

Altered Chemokine Th1/Th2 Balance in Addison's Disease: Relationship with Hydrocortisone Dosing and Quality of Life

Bertil Ekman, N Alstrand, Margareta Bachrach-Lindström, Maria C Jenmalm and Jeanette Wahlberg

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3 **Altered chemokine Th1 /Th2 balance in Addison's disease: relationship with**
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5 **hydrocortisone dosing and quality of life**
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10 ¹Bertil Ekman, ²Nils Alstrand, ³Margareta Bachrach-Lindström, ⁴Maria C Jenmalm, and

11
12 ¹Jeanette Wahlberg

13
14 ¹Department of Medicine and Health Sciences, Division of Endocrinology, Faculty of Health
15
16 Sciences, Linköping University, Linköping, Sweden, ²Department of Medicine and Health
17
18 Sciences, Faculty of Health Sciences, Linköping University, Linköping, County Hospital,
19
20 Kalmar, Sweden, ³Department of Medicine and Health Sciences, Division of Nursing Science,
21
22 Faculty of Health Sciences, Linköping University, Linköping, Sweden, ⁴Department of
23
24 Clinical and Experimental Medicine, Division of Inflammation Medicine, Faculty of Health
25
26 Sciences, Linköping University, Linköping, Sweden.
27
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45 Address for correspondence: Bertil Ekman, MD
46

47
48 Section of Endocrinology and Diabetology
49

50 University Hospital, S-581 85 Linköping, SWEDEN

51
52 Tel +46-(0)-101030000, Telefax +46-(0)-101033502.
53

E-mail Bertil.Ekman@lio.se

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5 **Abstract**

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7 Objective: The adrenalitis found in autoimmune Addison's disease (AAD) is considered
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9 having a Th1-driven pathogenesis. Circulating Th1- and Th2-associated chemokines
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11 responsible for the trafficking of leukocytes to inflammatory sites are markers for the
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13 Th1/Th2 balance.
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16 Aim: To assess if the same daily hydrocortisone dose of 30 mg given in either 2 or 4 doses to
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18 patients with AAD could affect the Th1/Th2 balance of circulating chemokines.

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20 Methods: Fifteen patients (6 women) with AAD were included in this randomised, placebo
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22 controlled, double blind cross-over study. Samples for chemokines, Th1-associated
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24 (CXCL10, CXCL11) and Th2-associated (CCL17, CCL22), were drawn 5 times during a 24-
25
26 h period at the end of each treatment period and analysed with Luminex. Seven control
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28 subjects did the same diurnal blood sampling once.
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31 Results: Subjects with AAD had higher median diurnal levels of the Th1-associated
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33 chemokines than controls, CXCL10 43 (33-56) pg/ml vs. 22 (19-34) pg/ml, $p < 0.01$) and
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35 CXCL11 (37 (29-48) pg/ml vs. 16 (9-24) pg/ml, $p < 0.001$), whereas no significant difference
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37 was found regarding the Th2-related chemokines. Similar chemokine levels were found when
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39 the same hydrocortisone dose of 30 mg was divided in 2 or 4 doses. Levels of CXCL11
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41 correlated negatively with scores of SF-36 domains (high score indicate better health) of
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43 General Health (GH) and total score for Physical Component Summary (PCS), and these
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45 negative correlations were most pronounced at 04:00 on the 2-dose regimen.
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49 Conclusions: Patients with AAD have a dominant Th1 chemokine profile that partially
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51 correlates to reduced quality of life.
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Introduction

Autoimmune Addison's disease (AAD) is a disease mainly caused by autoimmune destruction of the adrenals [1]. Chemokines, a large family of chemoattractants responsible for the trafficking of leukocytes to the site of inflammation, are involved in the pathogenesis of inflammatory and autoimmune diseases [1,2]. Chemokines play an important role in several endocrinological autoimmune diseases, such as Hashimoto's thyroiditis, Graves' disease, and type 1 diabetes mellitus [3]. Circulating Th1- and Th2-associated chemokine levels can be used as markers for the Th1/Th2 balance [4]. Thus, Th1 responses increase the levels of the CXCR3 ligands CXCL10 (interferon- γ inducible protein 10, IP-10) and CXCL11 (interferon- γ inducible T cell α chemoattractant, I-TAC), while Th2 responses enhance the CCR4 ligands CCL17 (thymus- and activation-regulated chemokine, TARC) and CCL22 (macrophage-derived chemokine, MDC) levels [5]. CXCR3 is preferentially expressed on Th1 cells and CCR4 on Th2 cells, thus further amplifying Th1 and Th2 immunity, respectively [6].

AAD often coexists with other autoimmune diseases such as T1D, hypothyroidism, pernicious anaemia, vitiligo and celiac disease in Autoimmune Polyglandular Syndrome type 2 (APS 2), and with hypoparathyroidism and mucocutaneous candidiasis in APS 1 [7].

Although different endocrine organs may at least in part share the mechanisms leading to the glandular autoimmune process, the role of chemokines in autoimmune adrenal insufficiency is poorly understood. The adrenalitis in AAD is considered, at least partly, having a cell mediated, and thus Th1 driven, pathogenesis [8,9]. Increased peripheral blood mononuclear cell IFN- γ responses to the steroidogenic cytochrome P450 enzyme 21-hydroxylase (21OH), an autoantibody target in PAI, was recently observed in AAD [10]. It has been noted that Th1-mediated diseases tend to lessen in severity during the shift towards a Th2-dominated

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3 immune response that occurs during pregnancy [11,12]. Thus, Th1-mediated autoimmune
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5 diseases can possibly be treated with therapy aiming for Th2-deviation, as tested in various
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7 studies using different modulatory substances such as progesterone [13]. Although not clearly
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9 proven in randomised placebo-controlled studies, accumulated data suggest that the immune
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11 modulatory effects of cortisol are more Th2-deviating than overall suppressive [14].
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13 The mechanisms underlying the increased fatigue reported in AAD are not fully understood,
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15 but the lack of restoration of a normal diurnal rhythm for cortisol is advocated as the main
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17 cause [15]. Supporting this theory, open studies have reported advantages on health related
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19 quality of life (HRQoL) in PAI if the same dose of hydrocortisone was divided in three
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21 instead of two doses [16,17], or given by continuous hydrocortisone infusion [18]. In
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23 addition, we have recently in a double blind manner showed that if the same hydrocortisone
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25 dose of 30 mg was divided either in four or in two doses, AAD patients blindly preferred four
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27 doses [19]. In this study our primary aim was to investigate the circulating Th1- and Th2-
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29 associated chemokine levels in AAD in comparison with healthy control subjects.
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31 Furthermore, we aimed to investigate if the same daily hydrocortisone dose divided in either
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33 two or four doses could affect the Th1/Th2 balance in AAD and how the chemokine levels
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35 were related to self evaluated health, sleep and fatigue.
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Subjects and Methods

Subjects

Fifteen patients (6 women and 9 men) with primary adrenal insufficiency of autoimmune origin (21-OH antibody positive) were included. The mean age at inclusion was 44.6 ± 15.7 yrs. The median duration of glucocorticoid replacement therapy since diagnosis was 12 yrs. (range 1-56 yrs.). The inclusion criteria were morning basal cortisol levels below 100 nmol/L or maximal plasma concentration of cortisol below 300 nmol/l after stimulation with synthetic ACTH (Synachten[®]), and an elevated plasma ACTH concentration determined in a morning sample. Only adult patients above 18 years of age were recruited and patients with diabetes mellitus were excluded. Fourteen of the patients were treated with fludrocortisone. Eleven of the patients had isolated AAD and four patients had in addition autoimmune thyroiditis treated with thyroxine. Seven healthy controls were included (5 women and 2 men; mean age 54.1 ± 10.2 years). Characteristics of patients and control subjects are given in Table 1.

Study design

This study was part of a randomised; placebo controlled, double blind cross-over study (EuduraCT number 2005-001768-30), assessing the effects of the same daily hydrocortisone dose given in either 2 or 4 doses to patients with AAD [19]. All patients were treated with a 30 mg standard dose of hydrocortisone during the whole study. The patients were randomised to receive either 20 mg hydrocortisone at 07:00 h and 10 mg at 16:00 h (two-dose regimen) or 10 mg at 07:00 h, 10 mg at 12:00 h, 5 mg at 16:00 h and 5 mg at 22:00 h (four-dose regimen). After 4 weeks they switched to the other treatment. Tablets were taken before meals except the evening dose. All patients received tablets four times a day, either hydrocortisone or placebo, to ensure the double blind study procedure. To accomplish this, our pharmacy (Apoteket AB, Produktion & Laboratorier 141 75, Kungens Kurva, Stockholm, Sweden),

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3 produced 5 mg hydrocortisone capsules (HC) and identical placebo capsules (P). The four
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5 dose schedule was thus 2 HC + 2 P at 07:00 h, 2 HC at 12:00 h, 1 HC and 1 P at 16:00 h and 1
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7 HC at 22:00 h. The two dose schedule was 4 HC at 07:00 h, 2 P at 12:00 h, 2 HC at 16:00 h
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9 and 1 P at 22:00 h. Seven patients started with four doses and 8 patients with two doses.

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11 Diurnal profiles of cortisol in plasma and urine were collected at the end of each treatment
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13 and the patients filled out questionnaires, administered once at daytime at the end of each
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15 treatment. Chemokines were analysed in the samples collected at 16:00, 20:00, 04:00, 07:00
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17 and 10:30 h. The seven control subjects did the diurnal profiles of cortisol and chemokines
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19 once. The control subjects did not fill in the questionnaires.
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22 23 24 25 *Cortisol analyses*

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27 Serum cortisol was measured with a commercial kit by fluorescence immunoanalysis on
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29 AutoDelfia (Wallac Oy, Finland). The total coefficient of variation (CV) for the serum
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31 cortisol assay was 6 % for high values and 8 % for low values and the lower detection limit
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33 was 15 nmol/l. Urine cortisol was measured with a commercial kit by radio-immuno-assay
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35 (RIA) (Diagnostic Products Corporation, Los Angeles, CA, USA). The total CV for the free
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37 urine cortisol analysis was 16 %.
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40 41 42 43 *Chemokine analyses*

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45 The chemokines CXCL10, CXCL11, CCL17 and CCL22 were analysed on a Luminex¹⁰⁰
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47 instrument (Biosource, Nivelles, Belgium), as described in detail previously [20]. The data
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49 were acquired using the StarStation 2.3 software (Applied cytometry systems, Sheffield, UK)
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51 with 5-parametric-curve fitting. The detection limits were 6 pg/ml for CXCL10 and CCL22,
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53 17 pg/ml for CXCL11, and 2 pg/ml for CCL17. All samples were analysed in duplicates and
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55 the sample was re-analysed if the coefficient of variance (CV) was >15%.
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3 *Health related quality of life (HRQoL) and subjective well-being*

4 The patients filled in HRQoL questionnaires administered once at the end of each study
5 period. The questionnaires used were the Short Form-36 (SF-36) [21], the fatigue impact
6 scale (FIS) [22] and the Epworth Sleepiness Scale (ESS-scale) [23].
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14 *Ethics*

15 The study was approved by the local Ethics Committee and performed in accordance with the
16 Declaration of Helsinki. The patients were informed about the purpose of the study and gave
17 their written informed consent.
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28 *Statistics*

29 Statistical calculations were made using SPSS 20 software for Windows. Results are given as
30 medians and 25th to 75th percentiles or when appropriate mean \pm SD. For some comparisons
31 individual means of chemokine levels from all diurnal five-time points were calculated. Area
32 under the curve was calculated using the trapezoid method. Results are given as median and
33 25th to 75th percentiles (or range) or mean \pm SD when appropriate. Comparisons within groups
34 were made with Wilcoxon rank sum test for non-normally distributed data or paired 2-tailed t-
35 test if normally distributed data. Comparisons between groups were made with Mann-
36 Whitney U test for non-normally distributed data or unpaired 2-tailed t-test if normally
37 distributed data. Correlations were made with Spearman's (ρ) for non-normally distributed
38 data or Pearson's test (r) if normally distributed data. Statistical significance was considered at
39 the 5% level ($p < 0.05$).
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Results

Comparison of chemokine levels in AAD vs. controls

Chemokines were measured at 16:00 h, 20:00 h, 04:00 h, 07:00 h and 10:30 h both in AAD subjects and control subjects. Subjects with AAD had higher levels of the Th1-associated chemokines than controls calculated as individual means of chemokine levels from all diurnal five-time points, during both the 2-dose and the 4-dose regimen (Fig 1, Table 2). On the other hand no significant difference was found between AAD and controls regarding the Th2-related chemokines CCL17 and CCL22, neither with 2-dose nor with 4-dose treatment, Table 2. No significant differences were found in chemokine levels between the 2-dose and the 4-dose regimen (Table 2). Age or disease duration showed no significant correlations with any of the chemokine levels found in AAD subjects or control subjects. The presence of other concomitant autoimmune disease (four cases of autoimmune thyroiditis) did not significantly influence the chemokine levels in the AAD group.

Diurnal variation of chemokines in AAD and controls

In subjects with AAD no significant diurnal changes were observed in the Th1-associated (CXCL10, CXCL11) or in the Th2-associated (CCL17, CCL22) chemokines, neither during the 2-dose period nor during the 4-dose period. However, there was a significant higher CXCL11/CCL22 ratio at 04:00 during the 2-dose period 0.18 (0.13-0.28) compared with the 4-dose period 0.15 (0.1-0.24), $p = 0.017$.

In the control group, small diurnal differences were observed in plasma levels of Th1 associated chemokines, CXCL10 levels were significantly higher at 07:00 h compared to levels at 04:00 h: 26.6 pg/ml (22.4-34.1) vs. 19.9 pg/ml (17.4-25.7), $p = 0.018$, and also CXCL11 tended to be higher at 07:00 h compared to 04:00 h: 17.3 (range 8.6-43.8) vs. 8.6 pg/ml (range 8.6-29.5) pg/ml, $p = 0.068$. For the Th2 associated chemokines a slightly higher

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3 value at 07:00 of compared with 04:00 h was found for CCL22, 251 pg/ml (174-315) vs. 244
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5 pg/ml (143-265), $p = 0.018$, while CCL17 at 07:00 was 66.2 pg/ml (39.0-307), and at 04:00
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7 55.4 pg/ml (40.9-270), n.s.
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10 11 *Chemokines and relation to circulating cortisol levels*

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13 Laboratory data regarding diurnal plasma levels of cortisol are partly described elsewhere
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15 [19]. The 24-h area under the cortisol curve (24-h-cortisol_{AUC}) was significantly higher during
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17 four-dose regimen $6023 \pm 1398 \text{ nmol} \times \text{l}^{-1} \times \text{h}$ than both vs. two-dose regimen 4462 ± 1094
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19 $\text{nmol} \times \text{l}^{-1} \times \text{h}$ ($p < 0.0001$) and vs. the controls $4342 \pm 848 \text{ nmol} \times \text{L}^{-1}$, $p < 0.0001$. No
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21 difference was seen in 24-h urinary free cortisol (24-h UFC) between 4-dose 337 ± 173 vs. 2-
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23 dose 330 ± 93 , while the control subjects excreted significantly lower 24-UFC 174 ± 143
24
25 nmol/day , $p < 0.001$, vs. both 4-dose and 2-dose respectively. None of the Th1 or Th2
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27 chemokines measured during the 2-dose period or 4-dose period correlated directly with
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29 cortisol neither at any time point measured, nor with 24-h-cortisol_{AUC} or 24-UFC.
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37 *Questionnaires at the end of each study period*

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39 When the values of all 5 time points both for 2-dose and 4-dose measurements for CXCL11
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41 were calculated together negative correlations were observed between CXCL11 levels and the
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43 SF-36 domain General Health and Physical component summary (PCS) (Table 3). These
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45 correlations were more marked during the 2-dose period, with some significant correlations
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47 disappearing during the 4-dose period (Table 3). Although the negative correlations were
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49 found at all time points during the 24-h period, the correlations between CXCL11 and
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51 domains of the SF-36 were strongest at 04:00 (Fig 2). At this time point, there were also weak
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53 negative correlations during the 4-dose treatment (Fig 2).
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3 When the median values of all 5 time points both for 2-dose and 4-dose measurements for
4 CXCL11 were calculated together, a weak positive correlation $\rho = 0.41$, $p = 0.033$ were
5 found with the ESS sum, indicating higher risk of daily sleepiness (Table 3). No significant
6 correlation was found between CXCL11 and the fatigue impact scale (FIS) if all time points
7 were calculated together both for 2-dose and 4-dose (Table 3).
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13 The positive correlation between CXCL11 and the ESS sum remained at 04:00, $\rho = 0.40$, $p =$
14 0.042 and in addition a borderline positive correlation with FIS sum (high values indicates
15 increased fatigue) 0.34 , $p = 0.087$ was recorded. No significant correlations were observed
16 between CXCL10 and CCL17 and any questionnaire, while for CCL22 and the SF-36 domain
17 General health a significant correlation was found, $\rho = -0.5$, $p < 0.001$, (most prominent
18 during the 4-dose period). Age and disease duration showed no significant relationships with
19 individual scores measured in the questionnaires.
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Discussion

In the present study, AAD was associated with increased Th1 activity, with elevated CXCL10 levels in line with previous studies [1,24,25]. The elevation of CXCL11 in AAD compared to control subjects has not been described before and this is contrary to a recent report where CXCL11 levels were not statistically significantly different in patients and controls [25], but in concordance with elevated CXCL11 levels found in autoimmune thyroiditis [26]. The IFN- γ inducible chemokines (CXCL10, CXCL11) bind to a common receptor, CXCR3, preferentially expressed on Th1 cells, and play important roles in the pathophysiology of autoimmune disease [27]. Almost all research focus has been on CXCL10, although CXCL11 has the highest binding affinity to the CXCR3 receptor and its role in AAD and other autoimmune endocrine diseases has not been fully explored [27].

As the immune modulatory effects of cortisol seems to be more Th2-deviating than overall suppressive [14], one could hypothesize that an increased cortisol level or enhanced cortisol effect should lower the Th1 response. Recently we reported that a four-dose regimen in patients with AAD gave an increased availability of cortisol with significantly higher 24-h-cortisol_{AUC} and an enhanced effect shown by lower morning ACTH levels compared to the same dose divided in two doses [19]. However, in the present analysis we could not confirm the hypothesis that an increased 24-h cortisol exposure should changed the ratio between Th1 chemokines and Th2 related chemokines to a more Th2 deviating status. The absence of correlations between the chemokine and cortisol levels in our patients is intriguing, since cortisol is found to suppress Th1 immune responses [14]. The patients had also a rather high substitution dose of 30 mg compared with 15-20 mg/day proposed in recent years [28]. Our patients probably have no functioning adrenals, since clinical disease appears only after over 90% of the glands has been destroyed [29] and all our patients had elevated ACTH levels and low morning cortisol levels before tablet intake. One can thus argue that the immunological

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3 process responsible for the tissue destruction, via lymphocyte infiltration [30], no longer
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5 should be active. This paradox could be due to a possible reduction of sensitivity to the
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7 immunomodulatory effect of cortisol among patient with AAD, as in accordance with
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9 previous findings [1], showing that CXCL10 is induced in the adrenals under influence of
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11 pro-inflammatory cytokines. The increased Th1 activity is also in concordance with the
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13 enhanced IFN- γ secretion to the candidate autoantigen 21-OH in AAD [10]. Our results
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15 indicate that conventional substitution with 2-4 daily doses of hydrocortisone could not
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17 normalize the disturbed Th1/Th2 balance in AAD.
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22 Our study with randomised, placebo controlled, double blind crossover design allowed us to
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24 study patient preference. We found a better preference and a tendency of improvement in all
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26 subscales in SF-36 for four doses [19], indicating that the better diurnal coverage of cortisol in
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28 some way was better for our patients. However, the stronger negative correlation between
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30 Th1 associated CXCL11 and subscales of SF-36 during the two-dose period indirectly
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32 indicate that a dosing scheme better covering the day (i.e. four doses) could have beneficial
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34 effects on health in AAD via the Th1/Th2 balance. Moreover, our findings that high levels of
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36 CXCL11 were associated with lower scores in SF-36 and increased ESS sum (indicating daily
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38 sleepiness), could also suggest that other mechanisms than inadequate cortisol replacement
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40 therapy could cause fatigue and decreased quality of life found in AAD [31]. In animal
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42 models fatigue appears to arise when some stimulus induces increased proinflammatory
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44 cytokines in the brain and/or activated microglia or by direct cytokine transport across the
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46 blood-brain barrier [32]. Consequently, as many hypothyroid associated symptoms could
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48 remain despite optimal replacement therapy, like in autoimmune thyroiditis [33], the
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50 autoimmunity in itself may cause disease symptoms in autoimmune diseases including AAD.
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3 Circadian rhythms are controlled by a pacemaker in the suprachiasmatic nuclei (SCN) in the
4 hypothalamus. A clinical report of a case study with damage to the circadian pacemaker in the
5 SCN suggests that such damage does leave a person feeling fatigued [34]. The lack of the
6 normal cortisol peak during the early morning can not be restored with conventional daily
7 dosing with 2-4 doses short acting hydrocortisone [19,35] or with a newly developed long
8 acting modified release hydrocortisone tablet [36]. Therefore most patients on daily
9 hydrocortisone replacement therapy have hypocortisolism during the early morning.
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11 Our results with a stronger negative correlation between Th1 associated CXCL11 and
12 subscales of SF-36 during the night also support the theory of nocturnal hypocortisolism as
13 the dominant cause of reduced quality of life in patients with AAD. Further supporting this
14 theory is the study by Lovas et al., in which the circadian cortisol rhythm in AAD was
15 restored with continuous hydrocortisone infusion [18]. The patients in that study scored over
16 all better in SF-36 scores, with significant improvement in physical functioning and vitality
17 subscales. This is in agreement with our results as high levels of CXCL11 were negatively
18 correlated with lower scores on SF-36 domains for General Health (GH) and total score of
19 Physical Component Summary (PCS), and these correlations were most pronounced at 04:00
20 h on the 2-dose regimen (with lowest nocturnal cortisol administration). Moreover, low levels
21 of cortisol during the night could also interfere negatively with sleep and Garcia-Borreguero
22 et al found [37] that cortisol plays a positive, permissive role in REM sleep regulation and
23 may help to consolidate sleep. One striking finding is that it seems that the physical
24 components seem more negatively affected in our and other studies [18] than the mental
25 components. Shortcomings of our study exist with relatively low number of subjects due to
26 the complicated study design and some of the findings between chemokines and the
27 questionnaires could therefore be due to multiple testing. On the other hand, despite the
28 double blind design used in our study, which allows us to compare differences in HRQoL,
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3 some of the effects could have been masked by the fact that our AAD subjects have normal
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5 SF-36 scores compared with the Swedish background population [38], which are in contrast
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7 to a large German study showing impaired HRQoL in subjects with primary or secondary
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9 adrenal insufficiency [39]. Importantly the strongest correlations between CXCL11 and
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11 HRQoL scores were at night, which encourage to further improvements of hydrocortisone
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13 tablets aiming to peak in the early morning [40], or further evaluation of continuous
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15 hydrocortisone infusion [18]. In parallel one must examine whether autoimmunity itself
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17 causes disease symptoms independent of hormonal effects and we suggest CXCL11 as a new
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19 marker for further investigations in AAD.
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25 In conclusion, patients with AAD have a dominant Th1 chemokine profile that partially
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27 correlates to impaired quality of life. We could not demonstrate any direct effect on the
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29 Th1/Th2 balance by changing from two to four daily doses of cortisol.
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Contributor ship statement

JW designed the study analysis and drafted the manuscript together with BE and NA.

MCJ stood for expertise regarding chemokine assessments and MB-L for the expertise regarding questionnaires. MCJ and MB-L reviewed and revised the manuscript for intellectual content. All authors approved the final manuscript

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Legends to figures

Fig 1. Combined values of all measurements at 16:00, 20:00, 04:00, 07:00, 10:30 for CXCL10 and CXCL11 during 2-dose and 4 dose hydrocortisone regimen in 15 patients with Addison's disease compared with seven healthy control subjects. Medians, 25th and 75th as well as outliers are indicated. *p < 0.05, ** p < 0.01 *** p <0.001. Mann-Whitney U test.

Fig 2. Data shown for 15 subjects with Addison's disease treated with twice daily or four times daily hydrocortisone doses. Spearman's rank order correlation coefficient (rho) for CXCL11 measured at five times during the 24-h versus the SF-36 domains

General Health (b) and Physical Component Summary (a). The white bars represent the values calculated together for both two doses and four doses. The hatched bars represent the two-dose regimen and the black bars the four-dose regimen.

*p < 0.05, ** p < 0.01 *** p <0.001.

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Table 1. Anthropometric data and laboratory tests during treatment with twice daily or four daily hydrocortisone doses in 15 subjects with autoimmune Addison's disease (AAD). In addition seven healthy controls were included.

Data shown as mean \pm 1 S.D.

	AAD		Controls
N	15		7
M/F	9/6		2/5
Age (years)	44.6 \pm 15.7	n.s	54.1 \pm 10.1
Weight (kg)	74.9 \pm 14.2	n.s	76.3 \pm 14.1
BMI (kg/m ²)	23.9 \pm 3.3	n.s	27.3 \pm 4.3
W/H-ratio	0.93 \pm 0.06	n.s	0.90 \pm 0.09

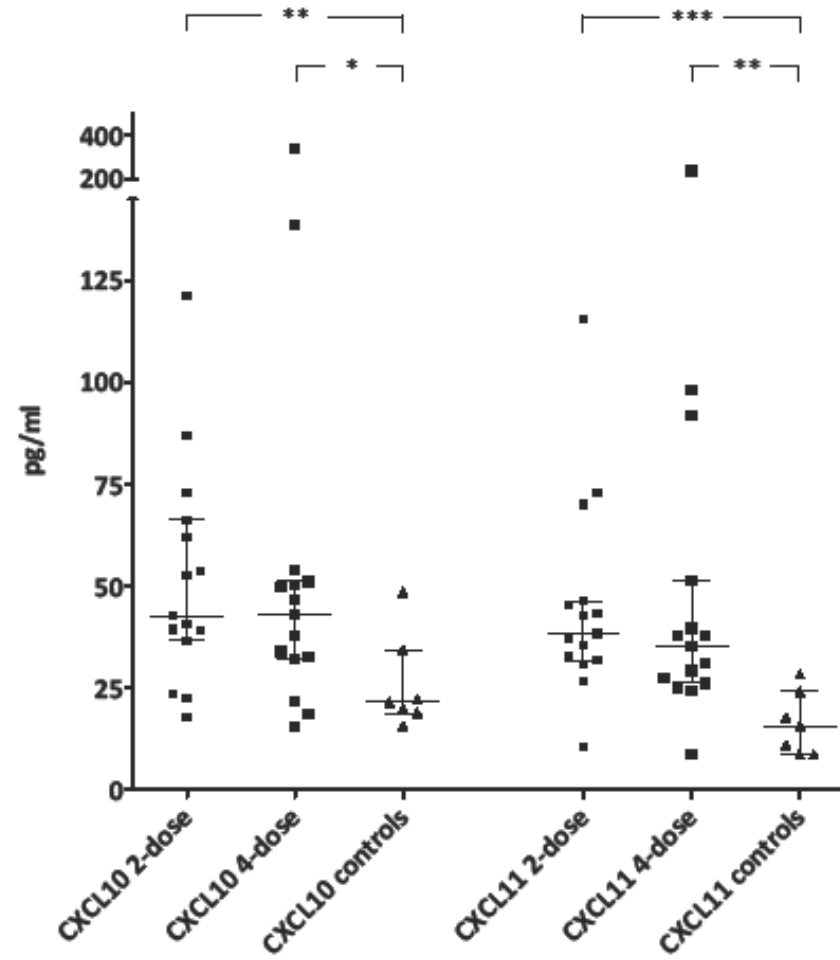
Table 2. Combined values of all chemokine measurements at 16:00, 20:00, 04:00, 07:00 and 10:30 h. Data calculated from both regimens together and each treatment regimen separately in 15 subjects with Addison's disease substituted with twice daily or four times daily hydrocortisone doses. For comparison same measurements were performed in seven controls. Data shown as median (25th - 75th percentile). * p < 0.05, ** p < 0.01 *** p < 0.001 vs. control subjects. Mann-Whitney U test.

pg/ml	Controls	2-dose + 4-dose	2-dose	4-dose
CXCL10 - Th1	22 (19 – 34)	**43 (33-56)	**43 (37 – 66)	*43 (32– 51)
CXCL11 - Th1	16 (9 – 24)	***37 (29-48)	***38 (32 - 46)	**35 (26 – 52)
CCL22 - Th2	214 (173 – 281)	209 (133-259)	227 (127 – 266)	197 (136 – 258)
CCL17 - Th2	60 (46 - 247)	104 (74-133)	106 (75– 157)	103 (74 – 129)

Table 3. Data shown for 15 subjects with Addison's disease treated with twice daily or four times daily hydrocortisone doses. ESS sum, FIS sum and SF-36 domains are correlated against CXCL11 expressed as combined median values of all measurements at 16:00, 20:00, 04:00, 07:00, 10:30 h for both the 2-dose and 4-dose treatment calculated together and for each treatment regimen separately. *p < 0.05, ** p < 0.01. rho= Spearman's rank order correlation coefficient.

Questionnaires	CXCL11 2-dose + 4-dose		CXCL11 2-dose		CXCL11 4-dose	
	rho	p	rho	p	rho	p
ESS sum	0.41	0.033*	0.43	0.14	0.37	0.20
FIS sum	0.09	0.65	0.45	0.13	-0.19	0.52
SF-36						
PF, Physical Functioning	-0.33	0.08	-0.27	0.33	-0.49	0.06
RP, Role Physical	-0.1	0.59	-0,31	0.27	0.22	0.45
BP, Bodily Pain	-0.19	0.32	-0.19	0.5	-0.12	0.68
GH, General Health	-0.49	0.006**	-0.66	0.007**	-0.34	0.21
VT, Vitality	-0.1	0.62	-0.44	0.1	0.24	0.38
SF, Social Functioning	0.03	0.86	-0.23	0.42	0.23	0.42
RE, Role Emotional	0.28	0.13	0.23	0.42	0.37	0.17
MH, Mental Health	0.12	0.52	-0.26	0.35	0.48	0.073
Physical Component Summary (PCS)	-0.50	0.004**	-0.55	0.032*	-0.41	0.13
Mental Component Summary (MCS)	0.34	0.064	0.038	0.89	0.60	0.019*

Fig 1



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