Clinical implications of immunogenicity

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Chapter 3

Clinical response to adalimumab: the relationship with anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis

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ABSTRACT

Background. A substantial proportion of rheumatoid arthritis (RA) patients does not respond or loses initial response to adalimumab therapy. One of the explanations for non-response could be that patients develop anti-adalimumab antibodies. Therefore, we evaluated the incidence of antibody formation against adalimumab and the association with serum adalimumab concentrations and clinical response.

Methods. In a cohort of 121 consecutive RA patients treated with adalimumab we measured serum adalimumab concentrations and antibodies against adalimumab together with clinical response parameters before and up to 28 weeks after the start of adalimumab treatment.

Results. Anti–adalimumab antibodies were detected in 21 patients (17%) during 28 weeks of treatment. EULAR non-responders significantly more often had anti-adalimumab antibodies than good responders (34% vs. 5%), ($P = 0.032$). Patients with anti-adalimumab antibodies had less improvement in DAS28 score (mean ± SD, delta DAS28: 0.65 ± 1.35) than patients without anti-adalimumab antibodies (mean delta DAS28: 1.70 ± 1.35), ($P = 0.001$). Patients with anti-adalimumab antibodies during follow-up had lower serum adalimumab concentrations at 28 weeks than patients without anti-adalimumab antibodies (median 1.2 mg/L , range 0.0 - 5.6 vs. median 11.0 mg/L, range 2.0 - 33.0, respectively), ($P < 0.001$). Good responders had higher serum adalimumab concentrations than moderate ($P = 0.021$) and non-responders ($P = 0.001$). Concomitant methotrexate use was lower in the group with anti-adalimumab antibodies (52%) than in the patient group without antibodies (84%), ($P = 0.003$).

Conclusions. Serum antibodies against adalimumab are associated with lower serum adalimumab concentrations and non-response to adalimumab therapy.

INTRODUCTION

A substantial proportion of rheumatoid arthritis (RA) patients still have persistent disease activity or flare of disease activity despite treatment with TNF-α blocking therapy.[1] An explanation could be that antibodies are formed against these therapeutic agents. In RA and Crohn's disease patients with human anti-chimeric antibodies (HACAs) to infliximab have been described. Initially, the clinical significance of these antibodies was uncertain. However, recent data on Crohn's disease indicate that these anti-infliximab antibodies are associated with allergic reactions and a shorter duration of response.[2] In RA patients the development of antibodies against infliximab is associated with a reduced response to infliximab after treatment for an extended period of time.[3] Simultaneous immunosuppressive therapy has shown to reduce HACA formation.[2, 4]

Adalimumab is a fully human antibody and, therefore, thought to be less immunogenic than chimeric antibodies.[5] Nevertheless, it has previously been suggested that human anti-human antibodies (HAHAs) against adalimumab may develop as well, although the data are limited. Anti-adalimumab antibodies were found in 12 % of RA patients on adalimumab monotherapy in a dose of 40 mg every other week.[6, 7] Contradictory results were reported with regard to the influence of these antibodies on clinical response.[6, 7] Recently, we found high anti-adalimumab concentrations in an RA patient, which was associated with undetectable serum adalimumab levels and a diminished clinical response.[8] This case report suggested that HAHA formation could play an important role in some patients not responding to adalimumab therapy. This emphasizes the need for further investigation with standardized analysis techniques into the role of antibody formation on clinical response. Therefore, we evaluated adalimumab and anti-adalimumab antibody levels in relation to clinical response in a cohort of RA patients up to 28 weeks after initiation of treatment.

PATIENTS AND METHODS

Patients. This prospective observational cohort study consisted of 121 consecutive RA patients treated with adalimumab therapy at the Departments of Rheumatology of the Jan van Breemen Institute and the Academic Medical Center, Amsterdam. All patients fulfilled the American College of Rheumatology 1987 revised criteria for RA, and had active disease, indicated by a disease activity score in 28 joints (DAS28) of $\geq$ 3.2 despite earlier treatment with two Disease-Modifying Anti-Rheumatic Drugs (DMARDs) including methotrexate (MTX) at a dosage of 25 mg weekly or at the maximal tolerable dosage, according to the Dutch consensus statement on the initiation and continuation of TNF blocking therapy in RA.[9] Patients were treated either with adalimumab and concomitant DMARD therapy or with adalimumab monotherapy. All patients used adalimumab 40 mg subcutaneously every other week. In patients with an inadequate response as judged by the treating rheumatologist, the dosing frequency of adalimumab could be increased to 40 mg per week. The study was approved by the Medical Ethics Committee of the Slotervaart Hospital, BovenIJ Hospital, the Jan van Breemen Institute, and the Academic Medical Center/University of Amsterdam. All patients gave written informed consent.

Clinical Response. Disease activity was assessed at baseline and after 4, 16 and 28 weeks of therapy using the DAS28 score.[10] Clinical response was assessed by the European League Against Rheumatism (EULAR) response criteria and the change in DAS28 score (delta DAS28).[11] Serum

samples were collected just prior to an injection with adalimumab at baseline, and after 4, 16 and 28 weeks.

Measurement of adalimumab concentrations. Trough serum adalimumab concentrations were measured by enzyme linked immunosorbent assay (ELISA), based on the principle that adalimumab is captured via its ability to bind TNF-α. Adalimumab was quantified as described previously for infliximab measurement with one modification.[12] Adalimumab binding was assessed by incubation with biotinylated rabbit IgG directed to the adalimumab idiotype. Detection limit of the assay is about 0.001 mg/L.

Measurement of antibodies against adalimumab. Anti-adalimumab was detected with a newly developed radio immunoassay (RIA). The assay measures specific high avid IgG antibodies against adalimumab by an antigen binding test as described previously for the detection of antibodies against infliximab.[3] Serum (1µL/test) was pre-incubated with Sepharose-immobilized protein A (1 mg/test; Pharmacia, Uppsala, Sweden) in Freeze buffer (Sanquin, Amsterdam, the Netherlands). Non-bound serum components were removed by washing before 50 µl of ^{125}I-labeled F(ab)'2 fragment of adalimumab was added. After overnight incubation non-bound radiolabel was washed away and Sepharose-bound radioactivity was measured. Test results were converted into arbitrary units per milliliter (AU/ml) by comparison with dilutions of a reference serum. The mean cut-off value was set at 12 AU/ml (mean + 6 SD of the pre-treatment values) which was derived from 100 healthy donors. In agreement with this all sera of patients in this study contained less than 12 AU/ml of anti-adalimumab before they received their first adalimumab injection. Assay specificity was demonstrated by the absence of anti-adalimumab in 25 sera containing high-titer anti-infliximab. Because the presence of adalimumab interferes with the assay, it is to be expected that in patients with high levels of adalimumab, anti-adalimumab is underestimated or undetectable. The antibody test was considered positive when the antibody concentration exceeded 12 AU/ml and the adalimumab concentration was 5 mg/L or less. We occasionally observed patients with anti-adalimumab simultaneous with serum levels of adalimumab. Adalimumab and antibody concentrations were measured at baseline and 28 weeks after initiation of treatment. At week 4 and 16, antibody concentrations were determined only in serum of patients with an adalimumab concentration of less than 5 mg/L and in patients with anti-adalimumab at week 28.

Statistical analysis. For differences between groups we used the independent samples T test or Mann-Whitney U as appropriate. For differences between paired samples we used paired samples T test or Wilcoxon signed rank test. The threshold for significance was set at $P < 0.05$. We used multiple logistic regression analysis to investigate the relationship between EULAR response and adalimumab concentrations as well as the presence of anti-adalimumab antibodies and the influence of sex, age, methotrexate and prednisone use and dose on this relationship.

To analyze clinical response in patients with and without antibodies after 28 weeks of treatment we used last observation carried forward for patients who stopped treatment due to non-response or adverse events. In a sub analysis the effects of increasing the adalimumab dosing frequency to 40 mg weekly were studied with the data obtained after the more frequent dosing.

RESULTS

Patient characteristics. The majority of the 121 patients who entered the study was female (79%) and mean (± standard deviation, SD) age was 53 ± 13 years (Table 1). At baseline patients had active disease as indicated by a mean DAS28 score of 5.3 ± 1.1. Mean disease duration was 12 ± 10 years and on average patients had failed 3.6 ± 1.6 prior DMARDs. Most patients used concomitant MTX (79%) with a mean dose of 19.4 ± 7.4 mg/ week, and 34% used prednisone at a mean dose of 7.9 ± 4.3 mg/day. Fourteen (12%) patients used sulphasalazine and/or hydroxychloroquine in combination with MTX, two (2%) patients used concomitant hydroxychloroquine only and 24 (20%) patients were on adalimumab monotherapy.

Table 1. Demographic and clinical characteristics at baseline*

	Total patient population	Patient with anti-adalimumab antibodies	Patients without anti-adalimumab antibodies
	n = 121	n = 21	n = 100
Demographics			
Age, years	53 ± 13	51 ± 16	54 ± 12
Female, no. (%)	95 (79)	17 (81)	78 (78)
DMARD therapy			
Prior DMARDs	3.6 ± 1.6	3.4 ± 1.6	3.7 ± 1.6
Prior Biologicals	34 (28)	10 (48)	24 (24)
Methotrexate use, no. (%)	95 (79)	11 (52)	84 (84)
Methotrexate dose (mg/wk)	19.4 ± 7.4	17.0 ± 8.6	19.7 ± 7.3
MTX plus other DMARD use, no. (%)	14 (12)	1 (5)	13 (13)
No concomitant DMARD, no. (%)	24 (20)	9 (43)	15 (15)
Prednisone use, no. (%)	41 (34)	9 (43)	32 (32)
Prednison dose (mg/day)	7.9 ± 4.3	7.1 ± 2.3	8.1 ± 4.7
Disease status			
Disease duration (years)	12 ± 10	11 ± 8	12 ± 11
Rheumatoid factor positive, no. (%)	93 (77)	16 (76)	77 (77)
Erosive disease, no. (%)	94 (78)	18 (86)	77 (77)
Nodular disease, no. (%)	29 (24)	5 (24)	24 (24)
Erythrocyte sedimentation rate (mm/h)	32 ± 25	42 ± 34	30 ± 23
C-reactive protein (mg/dl)	24 ± 28	34 ± 38	22 ± 24
DAS28	5.3 ± 1.1	5.5 ± 1.2	5.3 ± 1.1

* Mean values ± SD, or percentages are shown.
The percentage of patients using concomitant MTX was significantly lower in patients with antibodies against adalimumab compared to patients without such antibodies P = 0.003. The mean (± SD) MTX dose did not differ significantly between antibody positive and negative patients.

Clinical response. After 28 weeks follow-up 109 of 121 patients were still on adalimumab treatment. In ten patients dosing frequency was increased. Of the 99 patients who did not have an increased adalimumab dosing frequency, 77 (78%) were classified as responder according to the EULAR response criteria. The responders consisted of 34 (34%) good responders and 43 (43%) moderate responders. The mean improvement in DAS28 was 1.7 ± 1.3.

Twelve patients stopped treatment before week 28, of whom 6 (5%) due to inefficacy (as concluded by the treating rheumatologist), and 4 (3.3%) due to side effects (chronic coughing, flu like symptoms, palpitations, dysuria, and fever of unknown origin). In one patient (0.8%) adalimumab was

stopped because of a suspected malignancy, and one patient (0.8%) died of an unknown cause after 16 weeks of treatment.

Antibodies against adalimumab. During 28 weeks follow-up, antibodies were detected in 21 (17%) patients. Nine (7.8%) patients were found to be positive for anti-adalimumab at week 4 after start of treatment, six (5.0%) patients at week 16, and another six (5.0%) patients at week 28. The serum concentrations of anti-adalimumab had two clusters that could be separated at a cut off value of 100 AU/ml. Ten of 21 patients had antibody concentrations that stayed below 100 AU/ml at all time points [range 13-57 AU/ml] and 11 patients had antibody concentrations above 100 AU/ml [range 115-10344 AU/ml]. When antibody concentrations of 100 AU/ml or more were present, these remained high during treatment and concentrations increased further over time. Antibody concentrations between 12 and 100 AU/ml were less stable over time and were intermittently detectable or undetectable.

The percentage of patients using concomitant MTX was significantly lower in patients with antibodies against adalimumab (52%) compared to patients without (84%) such antibodies ($P = 0.003$), (Table 1). The mean MTX dose did not differ significantly between anti-adalimumab positive and negative patients. The mean MTX dose was 19.7 ± 7.3 mg/week in the patients without anti-adalimumab antibodies, 21.9 ± 6.3 mg/week in the low antibody group (12-100 AU/ml), and 14.3 ± 8.9 mg/week in patients with high anti-adalimumab antibody concentrations (>100 AU/ml). A trend towards a lower MTX dose was seen in the group with high antibody concentrations > 100 AU/ml ($P = 0.056$). Patients with concomitant MTX had a lower rate of antibody development than patients on adalimumab monotherapy (12% vs. 38%).

Adalimumab concentrations. At 28 weeks median trough adalimumab concentration was 10 mg/L and varied from undetectable to 28 mg/L in patients with adalimumab in a dose of 40 mg every other week. In patients who used 40 mg weekly, median trough adalimumab concentration was 12 mg/L and varied from 8 to 43 mg/L.

The course of the median trough adalimumab levels over 28 weeks of adalimumab is shown in Figure 1. In patients with anti-adalimumab antibodies serum adalimumab concentrations were significantly lower compared to patients without anti-adalimumab antibodies (median 1.2 mg/L, range 0.0-5.6 vs. median 11.0 mg/L, range 2.0-33.0), ($P < 0.001$).

Clinical response and anti-adalimumab antibodies. EULAR non-responders had significantly more often anti-adalimumab than good responders ($P = 0.006$). After adjusting for sex and MTX dose, the presence of anti-adalimumab antibodies remained a significant determinant of EULAR response ($P = 0.032$). EULAR non-responders significantly more often had anti-adalimumab than moderate responders ($P = 0.039$). However, this difference between non and moderate responders was no longer present after adjusting for MTX use ($P = 0.148$). The difference between good and moderate responders did not reach statistical significance.

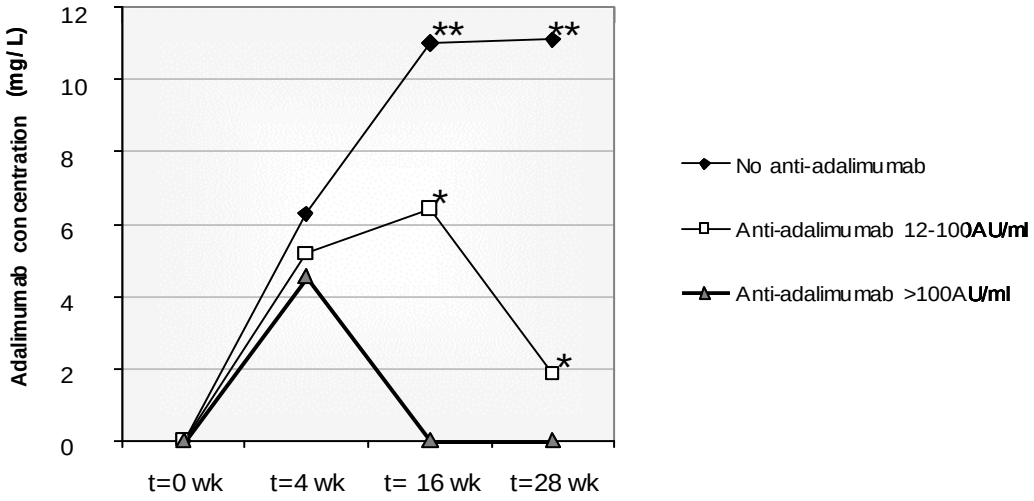

Figure 1. Median serum trough adalimumab concentrations (mg/L) over time in patients with anti-adalimumab antibody concentrations of 12-100 AU/ml, and >100 AU/ml compared to patients without anti-adalimumab antibodies. Adalimumab concentrations were significantly lower in patients with high anti-adalimumab antibody levels compared to low *($P < 0.001$) or absent antibodies** ($P < 0.001$) at week 16. The difference in adalimumab concentrations between low antibody levels and no antibodies was also significant ($P = 0.001$). At week 28 differences between all groups were highly significant ($P < 0.001$).

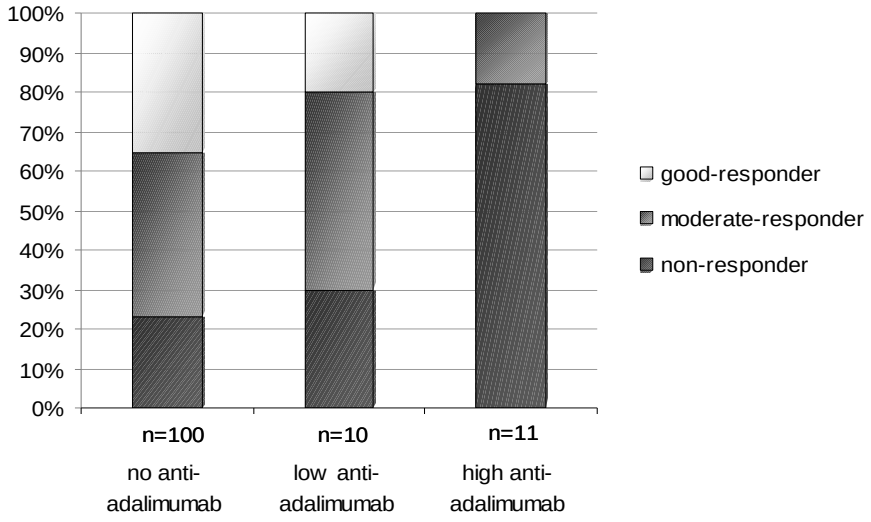

Figure 2. The relationship between EULAR response and presence or absence of anti-adalimumab antibodies is shown. Of the 11 patients with anti-adalimumab antibody concentrations above 100 AU/ml 9 patients (82%) were non-responder and 2 patients were moderate responder (18%). Of the 10 patients with anti-adalimumab levels between 12-100 AU/ml only 3 patients were non-responder (30%), 5 were moderate responder (50%) and 2 were good responder (20%). Of the 100 patients in whom no antibodies against adalimumab were detected during the 28 weeks follow-up, 23 (23%) were non-responder, 41 (41%) moderate, and 35 (35%) good responder. EULAR non-responders had significantly more often anti-adalimumab antibodies than good responders ($P = 0.006$) and moderate responders ($P = 0.039$), and most non-responders were found in the group with high (>100 AU/ml) anti-adalimumab concentrations compared to the group with low (12-100 AU/ml) anti-adalimumab concentrations.

Of the 11 patients with anti-adalimumab concentrations above 100 AU/ml 9 patients (82%) were non-responder and 2 patients were moderate responder (18%). In patients with anti-adalimumab levels between 12-100 AU/ml (n = 10) only 3 patients were non-responder (30%), 5 were moderate responder (50%) and 2 were good responder (20%). Mean improvement in DAS28 in these patients was 0.20 ± 0.98. Of all patients in whom no antibodies against adalimumab were detected during the 28 weeks follow-up, 23 (23%) were non-responder, 41 (41%) moderate, and 35 (35%) good responder . The relationship between EULAR response and presence or absence of anti-adalimumab is shown in figure 2. Patients with anti-adalimumab had significantly less improvement in DAS28 (mean delta DAS28: 0.65 ± 1.35) than patients without anti-adalimumab (mean delta DAS28: 1.70 ± 1.35) (P = 0.001). The relationship between DAS28, adalimumab, and anti-adalimumab concentrations is shown in figure 3.

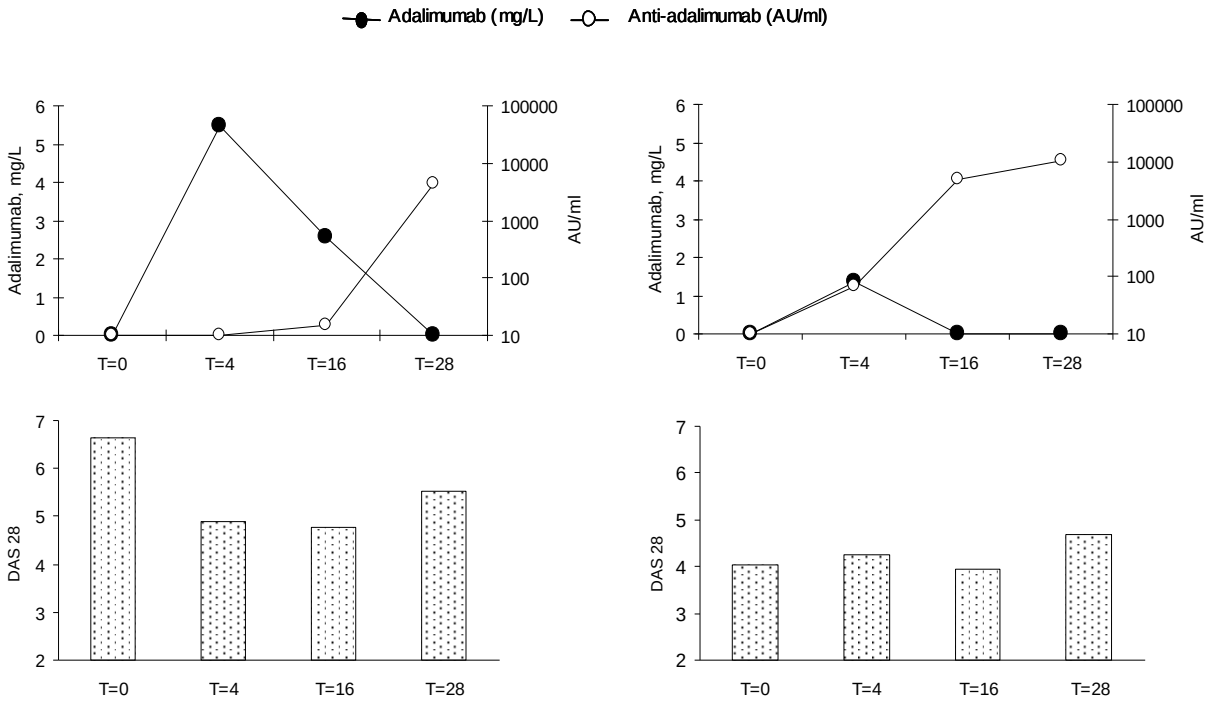

Figure 3. Serum adalimumab concentrations in relationship to anti-adalimumab antibody concentrations and DAS28. The graphs represent two panels for two individual patients who had high anti-adalimumab concentrations (>100 AU/ml) during 28 weeks follow-up. The X-axis represents time in weeks, the left Y-axis represents adalimumab concentration in mg/L. The right Y-axis represents the anti-adalimumab antibody concentration in AU/ml on a log-scale. The DAS28 score is presented in bars.

Clinical response and adalimumab concentrations. EULAR non-responders had significantly lower serum adalimumab concentrations than good responders (median 5.4 mg/L, range 0.0 - 21.2 vs. 9.8 mg/L, range 0.0 - 33.0), (P = 0.001). Furthermore, non-responders had lower serum adalimumab levels than moderate responders (P = 0.043). However, this difference was no longer significant after adjusting for MTX use (P = 0.098). The relationship between EULAR response and adalimumab concentrations is shown in figure 4. Good responders had significantly higher serum adalimumab concentrations than moderate responders (P = 0.021).

Prednisone and MTX dose remained relatively stable in 17 of 21 patients who tested positive for anti-adalimumab. In two of the 21 patients low dose prednisone (5.0 and 7.5 mg/day) was stopped. The MTX dose (5 mg/week) was discontinued in one of the 21 patients, and in another patient MTX (15 mg/week) was started simultaneously with adalimumab.

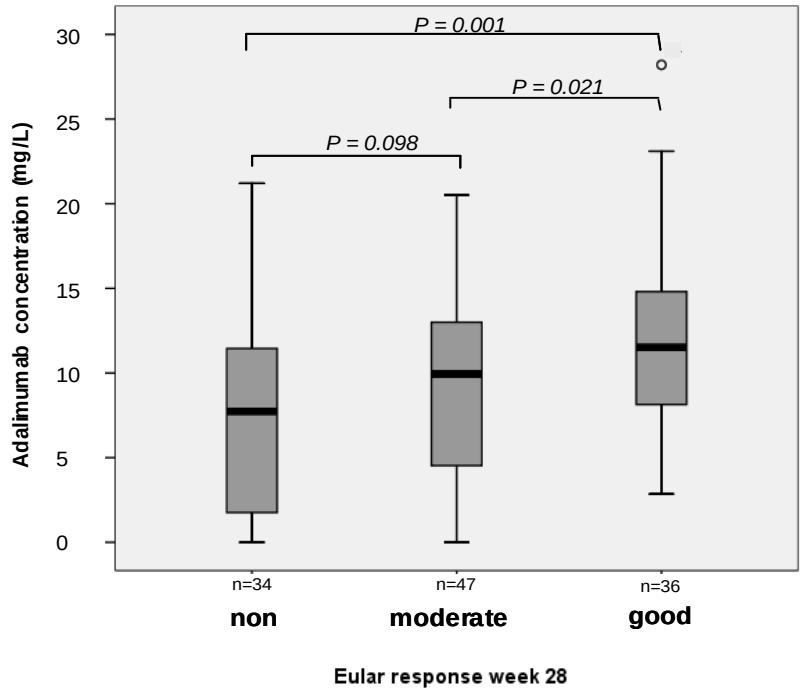

Figure 4. Serum adalimumab concentrations (mg/L) in non-responders (n= 34), moderate (n= 47), and good responders (n= 36), according to the EULAR response criteria at week 28. EULAR non-responders had significantly lower serum adalimumab concentrations than good responders (median 5.4 mg/L, range 0.0 - 21.2 vs. 9.8 mg/L, range 0.0 - 33.0), (P = 0.001). Non-responders had lower serum adalimumab levels than moderate responders (P = 0.043). However, this difference was no longer significant after adjusting for MTX use (P = 0.098). Good responders had significantly higher serum adalimumab concentrations than moderate responders (P = 0.021). The boxplot presents the median and interquartile range, the whiskers represent the 95th percentiles.

Increased dosing frequency of adalimumab. In ten patients (8,3%) the dosing frequency of adalimumab was increased to 40 mg weekly while concomitant MTX and prednisone dose remained stable. At the last visit before increasing the dosing frequency, 7 patients were non-responder, 1 moderate, and 2 good responder (who subjectively perceived inefficacy). In 5 of the seven non-responders anti-adalimumab antibodies were detectable with antibody concentrations ranging from 39-184 AU/ml. The anti-adalimumab was no longer detectable after increasing the dosing frequency and adalimumab concentrations significantly increased from a mean concentration of 2.0 mg/L ± 1.8 to 15.0 mg/L ± 8.0 (P = 0.043). Clinical response improved in the non-responders with a mean decrease in DAS28 of 1.7 ± 1.2. The effects of increasing the dosing frequency on DAS28, adalimumab, and anti-adalimumab concentrations is shown in figure 5.

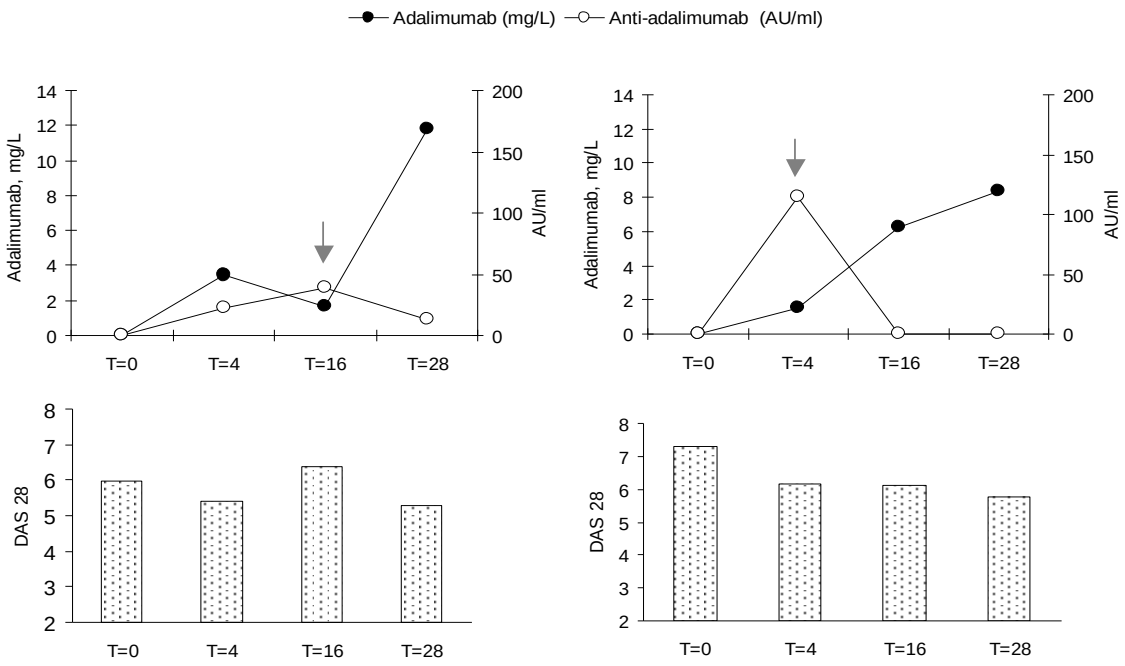

Figure 5. Changes in DAS28, adalimumab concentration, and anti-adalimumab antibody concentration after increasing the dosing frequency in two individual patients. In both non-responders the dosing frequency was increased to once per week after which an increase in adalimumab concentration was observed (see arrow for time of dose increase). The X-axis represents time in weeks, the left Y-axis represents the adalimumab concentration in mg/L. The right Y-axis represents the anti-adalimumab antibody concentration in AU/ml. The DAS28 score is presented in bars.

DISCUSSION

After 28 weeks of therapy anti-adalimumab antibodies were found in 17% of the patients. Our results show that the presence of anti-adalimumab is associated with diminished adalimumab concentrations and that especially high anti-adalimumab concentrations are associated with the absence of detectable serum adalimumab. Furthermore, the presence of anti-adalimumab as well as low serum adalimumab concentrations is associated with a diminished clinical response.

For infliximab HACAs were found in 29% of the patients during 30 weeks follow-up.[3] Infliximab is a chimeric monoclonal antibody, with a human constant region and murine variable regions. In adalimumab, a “fully human” antibody, the constant and variable regions are both “human”. Therefore, one can expect adalimumab to be less immunogenic. However, when comparing the relative immunogenicity we have to take into account not only the extent of humanization but also the differences in dosing schedules and route of administration of these two monoclonal antibodies.[13] It is doubtful that any biological therapy will be completely without immunogenicity, but some may be relatively more immunogenic than others.

Anti-adalimumab antibodies were described previously in 12% of patients using adalimumab monotherapy 40 mg every other week for 26 weeks.[7] In that study, no significant difference in ACR 20 response was found between patients with and without antibodies. In contrast, our data show that the presence of especially high concentrations of antibodies against adalimumab is associated with EULAR non-response. Other data confirm our finding that a diminished clinical response is seen in patients with detectable antibodies compared to patients without antibodies.[6] Differences between studies might be due to the use of different assays. Therefore, to come to an agreement on the immunological and clinical importance of antibody formation it will be essential to further standardize the assays used for studying immunogenicity. Obviously, this is not only important for adalimumab but for all therapeutic monoclonal antibodies.

During 28 weeks follow up high anti-adalimumab antibody concentrations (> 100 AU/ml) were associated with low serum trough adalimumab concentrations. The reduced adalimumab levels in these patients is probably due to an increased clearance of adalimumab via the formation of adalimumab–anti-adalimumab immune-complexes. This notion is supported by recent observations in RA patients receiving 99 Technetium-labeled infliximab.[14] In one HACA positive non-responder the formation of small immune complexes could be detected which coincided with a fast blood clearance and high uptake of infliximab in the liver and spleen. Without adalimumab in the circulation, its function would logically be impaired. It is possible that a patient has adalimumab in the circulation in the interval between two dosings, and the adalimumab concentration drops below detection limit just prior to the next adalimumab injection. In that case, it would be possible for a patient to have a good response based on adequate adalimumab concentrations in the two week dosing interval despite the presence of antibodies against adalimumab and undetectable trough concentrations prior to the next injection. However, the fact that most patients with anti-adalimumab concentrations above 100 AU/ml were non-responders indicates that overall serum adalimumab concentrations were very likely to be too low to have a therapeutic effect.

Since there is competition for the assay in the presence of adalimumab the incidence of anti-adalimumab may be underestimated in all patients. Anti-adalimumab antibodies could cause an increased clearance and lower adalimumab concentrations in some patients, while the antibodies are not yet detectable. This might be one of the explanations for the fact that serum adalimumab concentrations were significantly higher in EULAR good responders compared to moderate responders.

The question rises what causes anti-adalimumab formation in some patients, but not in others, and what could explain the difference between patients with high and low anti-adalimumab levels. It is likely that immunosuppression with MTX reduces anti-adalimumab formation as was shown previously for anti-infliximab antibodies.[4, 6] Our data confirm the notion that concomitant MTX use reduces the rate of antibody development .

Anti-adalimumab antibodies were no longer detectable in all 5 patients with anti-adalimumab antibodies in whom the dosing frequency was increased. Increasing the dosing frequency might overload the capacity of the immune system to produce anti-adalimumab, or alternatively induce immunotolerance. Interestingly, the disease activity (mean DAS28) improved considerably in these patients. It can be speculated that continuation of treatment with increased dosing frequency of adalimumab can be effective in some patients with anti-adalimumab antibody titers, however, dose escalation is not effective in all patients.[8, 15] In summary, these results show that anti-adalimumab antibodies are associated with diminished adalimumab concentrations, which may be related to an increased clearance of serum levels of adalimumab. Furthermore, a clear association was found between the presence of anti-adalimumab antibodies and a diminished clinical response, indicating that the formation of anti-adalimumab antibodies can be an explanation for non-response to adalimumab therapy. Quantification of serum adalimumab and anti-adalimumab antibody concentrations and learning more about the underlying factors of influence on these concentrations should lead to a more individualized and optimized treatment in the future.

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Reference list

(1) Olsen NJ, Stein CM. New drugs for rheumatoid arthritis. *N Engl J Med.* 2004;350(21):2167-2179.

(2) Baert F, Noman M, Vermeire S, Van AG, D' HG, Carbonez A, *et al*. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003;348(7):601-608.

(3) Wolbink GJ, Vis M, Lems WF, Voskuyl AE, de Groot E, Nurmohamed MT, *et al*. Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006;54(3):711-715.

(4) Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, *et al.* Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1998;41(9):1552-1563.

(5) Hwang WY, Foote J. Immunogenicity of engineered antibodies. *Methods* 2005;36(1):3-10.

(6) http:, www.fda.gov. Adalimumab Product Approval Information. 20-12-2002.

(7) van de Putte LB, Atkins C, Malaise M, Sany J, Russell AS, van Riel PL, *et al.* Efficacy and safety of adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed. *Ann Rheum Dis* 2004;63(5):508-516.

(8) Bartelds GM, Wolbink GJ, Stapel S, Aarden L, Lems WF, Dijkmans BAC, *et al.* High levels of human anti-human antibodies to adalimumab in a patient not responding to adalimumab treatment. *Ann Rheum Dis* 2006;65(9):1249-1250

(9) Furst DE, Breedveld FC, Kalden JR, Smolen JS, Burmester GR, Bijlsma JW, *et al.* Updated consensus statement on biological agents, specifically tumour necrosis factor {alpha} (TNF{alpha}) blocking agents and interleukin-1 receptor antagonist (IL-1ra), for the treatment of rheumatic diseases, 2005. *Ann Rheum Dis* 2005;64(Suppl 4):iv2-14.

(10) Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;38(1):44-48.

(11) van Gestel AM, Anderson JJ, van Riel PL, Boers M, Haagsma CJ, Rich B, *et al*. ACR and EULAR improvement criteria have comparable validity in rheumatoid arthritis trials. American College of Rheumatology European League of Associations for Rheumatology. *J Rheumatol* 1999;26(3):705-711.

(12) Wolbink GJ, Voskuyl AE, Lems WF, de GE, Nurmohamed MT, Tak PP, *et al.* Relationship between serum trough infliximab levels, pretreatment C reactive protein levels, and clinical response to infliximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005;64(5):704-707.

(13) Lobo ED, Hansen RJ, Balthasar JP. Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci* 2004;93(11):2645-2668.

(14) van der Laken CJ, Voskuyl AE, Roos JC, Stigter van WM, de Groot ER, Wolbink G, *et al.* Imaging and serum analysis of immune complex formation of radiolabeled infliximab and anti-infliximab in responders and non-responders to therapy for rheumatoid arthritis. *Ann Rheum Dis.* Published online first: 22 june 2006. doi:10.1136/ard.2006.057406v1.

(15) Breedveld FC, Weisman MH, Kavanaugh AF, Cohen SB, Pavelka K, van Vollenhoven R *et al.* The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. *Arthritis Rheum* 2006;54(1):26-37.