observed a significant difference in cortisol values between patients with less than or equal seven days distance to the last steroid intake and more than seven days distance (p=<0.0001). All children that had received dexamethasone or had received posaconazole prophylaxis during treatment, had random cortisol levels < 18 µg/dl in stress.

Previous studies have shown that adrenal insufficiency occurs commonly in the first days after cessation of corticosteroid therapy in childhood ALL and that its exact duration is unclear. Therefore, it is recommended to consider steroid replacement therapy during periods of serious stress to reduce the risk of complications. However, all of these studies assessed the adrenal function in children without fever at regular time points after cessation of the steroid therapy and none explicitly assessed the adrenal function in this patient cohort under stress. [42] Additionally, it was the first study that analysed the adrenal function in children receiving chemotherapy that were steroid naïve. We therefore think that our study addressed important and clinical relevant questions in this patient cohort.

All our patients were admitted due to fever which should be a sufficient stimulus for the HPA axis to produce cortisol. Hence, we decided to determine random cortisol,

ACTH and renin levels at admission, regardless from the time. We decided against additional dynamic tests (e.g. low dose corticotropin stimulation test), since repeated blood draws from central venous lines are an infectious hazard and we did not want to delay prompt antibiotic treatment in this high-risk patient cohort.

We defined a random cortisol level < 18 µg/dl as adrenal insufficiency in our patients, since a cortisol level ≥ 18 µg/dl would be expected in patients under stress. Additionally, it is the expected value to rule out adrenal insufficiency in low dose corticotropin stimulation test. [21, 52, 61] Current guidelines for the diagnosis and management of adrenal insufficiency in critical ill patients suggest a random plasma cortisol < 10 µg/dl. [55] We analysed our data under application of this limit. The overall incidence was 50.7% (95% CI, 39.1 – 62.3%) and the distribution in the group with and without steroids was nearly identical. However, our patient cohort differed since the patients were not septic or hemodynamic instable. Therefore, we think a cortisol < 18 µg/dl should be used to screen for adrenal insufficiency in paediatric, oncologic patients with fever.

This study has some weaknesses. Firstly, we did not have a control group of healthy children with fever. Secondly, as no HPA axis evaluation was performed before the patients were included in this study, an adrenal insufficiency could already have been present at the time of the cancer diagnosis. However, we found no reports of adrenal suppression at the time of diagnosis. [35, 37, 40] Thirdly, this study was a prospective, explorative single-centre study and future studies should evaluate our results in a multicentre setting.

Our patient cohort included 75 paediatric, oncologic patients treated with chemotherapy, excluding only patients with cranial irradiation or previous known HPA axis insufficiency. Although this cohort is very heterogeneous, we think it is a benefit of this study since it represents the usual distribution of oncologic entities in children apart from the brain tumours. [68] Another benefit, in our view is, that we included not only children with acute lymphoblastic leukaemia but also patients with Langerhans cell histiocytosis, Non-Hodgkin- and Hodgkin’s lymphoma. High dose steroid therapy is an important element of their treatment as well. With the exception of the study of Spiegel et al. in 1979, previous studies evaluated the adrenal function merely in acute lymphoblastic leukaemia patients. [35-40, 69] On the other hand, one could

argue that by including more entities, the respective corticosteroid derivative taken and duration of therapy was very mixed in our cohort.

58 of 75 patients included had cortisol level < 18 µg/dl under stress (77.3%; 95% CI, 67.6 – 87.0%). The incidence of adrenal insufficiency defined by a cortisol < 18 µg/dl was 78.7% in the cohort with steroids and 75% in the cohort without steroids in their therapy protocol. The difference was not significantly different (p=0.7789). The median cortisol level was 9.0 µg/dl [range 0.2 – 31.1] in patients with steroids versus 11.4 µg/dl [range 3.6 – 39.6] in patients without steroids in their therapy.
Only a few studies have evaluated the adrenal function during fever or infection in children with cancer and all of these studies were in patients with steroids in their therapy. Kuperman et. al performed weekly low dose ACTH tests in 16 patients with prednisolone and 13 patients with dexamethasone treatment until eight weeks after cessation of glucocorticoid treatment. Ten patients in both groups were admitted at least once due to fever. In five out of 15 (33.3%) episodes in the prednisolone group and one out of 15 (6.6%) episodes in the dexamethasone group, the patients had inadequate ACTH responses during stress. There was no correlation between the presence of infection or stress and the response to the low dose ACTH test. This study reported a remarkable lower frequency of adrenal insufficiency under stress, however, the patient size was smaller and there was no information on the distance from the last steroid intake at the time of fever. [36] Mahachoklertwattana et al. reported that all four out of 24 patients that were admitted with fever, had inappropriately low cortisol levels during this period with persistence of the adrenal suppression up to twenty weeks after cessation of the steroid therapy in three of the four patients. [38]

Regarding patients with cortisone in their treatment protocol, only two of 37 patients with inadequate cortisol response had high ACTH values [> 50 pg/ml]. The remaining 35 patients had either low or normal ACTH values. Since we see a notable higher rate of high ACTH in patients with an adequate cortisol response, we conclude that the HPA axis was still suppressed in these patients, leading to an insufficient stimulation of the adrenal gland. Cunha et al. reported a statistically significant baseline ACTH reduction between pre-treatment levels and levels taken with the 8th day and 28th day of dexamethasone therapy in children with ALL. However, baseline

ACTH levels obtained two days and one month after cessation of dexamethasone were not significantly different from pre-treatment levels. [34] Thus, different to our cohort, these patients had a fast recovery from the HPA axis suppression.

21 of 28 patients without corticosteroids in their treatment protocol had cortisol values < 18 µg/dl under stress. None of these 21 patients had a high ACTH levels [>50 pg/ml] despite the low cortisol level. We think that either the fever stimulus was not enough stress to elicit a higher ACTH response or the multiagent chemotherapy the patients received have influenced the adrenal function. Even though none of the chemotherapy agents administered has been reported to have an impact on the adrenal function, a general inhibitory effect on the HPA axis cannot be excluded. However, we do not know, if a cortisol < 18 µg/dl is an inadequate response in this patient cohort or if it is a physiologic low level. It is important to further validate our observation in patients without steroids in their therapy, since it would be hazardous to oversee this potentially fatal complication in this patient cohort.

50 of 75 patients (66.7%; 95% CI, 55.8 – 77.6%) presented with high renin levels [>23.9 ng/l] under stress. Electrolyte abnormalities were observed in eleven, arterial hypotension in two of the 75 patients. We explain the incidence of high renin [>23.9 ng/l] under stress in patients without noticeable vital parameter changes or electrolyte imbalances with the high sensitivity of the renin-angiotensin-aldosterone-system to small blood pressure or electrolyte changes. It is known that renin elevation is present before serum electrolytes become abnormal. [53]

Interestingly, all patients that took dexamethasone had an adrenal insufficiency under stress. These patients were treated due to B cell/ T cell lymphoma or ALL relapse. Only four of the eleven patients had a distance of less than or equal seven days to the last corticosteroid intake. Thus, we do not think that the high incidence of adrenal insufficiency under DXM can be explained by the distance to the last cortisone intake. Another observation speaks for the strong influence of dexamethasone on the HPA axis. 28 of the 47 patients with corticosteroids were treated in the COALL 08/09 protocol due to acute lymphoblastic leukaemia. 22 patients were included after the remission induction phase with methylprednisolone and seven patients during the reinduction phase or high-risk protocol having been treated with methylprednisolone

as well as dexamethasone. The incidence of adrenal insufficiency was 63.6% and 85.7%, respectively. Thus, dexamethasone treatment seems to have a great influence on the HPA axis. Previous studies have compared the occurrence of adrenal insufficiency between prednisolone and dexamethasone in paediatric, oncologic patients. Two randomized controlled studies found no difference, one observational study demonstrated earlier recovery in children receiving prednisolone. [36, 37, 41] In our cohort, 85.7% patients with prednisolone had cortisol levels < 18 µg/dl under stress. Regarding the occurrence of adrenal insufficiency, we could not observe a significant difference between the different derivatives. However, we conclude that dexamethasone stands out as a risk factor in our cohort and clinical and laboratory follow up is necessary after dexamethasone withdrawal.

Various studies have shown that the adrenal suppression after corticosteroid therapy is always present immediately after cessation and persists up to several months. Felner et al. observed a recovery of the adrenal function in all ten ALL children after eight weeks, Petersen et al. reported a suppressed function up to eight months later in three of 17 ALL children. [35, 39] On the other hand, all patients in the study by Cunha et al. showed a recovery of adrenal reserve one months after withdrawal. [34] In our study, the cortisol values under stress correlated significantly with the distance to the last intake. 93.8% of the patients that had less than or equal seven days distance to the last intake, had an inadequate cortisol response under stress. In contrast, the incidence was 61.1% in patients with a distance of more than 14 days. Thus, we observed the same trend as in previous reported studies. Since every patient was included only once in our study, we had no follow up blood values and no information on the duration of adrenal insufficiency. We saw a significant difference in the absolute cortisol values between patients with less than or equal seven days and more than seven days. We could not observe a significant difference when we compared patients with less than or equal 14 days and more than 14 days. Therefore, we think that patients with a distance of less than or equal seven days are almost never able to mount an adequate response under stress. These patients would probably benefit from hydrocortisone replacement under stress.

Another important observation of our study is the high incidence of adrenal insufficiency in patients under posaconazole prophylaxis. All seven patients in our

cohort had cortisol level < 18 µg/dl under stress. Three of them had steroids in their treatment, four of them were steroid naïve. Fluconazole therapy was evaluated as a risk factor for persistence of AI in two cohort studies in children treated for ALL, but no data exist on posaconazole and adrenal suppression in children. [39, 41] However, there are two case reports in adults that report about posaconazole related adrenal insufficiency, one in a patient with diabetes mellitus and another in a patient with chronic myelomonocytic leukaemia. [70, 71] A possible explanation for a prolonged suppression of the adrenal gland might be the inhibition of the cytochrome P450-dependent CYP3A4 that can lead to a decreased hepatic metabolism of synthetic glucocorticoid.[72] A therapy switch to posaconazole in a 51 year old woman with common variable immunodeficiency syndrome, who had received itraconazole and inhaled fluticasone for seven years, led to the new development of Cushingoid symptoms. [73] Our data needs to be confirmed in other studies since the patient size is too small to draw final conclusion. However, we would recommend considering hydrocortisone replacement therapy in paediatric, oncologic patients under posaconazole prophylaxis under stress.

This prospective, explorative study helps to better estimate the incidence of adrenal insufficiency in paediatric, oncologic patients under stress. Taken all our results into account, we believe that the incidence of adrenal insufficiency in paediatric, oncologic patients is underrated. Corticosteroid therapy might suppress the HPA axis in children with acute lymphoblastic leukaemia, lymphoma or LCH and the exact duration cannot be predicted. We saw a significant difference in cortisol values in patients with a distance of less than or equal seven days compared to more than seven days. Additionally, all our patients who took dexamethasone or posaconazole prophylaxis had an inadequate cortisol response. Patients with solid tumours or acute myeloid leukaemia that were corticosteroid naïve also presented with an astonishingly high incidence of adrenal insufficiency.

We think that more paediatric, oncologic patients would benefit from hydrocortisone substitution under stress as previously assumed. As a consequence of our experience in this study, we would recommend determining random cortisol level in every paediatric, oncologic patient admitted with fever. We think it would be a reasonable approach to administrate hydrocortisone stress doses (30 mg/m^2 in three doses) in patients under posaconazole prophylaxis, after dexamethasone therapy

(regardless of the distance) or with a distance of less than or equal seven days to the last corticosteroid intake. We would then suggest continuing the hydrocortisone replacement therapy if the random cortisol level is < 18 µg/dl. Besides, we would recommend administrating hydrocortisone stress doses in all other patients without one of the above risk factors if their cortisol value is < 18 µg/dl at admission. The aim of this approach would be to see, if the clinical course of the patients receiving hydrocortisone replacement therapy, changes and if a cortisol level ≥ 18 µg/dl is necessary in this patient cohort under stress. This proposed approach and the collected data is based on the experience of a single-centre, explorative study with limitations and has to be confirmed and examined in further studies.

6 Conclusion

Adrenal insufficiency represents a possible life-threatening adverse effect of chemotherapy and its incidence is not well known in oncologic children. Hypothalamic-pituitary-adrenal (HPA) axis suppression may result from glucocorticoid treatment in leukaemia and lymphoma patients but may also occur due to other chemotherapeutic regimens for solid tumours. The aim of this explorative, prospective single-centre study was to evaluate the incidence of adrenal insufficiency in children treated with chemotherapy under stress (fever). Adrenal insufficiency was hereby defined by a random cortisol level < 18 µg/dl (= 497 nmol/l). We further wanted to assess whether there is a difference in the occurrence rate between patients receiving glucocorticosteroid in their treatment and those who are steroid naive.

We prospectively analysed the random cortisol, adrenocorticotropic hormone (ACTH) and renin level in 75 paediatric patients (age 1 months – 18 years) treated with chemotherapy at the Dr. von Hauner Children's Hospital from 2015-2018 who were submitted to the hospital due to fever or developed fever during an inpatient stay. Children with known suppression of the HPA axis, cranial irradiation or age < one months were excluded from the study. Test results were not available during the study.

58 of 75 (77.3%) children treated with chemotherapy had an inadequate low cortisol level (< 18 µg/dl) during stress. There was no significant difference in the incidence rate between the patients who received glucocorticoids in their therapy and those who were glucocorticoid naïve (p=0.7789). We observed a direct correlation between low cortisol and the distance from the last corticosteroid intake (p=<0.0001). A distance of less or equal than seven days distance to the last corticosteroid intake revealed significantly lower cortisol levels than a distance of more than seven days. (p=<0.0001) All patients treated with dexamethasone or under posaconazole prophylaxis mounted an inadequate adrenal response under stress.

Taken together, the incidence of adrenal insufficiency in paediatric, oncologic patients seems to be higher than previously assumed. We were able to identify dexamethasone therapy, a distance of less than seven days to the last corticosteroid intake as well as posaconazole prophylaxis as a risk factor for adrenal insufficiency. We think it is a reasonable approach to determine random cortisol level in every paediatric, oncologic patient with fever and to consider the administration of

hydrocortisone stress dose in patients with risk factors or inadequate cortisol level under stress. Since this is a single-centre, prospective, explorative study, further studies are needed to confirm our observations and to gain a better understanding about this serious complication in children with cancer.

7 Zusammenfassung

Die Nebenniereninsuffizienz stellt eine möglicherweise lebensbedrohliche Nebenwirkung unter Chemotherapie dar und ihre genaue Inzidenz ist bei pädiatrischen Krebspatienten nicht bekannt. Die Einnahme von Glukokortikoiden im Rahmen der Therapie von Leukämien oder Lymphomen einerseits, aber auch Chemotherapie zur Behandlung von soliden Tumoren andererseits, kann zu einer Unterdrückung der Hypothalamus-Hypophysen-Nebennieren-Achse führen. Das Ziel dieser prospektiven, explorativen, monozentrischen Studie ist es, die Inzidenz der Nebenniereninsuffizienz bei pädiatrischen, onkologischen Patienten unter Chemotherapie in febrilen Episoden zu eruieren. Die Nebenniereninsuffizienz wurde dabei durch einen zufälligen Cortisol-Spiegel < 18 µg / dl (= 497 nmol / l) definiert. Wir wollten ferner abschätzen, ob es einen Unterschied in der Häufigkeit des Auftretens zwischen Patienten gibt, die Glucocorticosteroid während ihrer Behandlung erhalten haben, und solchen, die steroid-naiv sind.

Wir bestimmten von 2015 bis 2018 bei insgesamt 75 Kindern (1 Monat bis 18 Jahre alt), die aufgrund einer onkologischen Erkrankung im Dr. von Hauner'schen Kinderspital mit Chemotherapie behandelt wurden und mit Fieber stationär aufgenommen wurden oder Fieber während ihres Aufenthaltes entwickelten, Kortisol- und ACTH-Spiegel und die Reninaktivität. Kinder mit bekannter Unterdrückung der Hypothalamus-Hypophysen-Nebennierenrinden-Achse, nach Schädelbestrahlung oder Alter <1 Monat wurden von der Studie ausgeschlossen. Testergebnisse waren während der Studie nicht verfügbar.

58 von 75 (77.3%) Kinder, die mit Chemotherapie behandelt wurden, zeigten einen inadäquaten Kortisol Spiegel (< 18 µg/dl) im Stress. Es gab keinen signifikanten Unterschied in der Inzidenzrate zwischen den Patienten, die Glukokortikoide in ihrer Therapie erhielten, und denen, die steroid-naiv waren (p=0.7789). Wir beobachteten eine direkte Korrelation zwischen niedrigen Kortisol Spiegeln und dem zeitlichen Abstand zur letzten Glukokortikoid Einnahme (p=<0.0001). Ein Abstand von weniger als oder gleich sieben Tagen bis zur letzten Kortikosteroid Einnahme ergab signifikant niedrigere Kortisol Werte als eine Entfernung von mehr als sieben Tagen. (p=<0.0001) Bei allen Patienten, die mit Dexamethason oder einer Posaconazol-Prophylaxe behandelt wurden, trat unter Stress eine unzureichende Nebennierenreaktion auf.

Insgesamt scheint die Inzidenz von Nebenniereninsuffizienz bei pädiatrischen, onkologischen Patienten höher zu sein als bisher angenommen. Wir konnten die Dexamethason-Therapie, einen Abstand von weniger als sieben Tagen bis zur letzten Kortikosteroid-Einnahme, sowie eine Posaconazol-Prophylaxe als Risikofaktor für eine Nebenniereninsuffizienz identifizieren. Wir glauben, dass es ein vernünftiger Ansatz ist, den Kortisolspiegel bei jedem pädiatrischen, onkologischen Patienten mit Fieber zu bestimmen und die Verabreichung von Hydrocortison-Stressdosis bei Patienten mit Risikofaktoren oder unzureichendem Kortisolspiegel unter Stress in Betracht zu ziehen. Da es sich um eine prospektive, monozentrische, explorative Studie handelt, sind weitere Studien erforderlich, um unsere Beobachtungen zu bestätigen und noch ein besseres Verständnis dieser schwerwiegenden Komplikation bei krebskranken Kindern zu erlangen.

8 Supplements

Table A.

Inclusion number	Study number	Corticosteroid intake Yes (1)/ No (0)	Cortisol (µg/dl)	ACTH [10-50 g/dl]	Renin [1.7 - 23.9 ng/l]
1	8	0	25.4	79.4	83
2	12	1	16	6.1	148
3	14	1	10.2	3.7	65
4	21	1	7.8	15.6	46
5	23	1	19.5	4.8	7.7
6	28	0	7	6.9	71
7	30	1	29.2	148	9.7
8	40	1	16.2	25.3	100
9	45	0	13.1	32.7	130
10	4	0	8.1	25.2	38
11	24	1	14.1	31.8	3
12	29	1	0.4	11.4	220
13	3	0	7.5	18.9	57
14	33	0	9.4	21.3	<1
15	37	0	9.6	5.8	3.3
16	42	1	7.8	24.2	30
17	51	0	39.6	308	5.6
18	55	1	1	7.9	32
19	74	0	22.8	44.7	13
20	58	1	0.2	3.7	42
21	60	0	12.1	13	50
22	62	0	19.4	13.7	31
23	78	1	11.9	601	12
24	74	1	7.3	43.6	59
25	91	1	2.8	79.6	164
26	86	1	25.8	20.4	60
27	68	1	9.7	10	143
28	100	0	8.3	9.3	24
29	96	1	0.6	7.8	113
30	90	1	15.3	16.8	96
31	95	1	1.6	6.8	59
32	88	1	7.4	4.4	43
33	92	1	9	13.9	9
34	82	1	10.8	5.7	16
35	98	1	8	16.5	57
36	97	0	11.6	22.6	32
37	41	1	6.1	8.5	33
38	104	0	8.2	14.2	84
39	107	1	8.7	7.2	17
40	103	0	27.2	21.4	2.4
41	106	0	12.3	2.7	230
42	105	1	5.1	3.4	56

43	84	1	14.7	21.6	6.7
44	102	1	10.8	5.1	56
45	109	1	2.9	6.3	127
46	126	0	9	9.9	20
47	130	1	31.1	6.2	192
48	10	0	13.4	12.1	26
49	128	1	18.9	7.1	21
50	108	0	8.5	13.4	18
51	141	1	20.8	127	104
52	145	1	4.5	13	5.7
53	147	1	5.7	10.5	88
54	120	1	15.6	31.5	71
55	146	1	19.8	5.9	14
56	155	1	6.9	5.6	19
57	114	1	25.5	35.6	42
58	72	0	11.2	27.5	64
59	160	0	13.4	23.5	86
60	157	1	19.3	6.6	127
61	163	0	8.6	8.9	97
62	22	0	6.5	15.2	96
63	123	1	11.1	22.7	2.2
64	162	0	15.9	9.1	11
65	149	1	4.2	4.9	67
66	136	0	8.7	15.7	3.4
67	177	1	12.8	23.4	105
68	156	0	18.5	7.6	4.8
69	179	1	3.6	8.8	43
70	187	0	3.6	14	32
71	182	1	19.7	157	2.3
72	194	0	19.6	26.4	26
73	207	1	4.8	5	56
74	199	1	6.7	11.1	14
75	197	1	6.1	11.1	30

9 List of abbreviations

ACTH	Adrenocorticotropic hormone
AI	Adrenal insufficiency
AML	Acute myeloid leukaemia
ALL	Acute lymphoblastic leukaemia
BSA	Body surface area
CAI	Central adrenal Insufficiency
CBG	corticosteroid-binding globulin
CIRCI	Critical illness related corticosteroid insufficiency
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
EDTA	Ethylenediaminetetraacetic acid
Etc.	et cetera
Fig.	Figure
g	gram
Gy	gray
HPA	Hypothalamic-pituitary-adrenal axis
IL	Interleukin
ITT	Insulin tolerance test
Kg	kilogramme
LCH	Langerhans cell histiocytosis
LDSST	Low dose Synacthen stimulation test
µ	micro (10^{-6})
M	Mean
PAI	Primary adrenal insufficiency
POMC	pro-opiomelanocortin
SD	standard deviation
SDSST	standard dose Synacthen stimulation test
Tab.	Table

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12 Affidavit

Boekstegers, Ann-Madeleine

Surname, first name

I hereby declare, that the submitted thesis entitled

Incidence of adrenal insufficiency in paediatric, oncologic patients with fever during chemotherapy

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

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