Alleviation of morning joint stiffness by low-dose prednisone in rheumatoid arthritis is associated with circadian changes in IL-6 and cortisol

Aim: The effect of prednisone on the morning joint stiffness of rheumatoid arthritis (RA) is enhanced by night-time (2 am) administration. It has been hypothesized that this may be due to suppression of the pathological early-morning rise in plasma IL-6, but this has not yet been measured. A theoretical disadvantage of night-time prednisone is increased suppression of the hypothalamic–pituitary–adrenal axis and reduced peak plasma cortisol levels, usually attained at approximately 7 am. This study measured 24-h variations in IL-6, other cytokines and cortisol in patients before and after a 2-week course of night-time prednisone to address both these questions. **Materials & methods:** Nine patients with active RA were clinically assessed and had 24-h blood sampling before and after a 2-week course of timed-release tablet (TRT) prednisone (5 mg per day). Patients took the TRT orally at 10 pm and the prednisone was released at 2 am. Changes in circadian variation in cortisol and IL-6 and clinical measures were compared using regression modeling and Wilcoxon matched-pairs signed-rank test. Cytokines IL-1 receptor antagonist, IL-1β, IL-4 and TNF were also measured. **Results:** Significant alterations in the circadian profiles and concentrations of IL-6 and cortisol were observed following TRT prednisone. The estimated peak value of IL-6 fell from 42.2 to 21.3 pg/ml and occurred earlier (8:05 am compared with 1:21 am; $p < 0.005$). Following TRT prednisone, the peak value of cortisol increased from 14.1 to 19.3 μg/dl and the trough fell from 2.9 to 2.1 μg/dl ($p < 0.001$). Clinical symptoms, particularly morning stiffness ($p = 0.028$), were reduced, but in three patients with high concentrations of IL-1 receptor antagonist, IL-1β, IL-4 and TNF, neither IL-6 nor morning stiffness changed. **Conclusion:** Prednisone released at 2 am does suppress the pathological early-morning rise in plasma IL-6 in RA. The nocturnal rise in plasma cortisol was not suppressed but was enhanced, consistent with a changing relationship between hypothalamic–pituitary–adrenal axis and immune system activation. A subset of patients with high concentrations of several cytokines did not respond to prednisone.

KEYWORDS: circadian variation ▪ cortisol ▪ early-morning stiffness ▪ interleukin-6 ▪ rheumatoid arthritis

Lynsey L Clarke*[1], David S Jessop[1], Linda P Hunt[1], Rainer H Straub[1], Mark G Perry[1] & John R Kirwan[1]

[1]Bristol Academic Rheumatology Unit, The Courtyard, Bristol Royal Infirmary, Bristol, BS2 8HW, UK
**Author for correspondence: Tel.: +44 117 342 2515 Fax:+44 117 342 3841 lynsey@doctors.org.uk*

Since their discovery [1], glucocorticoids have been used to treat rheumatoid arthritis (RA) through powerful anti-inflammatory [2,3] and anti-erosive [4,5] properties. In RA, symptoms such as pain, early-morning joint stiffness and joint swelling have a circadian rhythm, with maximum activity in the morning and minimum activity in the afternoon [6,7]. This phenomenon, often described as early-morning stiffness (EMS), is so characteristic of RA that it is considered a basic feature pointing to inflammatory polyarthritis [8]. The synovitis of RA is driven in part by deregulation of the inflammatory process and production of proinflammatory cytokines such as IL-6 and TNF [9].

Early studies by Arvidson *et al.* demonstrated that overnight plasma IL-6 is reduced by 15–20 mg of prednisolone [10] and that lower doses (5–7.5 mg) are more effective at lowering early-morning IL-6 when given at 2 am compared with 7 am [11]. In a recent clinical trial, Buttgereit *et al.* tested the timed-release formulation of prednisone in patients with RA against standard-release prednisone given in the morning [12]. In this timed-release tablet (TRT) formulation, prednisone is released into the gut lumen 4 h (±15 min) after ingestion. This allows patients to take the medication at 10 pm (before going to bed), while drug delivery occurs at 2 am, similar to the Arvidson study but without the need to wake the patients [13]. A significant reduction in early-morning joint stiffness was detectable in the TRT group after 2 weeks and was sustained for 12 weeks. In single morning blood samples taken at 12 weeks, IL-6 was also reduced.

A wide range of systemic proinflammatory actions have been ascribed to IL-6 [14]. In RA patients, IL-6 is substantially elevated in serum and synovial fluid compared with healthy controls [15,16]. Serum IL-6 shows a clear circadian pattern of secretion, with a markedly exaggerated overnight peak reaching a maximum

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around 8 am [10,13,17,18], synchronous with the early-morning inflammatory activity in patients. Therefore, the rise in IL-6 in the early morning has been implicated in the mechanisms underlying EMS. EMS in RA has also been associated with an impaired ability of the hypothalamic–pituitary–adrenal (HPA) axis to secrete adequate amounts of cortisol and to maintain a normal circadian rhythm in response to elevated blood proinflammatory cytokines, particularly at night [19–23]. However, although subtle abnormalities have been reported, the HPA axis and cortisol production in RA is similar to that in normal subjects. Consequently, there is a tonic 'underactivity' of the HPA axis in responding to inflammatory processes in RA, possibly owing to reduced sensitivity to proinflammatory cytokines at the hypothalamic or pituitary level or reduced adrenal sensitivity to adrenocorticotrophic hormone [24,25]. The HPA axis in normal human subjects is stimulated by IL-6. In volunteers, injection of recombinant IL-6 stimulated the production of hypothalamic corticotrophin-releasing factor and increased cortisol release [26]. IL-1 and IL-6 stimulate adrenocorticotrophic hormone release from pituitary cells in culture and IL-6 stimulates cortisol production from cultured adrenal cells, illustrating a potential for multilevel activation by IL-6 of the HPA axis [27]. Therefore, the HPA axis and cortisol secretion in RA patients might be expected to be reset at a higher level of activity in response to elevated IL-6. A defective response of the HPA axis to elevated levels of IL-6 in RA is suggestive of an imbalance in bidirectional interactions between the neuroendocrine and immune systems, which may make a major contribution towards the pathology of RA. Exogenous glucocorticoids suppress the HPA axis and plasma cortisol and it is unclear if nocturnal administration would make this worse by suppressing the physiological morning cortisol rise, or improve it by reducing immune system activation.

We designed this study to test the hypothesis that the significant improvement in EMS in RA patients observed after 2 weeks of TRT treatment [12] is correlated with a reduction in IL-6 production and to discover whether plasma cortisol concentrations are suppressed or enhanced.

Materials & methods

Patients & assessments

The study was approved by the Central and South Bristol Research Ethics Committee UK and was registered with Controlled-Trials.com; ISRCTN 17552423. Nine patients aged 50–80 years with active RA [8] recruited within the University of Bristol Academic Rheumatology Unit at Bristol Royal Infirmary (Bristol, UK) gave informed written consent. Based on previous studies within the department and other published research it was felt that significant alterations in IL-6 circadian variation could be detected in nine to 12 patients [13]. In the event, nine patients were recruited within the study period. Active disease was defined by more than three tender and swollen joints, pain of at least 30 mm as measured on a 100 mm visual analog scale (anchors were no pain to severe pain), C-reactive protein of at least 15 mg/l or erythrocyte sedimentation rate (ESR) of at least 30 mm/h. All patients had erosions on x-rays of hands and feet and all were on stable medical therapy. Patients had received no glucocorticoids by any route in the preceding 3 months and had never been treated with TNF antagonists. Treatment with disease-modifying drugs and other medications had been stable for at least 3 months. Patients underwent further assessment including reporting average duration of EMS over the previous 3 days in minutes, self-reported disability (Health Assessment Questionnaire) [28] and Disease Activity Score (DAS)28 [29]. Patients were admitted on two occasions. A venous cannula was inserted, usually in the antecubital fossa, at least 1 h before blood samples were taken over the following 24 h. Blood samples were taken at 4:30 pm, 6 pm, 7:30 pm, 9 pm, 10 pm, 10:45 pm, 11:45 pm, 12:45 am, 1:45 am, 2:45 am, 3:45 am, 4:45 am, 6 am, 7 am, 8 am, 9:30 am, 11:30 am, 1 pm, 2:30 pm and 4:30 pm. Sampling was more frequent during periods in which previous work had suggested there would be the greatest change [10,13], therefore these intervals were not equally spaced. Overnight samples were taken with as little disturbance to the patient as possible. Following the first admission for blood sampling, the patients took 5 mg of TRT prednisone each evening at 10 pm for 2 weeks and the clinical assessments and overnight blood sampling were then repeated.

Laboratory measurements

Samples were collected into chilled ethylenediaminetetraacetic acid blood collection tubes and centrifuged at 5750 rpm for 7 min immediately after collection. Plasma was separated and stored at -20°C before transfer to -80°C within 25 h of collection. Cytokines were measured by Luminex Multiplex Detection technology using Beadlyte reagents (Lincoplex, MO, USA). The inter- and intra-assay coefficients of variation with this technique are below 10% for

all cytokines measured. Lower limits of detection were 0.79 pg/ml for IL-6, 2.87 pg/ml for IL-4, 0.32 pg/ml for IL-8, 0.41 pg/ml for IL-10, 0.22 pg/ml for TNF, 7.47 pg/ml for IL-1 receptor antagonist (IL-1ra) and 0.19 pg/ml for IL-1β.

Plasma cortisol was measured by Radioimmunometric assay (Coulter Immunotech, Marseilles, France). Intra- and inter-assay coefficients of variation were below 10%. Lower limit of detection was 0.3 µg/dl and cross-reactivity with prednisolone was less than 6%. Prednisone and prednisolone were therefore measured in all samples from the post-treatment night using the high-performance liquid chromatography tandem mass spectrometry method. Lower limits of detection were 237 ng/l for prednisone and 986 ng/l for prednisolone. Cortisol measurements in each sample were then corrected by subtraction of 6% of the measured prednisolone at each time point to allow for maximum possible cross-reactivity.

Statistical analysis

Cortisol and cytokine measurements were logarithmically transformed (log base 10) prior to analysis to reduce skewness and render their distributions more 'normal'. Zero values were replaced by the lower limit of detection to allow log transformation. Geometric means (antilog of the mean log values) and ranges were used for data summary. Preliminary analysis included repeated measures of analyses of variance (ANOVA) to identify cytokines showing circadian variation over either the pre- or post-treatment 24-h periods. Those measures showing circadian variation were further investigated by statistical modeling techniques to assess differences in the 24-h patterns before and after TRT medication. We used random coefficient cubic regression or harmonic models, depending on the shape of the 24-h profile.

The 'random coefficient' cubic regression model allowed regression coefficients (up to power 3) to vary between patients and a mean profile to be estimated; such models have previously been used by us [13]. We further allowed for variation between the two nights within the same patient [30]. Models were fitted using the 'proc MIXED' facility in the software package SAS (SAS v 9.1, SAS Institute Inc., NC, USA).

Random coefficient harmonic models can be fitted using linear or nonlinear approaches [31]. Despite subtle differences between the approaches, we obtained similar results (data not shown) and describe only the former. Harmonics up to the third were used as they were believed to be sufficient for any number of observations per day [32]. For example, a harmonic model for a given individual's cortisol was:

$$\log_{10}Cortisol = \mu + \alpha_1 Cos(2\pi t - \alpha_2) + \alpha_3 Cos(4\pi t - \alpha_4) + \alpha_5 Cos(6\pi t - \alpha_6)$$

where t was the fraction of a day from midnight (i.e., t = hours from midnight/24, thus ranging from -7.5/24 to 16.5/24). This was converted into the linear model:

$$\log_{10}Cortisol = \mu + \beta_1 r_1(t) + \beta_2 s_1(t) + \beta_3 r_2(t) + \beta_4 s_2(t) + \beta_5 r_3(t) + \beta_6 s_3(t)$$

by using the following functions of t as predictors:

$$r_1(t) = Cos(2\pi t); s_1(t) = Sin(2\pi t); r_2(t) = Cos(4\pi t); s_2(t) = Sin(4\pi t); r_3(t) = Cos(6\pi t); s_3(t) = Sin(6\pi t)$$

The values of µ and the βs were assumed to vary normally across patients. The same method of fitting was used as for the random coefficient cubic regression (above).

Clinical measures at baseline and the end of week 2 were compared using the nonparametric Wilcoxon matched-pairs signed-rank tests. Spearman's correlation coefficients were calculated to relate changes in mean cytokine levels and clinical measures after 2 weeks of TRT treatment.

Results

Clinical data

Nine patients took part and are described in Tables 1 & 2 (one patient was included with a slightly lower pain score of 15). Although this study was powered to detect changes in IL-6 rather than small changes in clinical measures, there was nevertheless a statistically significant reduction in mean duration of EMS (p = 0.028), pain (p = 0.008) and ESR (p = 0.012) after 2 weeks of TRT treatment (Table 2). The three patients with baseline values of only 60 min showed no change in EMS.

Preliminary analysis (not shown) found that IL-6 was the only cytokine to show circadian variation. The cubic regression model gave the best fit for IL-6 and better accommodated a small, but statistically significant, upwards 24-h drift. There was negligible between-patient variation in the cubic term and this term was regarded as 'fixed' in the random coefficient model. A few values of IL-6 below the limit of detection for IL-6 (<0.79) were arbitrarily assigned values of 0.5 in these analyses, although similar results were obtained when they were set to 0.79.

Table 1. Individual patient characteristics.

Patient	Sex	Age (years)	Years diagnosed	Rheumatoid factor titer	Erosions on x-ray	Taking NSAIDs	Current DMARD
A	F	51	22	160	Yes	Yes	None
B	F	59	17	–	Yes	Yes	SS
C	M	69	17	40	Yes	Yes	MTX
D	F	74	28	–	Yes	No	MTX
E	F	54	27	–	Yes	Yes	MTX
F	M	79	0	–	Yes	No	MTX
G	F	61	16	640	Yes	Yes	MTX
H	F	76	24	2560	Yes	Yes	None
I	M	60	2	320	Yes	Yes	LEF

Blank cell indicates rheumatoid factor negative.
F: Female; LEF: Leflunomide; M: Male; MTX: Methotrexate; SS: Sulphasalazine.

The estimated mean IL-6 profile before and after treatment is shown in Figure 1. Following treatment there were significant changes with respect to the linear, quadratic and cubic coefficients, but not the mean at baseline (which here was taken as midnight). Taking all four components together indicated a significant change in shape ($p < 0.005$; likelihood ratio test), with a reduction in the peak mean value from 42.2 to 21.3 pg/ml (Figure 1B). This peak also occurred earlier in time following TRT treatment (8:05 am shifted to 1:21 am).

Changes in 24-h mean IL-6 and EMS were correlated ($r = 0.81$; $p = 0.01$), suggesting a link between these two variables. The change in mean IL-6 and ESR was also correlated ($r = 0.73$; $p = 0.025$).

There was a significant change in the mean pattern of cortisol following TRT medication ($p < 0.001$), with estimated peak cortisol changing from 14.3 to 19.3 µg/dl and estimated trough changing from 2.8 to 2.1 µg/dl (Figure 2). Harmonics up to the third were needed to fully describe the shape but in the random coefficient models it was only possible to allow coefficients of the first and second harmonics to vary amongst patients, coefficients for the third thus being 'fixed'. When the mean curves are shown on the same graph (Figure 2B), afternoon and evening cortisol shows suppression, as might be expected with exogenous glucocorticoid use. However, the overnight/early-morning peak appears amplified even after correcting for any cross-reactivity with the TRT medication.

Three patients had high concentrations of IL-1β, IL-1ra, IL-4 and TNF and these patients did not show improvement in their EMS after taking the TRT medication (mean 24-h values shown in Figure 3). There were no clinical features that distinguished these patients from the patients who showed improvement in EMS, although the rheumatoid factor titer was higher in the former group. These cytokines were below the limit of detection in most patients and in those in whom they were measurable no circadian variation was detected. In addition, there was no suppression of these cytokines after 2 weeks of TRT medication.

Table 2. Median values (range) for clinical measures before and after 2 weeks of timed-release tablet treatment.

Variable	Pre-TRT	Post-TRT	p-value
PV	1.78 (1.54–2)	1.71 (1.48–1.9)	0.53
ESR	40 (10–80)	34 (4–54)	0.012†
CRP	14 (9–76)	9 (9–75)	0.018†
TJC (28 joints)	16 (7–25)	15 (3–20)	0.15
SJC (28 joints)	9 (3–13)	4 (1–15)	0.068
EMS (min)	180 (60–480)	60 (10–60)	0.028†
Pain (1–100 mm)	68 (15–80)	40 (6–73)	0.008†
DAS-28 (CRP)	5.52 (5.09–6.28)	4.46 (3.71–6.87)	0.028†
HAQ	1.875 (1.75–2.88)	1.875 (1.63–3)	0.11
Mean improvement in EMS (6 patients)		54%	0.01†

†Results statistically significant at the 5% level.
p-values are for the comparison of pre- and post-treatment values using Wilcoxon matched-pairs signed-rank test.
CRP: C-reactive protein; DAS: Disease Activity Score; EMS: Early-morning stiffness; ESR: Erythrocyte sedimentation rate; HAQ: Health Assessment Questionnaire; PV: Plasma viscosity; SJC: Swollen joint count; TJC: Tender joint count; TRT: Timed-release tablet.

Discussion

We have demonstrated that the alleviation in morning stiffness in RA patients following nighttime administration of prednisone is closely associated with a decrease in plasma levels and an alteration in the circadian rhythm of IL-6. Tablets timed to release a low dose of prednisone at 2 am resulted in significant normalization of the circadian rhythm of IL-6, with an approximately 50% reduction in peak levels. Reduced secretion of this strongly proinflammatory

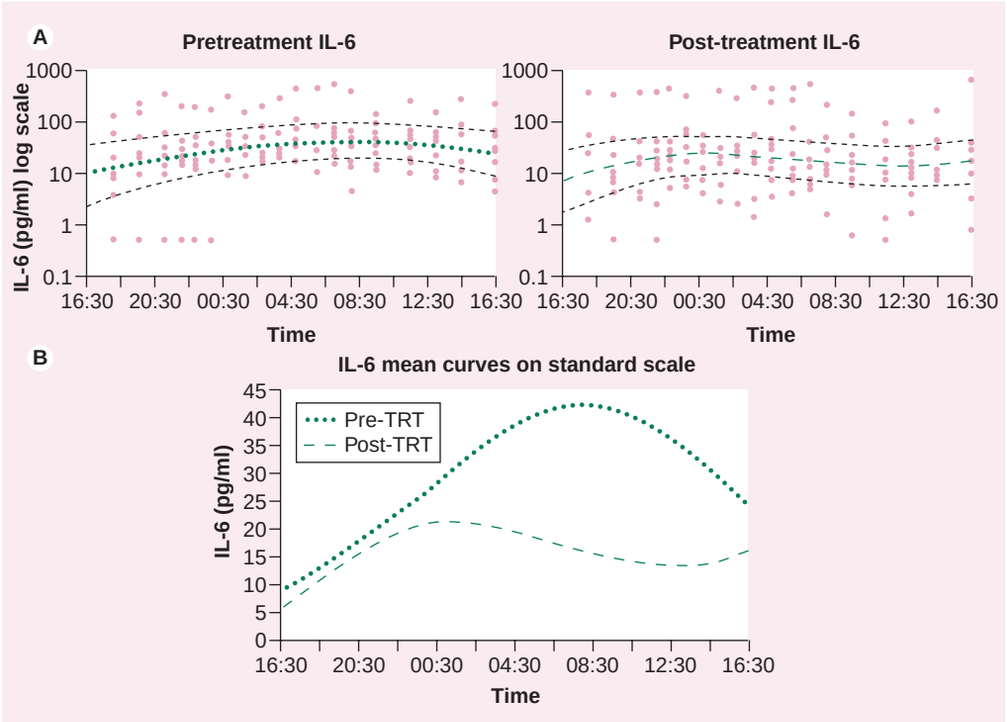

Figure 1. IL-6 concentrations over 24 h. (A) Estimated mean IL-6 concentrations in nine patients before timed-release tablet prednisone (dots) and after TRT prednisone (dashes) with 95% CIs shown with lighter type broken lines. The absolute concentrations for all patients are shown as background dots on the logarithmic scale. Equations for the curves shown are: Pretreatment: $\log_{10}(\text{IL-6}) = 1.4334 + 1.0847 \times t - 1.3636 \times t^2 - 0.4848 \times t^3$; Post-treatment: $\log_{10}(\text{IL-6}) = 1.3188 + 0.3323 \times t - 3.2563 \times t^2 + 3.6636 \times t^3$, where 't' is a fraction of a day from midnight (see materials and methods section in text). **(B)** Estimated mean IL-6 concentrations on a standard scale demonstrating difference in shape between pre- and post-TRT prednisone treatment curves.
TRT: Timed-release tablet.

cytokine is consistent with a functional role for IL-6 in mediating the anti-inflammatory effects of prednisone in RA, which may be a direct link or operate via other factors.

Although this study has few patients and was not designed to detect clinically significant changes, duration of EMS in the patient group as a whole was significantly reduced following TRT treatment. This further demonstrates the importance of timing of relatively low doses of glucocorticoid treatment in improving symptoms. In addition, as the reduction in EMS following TRT treatment correlated with a reduction in IL-6 and none of the other cytokines measured had reduced levels following TRT, this suggests that the change in EMS is specifically linked with IL-6. It is not possible to say if this is a direct effect on IL-6 release from lymphocytes or via other factors not measured within this study. This study examined circulating cytokines but many cytokines act in a paracrine fashion and we have not examined cytokines released and acting at the local intra-articular level.

We observed in detail a circadian rhythm in blood IL-6 in RA patients before treatment similar to that which has been previously reported in outline [10,13,17,18]. The peak value for IL-6 at 8 am prior to TRT treatment accords well with other reports [10,13,18] and is synchronous with the major symptoms of EMS. In addition to a reduction in IL-6 following TRT treatment, a shift in peak value in the circadian rhythm of IL-6 was observed. The shift in peak IL-6 to around 1 am that we observe in TRT patients has not been previously reported following glucocorticoid treatment and may be significant in ameliorating EMS.

We also observed a significant change in the circadian profile of cortisol following TRT, with an increase in the slope of the cortisol rise and a higher peak cortisol concentration. Very few studies with cortisol data from frequent overnight sampling of RA patients have been published. The circadian profile of cortisol in our pre-TRT results shares a similar pattern with the cortisol reported in five untreated RA patients by Crofford *et al.* with slightly elevated night-time

levels and a putative flattened slope in RA, compared with five healthy controls [18]. A flattened cortisol circadian rhythm has been reported as characteristic of a range of chronic illnesses such as cancer and depression [33,34] and a significant sharpening of the profile, with lower trough and higher peak values, may be more representative of normal healthy humans.

Although the improvement in EMS following TRT is undoubtedly primarily due to the anti-inflammatory effects of prednisone (or prednisolone as the active metabolite), it is also conceivable that endogenous cortisol contributes to this effect. Fast feedback inhibitory effects of glucocorticoids have long been recognized to not be primarily dependent on absolute concentrations but rather on rate of change [35,36]. Glucocorticoids exert potent inhibitory effects on the secretion of proinflammatory cytokines such as IL-6. The slower rate of increase in blood concentrations of cortisol between 2 am and 6 am, which we have observed in RA patients prior to TRT, may blunt this inhibitory effect. Therefore, the steeper rate of increase in blood cortisol between 2 am and 6 am in patients following TRT is consistent with a contribution towards reducing blood IL-6 concentrations and contributing to an alleviation of EMS. Further research is necessary to determine whether normalizing the circadian rhythm of cortisol in RA by increasing the amplitude of the early-morning rise through modified-release glucocorticoid preparations may be clinically beneficial [37].

Not all patients responded to the medication either clinically or with a reduction in IL-6 as three of the nine patients had no change in either. These same three patients also had high levels of IL-1β, IL-1ra, IL-4 and TNF. The significance of this finding is not clear and in this small group of patients it was not related to medication, comorbidities or disease duration. Rheumatoid factor titer may be relevant as the three patients who did not respond were those with the highest rheumatoid factor. It would be interesting to know

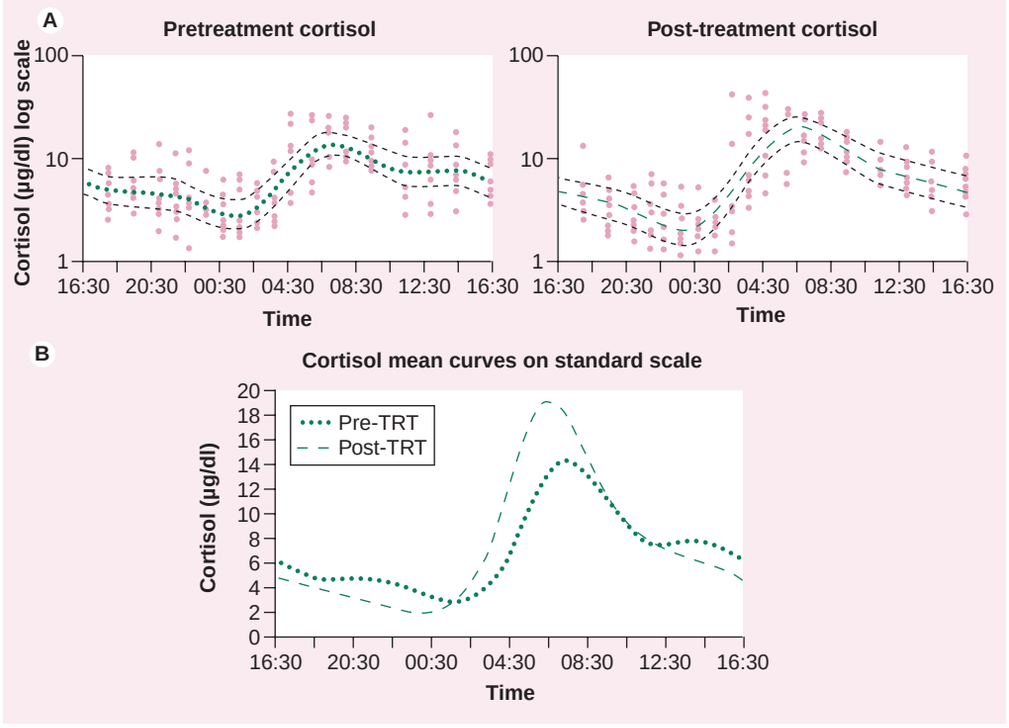

Figure 2. Cortisol concentrations over 24 h. (A) Estimated mean cortisol concentrations in nine patients before TRT prednisone (dots) and after TRT prednisone (dashes) with 95% CIs shown with lighter-type broken lines. The absolute concentrations for all patients are shown as background dots on the logarithmic scale. Equations for the curves shown are: Pretreatment: $\log_{10}$Cortisol = 0.7919 - 0.2141 Cos(2π t) + 0.1112 Sin(2π t) - 0.09970 Cos(4π t) - 0.07157 Sin(4π t) + 0.02655 Cos(6π t) - 0.08086 Sin(6π t); Post-treatment: $\log_{10}$Cortisol = 0.7703 - 0.2525 Cos(2π t) + 0.2658 Sin(2π t) - 0.17498 Cos(4π t) - 0.02535 Sin(4π t) - 0.02452 Cos(6π t) - 0.05579 Sin(6π t), where 't' is a fraction of a day from midnight (see materials and methods section). **(B)** Estimated mean cortisol concentrations in nine patients on a standard scale demonstrating difference in shape between pre- and post-TRT prednisone treatment curves.
TRT: Timed-release tablet.

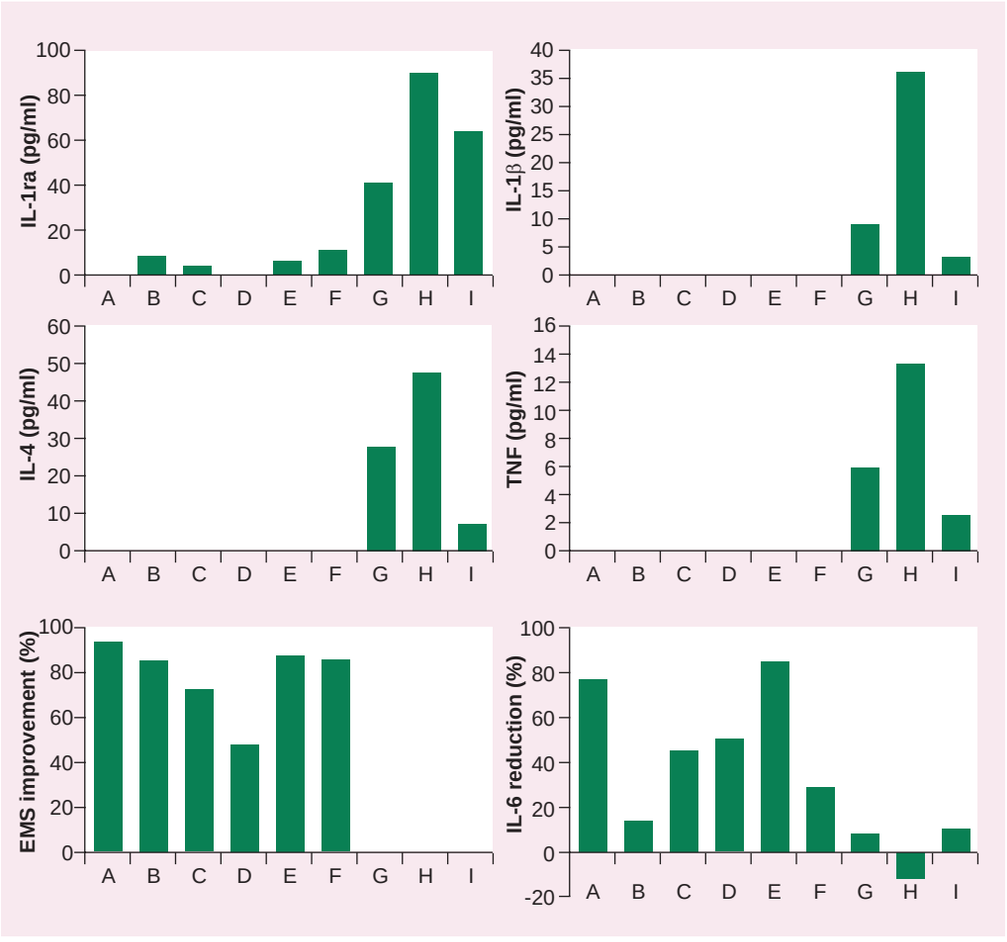

Figure 3. Mean 24-h pretreatment values for IL-1 receptor antagonist, IL-1β, IL-4 and TNF with individual patients labeled A–I. EMS and IL-6 charts show percentage improvement (reduction) in pre- versus post-timed-release tablet prednisone treatment with individual patients labeled A–I.
EMS: Early-morning stiffness; IL-1ra: IL-1 receptor antagonist.

anticyclic citrullinated peptide antibody status, although this was not measured in this study. It is possible that this different cytokine profile is a marker of a specific subset of RA patients and/or of glucocorticoid resistance. A wider survey of blood cytokines in a range of patients with different clinical characteristics and different responses to glucocorticoid therapy would address this issue.

Conclusion

We have demonstrated that overnight and early-morning plasma IL-6 is significantly decreased after 2 weeks of 5-mg TRT prednisone and that this change is correlated with improvement in EMS. We have also shown that the circadian rhythm of cortisol is not suppressed, but is enhanced. The dynamic interplay of IL-6 and cortisol in terms of amounts secreted and circadian profiles is evidence for a coordinated neuroimmune response to inflammation, which may be crucial in regulating EMS in RA. We propose that these changes in IL-6 and cortisol during the early morning, prior to the usual onset of EMS, are functionally important in mediating the improvement in EMS following prednisone in RA patients.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Early-morning stiffness (EMS) is characteristic in rheumatoid arthritis (RA).
- EMS and IL-6 are likely to be linked.
- IL-6 has a circadian rhythm of variation in RA, with peak levels occurring in the early morning.
- Alleviation in EMS following night-time timed-release tablet (TRT) prednisone is closely correlated with decreased plasma IL-6 on waking.
- In this study, 5 mg of TRT prednisone suppressed plasma IL-6 measured throughout the night.
- 5 mg of TRT prednisone does not cause suppression of the overnight cortisol peak.
- The rate of increase and the peak level of overnight cortisol is enhanced by 5 mg of TRT prednisone.
- 'Nonresponders' have high levels of other plasma cytokines.

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