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# Improved adalimumab dose decision with comprehensive diagnostics data

M. Zänker[1], G. Becher[2], O. Arbach[3], M. Maurer[3], B. Stuhlmüller[3], A. Schäfer[4], P. Strohner[4], J. Brand[4]

*[1]Immanuel Klinikum Bernau Herzzentrum Brandenburg, Bernau, MHB, Germany; [2]BecherConsult GmbH, Bernau, Germany; [3]Charité, Berlin, Germany; [4]BioTeZ Berlin-Buch GmbH, Berlin, Germany.*

*Michael Zänker, Dr. med.*
*Gunther Becher, Dr. med.*
*Olga Arbach, Dr. med.*
*Marcus Maurer, Prof. Dr. med.*
*Bruno Stuhlmüller, Dr. rer. nat*
*Astrid Schäfer, Dipl. LMChem*
*Pavel Strohner, Dr. rer. nat*
*Janko Brand, Dr. rer. nat*

*Please address correspondence to:*
*Dr Michael Zänker,*
*Immanuel Klinikum Bernau,*
*Ladeburger Strasse 17,*
*16321 Bernau, Germany.*
*E-mail: m.zaenker@immanuel.de*
*and*
*Dr Janko Brand,*
*BioTeZ Berlin-Buch GmbH,*
*Robert-Rössle Strasse 10,*
*13125 Berlin, Germany.*
*E-mail: j.brand@biotez.de*

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## ABSTRACT

**Objective.** *Monoclonal antibodies are important in the treatment of rheumatoid arthritis (RA). This is the first trial to monitor the effect of adalimumab dose escalation in persistently active RA. The aim of this study was to identify the response to adalimumab to improve the basis for making decision in relation to actual drug capacity in serum.*

**Methods.** *The disease activity of RA patients was assessed with CDAI and $DAS_{28}$ before administration of additional 40 mg adalimumab one week after standard injection. Serum samples were analysed using the recoveryELISA technology, a combination of sandwich ELISA and competitive assay. The recoveryELISA measure the concentrations of free TNF-α, drug level, and the remaining active adalimumab in the patients' sera. An adalimumab concentration of 5.0–10.0 g/mL was defined as the targeted therapeutic window.*

**Results.** *Five of 8 patients achieved moderate EULAR response by dose escalation. The results of the free adalimumab and TNF-α neutralisation measurements allowed a separation of the cohort (n=17) into three groups. Group 1 represents 18% of the patients with free adalimumab level higher 30.0 µg/mL and TNF-α neutralisation above 95%. Group 2 (47%) consists of patients within the therapeutic window with balanced free adalimumab and TNF-α neutralisation values. Group 3 contains 35% of the cohort with low concentrations of free adalimumab and lowest remaining TNF-α-neutralisation capacity. Anti-drug antibodies were detected in four patients but did not prevent response to treatment.*

**Conclusion.** *Drug and antigen monitoring using recoveryELISA may support dose decision to avoid unnecessary switch in medication or possible overtreatment.*

## Introduction

Rheumatoid arthritis (RA) is associated with chronic inflammation, functional disability, and comorbidity resulting in reduced life expectancy (1, 2). Despite recent advances, even biological DMARDs fail to achieve sustained remission in many patients (3, 4). Reasons for inadequate response include neutralising anti-drug antibodies (ADA) and sub-therapeutic drug levels (5, 6). Most of the TNF-α inhibitors are prescribed at standard doses, irrespectively of body weight, disease state, and age. Since some RA patients report intermittent disease activity several days prior to their next injection and patients with high levels of free TNF-α may benefit from an increased dose of TNF inhibitors, *e.g.* adalimumab (7), it is one option to increase the drug level by additional weekly injection (boost) before switching the drug.

Since biologics remain cost expensive and may cause side effects, such dosing decisions should be studied in more detail, allowing a better estimate of therapeutically optimised doses.

The synovial inflammation is largely tumour necrosis factor- (TNF-α) dependent and treatment with TNF-α inhibitors has proved to be efficient in approximately two-thirds of RA patients. However, in a substantial proportion of RA patients, anti- TNF-α treatment does not lead to a satisfactory clinical improvement or fail to sustain a clinical response over time

The *recovery*ELISA as combined sandwich ELISA and competitive assay uses a labelled adalimumab competing with the therapeutic antibody (adalimumab) for the same binding epitope on TNF-α. This allows the simultaneous detection of free TNF-α, free therapeutic antibody and the calculation of the TNF-α neutralisation capacity in one test system (8).

The aim of this study was to monitor drug and antigen levels using the *recovery*ELISA and to evaluate whether this test may help in clinical decision making to adjust the adalimumab dosage.

## Patients and methods

Seventeen patients treated with stable dosing of adalimumab (40 mg biweekly), methotrexate (15 mg weekly) and steroids (2.5–7.5 mg daily) for more than 3 months and fulfilling the ACR/EULAR classification criteria of RA (9) were consecutively recruited. The study protocol was approved by the ethics board of the Medical Associa-

**Table I.** Demographics, RA disease parameters, and results of recoveryELISA in the study cohort and the subgroups. (significance levels *$p$<0.05; **$p$<0.01; ***$p$<0.001 in Fisher's exact test (categorical data) and $t$-test (parametric data)).

| | All n=17 | Non-Boosted n=9 | Boosted n=8 | Supra-therapeutic ADA-Levels n=3 | Therapeutic Window ADA-Levels n=8 | Sub-therapeutic ADA-Levels n=6 |
|---|---|---|---|---|---|---|
| Age (years), mean ±SD | 57 ± 15 | 60 ± 17 | 54 ± 14 | 56 ± 12 | 54 ± 17 | 62 ± 15 |
| Female sex, (n) % | (11) 65% | (5) 56% | (6) 75% | (2) 66% | (6) 75% | (3) 50% |
| Body weight (kg), mean ±SD | 73 ± 18 | 81 ± 20* | 65 ± 11 | 65 ± 5 | 67 ± 13 | 87 ± 23 |
| RA duration (years) mean ±SD | 19 ± 16 | 22 ± 15 | 16 ± 16 | 13 ± 10 | 20 ± 19 | 15 ± 13 |
| Adalimumab pre-treatment (months), mean ±SD | 44.7 ± 40.8 | 52.6 ± 39.6 | 32.8 ± 44.6 | 53.3 ± 57.0 | 43.9 ± 46.3 | 37.3 ± 35.0 |
| Adalimumab is 2nd bDMARD, (n) % | (5) 29% | (5) 56% | 0 | (2) 67% | (1) 13% | (2) 33% |
| Anti-drug-antibody positive, (n) % | (4) 24% | (3) 33% | (1) 13% | (2) 67% | (1) 13% | (1) 17% |
| CDAI baseline, mean ±SD | 9.8 ± 7.7 | 4.1 ± 4.8*** | *16.2 ± 4.7*** | 10.7 ± 3.5 | 10.6 ± 8.4 | 8.3 ± 9.2 |
| CDAI day 14, mean ±SD | 7.5 ± 5.1 | 4.1 ± 4.2** | *11.3 ± 3.1** | 7.3 ± 4.7 | 8.3 ± 4.4 | 6.5 ± 6.8 |
| ESR (mm/h) mean ±SD | 17 ± 16 | 20 ± 17 | 11 ± 8 | 31 ± 24 | 11 ± 8 | 18 ± 29 |
| $DAS_{28}$ baseline, mean ±SD | 2.9 ± 1.2 | 2.5 ± 1.2 | 3.5 ± 1.1 | 3.6 ± 1.0 | 2.9 ± 1.3 | 2.7 ± 1.3 |
| $DAS_{28}$ day 14, mean ±SD | 2.6 ± 1.0 | 2.3 ± 1.2 | 2.9 ± 1.0 | 2.9 ± 1.2 | 2.3 ± 1.0 | 2.7 ± 1.2 |
| Moderate EULAR response | (6) 35% | (1) 11% | (5) 63% | (2) 66% | (3) 38% | (1) 17% |
| Free adalimumab baseline mean ±SD | 9.9 ± 10.7 | 10.3 ± 11.4 | 9.6 ± 10.3 | 31.7 ± 2.9** | 7.1 ± 1.6*** | 2.9 ± 0.8*** |
| Free adalimumab, day 14, mean ±SD | 10.1 ± 10.2 | 9.3 ± 11.7 | 10.6 ± 9.6 | 32.0 ± 2.8** | 8.2 ± 2.4* | 2.6 ± 0.6* |
| Free TNF-α baseline ±SD | 8.5 ± 6.2 | 8.3 ± 5.6 | 8.7 ± 7.2 | 1.0 ± 0.0*** | 6.4 ± 1.8*** | 15.0 ± 5.3*** |
| Free TNF-α day 14 ±SD | 8.3 ± 5.7 | 9.2 ± 5.3 | 7.5 ± 5.7 | 1.0 ± 0.0*** | 5.6 ± 1.7*** | 14.5 ± 3.8*** |
| TNF-α neutraliation, baseline, mean ±SD | 91.5 ± 6.2 | 91.5 ± 5.6 | 91.3 ± 7.2 | 99.0 ± 0*** | 93.6 ± 1.8*** | 85.0 ± 5.3*** |
| TNF-α neutralisation, day 14 mean ±SD | 92.1 ± 5.9 | 91.9 ± 5.8 | 92.5 ± 6.2 | 99.3 ± 0.6*** | 94.4 ± 1.7*** | 85.6 ± 3.8*** |

tion of Brandenburg. All patients gave written consent according to the Helsinki declaration.

Clinical Disease Activity Index (CDAI) and Disease Activity Score-28 ($DAS_{28}$) based on erythrocyte sedimentation rate (ESR) (9) were measured at baseline, day 7 and day 14. $DAS_{28} \geq 2.6$ were considered as active RA and improvement of >0.6 was defined as moderate EULAR response (10). Patients with persistent clinical disease activity, *i.e*. CDAI score of >10 (summarising tender/swollen joints, patients and physicians global assessment), were treated by an additional injection of 40 mg adalimumab at day 7 (boost).

To allow a quantification of free adalimumab, TNF-α and TNF-α neutralisation capacity serum samples were prepared as recently described (11, 12). The *recovery*ELISA (BioTeZ Berlin-Buch, Germany) was carried out with different antigen-additions for one sample; this requires a multidimensional assay calibration. The analysis uses a non-linear regression Marquardt-Levenberg algorithm, the Michaelis-Menten model for enzyme kinetics and the Langmuir isotherm for surface binding. Measurement of anti-drug autoantibodies (ADA) was carried out by Medizinisches Labor Oldenburg using IDK*monitor* Adalimumab total ADA Elisa K9651 (Immundiagnostik, Germany).

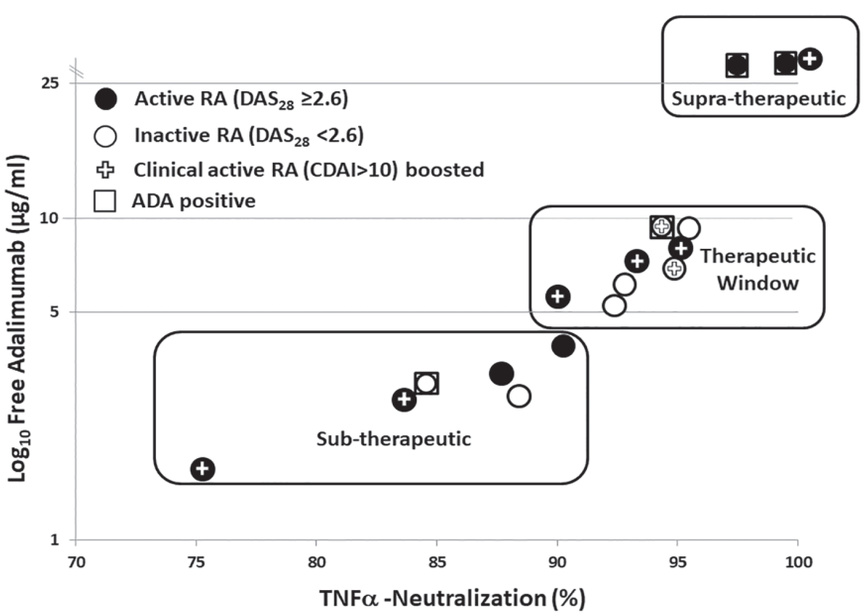


**Fig. 1.** Free adalimumab concentration (µg/mL) and TNF-α neutralisation (%) for all patients at baseline; filled circles indicate RA active patients (DAS28 ≥2.6); circles indicate RA inactive patients (DAS28 <2.6); crosses indicate clinical active (CDAI>10) and therefore boosted patients; squares show anti-drug antibodies (ADA) positive (AU/ml >10) patients.

## Results

In total, 17 RA patients at a mean±SD age of 57±15 years were studied. Eleven (65%) of them were female. The mean disease duration was 19±16 years, and the mean $DAS_{28}$ at baseline was 2.9±1.2. Duration of adalimumab pre-treatment was 45±41 months. For 5 patients, adalimumab was the first bDMARD, for all other the second (Table I). Whereas 41% of the patients

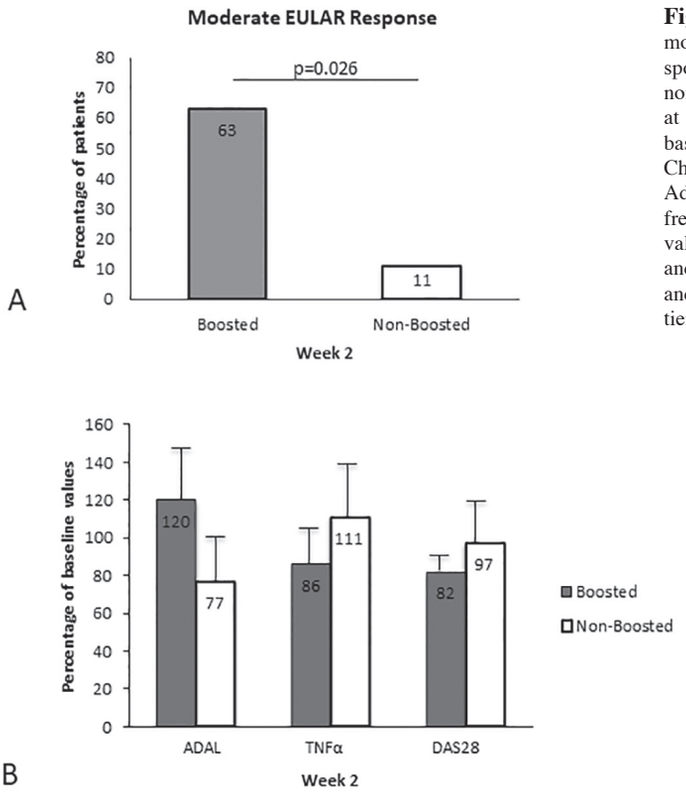


**Fig. 2.** Percentage of moderate EULAR response in boosted and non-boosted patients at week 2 compared to baseline (**A**).
Change of mean (SD) Adalimumab (ADAL), free TNF-α, and DAS28 values between baseline and week 2 in boosted and non-boosted patients (**B**).

were in $DAS_{28}$ remission, 59% exhibited low or moderate disease activity with a mean $DAS_{28}$ of 3.8±0.8. Eight patients with CDAI>10 were treated with an additional adalimumab injection (Table I, Fig. 1).

Boost injections at day 7 led to a 20% mean decrease of $DAS_{28}$ at day 14 ($DAS_{28}$ of 3.5±1.0 *vs*. 2.8±1.0). Seven of the 8 boosted patients responded clinically, 5 (61%) reached EULAR moderate response criteria, while only 1 patient (11%, $p$=0.026) of the regularly treated group presented a decrease of $DAS_{28}$ (Fig. 2A). CDAI of the boosted patients significantly decreased from 16.2±4.7 to 11.3±3.1 ($p$=0.03). When compared to regularly treated patients, adalimumab boosted patients were more often females with lower mean weight, age and ESR values, but higher $DAS_{28}$ scores at baseline (Table I).

The higher the free adalimumab baseline level, the higher TNF-α neutralisation capacities were found (Fig. 1). Clinically active patients had lower baseline levels of free adalimumab and developed an increase in adalimumab levels and a reduction of free TNF-α after the boost (Fig. 2B).

Interestingly, only 47% of the patients matched the therapeutic window of adalimumab concentration (5.0–10.0 μg/mL (5)), whereas 18% of the patients exceeded this range and 35% did not achieve the recommended drug concentration. So the cohort was divided into three subgroups with sub-therapeutic, supra-therapeutic, and therapeutic adalimumab levels (Fig. 1). All groups showed comparable values in demographics. Interestingly, in the sub-therapeutic group a trend to higher mean body weight was found (Table I).

In the group with therapeutic adalimumab levels, only 38% (3 out of 8) of the patients had a $DAS_{28}$≥2.6. Furthermore all of them were boosted and achieved moderate EULAR response.

In the group with sub-therapeutic drug levels, all patients with increased free TNF-α concentrations and consequently the lowest levels of TNF-α neutralisation were found. Four out of six patients (67%) had a $DAS_{28}$ ≥2.6. Two of them were selected for boost, one achieved moderate EULAR response.

In the group with supra-therapeutic drug levels, all patients had high free adalimumab >30.0 μg/mL and TNF-α neutralisation rates >95%. Nevertheless, all patients had $DAS_{28}$ ≥2.6 (Fig. 1), whereas only one patient was classified as clinically active. This boosted patient, as well as another regularly treated patient, achieved moderate EULAR response.

ADA were detected in four patients, of which one was clinically active and responded partially to boost injection with a decrease in CDAI (-1.0; 91% baseline) and DAS28 (-0.3; 85% baseline). The time of adalimumab pretreatment of these patients was 7 months (active RA), 6, 7, and 117 months, respectively. For all, adalimumab was the first bDMARD.

## Discussion

In this proof of concept study, we aimed to evaluate the *recovery*ELISA technology, allowing the simultaneous detection of free TNF-α free therapeutic antibody concentrations, and TNF-α neutralisation capacity in one test (8), as potential prognostic laboratory markers for adjustment of adalimumab dosage decision in patients with active RA.

Dose adjustments (boost) were decided by clinical activity (CDAI) leading to improvement of clinical symptoms in 7 of 8 patients and significantly increased rate of moderate EULAR response (63%) at day 14 (Fig. 2A). A partial mismatch was obtained between $DAS_{28}$ definition and the CDAI. Of 10 patients with $DAS_{28}$≥2.6 only 6 were found clinically active with CDAI>10 and 2 of 8 boosted patients with CDAI>10 had $DAS_{28}$<2.6. Due to the different components of the $DAS_{28}$ score, limited number of analysed joints, and low specificity of included ESR, such mismatch is not surprising and known from daily practice.

The *recovery*ELISA assessing the free TNF-α concentration and TNF-α neutralisation capacity gives a more detailed serological picture before and after therapy adjustments. Based on adalimumab serum levels, the test identified three subgroups of patients: ranging in therapeutic dosage, under- or over-treated with adalimumab. Interestingly, only 47% of patients matched recommended therapeutic levels of adalimumab despite using standard dosage. This confirms recently published data

(13) where only 52% of the patients remained in the therapeutic window. Secondly, the assay yielded concordant results in three tested parameters: the patient group with high adalimumab levels in the supra-therapeutic range displayed lowest free TNF-α levels and highest TNF-α neutralisation capacity while concordantly patients with sub-therapeutic antibody levels showed highest free TNF-α levels and lowest TNF-α neutralisation capacity.

The identification of patients with increased free TNF-α concentration offers additional serological markers for the rheumatologist to decide for dose adjustment. Simultaneously, *recovery*-ELISA allows monitoring the TNF-α neutralisation capacity in patient's sera during therapy and particularly after dose adjustment. Additionally, the higher body weight found in the sub-therapeutic group might contribute to low bioavailability of adalimumab, suggesting that dosage adaption is dependent on patient's body weight.

Single adalimumab boosts lead to an increase in adalimumab levels and TNF-α neutralisation and decrease in free TNF-α correlating with clinical improvement, yet did not reach statistical significance, nor therapeutic levels in all patients. Best amount and frequency of dosage adjustment has to be studied in future trials. In the supra-therapeutic group, saturated adalimumab serum levels were observed. Regularly treated and boosted patients exhibited moderate disease responses, suggesting that disease activity and therapeutic response might be TNF-α-independent. Thus, adalimumab boost should be critically reconsidered, as saturating levels of adalimumab might be harmful, leading to immunosuppression, increased risk of infection (14) and additional costs for the health care system (13, 15).

Clear limitations of this proof of concept study include the small number of patients in the cohort, leading to insufficient power for subgroup analysis.

## Conclusions

The *recovery*ELISA technology offers a test system to simultaneously evaluate free TNF-α, serum drug levels and the neutralisation capacity of the drug, providing help to decide whether the drug is present in recommended dosage or out of recommended range in individual patients. This may facilitate clinical decision-making based on drug and antigen monitoring by offering potential prognostic laboratory markers for a dosage adjustment in patients with active RA while avoiding expendable and harmful doses that are ineffective.

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