Effects of treatment with etanercept *versus* methotrexate on sleep quality, fatigue and selected immune parameters in patients with active rheumatoid arthritis

J. Detert[1], R. Dziurla[1,2], P. Hoff[1,2], T. Gaber[1-3], P. Klaus[1], H. Bastian[1], T. Braun[1], S. Schellmann[1,2], T. Penzel[4], I. Fietze[4], P.-A. Loeschmann[5], P. Jaehnig[6], R.H. Straub[7], G.R. Burmester[1], F. Buttgereit[1]

[1]Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin; [2]German Rheumatism Research Center (Deutsches Rheumaforschungszentrum, DRFZ), Berlin; [3]Berlin-Brandenburg Center for Regenerative Therapies (BCRT), alliance between the Charité-Universitätsmedizin Berlin and the Helmholtz Association, Berlin; [4]Sleep Medicine Center, Charité-Universitätsmedizin Berlin; [5]Medical Director Global Innovative Pharma, Pfizer Pharma GmbH, Berlin, Germany; [6]pj statistics, Berlin, Germany; [7]Laboratory of Experimental Rheumatology and Neuroendocrino-Immunology, Department of Internal Medicine I, University Hospital Regensburg, Regensburg, Germany.

Abstract

Objective

To compare sleep quality, disease activity and patient-reported outcomes such as fatigue and immune parameters in patients with rheumatoid arthritis treated with etanercept (ETA) or methotrexate (MTX).

Methods

Of 36 patients (28-joint Disease Activity Score, $DAS28_{CRP} \geq 3.2$) in this 16-week (w), open, prospective study, 19 (11 women) received MTX 12.5–17 mg/w, and 17 (14 women) received ETA 25 mg x 2/w, alone or in combination with MTX. Clinical ($DAS28_{CRP}$, visual analogue scale), laboratory (C-reactive protein [CRP]), sleep (polysomnography), functional (Multidimensional Fatigue Inventory; Health Assessment Questionnaire-Disability Index (HAQ-DI); 36-item Short-Form Health Survey (SF-36), immunological (humoral/cellular) and neuroendocrine (hormonal) parameters were recorded at baseline (BL), w8 and w16.

Results

BL characteristics did not differ significantly between the ETA and MTX groups except disease duration: mean age (years): 48.6±8.8 vs. 49.4±16.6; mean disease duration (months): 19.6±46.3 vs. 81.2±79.2; and $DAS28_{CRP}$: 4.4±0.9 vs. 4.4±1.7, respectively. $DAS28_{CRP}$, SF-36, and HAQ-DI improved significantly in both groups from BL to w16 ($p \leq 0.05$).
The $DAS28_{CRP}$ improvements at w16 (mean changes -1.8 in the ETA group, and -1.4 in MTX group), were not statistically significant from each other. The absolute values of sleep efficiency, total sleep time, and stage 2 sleep duration increased significantly in the ETA group, but no significant changes were reported in the MTX group.

Conclusion

Both therapies improved disease activity, CRP, SF-36 and HAQ-DI, with faster, more pronounced changes in $DAS28_{CRP}$ in the ETA group, which alone had significantly improved sleep parameters.

Key words

treatment with etanercept versus methotrexate, rheumatoid arthritis, sleep quality, fatique, immune response, endocrinology, interleukin

Jacqueline Detert, MD, MHBA
René Dziurla, MD
Paula Hoff, MD
Timo Gaber, Dr. rer. nat.
Pascal Klaus, MD
Hans Bastian, MD
Tanja Braun
Saskia Schellmann, MD
Thomas Penzel, MD
Ingo Fietze, MD
Peter-Andreas Loeschmann, MD
Peter Jaehnig
Rainer H. Straub, MD
Gerd R. Burmester, MD
Frank Buttgereit, MD

Please address correspondence to:
Jacqueline Detert, MD, MHBA,
Charité-Universitätsmedizin Berlin,
Department of Rheumatology
and Clinical Immunology,
Charitéplatz 1,
10117 Berlin, Germany.
E-mail: jacqueline.detert@charite.de

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease that primarily affects joints, leading to pain, swelling, joint destruction, deformities and disability. Key factors in RA are pro-inflammatory and anti-inflammatory cytokines, which follow circadian rhythms (1, 2). Among others, tumour necrosis factor (TNF) is part of a complex biochemical cascade regulating sleep (3). Extra-articular symptoms such as poor sleep quality and fatigue occur, contributing to reduced quality of life (QoL) and physical function. In fact, chronic sleep disturbance is associated with increased morbidity and mortality (4). A relationship between sleep restriction and sensitivity to pain has also been reported (5), and sleep disturbance may result in increased musculoskeletal pain in patients with RA (6, 7).

Fatigue is common among RA, with clinically relevant levels present in 41% of patients (8). Fatigue has been linked with discomfort, including joint pain and sicca symptoms, and poor sleep (8) and several studies have found sleep fragmentation in RA patients, contributing to inefficient sleep, frequent awakenings and poor sleep quality (9-13). Sleeping problems are reported by 50–75% of RA patients and are thus two to three times more prevalent among RA patients than the general population (13). Patients commonly report difficulty falling and staying asleep, non-restorative sleep and excessive daytime sleepiness (14). Among possible causes of sleep disturbance among patients with RA are inflammatory disease activity and arthralgia, which may cause to greater pain, disease activity and mood disturbance (15). Authors of recent publications agree that the relationship between sleep disturbance and pain is most likely bidirectional (16–18). However, the translational importance and the nature of this relationship is not well understood (17). Further experimental and clinical studies are therefore needed to understand this relationship.

It can be hypothesised that treating RA with conventional synthetic (csDMARD) and biologic disease-modifying anti-rheumatic drugs (bDMARD) should improve not only disease activity and articular symptoms but also important extra-articular manifestations such as fatigue and sleep disturbance. The results of a pilot study showed that tocilizumab (TZB) decreased the disease activity as well as the fatigue, and functional status improved significantly. The authors concluded that increase of sleep quality after TZB treatment in RA patients does not appear to directly result from decreased disease activity, suggesting that aberrant interleukin (IL)-6 regulation is associated with sleep disturbances (19, 20). Other authors demonstrated that neutralising TNF activity is associated with a significant reduction of objective sleepiness in obstructive sleep apnea (19). Moreover, it has been reported that most patients with moderate-to-severe psoriasis have impaired sleep and that treatment with etanercept (ETA) significantly improves sleep in patients This open, prospective study involved patients with active RA according to phase I or II of the RA management algorithm based on the European League Against Rheumatism (EULAR) recommendations (21). Effects of therapies with methotrexate (MTX) and ETA (alone or combined) on sleep quality, as determined by polysomnography (PSG) and other clinical and laboratory surrogate parameters were assessed.

Methods

Trial design

In this 16-week (w), prospective, non-randomised study, eligible patients received ETA (2 x 25 mg weekly as monotherapy or combination therapy with MTX) or MTX (12.5–17 mg weekly) between March 2007 and July 2009. The protocol was approved by the ethics committee of Charité-Universitätsmedizin Berlin. The trial is registered at http://register.germanctr.de, number DRKS00000150.

Participants

Patients aged 18–70 years with a RA diagnosis according to the criteria of the American College of Rheumatology (1987) were eligible for inclusion

(22). Active disease (28-joint Disease Activity Score, $DAS28_{CRP}$, ≥3.2, morning stiffness ≥30 min) and meeting the criteria for initiation of either ETA or MTX according to German treatment guidelines were required. Stable non-steroidal anti-inflammatory drug and glucocorticoid treatment was required before and during the trial (prednisone or equivalent ≤10 mg/day). In addition, other medications, namely Sulfasalazine and Hydroxychloroquine, and their dosages are shown in Table I. The detailed screening procedure to prevent dropout after the first night of PSG is described in supplemental methods (Supp. 1). available at *www.clinexprheumatol.org*. All patients were assessed for clinical, laboratory and sleep parameters by PSG at BL, w8 and w16. Secondary and/or accompanying rheumatic diseases (such as secondary Sjögren's syndrome or fibromyalgia) were not defined as being an exclusion criterion. However, in case the study team became aware of such conditions, they were recorded as part of the patient history.

Outcomes and follow-up

At the screening and study visits (BL, w8,w16), patients underwent physical examination, including parameters to determine disease activity based on C-reactive protein ($DAS28_{CRP}$) (23) with tender joint count (TJC) and swollen joint count (SJC); 20-item Multidimensional Fatigue Inventory (MFI–20) (24); Health Assessment Questionnaire-Disability Index (HAQ-DI) (25); QoL 36-item Short-Form Health Survey (SF-36) (26, 27); laboratory tests such as erythrocyte sedimentation rate (ESR), CRP, rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA); safety parameters such as blood cell count, and liver and kidney values; and global disease activity of physician, patients assessed pain and global disease activity with 0–100 mm visual analogue scales (VAS). The primary outcome variables were measured at w16 and included assessment of sleep parameters by PSG, fatigue by MFI-20, and well-being by SF-36 and $DAS28_{CRP}$. Secondary end points evaluated after w8 and/or w16 were $DAS28_{CRP}$, sleep parameters, fatigue (24), joint count, VAS, CRP, HAQ-DI and SF-36. The evaluation of primary and secondary outcomes was performed at the same time of the day, *i.e.* between 8 and 9 am after the end of the PSG.

Sleep

Nocturnal in-laboratory PSG was performed at baseline, w8 and w16 using the Embla N7000 (TNI Medical) and Alice 4 (Philips) systems. Each PSG study night was preceded by one adaptation night. During the first adaptation night, obstructive sleep apnoea and periodic limb movements (PLM) were assessed. Bedtime was between 10 pm and 7 am with a standardised bedtime of 8 hours which could vary from 10 pm to 11 pm and from 6 am to 7 am dependent on individual preferences. The following parameters were recorded with each PSG: brain activity (electroencephalography), eye movements (electro-oculography), muscle activity (electromyography), heart rhythm (electrocardiography) and respiration (respiratory airflow, respiratory effort, and flow signal via nasal cannula and peripheral pulse oximetry).

PSG was performed according to international standards and analysed visually by a trained sleep technician using Rechtschaffen and Kales criteria (28). Sleep parameters calculated were sleep efficiency (total sleep time [TST]/total recording time x 100; min, percentage) (29); sleep stages (S1 to S4, rapid eye movement, REM; min, percentage); wake time before sleep onset (min); wake time after sleep onset (WASO; min); number of sleep stage changes; and TST (min). The authors include measure of apnoea-hypopnoea index (AHI), and PLMs/PLM index (PLMI) as well as other PSG measurements in the methods but this follow data is not reported in the results. Sleep-disordered breathing (obstructive, central and mixed apnoea and hypopnoea) was measured (see exclusion criteria) using standard criteria (30) to calculate the AHI. The PLMI was calculated according to standard criteria (31). PLM arousal index (PLMAI) was detected additionally if PLMI was greater than 20 per hour of sleep.

Blood samples, multichannel flow cytometry and measurements of cytokines and neuroendocrine-immunological parameters

Peripheral blood samples and serum were collected at each visit for fluorescence–activated cell sorting (FACS) analysis. Serum tubes were centrifuged and serum stored at -80°C until cytokine/hormone analysis could be performed. Peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation (Ficoll™ , PAA Laboratories GmbH, Cölbe, Germany) from lithium heparin tubes. Blood sampling was always performed at the same time, *i.e.* between 8 and 9 am in order to minimise circadian effects.

Details on measured cellular and humoral parameters, including the techniques used, appear in supplementary data (Supp. S2) (32).

Sample size and statistical analyses

Sleep efficiency was the primary outcome parameter. According to Ohayon *et al.* the sleep efficiency varies from 95% to 82% (age from 18–70 years) as studied here (33). We assumed a BL sleep efficiency of approximately 80% in both groups and an improvement in sleep efficiency to 88% in the control group (MTX) and to 95% in the intervention group (ETA), respectively. Thus, the expected difference between the two treatment groups (considered as normally distributed) to be detected was 7 percentage points. A minimal sample size of 15 patients per group already resulted in 95% power to detect an effect size of 1.4 when using a two-group t test with a 0.05 two-sided significance level of 5% for the difference (*i.e.* 7%) of two independent means of sleep efficiency with a common standard deviation (SD) of 5. In this study, 20 patients per group were enrolled.

No imputations or changes to the original data were performed besides replacing values below limit of quantitation (LoQ) by half the LoQ value for laboratory variables. Observed data and changes from BL (CfB) were summarised using descriptive statistics and visualised in box plots. For CfB, *p*-values of a one-sample *t*-test (H0: CfB=0) were calculated. Group differ-

ences together with the respective 95% CIs and the corresponding *p*-values of two-sample *t*-tests (H0: MTX minus ETA=0) were derived by using AN(C)OVA methods. No adjustment for multiplicity was performed.

Results

Patient and disease parameters at baseline and change from baseline to w16

Forty patients participated in this trial, among which 36 could be evaluated after w16 as one patient withdrew consent after screening and three patients after the first PSG. All but one patient in the ETA group received ETA plus MTX.

BL characteristics for the two groups did not differ significantly except for disease duration ($p \leq 0.001$, Table I; Fig. 1; Table SI). $DAS28_{CRP}$, SJC and $VAS_{disease\text{-}activity}$ improved significantly in both groups to w16 (CfB: $p \leq 0.01$) (Fig. 1; Table SI). The difference in $DAS28_{CRP}$ improvements at w16 (mean $DAS28_{CRP\text{-}ETA}$=-1.8, mean $DAS28_{CRP\text{-}MTX}$=-1.4) was not statistically significant as well as the difference in mean reductions of CRP (p=0.943). Within the MTX group (CfB: $p \leq 0.1$), and within the ETA group (CfB: $p \leq 0.02$) demonstrated a significant reduction in mean CRP to w16. At the end of trial, $SF\text{–}36_{total}$ (p=1.0) and HAQ–DI (p=0.88) showed no significant differences between the both group. However, within both groups significant CfB to w16 of $SF\text{-}36_{total}$ (p_{ETA}=0.01; $p_{MTX} \leq 0.01$) and HAQ-DI (p_{ETA}=0.01; $p_{MTX} \leq 0.01$) were observed (Fig. 2; Table SII) (34–36).

Sleep parameters

In ETA patients, mean sleep efficiency (BL=79.3% *vs*. w16=6.5%; $p_{CfB} \leq 0.01$), TST (BL=432.5 min *vs*. w16=447.4 min; $p_{CfB} \leq 0.1$) and mean S2 (BL=130.8 min *vs*. w16=167.9 min; $p_{CfB} \leq 0.01$) increased significantly to w16, respectively (Fig. 3; Table II; Fig. S1; Table SIII). Thus, WASO value was shortened (BL=75.5 min, w16=40.3 min; $p_{CfB} \leq 0.01$) (Table SIII).

In the MTX group, the absolute values of the sleep efficiency (BL=84.0% *vs*. w16=83.0%; p_{CfB}=0.71), TST and duration of all sleep phases (S1-S4, REM) and WASO showed no significant CfB to w16 (Table SIII). Also, sleep efficiency remained stable in the MTX-group (Fig. 3). The effects on changes in sleep efficiency described in both groups were found to be influenced by treatment of patients at w16 (p_{CfB}=0.033), but not by age (p=0.231). The effects on changes in S2 described in both groups were found to be influenced by treatment of patients at w16 (p_{CfB}=0.062).

Effects on fatigue

In the ETA group only, the MFI–20 values for both physical (p_{CfB}=0.02) and mental fatigue (p_{CfB}=0.05) were substantially reduced from BL to w16, respectively (Fig. 2; Table SII). However, in both groups, MFI-20 in all categories at each time point was assessed higher than in published reference data of healthy (35). HAQ–DI ($p_{CfB\text{-}MTX} \leq 0.01$; $p_{CfB\text{-}ETA} \leq 0.01$), $SF\text{-}36_{total}$ ($p_{CfB\text{-}MTX} \leq 0.01$; $p_{CfB\text{-}ETA}$=0.01) and $SF\text{-}36_{physical}$ ($p_{CfB\text{-}MTX} \leq 0.01$; $p_{CfB\text{-}ETA} \leq 0.01$) improved significantly in both groups from BL to w16 except for the $SF\text{–}36_{mental\text{-}health}$ with no differences ($p_{CfB\text{-}MTX}$=0.13; $p_{CfB\text{-}ETA}$=0.39) between the groups (Fig. 2; Table SII).

Table I. Demographic and clinical characteristics at baseline.

	n	mean±SD	range	difference ETA *vs*. MTX	
				95%CI	*p*
Demographic characteristics					
Patients					
MTX	19 (♀:11; ♂8)				0.112
ETA	14 (♀14; ♂3)				
Mean age (years)					
MTX	19	48.6 ± 8.8	31≤48≤66	44.3;52.8	0.637
ETA	17	49.4 ± 13.6	22≤51≤71	42.5; 56.3	
Mean disease duration (months)					
MTX	18	19.6 ± 46.3	22≤2≤184	-3.5:42.6	≤0.001
ETA	17	81.2 ± 79.2	7≤39≤237	40.5; 121.9	
Treatment regimes					
MTX (mg/weekly)					
MTX	19	15 ± 0	0≤15≤20		
ETA	12	13.8 ± 4.2	10≤10≤20		
Prednisolone (mg/d)					
MTX	6	6.2 ± 2.6	5 ≤ 5 ≤10		
ETA	8	4.7 ± 0.9	2.5≤2.5≤5		
Other treatments in ETA-group					
ETA mono, (mg/weekly)	1	50			
ETA and Sulfasalazine,(mg/d)	2	2.000			
ETA and Hydroxychloroquine (mg/d)		1400			
Laboratory and clinical characteristics					
Rheumatoid factor IgA (IU)					
MTX	12	30.5 ± 45.1	0.1≤122.5≤2.9	1.8;59.2	0.507
ETA	9	180.9 ± 468.8	0.2≤32≤1,430	-178.5;541.2	
Rheumatoid factor IgM (IU)					
MTX	16	335.1 ± 717.5	0.1≤122.5≤2,910	-47.2;717.4	0.539
ETA	16	359.7 ± 459.8	0.8≤139≤1,387	604.7; 604.7	
ACPA (IU)					
MTX	16	327.7 ± 377.2	1.1≤183.5≤1,000	126.7; 528.7	0.845
ETA	15	270.8 ± 320.9	1≤145≤1,000	93.2; 448.5	
Morning stiffness (min)					
MTX	10	113.5 ± 108.9	30≤60≤390	35.6; 191.4	0.895
ETA	12	102.9 ± 77.9	30≤90≤300	53.4; 152.4	
$DAS28_{CRP}$					
MTX	18	4.4 ± 0.9	2.7≤4.3≤5.7	3.9; 4.8	0.994
ETA	17	4.4 ± 1.7	1.7≤3.8≤7.5	1.9; 5.2	

ACPA: anti-citrullinated protein antibodies; CI: confidence interval; CRP: C-reactive proteine; $DAS28_{CRP}$: 28-joint disease activity score with CRP; ETA: etanercept; Ig: immunoglobulin; IU: international units; M: mean; MTX: methotrexate; min: minutes; n: number; *p*: *p*-value; SD: standard deviation; V: variables compared between MTX and ETA groups.

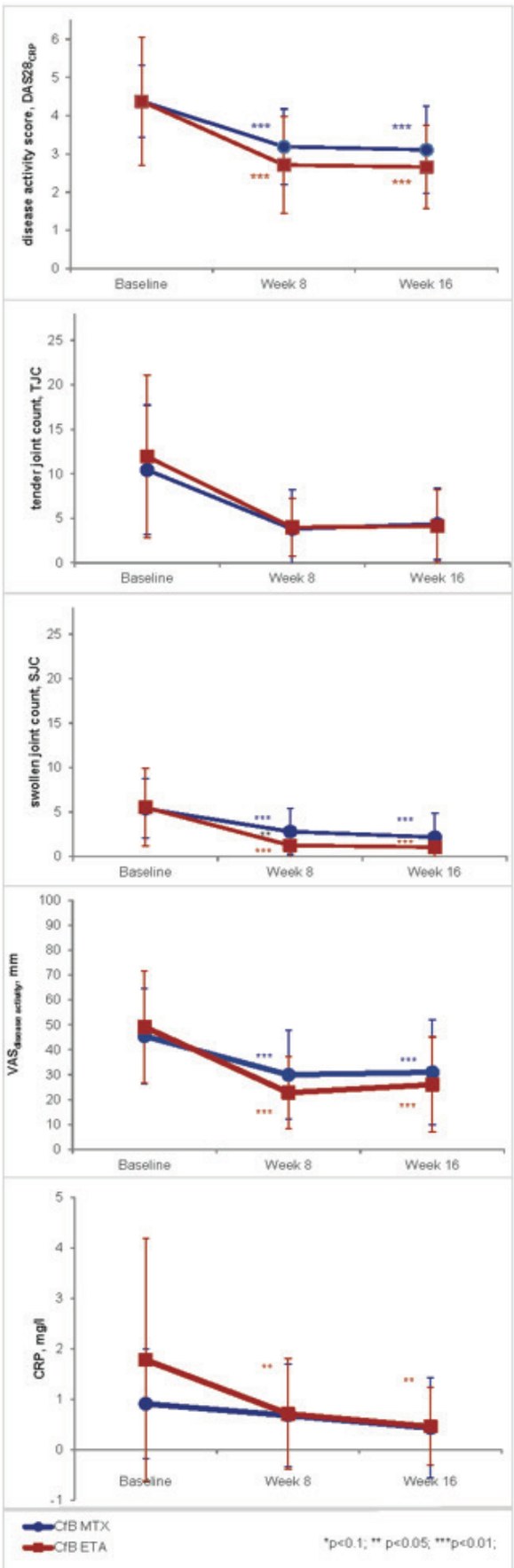

Fig. 1. Clinical and selected laboratory parameters. CfB: change from baseline; CRP: C-reactive protein; $DAS28_{CRP}$: 28-joint Disease Activity Score; ETA: etanercept; MTX: methotrexate; VAS: visual analogue scale; black flags, comparison ETA *vs*. MTX at visit.

Neuroendocrine-immunological parameters

In the ETA group, serum levels of IL-10 ($p_{CfB\text{-}w16} \leq 0.01$), IL-17 ($p_{CfB\text{-}w16} \leq 0.01$), MIP-1β ($p_{CfB\text{-}w16} \leq 0.01$) and lymphocytes ($p_{CfB\text{-}w16} \leq 0.01$) increased to w16. ETA treatment resulted in a not statistical significant reductions of IL-4 ($p_{CfB\text{-}w16}=0.93$), IL-7 ($p_{CfB\text{-}w16}=0.94$) and TNF levels ($p_{CfB\text{-}w16}=0.32$) to w16. CD3+/CD8+ cytotoxic T cells decreased in the MTX group at w16 ($p_{CfB\text{-}w16}=0.03$). The CfB at w16 between groups was significant for IL–10 ($p_{CfB\text{-}w16}=0.016$), IL-17 ($p_{CfB\text{-}w16}=0.0005$), MIP-1β ($p_{CfB\text{-}w16} \leq 0.001$) and TNF ($p_{CfB\text{-}w16}=0.004$). In both groups, the proportion of neuropeptide Y (NPY) were reduced from BL to w8 ($p_{CfB\text{-}MTX}=0.02$; $p_{CfB\text{-}ETA}=0.38$) and were significant in the MTX group. NPY subsequently numerical increased from w8 to w16. Levels of 17β-estradiol showed an increase to w16 during MTX therapy ($p_{CfB\text{-}MTX}=0.43$), but in the ETA group the level increased to w8 ($p_{CfB\text{-}ETA}=0.27$) and decreased to w16 ($p_{CfB\text{-}ETA}=0.42$). The difference between the groups was significant at w8 ($p_{CfB}=0.01$) but not at w16 ($p_{CfB}=0.19$). For additional results on humoral, cellular and hormonal parameters see supplemental material (Fig. S2; Table SIV).

Analyses, comparisons and relationships of the main interaction effects between MTX and ETA group

The statistical analyses (*e.g.* regression analyses, Pearson' correlation tests) of the changes described for cytokines, cells and hormones based on a small number of cases and without any adjustment with respect to multiple testing, whether a relationship to changes in disease activity, to changes in the various sleep parameters, to changes in fatigue symptoms and quality of life can be detected, showed neither statistical significance nor correlations.

Discussion

Reduced sleep quality and fatigue are frequently reported by patients with RA but rarely assessed in clinically (37, 38). Qualitative and quantitative studies on sleep quality have been rare, as they require extensive resources (11, 39–41). In active RA, pro–inflammato-

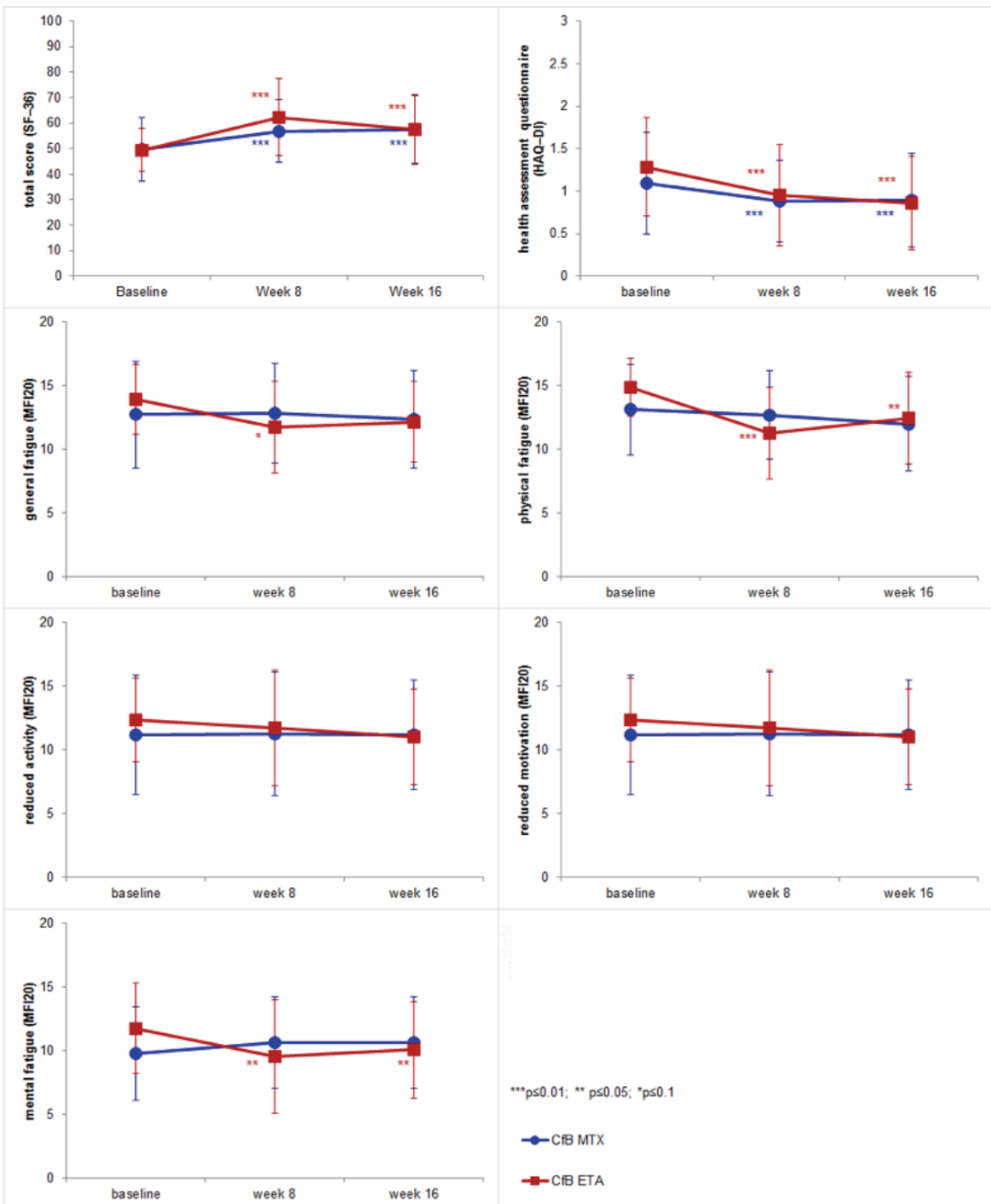

Fig. 2. Patient-reported outcome measures. CfB: change from baseline; ETA: etanercept; HAQ-DI: Functional Disability Index of the Health Assessment Questionnaire; MFI-20: 20-item Multidimensional Fatigue Inventory; MTX: methotrexate, SF-36: 36-item Short-Form Health Survey; black flags: comparison ETA *vs*. MTX at visit).

ry cytokines such as TNF lead to sleep disturbances, sleepiness, increased sleep pressure and subsequently to fatigue. TNF blockade is proved to be effective in reducing clinical signs and symptoms of joint disease thus improving the QoL (42, 43). However, the effects of TNF inhibitors on sleep quality have been investigated in a few studies only (19, 44), and the effects on fatigue are poorly understood (42, 43).

We hypothesised that ETA is able to develop the main effects by rapid and significant improvement of disease activity, in sleep quality and fatigue independent from co-treatment with MTX. All but one patient in the ETA group received ETA plus MTX. Therefore, we cannot identify differences between ETA with *vs*. without MTX, but report the incremental therapeutic effects as induced by ETA as compared to MTX alone. Besides the association of fatigue with pro–inflammatory cytokines, a relation with (mal)functioning of the hypothalamic-pituitary-adrenal axis has been suggested (1, 8). It has been hypothesised that depression, fatigue, physical fitness and sleep disturbances may influence cortisol levels (19, 45). Therefore, selected immune and hormone parameters were also determined. Eligible RA patients were given MTX or a bDMARD due to high disease activity (Fig. 1; Table SI). When interpreting data, it should be stressed that both treatments groups were similar in terms of comprising RA patients with active disease, but differed in terms of their

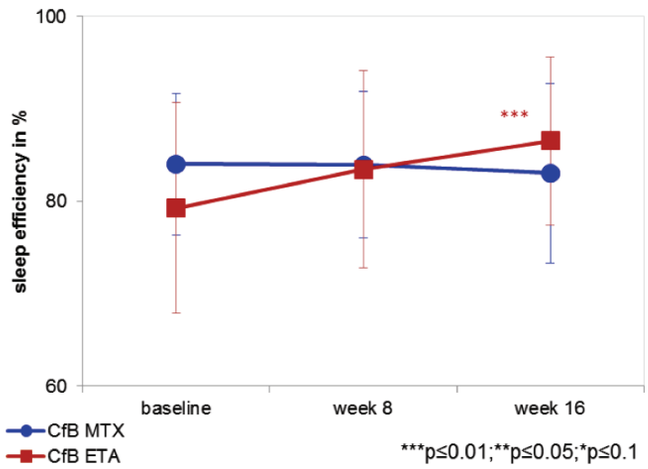

Fig. 3. Sleep efficiency. CfB: change from baseline; ETA, etanercept; MTX: methotrexate.

position (*i.e.* phase I or II) within the algorithm based on the EULAR recommendations on RA management (21).

For homogeneity, only the bDMARD ETA was used, either alone or combined with MTX. BL scores for SF–36, HAQ–DI and $VAS_{disease\text{-}activity}$ demonstrated severe limitations due to emotional distress (46, 47), bodily pain and sleep problems (Fig. 1-3; Table SI-SIII) (48). These limitations were present not only among study participants with long disease duration (ETA group; phase II patients) but also among patients newly diagnosed with RA (MTX group; phase I patients) and had a strong effect on QoL (Fig. 2; Table SII) (47).

The key observation was that ETA treatment led to significant improvement in sleep quality. Both TST and the S2 phase were significantly extended, the WASO value was shortened, and sleep efficiency increased significantly and in a clinically meaningful extent from BL to w16 (Fig. 3; Table SIII). In contrast, MTX group patients did not show any measurable changes in TST, duration of all sleep phases, WASO value or sleep efficiency. However, both groups showed already at baseline a good sleep efficiency, taking into account the age dependency of sleep changes (49, 50).

It should be noted that improvement in most sleep parameters could be observed as early treatment effects of ETA, *i.e.* at w8. The respective results underlying this observation are presented both as changes in absolute and percentage sleep parameters (Fig. 3; Fig. SI; Table SIII). Beneficial effects of ETA on sleep quality may well be underestimated due to the disease stages of the patients studied here. The improvement in sleep quality is probably a direct effect of the inhibition of TNF and other pro-inflammatory cytokines. Indeed, significant changes in cytokine levels were observed in both groups (Fig. S2; Table SIV). Pro-inflammatory cytokines such as IL-6 and TNF increased at the beginning of the study and decreased after successful treatment, whereas IL-17 increased and remained constant (Fig. S2; Table SIV). Interestingly, the suppression of TNF by ETA was much more pronounced than MTX illustrating the high treatment potential of ETA and perhaps partly explaining the lack of improvements in sleep quality in the MTX group. However, it should be noted, that the treatment groups showed different numerical BL concentrations of TNF, with higher concentrations in the ETA group most likely representing longer disease duration. We cannot exclude, however, that the study period was too short to detect the maximal therapeutic benefit of MTX.

Patients reported high levels of fatigue at baseline (Table SII). Indeed, high disease activity in RA affected self-reported fatigue as measured by the MFI-20 questionnaire (48, 51, 52). Patients in both groups differed from healthy subjects in all categories (Fig. 2). We showed that individual categories on the MFI-20 (physical and mental fa-

Table II. Selected sleep parameters (%).

	MTX group					ETA group					MTX minus ETA	
	baseline	week 8		week 16		baseline	week 8		week 16			
Sleep stage	% TST±SD	%TST±SD	CfB *p*	%TST±SD	CfB *p*	% TST±SD	%TST±SD	CfB *p*	%TST±SD	CfB *p*	CfB (adj.)	*p*
S1	16.6 ± 10.6	14.6 ± 9.8	0.52	14.2 ± 9.4	≤0.01	16.8 ± 9.8	12.7 ± 6.6	0.1	13.0 ± 6.0	0.1	w8: w16:	0.49 0.51
S2	34.7 ± 10.8	37.4 ± 13.2	0.44	33.6 ± 10.8	0.81	31.8 ± 13.2	35.0 ± 8.2	0.29	39.1 ± 8.6	≤0.01	w8: w16:	0.69 0.034
S3	10.4 ± 4.4	11.2 ± 4.0	0.88	9.5 ± 3.0	≤0.01	11.5 ± 6.7	11.7 ± 5.9	0.87	18.0 ± 7.4	≤0.01	w8: w16:	0.86 0.0004
S4	11.6 ± 8.4	13.2 ± 7.0	0.63	12.3 ± 6.2	≤0.01	9.5 ± 6.3	13.2 ± 6.1	0.15	14.6 ± 13.1	0.31	w8: w16:	0.75 0.78
REM	16.2 ± 5.0	17.4 ± 3.5	0.40	17.4 ± 3.5	≤0.01	17.3 ± 13.9	18.2 ± 5.0	0.57	18.2 ± 5.9	0.40	w8: w16:	0.56 0.57

CfB: change from baseline; w: week; %TST: percent of total sleep time; SD: standard deviation; MTX: methotrexate; ETA: etanercept; REM: rapid eye movement sleep; S1–S4: sleep stages 1-4.

tigue) improved significantly after treatment with ETA compared with MTX (Fig. 2; Table SII), contrary to previous assumptions (42). Decreased levels of fatigue may be due to improved sleep quality and TST.

In contrast to the study hypothesis, treatment effects on hormonal status were marginal (Fig. S2; Table SIV). Previous studies have demonstrated an increased sympathetic tone in patients with chronic inflammatory diseases. NPY is described as a reliable measure of the activity of the sympathetic nervous system, and a decrease in NPY levels after treatment would be expected (53). Indeed, at w8 our study showed a slight decrease in NPY levels in both groups, although it appeared to be more pronounced in the ETA group. It is unclear, however, why the NPY levels increased again slightly after w8. In contrast, a continuous increase during treatment with TNF blockers has already been described (54). Free testosterone levels did not change in either group. Serum levels of 17β-estradiol initially increased in both groups, which were attributed to the anti–inflammatory effects of ETA and MTX (54-57). Levels of 17β-estradiol increased from BL to w16 during MTX therapy, but in the ETA group, 17β–estradiol levels increased from BL to w8 and decreased from w8 to w16. Only marginal changes were observed for specific cell populations in the course of treatment (Table SIV).

Limitations of this study included the open, non-randomised design. Furthermore, the number of study participants, although based on power and sample size calculations was relatively small. Significant differences between the groups regarding disease duration were evident at BL. Although both treatment groups include patients with active RA, they differed in their position (*i.e.* either phase I or II) within the EULAR recommendations on RA management (21). This may well have influenced the comparative evaluation of cytokines, cell populations and hormones, because early and established RA represents distinct phases of inflammation. Thus, the distribution of parameters in newly diagnosed disease as well as their behaviours over time can differ from chronic disease. Similarly, all other recorded study parameters must be discussed with caution. The study design did not address considerations of the potential influence of circadian rhythms on data collection for each parameter. Thus, for example, the sensation of pain differs during the day, so the direct impact on quality of sleep is comparable only with shorter survey intervals over the day and night. We can also not rule out that rheumatic diseases secondary to RA (such as secondary Sjögren's syndrome or fibromyalgia) or other underlying accompanying diseases may have influence on our results although we have made every attempt to avoid this (see calculation of AHI).

Given these limitations, our trial should be seen as a pilot study in order to generate results, which facilitate the planning of future larger trials. Subsequent RCTs are clearly needed to better understand the factors influencing varying disease courses of RA.

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