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ORIGINAL RESEARCH

Serum etanercept concentrations in relation to disease activity and treatment response assessed by ultrasound, biomarkers and clinical disease activity scores: results from a prospective observational study of patients with rheumatoid arthritis

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ABSTRACT

Objectives To identify the therapeutic range for etanercept and to assess the incidence of anti-etanercept antibody formation.

Methods Associations between etanercept serum concentration and disease activity as well as treatment response were examined in a longitudinal observational study of rheumatoid arthritis patients starting etanercept. Disease activity was assessed by ultrasound (grey scale and power Doppler), 28-joint Disease Activity Score (DAS28), Simplified Disease Activity Index, plasma calprotectin and C reactive protein. Etanercept concentration and anti-etanercept antibodies were analysed using automated in-house fluorescence assays.

Results A total of 89 patients were included, whereof 66% were biological disease-modifying antirheumatic drug (DMARD) naïve and 91% used concomitant synthetic DMARD. At 3 months, the median etanercept concentration was 1.8 (IQR 1.1–2.5) mg/L. Longitudinal associations were found between etanercept concentration and disease activity assessed by plasma calprotectin, C reactive protein and DAS28, but not between etanercept concentration and improvement in disease activity by any of the parameters at 3, 6 or 12 months of treatment. Etanercept concentrations were not significantly different among patients who achieved response or remission, compared with non-response or non-remission. Hence, no therapeutic range could be identified. None of the patients developed anti-etanercept antibodies.

Conclusion Despite the use of sensitive and objective markers of inflammation, a therapeutic range could not be identified for etanercept. Hence, this study suggests that proactive therapeutic drug monitoring is unlikely to benefit rheumatoid arthritis patients treated with etanercept, but a potential benefit in certain clinical situations cannot be excluded.

Key messages

What is already known about this subject?
► Low drug concentrations and development of anti-drug antibodies are associated with lack of response to tumour necrosis factor alpha inhibitors.
► For etanercept, results from previous concentration-effect studies are conflicting and an optimal range of serum etanercept remains to be identified.

What does this study add?
► Novel sensitive and objective inflammatory markers, that is, grey scale and power Doppler ultrasound, plasma calprotectin and C reactive protein (CRP), were used alongside conventional clinical disease activity measures in this longitudinal observational study.
► Despite longitudinal associations between etanercept serum concentrations and plasma calprotectin, CRP and 28-joint Disease Activity Score, no clinically relevant therapeutic range could be identified.

How might this impact on clinical practice or further developments?
► Our results suggest that proactive therapeutic drug monitoring is unlikely to benefit rheumatoid arthritis patients treated with etanercept.

INTRODUCTION

Etanercept, a recombinant human soluble dimeric tumour necrosis factor (TNF) receptor (p75) fusion protein, is effective treatment of rheumatoid arthritis (RA).[1 2] However, a significant proportion of patients do not respond adequately to therapy.[1] Lack or loss of response to other TNF inhibitors

(TNFi) has been attributed to low serum drug concentrations (pharmacokinetic failure), which is sometimes caused by anti-drug antibodies (ADAb).[3–7] The role of personalised dosing of TNFi guided by therapeutic drug monitoring (TDM) is still controversial,[8 9] but observational data have suggested a rationale for TDM by demonstrating large variations in drug concentrations between individuals on standard dose, as well as concentration–effect relationships.[3–6]

For etanercept, however, results from previous concentration-effect studies are conflicting,[10–14] and an optimal therapeutic range for serum etanercept remains to be identified.[12–14] Previous studies are also conflicting with regard to the occurrence of ADAb. Etanercept is suggested to be less immunogenic than other TNFi, yet the reported prevalence of ADAb ranges from 0% to 13%.[15–17]

Concentration–effect relationships for TNFi have mainly been studied using composite clinical disease activity measures and conventional biochemical inflammation markers as outcome measures. Musculoskeletal ultrasound, including assessment of grey scale (GS) synovitis (combined score of hypertrophied synovia and joint effusion) and vascularisation of the synovitis by power Doppler (PD), is a frequently used and sensitive imaging technique for assessing disease activity in RA.[18–21] Calprotectin (S100A8/A9), a major granulocyte protein, has been found to have higher associations to joint inflammation, radiographic progression, clinical response to biological disease-modifying antirheumatic drugs (bDMARDs) and PD ultrasound synovitis, compared with C reactive protein (CRP) or Erythrocyte Sedimentation Rate (ESR).[22–24]

To evaluate the clinical value of TDM of etanercept in clinical strategy trials, the therapeutic target interval must be identified. Given the lack of consistent data, an examination including sensitive markers of inflammation was warranted.

The aims of this study were to identify the therapeutic range for etanercept and to assess the incidence of anti-etanercept antibody formation. To this end we used data from a well-characterised prospective observational study of patients starting etanercept with disease activity and treatment response assessed by ultrasound, sensitive biomarkers and conventional clinical disease activity measures.[25]

METHODS
The study population
This study uses data from a prospective observational study including patients with established RA starting treatment with a bDMARD between January 2010 and June 2013 (Anzctr.org.au identifier ACTRN12610000284066).[25] All patients met the 1987 revised American Rheumatism Association classification criteria.[26] The patients were assessed by ultrasound and clinical examination at inclusion and at 1, 2, 3, 6 and 12 months after initiation of bDMARDs. Non-trough serum samples were collected at the same time points. In this study, we included patients who started treatment with originator etanercept and who had available biobank samples and clinical disease activity and ultrasound data from two or more visits. All patients were given the standard dose of s.c. etanercept 50 mg weekly.

Patient and public involvement
Patient or public representatives were not involved in the study.

Clinical disease activity and response measures
The examinations of tender and swollen joint counts as well as the assessor's global evaluation of disease activity, were performed by two trained and experienced study nurses blinded for ultrasound results. The clinical composite scores for disease activity included the 28-joint Disease Activity Score (DAS28-ESR)[27] and the Simplified Disease Activity Index (SDAI) for RA.[28] Response was defined by EULAR responses.[29] Remission was defined by DAS28-ESR remission (DAS28-ESR <2.6)[30] and SDAI remission (SDAI ≤3.3).[31]

Ultrasound assessments
GS and PD were semiquantitatively scored on a 4-point scale (0=no, 1=minor, 2=moderate and 3=major), according to Outcome Measure in Rheumatology scores and the Norwegian ultrasound atlas.[20 32] The following 36 joints were scored: bilateral wrist (radiocarpal, midcarpal and radioulnar joints scored separately), metacarpophalangeal joints 1–5, proximal interphalangeal joints 2–3, elbow, knee, tibiotalar and metatarsophalangeal joints 1–5) and 4 tendon sheets (bilateral extensor carpi ulnaris and tibialis posterior),[20] (giving a maximum of either GS or PD sum score of 120). The ultrasound examinations were performed using an Antares Excellence version with at 5–13 MHz probe (Siemens Healthcare, Erlangen, Germany) by an experienced sonographer (HBH), blinded to the results of the clinical examination and laboratory results. PD ultrasound remission was defined as sum score PD=0.[21]

Biochemical inflammatory markers
Calprotectin was measured in EDTA plasma using a commercial ELISA (CALPRO AS, Lysaker, Norway) as described previously[24] and CRP was determined by the Roche Tina-quant CRP assay (Roche Diagnostics, Mannheim, Germany).

Measurement of serum etanercept concentration and ADAb
Etanercept concentrations were measured using an in-house time-resolved fluorescence assay automated on the AutoDELFIA (PerkinElmer, Waltham, Massachusetts, USA) immunoassay platform.[33–35] The assay uses recombinant tumour necrosis factor alpha (TNFα) as capture reagent. Drug binding to the TNFα solid phase is detected using a europium-labelled protein-A tracer reagent. The coefficient of variation of the assay used in

this study was less than 6% and when measuring two dilutions of an international reference standard (European Pharmacopoeia Reference standard, Etanercept CRS batch 1, Y0001969, concentrations 3.0 and 10.0 mg/L) a difference of <25% was observed between the calculated and measured concentrations. ADAb was detected by a drug-sensitive three-step antigen bridging assay, measuring ADAb able to cross-link the biotinylated solid-phase and europium-labelled tracer etanercept p75 receptor molecule.

Statistical analyses

Correlations between baseline variables and 3-month etanercept concentrations were assessed by multivariable linear regression analyses. Associations between etanercept concentrations and improvement in clinical composite scores for disease activity, GS/PD ultrasound scores and biochemical markers of inflammation, were assessed by separate multivariable linear regressions at each time point and linear mixed models for repeated measures. The models were adjusted for possible confounders (gender, and prior use of bDMARD (yes/no), baseline age and disease duration). The mixed model analyses used data from all five assessments with the covariates time-fixed at baseline. Robustness analyses were carried out for the models using log-transformed versions of plasma calprotectin and CRP, due to skewed distributions of the standardised residuals of these variables. Differences in etanercept concentrations for response versus non-response and remission versus non-remission, were assessed at each time point using independent samples Mann-Whitney U test or Kruskal-Wallis test and across time by logistic mixed models, adjusting for the same variables as described for the linear regression analyses. The predictive value of etanercept concentration at 1 or 3 months for response/remission at 6 and 12 months was assessed using receiver operating characteristics analyses. In the main cross-sectional analyses, the last observation carried forward (LOCF) approach was applied for missing laboratory, clinical composite scores or ultrasound data. Sensitivity analyses using completer data, that is, no imputations, were also performed. Imputation was not done prior to the mixed model analyses, as the model adjusts for missing data. Statistical analyses were performed using IBM SPSS Statistics, V.26 (IBM) and Stata V.16 (StataCorp).

RESULTS

Study population and baseline characteristics

A total of 89 patients with RA were included. Baseline demographics and clinical characteristics of the study population are shown in table 1. Notably, 81 out of 89 used concomitant conventional synthetic (cs) DMARD, whereof 73 used methotrexate (median weekly dose 15 (IQR 10–20) mg), 4 sulfasalazin and 4 leflunomide. Sixty-two patients (70%) completed 12 months follow-up, whereas 5 patients had discontinued etanercept before

Table 1 Baseline demographics and clinical characteristics of the study population

Rheumatoid arthritis	All (n=89)
Age, years, mean (SD)	51 (13)
Female, n (%)	68 (76)
Disease duration, years, median (IQR)	6.0 (2.5–12.2)
Body mass index, median (IQR), (kg/m^2)	24.0 (22.1–27.2)
RF-positivity, n (%)	57 (64)
ACPA positivity, n (%)	67 (75)
ESR, median (IQR), (mm/hour)	20 (9–30)
CRP, median (IQR), (mg/L)	5 (2–10)
Calprotectin, median (IQR), (µg/L)	977 (641–1590)
Tender joint count (28 joints), median (IQR)	3 (1–7)
Swollen joint count (28 joints), median (IQR)	4 (2–8)
DAS28-ESR, mean (SD)	4.2 (1.4)
SDAI, median (IQR)	15.9 (9.9–22.6)
Sum GS ultrasound score, median (IQR)	26 (16–36)
Sum PD ultrasound score, median (IQR)	8 (3–20)
Prior use of biological DMARD, n (%)	30 (34)
Concomitant conventional synthetic DMARD, n (%)	81 (91)
Methotrexate dose, median (IQR), (mg/week)	15 (10–20)
Prednisolone dose, median (IQR)	2.5 (0.0–5.0)

ACPA, anticyclic citrullinated peptide; CRP, C reactive Protein; DAS28, 28-joint Disease Activity Score; DMARD, disease-modifying anti rheumatic drug; ESR, erythrocyte sedimentation rate; GS, grey scale; PD, power Doppler; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index.

the 3-month visit and 14 patients discontinued before the 6-month visit. Reasons for discontinuation were lack of effect (n=11), adverse event (n=6), pregnancy (n=3), remission (n=2) and other reason (n=5). An overview of the study population and missing variables, which were replaced by LOCF in the main analyses, is shown in online supplemental figure S1.

Distribution of etanercept serum concentration

The etanercept concentration ranges/medians were as follows: range 0.0–5.1 mg/L/median 1.8 (IQR 1.1–2.5) mg/L at 3 months, range 0.0–6.2 mg/L/median 1.7 (IQR 0.8–2.3) mg/L at 6 months and range 0.0–5.0 mg/L, median 1.7 (IQR 0.8–2.5) mg/L at 12 months. The distribution at 3 months is shown in figure 1. Median etanercept concentrations at 3 months were comparable among the 81 patients on concomitant csDMARD and the 8 patients on etanercept monotherapy, 1.8 (IQR 1.1–2.5) mg/L, and 2.0 (IQR 1.5–2.7) mg/L, respectively. No significant correlations were found between baseline disease activity, body mass index or
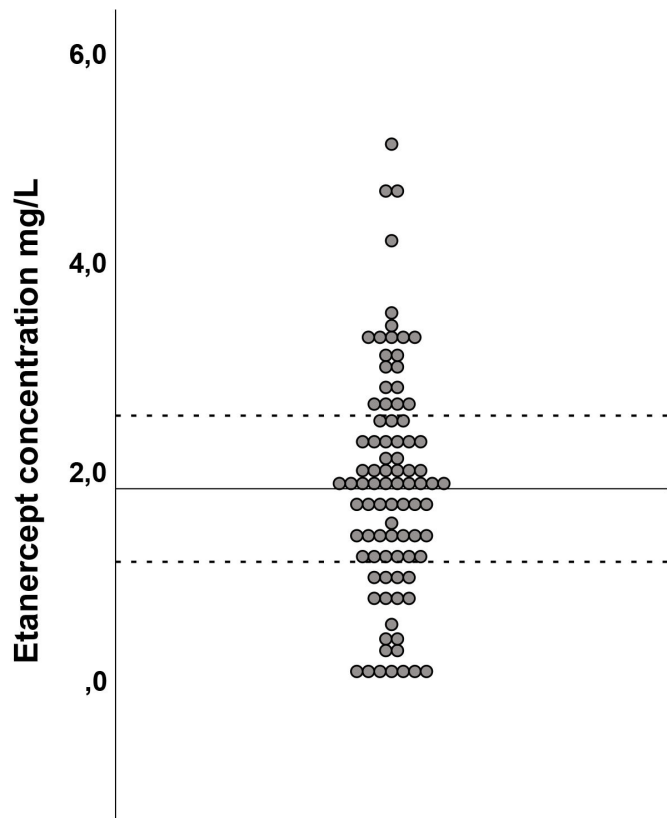
Figure 1 Distribution of etanercept serum concentrations at 3 months. Median (IQR) 1.8 (1.1–2.5) mg/L.

methotrexate dose, and 3-month etanercept concentrations. None of the patients developed anti-etanercept antibodies during follow-up.

Association between etanercept concentrations and improvement in disease activity

As shown in table 2 and figure 2A–F, etanercept serum concentration was not associated with improvement from

baseline in clinical disease activity, GS/PD ultrasound scores, plasma calprotectin or CRP at 3, 6 or 12 months of treatment, in the cross-sectional analyses. Sensitivity analyses using completer data yielded similar results (online supplemental table S1). In the longitudinal analyses, however, etanercept concentrations were significantly associated with improvement in DAS28, calprotectin and CRP from baseline (table 2). The robustness analyses of log-transformed values yielded consistent results, but a statistically significant association between etanercept concentrations and log plasma calprotectin at 3 months was revealed in the linear regression analysis (online supplemental table S3).

Association between etanercept concentration and response or remission

The results of the cross-sectional analyses are shown in figure 3A–D. Etanercept concentrations were not significantly different among patients who achieved EULAR good/moderate response or DAS28, SDAI or PD ultrasound remission, compared with those who did not achieve response or remission at 3 months. The results were consistent after 6 and 12 months of treatment (data not shown) and in sensitivity analyses using completer data (online supplemental figure S2).

The logistic mixed model analyses yielded consistent results for the binary response/remission outcomes (table 3).

Predictive value of serum etanercept concentration

As shown in table 4, no cut-off for a predictive value of etanercept concentration could be identified in relation to response after 6 and 12 months, as the area under curves were not significantly different from 0.5 in the receiver operating characteristics curve analyses. Sensitivity analyses using completer data yielded similar results (online supplemental table S2).

Table 2 Associations at 3, 6 and 12 months and in longitudinal analyses, between etanercept serum concentration and improvement from baseline in clinical disease activity, GS/PD ultrasound scores and biochemical inflammatory markers

	3 months			6 months			12 months			Mixed effect regression model	
	Median (IQR)/ mean (SD) change from baseline	β (95% CI)	P value*	Median (IQR)/ mean (SD) change from baseline	β (95% CI)	P value*	Median (IQR)/ mean (SD) change from baseline	β (95% CI)	P value*	β (95% CI)	P value†
Delta sum GS ultrasound score	−6.0 (−14.0, −1.0)	−1.1 (−3.2 to 1.0)	0.29	−7.0 (−13.5, −2.0)	−0.5 (−2.6 to 1.6)	0.64	−7.0 (−15.5, −1.0)	−2.2 (−4.6 to 0.1)	0.06	−0.4 (−1.2 to 0.4)	0.36
Delta sum PD ultrasound score	−3.0 (−12.0, −1.0)	−0.1 (−1.8 to 1.6)	0.88	−4.0 (−10.5,−1.0)	−0.4 (−2.4 to 1.5)	0.65	−4.0 (−12.0,−1.0)	−1.3 (−3.2 to 0.7)	0.20	−0.3 (−0.9 to 0.4)	0.42
Delta DAS28-ESR	−1.1 (1.0)	−0.2 (−0.4 to −0.0)	0.06	−1.2 (1.0)	0.0 (−0.2 to 0.2)	0.82	−1.2 (1.1)	−0.1 (−0.3 to 0.1)	0.31	−0.1 (−0.2 to −0.0)	**0.04**
Delta SDAI	−7.9 (−11.9, −3.4)	−1.5 (−3.0 to 0.0)	0.05	−8.3 (−12.3, 3.1)	−0.3 (−2.1 to 1.4)	0.72	−8.3 (−12.3,−3.1)	−0.8 (−2.4 to 0.9)	0.35	−0.3 (−0.9 to 0.3)	0.35
Delta Calprotectin (µg/L)	−477 (−992, −154)	−85 (−294 to 124)	0.42	−462 (−1091, −142)	−31 (−266 to 205)	0.80	−426 (−1053, −95)	−201 (−430 to 27)	0.08	−96 (−144 to −49)	**<0.001**
Delta CRP (mg/L)	−2 (−7, 0)	−1.1 (−3.6 to 1.5)	0.41	−1 (−7, 0)	0.1 (−2.5 to 2.7)	0.94	−2 (−7, 0)	−0.9 (−3.3 to 1.5)	0.46	−1 (−1 to 0)	**0.02**

Multivariable linear regression analyses (3, 6 and 12 months) and mixed effect regression model, adjusted for age, gender, disease duration and prior use of biological disease-modifying antirheumatic drug (yes/no), as time-fixed baseline variables.
P values in bold indicate statistically significant results.
*P value in cross-sectional linear regression analyses.
†P value in mixed effect linear regression model. Interaction test of time-by-serum etanercept concentration showed that the association did not vary significantly over time.
CRP, C reactive protein; DAS28, 28-joint Disease Activity Score; ESR, erythrocyte sedimentation rate; GS, grey scale; PD, power Doppler; SDAI, Simplified Disease Activity Index.
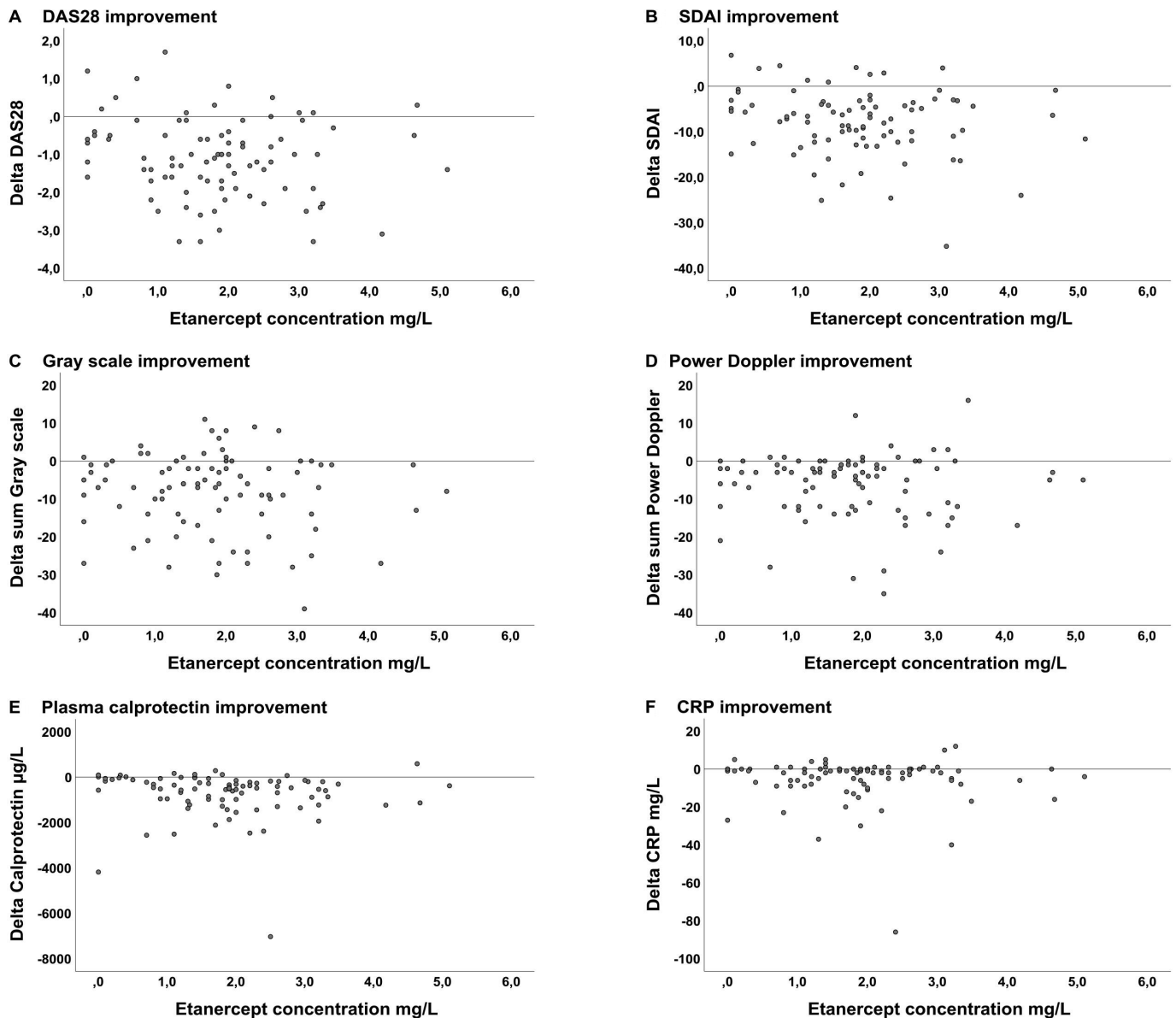
A   DAS28 improvement

B   SDAI improvement

C   Gray scale improvement

D   Power Doppler improvement

E   Plasma calprotectin improvement

F   CRP improvement

Figure 2   (A–F) Associations between improvement in clinical composite scores, ultrasound scores and laboratory variables and serum etanercept at 3 months. (A) Improvement in DAS28 from baseline to 3 months. (B) Improvement in SDAI from baseline to 3 months. (C) Improvement in sum grey scale from baseline to 3 months. (D) Improvement in sum power Doppler from baseline to 3 months. (E) Improvement in plasma calprotectin levels (µg/L) from baseline to 3 months. (F) Improvement in C reactive protein (CRP) levels (mg/L) from baseline to 3 months. DAS28, Disease Activity Score 28 joints; SDAI, Simplified Disease Activity Index.

DISCUSSION

In this prospective observational study of patients with RA starting etanercept treatment, we show that despite a large interindividual variation in serum etanercept concentrations, no therapeutic range could be identified. Importantly, analyses at 3, 6 and 12 months revealed no association between etanercept serum concentration and improvement in disease activity scores and no significant differences in etanercept concentrations among those who achieved response or remission compared with those who did not. The results were consistent in the cross-sectional analyses across different clinical assessments, ultrasound scores and sensitive biochemical markers of disease activity, as well as different time points

up to 12 months after initiation of treatment. In the longitudinal analyses, however, significant associations between etanercept concentration and improvement in DAS28, calprotectin and CRP were revealed. Finally, no patients developed antietanercept antibodies.

Based on the present data and conflicting results of previous studies, there is a weak association between etanercept concentrations and disease activity. However, the association is unlikely to be clinically relevant as no clinically applicable therapeutic range can be established. Assessments of individual patients over time might, however, be considered in selected patients and clinical situations. Consecutive measurements of etanercept serum concentrations are not easily integrated
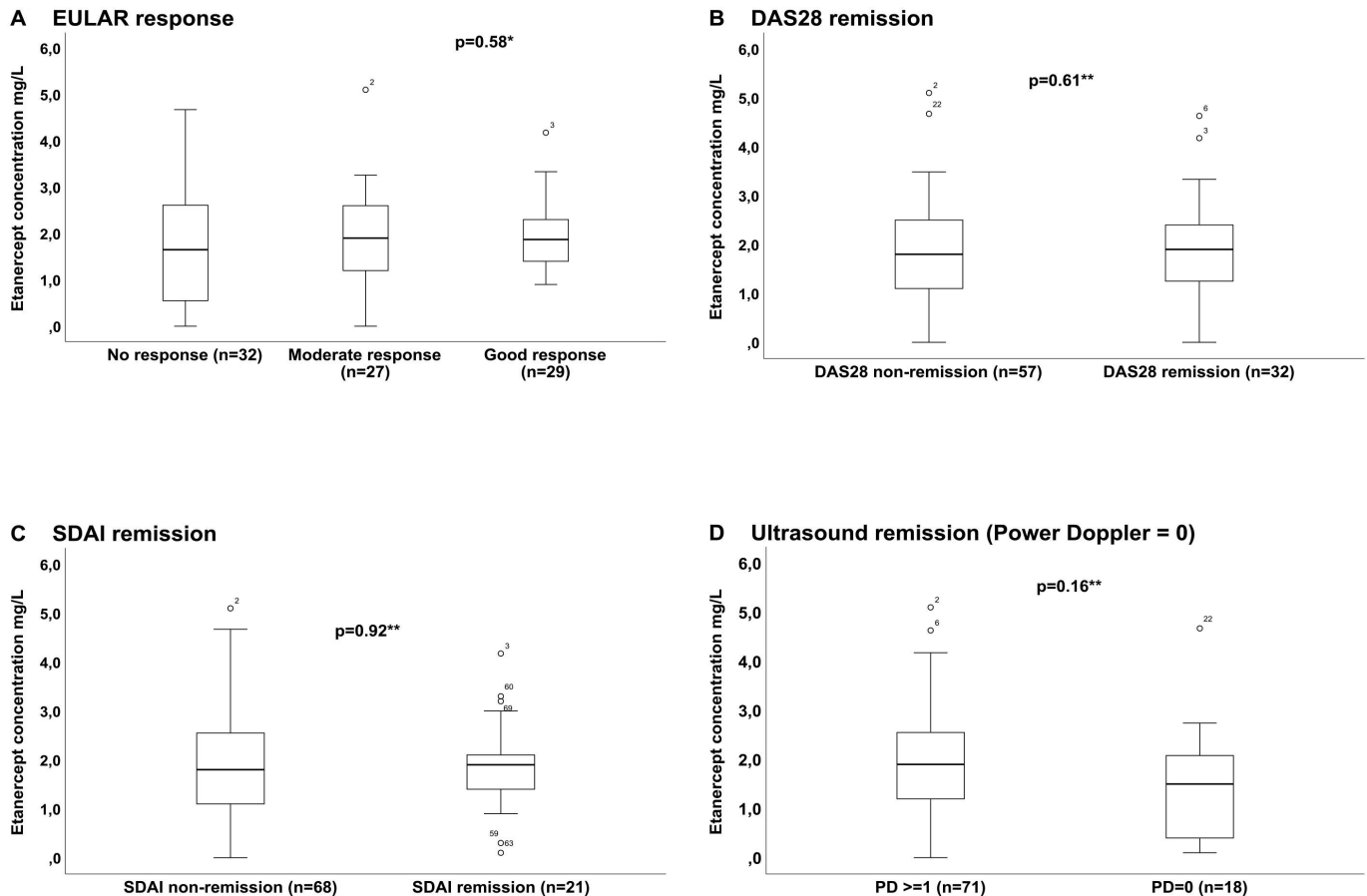
Figure 3 (A–D) Etanercept concentrations in patients with response/remission versus non-response/non-remission at 3 months. (A) EULAR good, moderate or non-response at 3 months. (B) DAS28 remission (DAS28 <2.6) vs non-remission (DAS28 ≥2.6) at 3 months. (C) SDAI remission (SDAI ≤3.3) vs non-remission (SDAI >3.3) at 3 months. (D) PD ultrasound remission (PD=0) vs PD ultrasound activity (PD ≥1) at 3 months. *Kruskal-Wallis test. **Mann-Whitney U test. DAS28, Disease Activity Score 28 joints; PD, power Doppler; SDAI, Simplified Disease Activity Index.

into clinical practice, and the clinical benefit or cost-effectiveness of such a strategy is in our opinion doubtful.

The discrepancies in results for some outcome measures, that is, improvement from baseline in DAS28, calprotectin and CRP in the cross-sectional versus longitudinal analyses are probably due to greater power in

Table 3 Longitudinal analyses for repeated measures for association between etanercept serum concentration and response or remission

	OR (95% CI)	P value
EULAR good/moderate response	1.37 (0.71 to 2.64)	0.35
DAS28 remission	0.86 (0.56 to 1.32)	0.49
Ultrasound remission (PD=0)	0.70 (0.46 to 1.05)	0.09
SDAI remission	0.99 (0.61 to 1.61)	0.96

Mixed effect logistic regression model, adjusted for age, gender, disease duration and prior use of biological disease-modifying antirheumatic drug (yes/no). OR EULAR good/moderate response versus non-response and remission, defined by DAS28-, PD=0 or SDAI remission, vs non-remission. Interaction test of time-by-serum etanercept concentration showed that the association did not vary significantly over time.
DAS28, Disease Activity Score 28 joints; PD, Power Doppler; SDAI, Simplified Disease Activity Index.

longitudinal analyses of continuous variables. Moreover, the effect sizes (regression coefficients (β)) were small and thus of uncertain clinical relevance. Plasma calprotectin and CRP are objective markers of inflammation that could be more sensitive to changes in inflammatory activity than clinical disease activity measures. In addition, calprotectin can induce production of important proinflammatory cytokines, including TNFα, through the nuclear factor kappa B (NF$_k$B)-pathway.[36] Consequently there could be a particular relationship between calprotectin and the measured serum concentrations of etanercept, mediated by the influence of calprotectin on the target of etanercept (TNFα).

The conflicting results in etanercept concentration–effect studies could be a result of different study populations, outcome measures or assay methods.[10–14] To our knowledge, only one previous study has assessed associations between TNFi serum concentrations and sensitive markers of inflammation, such as PD ultrasound synovitis and plasma calprotectin levels.[37] That study showed that both low etanercept serum concentration and higher calprotectin levels were associated with PD, but differed from our cohort as it included relatively few patients

Table 4 Predictive value of etanercept concentration at 1 or 3 months for response/remission at 6 and 12 months

	1-month etanercept concentration		3-month etanercept concentration	
	6 months, AUC (95% CI)	12 months, AUC (95% CI)	6 months, AUC (95% CI)	12 months, AUC (95% CI)
EULAR good/moderate response	0.50 (0.37 to 0.63)	0.51 (0.39 to 0.64)	0.54 (0.41 to 0.67)	0.50 (0.38 to 0.62)
DAS28 remission	0.46 (0.34 to 0.58)	0.50 (0.38 to 0.62)	0.49 (0.37 to 0.61)	0.51 (0.39 to 0.63)
SDAI remission	0.53 (0.40 to 0.66)	0.58 (0.45 to 0.71)	0.55 (0.43 to 0.67)	0.54 (0.41 to 0.66)
Ultrasound remission	0.53 (0.40 to 0.66)	0.53 (0.40 to 0.66)	0.40 (0.26 to 0.55)	0.36 (0.29 to 0.49)

AUC, area under curve; DAS28, Disease Activity Score 28 joints; SDAI, Simplified Disease Activity Index.

with RA on etanercept treatment and all patients were in DAS28 remission or low disease activity. PD ultrasound and calprotectin are considered sensitive markers of inflammation in joints and tendons. However, studies have not been able to demonstrate that routine use of PD ultrasound assessments improves patient outcomes in a treat-to-target tight control setting.[38] More sensitive ultrasound machines and some differences in ultrasound scoring systems are available now compared with when the data in this study were collected. However, the scoring system presently used has been shown to have very high reliability,[20] which supports the value of our ultrasound assessment.

Since previous publications have demonstrated concentration-effect relationships for other TNFi,[3–6] our overall finding that no clear and robust relationship could be concluded for etanercept might seem counterintuitive. We believe the main reason for this discrepancy is that etanercept pharmacokinetics are not affected by immunogenicity.[15 16] Furthermore, the shorter elimination half-life, compared with other TNFi, could contribute to variability in the concentration–effect curve. Another possible explanation, attributed to the smooth pharmacokinetic curve, could be that the target molecule, TNFα, is saturated throughout the injection interval in all etanercept-treated patients. The mean baseline disease activity was moderate among patients in our study, which could potentially affect our results. If all TNFα is neutralised, higher exposure is not associated to better effect of treatment. The smooth pharmacokinetic curve of etanercept could probably be attributed to the frequent dosing in combination with the slow absorption from the peripheral to the central compartment.[2 39] Considerable differences in binding to TNFα are unlikely, as previous studies have suggested similar affinities of etanercept, infliximab and adalimumab.[40 41]

None of the 89 patients had detectable anti-etanercept antibodies during the 12-month study period, using an antigen-bridging fluorometric assay. Owing to its structure, the TNF receptor (p75) fusion protein, etanercept, does not contain allogenic epitopes in the target binding domain. Although most studies report no formation of anti-etanercept antibodies,[15 16] which is in line with our findings, a few studies report a prevalence of anti-etanercept antibodies up to 13% in RA.[2 17 42 43] The anti-etanercept antibodies reported in these studies were non-neutralising and not associated with etanercept drug concentrations nor lack of response. Our ADAb assay uses etanercept p75 receptor molecules (without Fc) as both solid phase and tracer molecules, in order to prevent binding from unspecific, presumably non-neutralising, Fc-reactive antibodies (eg, rheumatoid factor).

The main strength of our study is the inclusion of several sensitive and objective markers of inflammation at all time points up to 12 months of etanercept treatment. In addition, we believe that using data from a real-life cohort and a feasible sample collection, contributes to the external validity of our results. The lack of data on adherence was a limitation in our study. Another possible limitation of this study was the random timing of serum sampling and the lack of data regarding the timing of sampling in relation to the last injection. The random timing could theoretically influence the results of the concentration–effect analyses. However, pharmacokinetic studies for etanercept have demonstrated that etanercept is slowly absorbed and eliminated from the circulation, with a half-life of 68±19 hours, and that the variation in serum concentrations is relatively small between injections.[2 39 44–46] Based on these observations, we believe that the random timing of sampling is unlikely to have a significant influence on our findings. Moreover, several studies have shown a benefit of pharmacologic testing of non-trough samples for other subcutaneous TNFi.[6 14 47] For subcutaneous and self-administered TNFi, non-trough sampling is more practical and probably more cost-effective than trough sampling, as the latter is often associated with challenging logistics. Concomitant use of csDMARD is a possible confounder when assessing concentration–effect relationships for TNFi, because it can affect drug levels directly through inhibition of disease activity and indirectly by preventing ADAb formation.[3 48 49] A high proportion of patients used concomitant csDMARD in our study, but in contrast to other TNFi, etanercept pharmacokinetics are probably less affected by methotrexate.[50] Moreover, our results were consistent with results from previous reports.[5] Missing data are often a challenge in longitudinal observational studies. Although we employed both the LOCF approach and completer data, as well as mixed effect models adjusting for missing data, we cannot rule out that the missing variables could represent a bias in our study.

In conclusion, this study suggests a weak association between serum etanercept concentrations and disease activity in individual patients over time. Despite the use of objective and sensitive markers of disease activity, we were not able to establish a therapeutic range to be used on a group level for patients with RA treated with etanercept. None of the patients developed anti-etanercept antibodies. The lack of a clinically applicable therapeutic range suggests that proactive TDM is unlikely to benefit patients with RA treated with etanercept in general, but a potential benefit in individual patients and certain clinical situations cannot be excluded.

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