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Chapter 4

The clinical relevance of immunogenicity in a long-term follow-up cohort of adalimumab treated rheumatoid arthritis patients

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ABSTRACT

Context. Short term data on the immunogenicity of monoclonal antibodies showed associations between the development of anti-drug antibodies and diminished serum drug levels and a diminished treatment response. Little is known about the clinical relevance of anti-drug antibodies against these drugs during long-term follow-up.

Objective. To examine the course of anti-drug antibody formation against fully human monoclonal antibody adalimumab and its clinical relevance during long-term (3 year) follow-up of patients with rheumatoid arthritis (RA).

Design, Setting and Patients. All two-hundred-seventy-two consecutive RA patients with active disease treated with adalimumab in an outpatient clinic were enrolled in a prospective observational cohort study between February 2004 and September 2008 at the initiation of treatment.

Main Outcome Measures. The disease activity was monitored at baseline and 4, 16, 28, 40, 52, 78, 104, 130 and 156 weeks. Trough serum samples were obtained at all visits. Serum adalimumab concentrations and anti-adalimumab antibody titres were determined at the end of follow-up using an ELISA and RIA, respectively. Patients were defined as positive for anti-adalimumab antibodies if titres were above 12 AU/ml on at least one occasion, in combination with serum adalimumab levels below 5.0 mg/L. Treatment discontinuation and the achievement of minimal disease activity and clinical remission was compared for patients with and without anti-drug antibodies.

Results. After three years 76 out of 272 patients (28%) developed antibodies against adalimumab (AAA), of whom 51 patients (67%) developed AAA during the first 28 weeks of treatment. Patients without AAA had significantly higher adalimumab concentrations compared to patients with both antibody titres from 13 to 100 AU/ml ($p<0.0001$) and higher than 100 AU/ml ($p<0.0001$). Patients with AAA more often dropped out of the study due to treatment failure ($p<0.0001$) and less often achieved minimal disease activity (DAS28 <3.2 ; $p<0.0001$) and clinical remission (DAS28 <2.6 ; $p<0.0001$) compared to patients without AAA.

Conclusions. The development of anti-drug antibodies jeopardizes the long-term efficacy of adalimumab treatment in rheumatoid arthritis patients in clinical practice. Therefore, monitoring of serum drug levels and anti-drug antibody titres in daily routine practice is recommended to adjust treatment regimens towards achieving long-term treatment goals.

INTRODUCTION

Short term data regarding the immunogenicity of monoclonal antibodies and the impact on treatment response have been reported for several conditions, such as inflammatory bowel disease, rheumatoid arthritis, psoriatic arthritis, psoriasis and multiple sclerosis. (1-7) Most studies comprised a period of 6 to 12 months, and showed that the presence of anti-drug antibodies was associated with low to absent serum drug levels and a diminished treatment response, or even exacerbation of the underlying disease.

These associations raise questions regarding the extent to which anti-drug antibodies influence treatment response or, in other words, how clinically relevant the development of anti-drug antibodies is. In addition, how anti-drug antibodies presence should direct our management has been a subject of debate. (8) These questions can be applied to all diseases in which biological therapeutics are used. Long-term immunogenicity studies could help elucidate the clinical impact of anti-drug antibodies. Up till now, long-term data on immunogenicity are scarce. In Crohn's disease one study described the long-term outcome of adalimumab treatment focused on immunogenicity. (9) It showed that adalimumab trough serum concentration was lower throughout the entire follow-up period (median follow-up of 20 months) in patients who discontinued therapy and was affected by the presence of antibodies against adalimumab. In patients who displayed an adalimumab trough concentration <0.33 $\mu\text{g/ml}$ at least once, sustained clinical benefit was decreased in comparison to patients never showing such low trough serum concentration. However, the authors warn that this should be interpreted with caution due to the limited number of patients.

The current study is the first to investigate the course of anti-drug antibody development and its clinical relevance measured by the effect on treatment discontinuation, disease activity, and remission during longterm follow-up. Therefore, we followed a cohort of 272 consecutive rheumatoid arthritis patients treated with adalimumab, and measured anti-adalimumab antibody (AAA) development to investigate the described outcome measures.

PATIENTS AND METHODS

Patients. This prospective observational cohort study consisted of 272 consecutive RA patients treated with adalimumab therapy at the Department of Rheumatology of the Jan van Breemen Institute, 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 ≥ 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. (10) 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 and the Jan van Breemen Institute. All patients gave written informed consent.

Clinical Response. Disease activity was assessed at baseline and after 4, 16, 28, 40, 52, 78, 104, 130 and 156 weeks of therapy using the DAS28 score. (11) The DAS28 score is based on the number of tender joints (TJC28) and the number of swollen joints (SJC28) in 28 joints, the erythrocyte

sedimentation rate (ESR in mm/hour) and patient's general health or global disease activity on a Visual Analogue Scale (VAS) of 100 mm. The DAS28 can then be calculated using the formula: $DAS28 = 0.56 * \sqrt{TJC28} + 0.28 * \sqrt{SJC28} + 0.70 * \ln(ESR) + 0.014 * VAS$. Clinical response was assessed by investigating the proportion of patients who achieved sustained minimal disease activity and remission. Minimal disease activity was defined as a DAS28 that stayed below 3.2 at all consecutive measurements after a certain time point, with a minimum of two measurements below 3.2 for patients who discontinued treatment prematurely. Remission was defined as a DAS28 that stayed below 2.6 at all consecutive measurements after a certain time point, with a minimum of two measurements below 2.6 for patients who discontinued treatment prematurely. Furthermore, dropout from the study including the reason for dropout were used as outcome parameters.

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.

Serum samples were collected just prior to an injection with adalimumab at baseline, and after 4, 16, 28, 40, 52, 78, 104, 130 and 156 weeks. The presence of AAA was determined at all time points between baseline and 156 weeks. AAA were detected with a radio immunoassay (RIA). (13) One micro liter of serum diluted in PBS/0.3% bovine serum albumin (BSA) (PA buffer) was incubated o/n with 1 mg Sepharose-immobilized protein A (GE healthcare, Chalfont St. Giles, UK) in a final volume of 800 μ l. Subsequently the samples were washed with PBS 0.005% Tween and specific ADA binding was detected by o/n incubation with 20.000 dpm (approximately 1 ng) ¹²⁵I labeled F(ab)₂ adalimumab diluted in Freeze buffer (Sanquin). Unbound label was removed by washing, and protein A bound radioactivity was measured. When binding was higher than 25% of the input, sera were further titrated. Antibody levels were compared to a standard serum containing anti-drug antibody levels and expressed in arbitrary units (AU). One AU corresponds to approximately 12 ng. The mean cut-off value was set at 12 AU/ml which was derived from 100 healthy donors. Assay specificity was demonstrated by the absence of AAA in 25 sera containing high-titres anti-infliximab antibodies from patients not treated with adalimumab. In the assays we did not find cross reactivity. Recently, patient sera were tested in a bioassay, which confirmed the specificity and validity of the RIA. Patients were defined as positive for anti-adalimumab antibodies if titres were above 12 AU/ml on at least one occasion, in combination with serum adalimumab levels below 5.0 mg/L. All baseline samples before the start of treatment were negative.

Statistical analysis. For differences between groups we used the independent samples T test or Mann-Whitney U as appropriate. The threshold for significance was set at $P < 0.05$. The generalized estimating equation (GEE) approach was used to analyze the course of serum adalimumab concentrations over time for patients with and without AAA. Furthermore, GEE was used to investigate the association between AAA and the DAS28 score over time. For estimating the proportion of patients who discontinued follow-up prematurely and the proportion that achieved minimal disease activity or remission we used a log rank test and Cox regression analysis to adjust for confounders.

Variables considered potential confounders were chosen from all available baseline variables and were determined for every analysis specifically. Variables were included in the regression model as confounders if the beta changed 10% or more after inclusion of the variable.

RESULTS

Of the 272 patients enrolled in the study, 148 (55%) completed follow-up. Median follow-up period was 156 weeks (interquartile range 40-156). Fifty-seven patients (21%) stopped due to treatment failure, 41 (15%) because of adverse events and 26 (10%) because of other reasons such as clinical remission (n=2), relocation (n=9), unwillingness to participate (n=6) and loss to follow-up (n=9) (Figure 1). Patient characteristics are shown in Table 1. There were differences between anti-adalimumab antibodies positive and negative patients at baseline regarding prior DMARD use, concomitant use of methotrexate and other DMARDs, disease duration, erosive disease, C-reactive protein and DAS-28 score.

Table 1. Demographic and clinical characteristics at baseline

	Total patient population n = 272	Patient with anti-adalimumab antibodies n = 76	Patients without anti-adalimumab antibodies n = 196
Demographics			
Age, years	54 ± 12	53 ± 13	54 ± 11
Female, no. (%)	219 (81)	62 (82)	157 (80)
DMARD therapy			
Prior DMARDs	3.1 ± 1.4	3.4 ± 1.5*	3.0 ± 1.3*
Prior Biologicals	75 (28)	25 (33)	50 (26)
Methotrexate use, no. (%)	202 (74)	41 (54)*	161 (82)*
Methotrexate dose (mg/wk)	25 (15-25)	18 (10-25)*	25 (15-25)*
DMARD use other than MTX, no. (%)	19 (7)	7 (9)	12 (6)
MTX plus other DMARD use, no. (%)	55 (20)	8 (11)*	47 (24)*
No concomitant DMARD, no. (%)	51 (19)	28 (37)*	23 (12)*
Prednisone use, no. (%)	91 (34)	27 (36)	64 (33)
Prednisone dose (mg/day)	7.5 (5-10)	7.5 (5-10)	5 (5-10)
Disease status			
Disease duration (years)	8 (3-17)	12 (5-18)*	8 (3-16)*
Rheumatoid factor positive, no. (%)	196 (72)	57 (75)	139 (71)
Anti-CCP positive, no. (%)	196 (72)	55 (72)	141 (72)
Erosive disease, no. (%)	201 (74)	63 (83)*	138 (70)*
Erythrocyte sedimentation rate (mm/h)	23 (11-42)	35 (18-60)*	21 (11-39)*
C-reactive protein (mg/L)	12 (5-29)	19 (7-46)*	11 (4-22)*
DAS28	5.2 ± 1.2	5.5 ± 1.1*	5.1 ± 1.3*

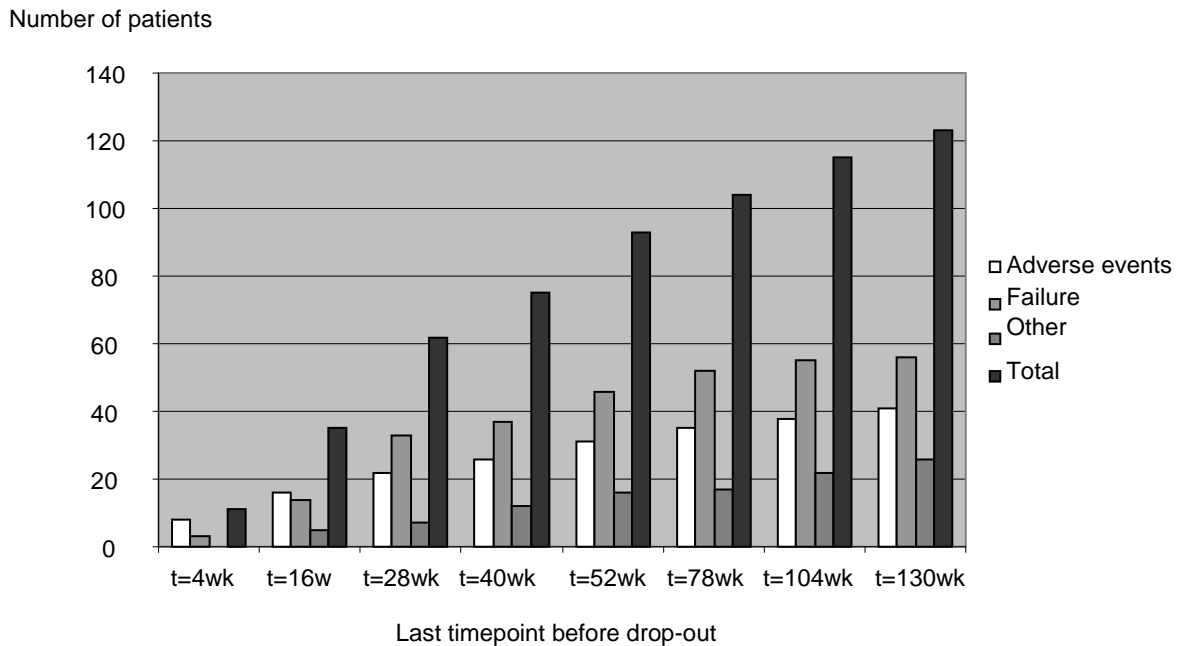
Mean values ± SD, Median (interquartile range) or numbers (percentages) are shown.

DMARD=disease modifying antirheumatic drug; MTX=methotrexate; CCP=cyclic citrullinated peptide;

DAS28=Disease activity score in 28 joints.

DMARDs other than MTX were sulphasalazine and/or hydroxychloroquine.

*There were significant differences between patients with and without anti-adalimumab antibodies for number of prior DMARDs (p=0.021), methotrexate use (p<0.0001), methotrexate dose (p=0.005), MTX plus other DMARD use (p<0.0001), no concomitant DMARD use (p<0.0001), disease duration (p=0.016), erosive disease (p=0.035), ESR (p<0.0001), CRP (p=0.001) and DAS28 (p=0.013).

Figure 1. Cumulative drop-out per time point and reason for discontinuation.

Antibodies against adalimumab. During 156 weeks follow-up, anti-adalimumab antibodies were detected in 76 (28%) patients. Figure 2 shows that 51 of 76 patients (67%) developed AAA during the first 28 weeks of treatment. The antibody test was considered positive when the antibody concentration exceeded 12 AU/ml and the adalimumab concentration was 5 mg/L or less. In 13 serum samples an antibody titre above 12 AU/ml together with an adalimumab concentration above 5 mg/L was detected and were therefore considered false positive for anti-adalimumab. Anti-adalimumab titres ranged from 13 to 17 AU/ml in these samples. The serum titres of anti-adalimumab positive patients had two clusters that could be separated at a cut off value of 100 AU/ml. Forty-five of 76 patients had antibody concentrations that stayed below 100 AU/ml at all time points [range 13-88 AU/ml] and 31 patients had antibody concentrations above 100 AU/ml [range 103-110000 AU/ml] at one or more time points. Figure 3 shows the median adalimumab concentrations for patients without antibodies and for patients with anti-adalimumab titres from 13 to 100 AU/ml and 100 AU/ml or higher. Patients without anti-adalimumab antibodies had significantly higher adalimumab concentrations compared to patients with both antibody titres from 13 to 100 AU/ml ($p < 0.0001$) and higher than 100 AU/ml ($p < 0.0001$), with regression coefficients of -4.5 (95%CI -6.0 to -2.9) and -7.1 (95%CI -8.4 to -5.8) respectively. Although data were not normally distributed, general estimate equation (GEE) was used without transformation of the data into logarithms for normality because the distribution of adalimumab serum concentrations was similarly skewed in all three groups compared.

Figure 2. Percentage of anti-adalimumab development over time.

Percentage of patients with anti-adalimumab antibodies

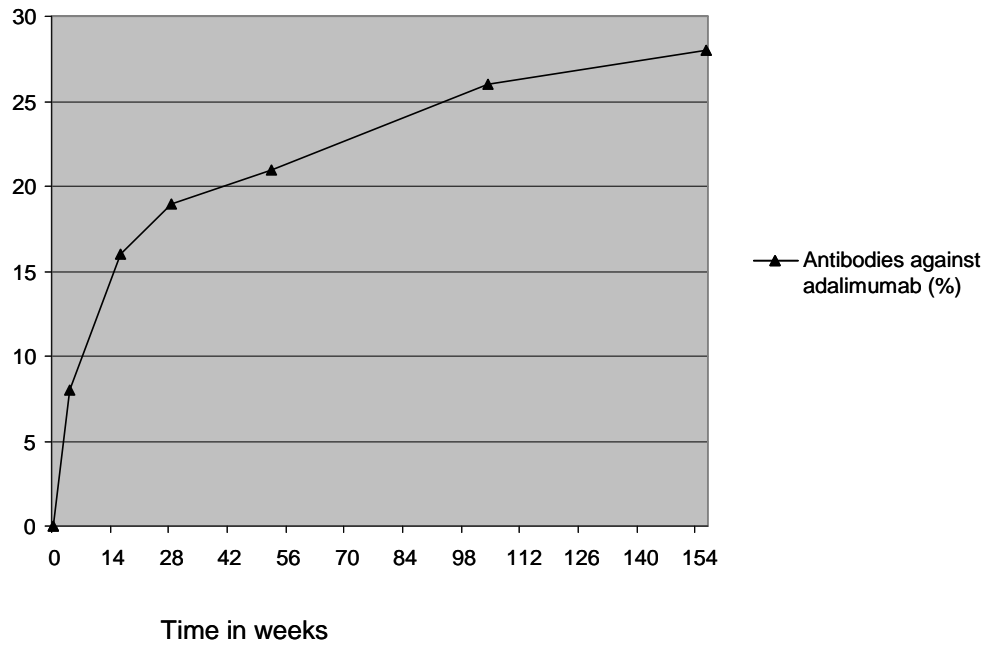
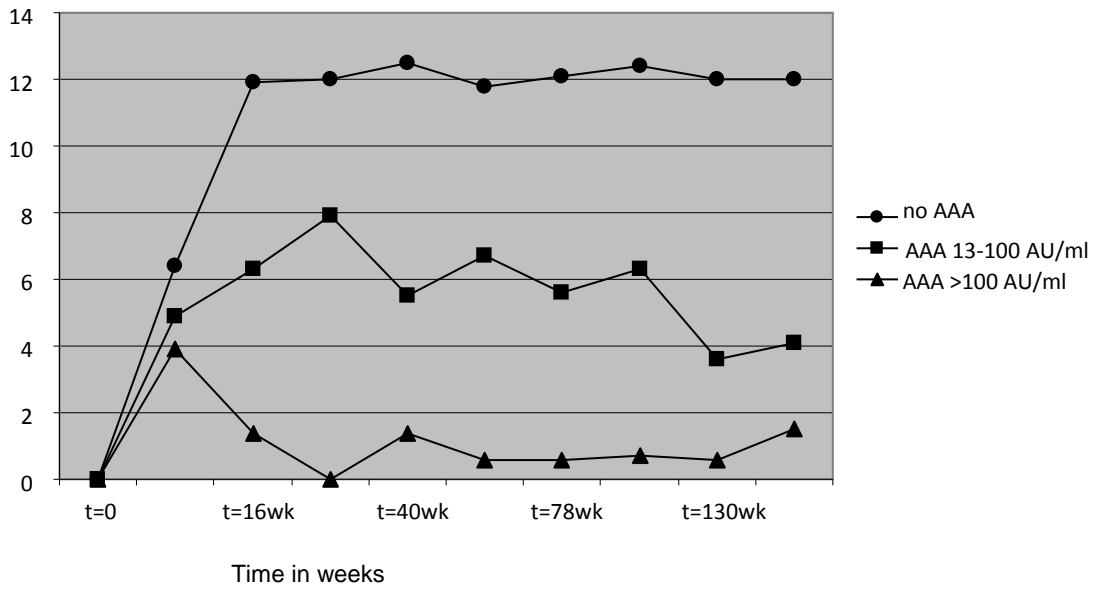


Figure 3. Median adalimumab concentrations per time point for patients without and with low and high anti-adalimumab titres.

Median adalimumab concentration (mg/L)



Clinical response and anti-adalimumab antibodies.

Discontinuation of treatment. Patients with detectable AAA more often interrupted adalimumab treatment, regardless the reason of drop-out, compared to patients without AAA in univariate analysis ($p=0.002$; figure 4A). However, after adjustment for confounders MTX dosage, baseline DAS28 and C-reactive protein the association between anti-adalimumab antibodies and drop-out appeared to be a trend (HR:0.7; 95%CI:0.5-1.0, $p=0.079$).

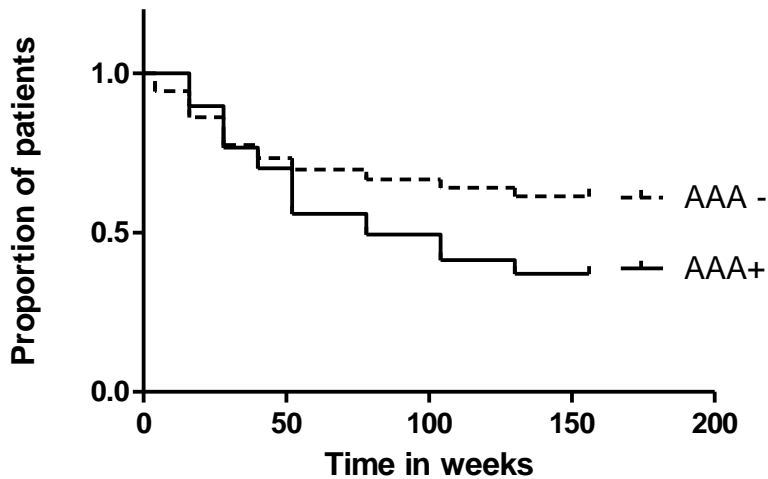


Figure 4A. Overall drop-out for patients with and without AAA ($p=0.002$).

When focusing on drop-out owing to treatment failure, patients with anti-adalimumab antibodies significantly more often dropped out of the study due to treatment failure compared to patients without anti-adalimumab in univariate analysis ($p<0.0001$; figure 4B) and after adjustment for confounders MTX use, number of previous DMARDs and C-reactive protein (HR:3.0; 95%CI:1.6-5.5, $p<0.0001$).

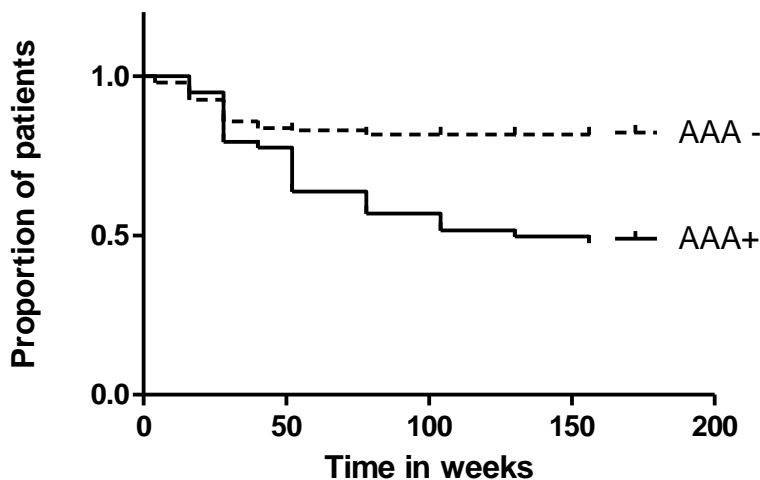


Figure 4B. Drop-out due to failure for patients with and without AAA ($p<0.0001$).

Disease activity score over time. GEE analysis demonstrated a significant association between the presence or absence of AAA and DAS28 score over time. Patients with AAA had a higher DAS28 score over time (and at all time points) compared to patients without AAA in univariate analysis ($P < 0.0001$; regression coefficient 0.8; 95%CI 0.57- 1.1). After adjustment for confounding variables ESR, MTX dosage and age this association remained significant, but the regression coefficient became smaller ($P = 0.001$; regression coefficient 0.4; 95%CI 0.2- 0.6).

Minimal disease activity. Patients with AAA less often achieved sustained minimal disease activity (DAS28 < 3.2) compared to patients without AAA (figure 5A; $P < 0.0001$) in univariate analysis and after adjustment for confounding variables MTX dosage, ESR and CRP (HR:3.6; 95%CI:1.8-7.2, $p < 0.0001$). Ninety-five of 196 patients without AAA achieved minimal disease activity, 8 of 45 patients with AAA titres from 13 to 100 AU/ml and 2 of 31 patients with AAA titres above 100 AU/ml. Both patients with high and low AAA titres achieved sustained minimal disease activity less often compared to patients without AAA (figure 5B; $P < 0.0001$).

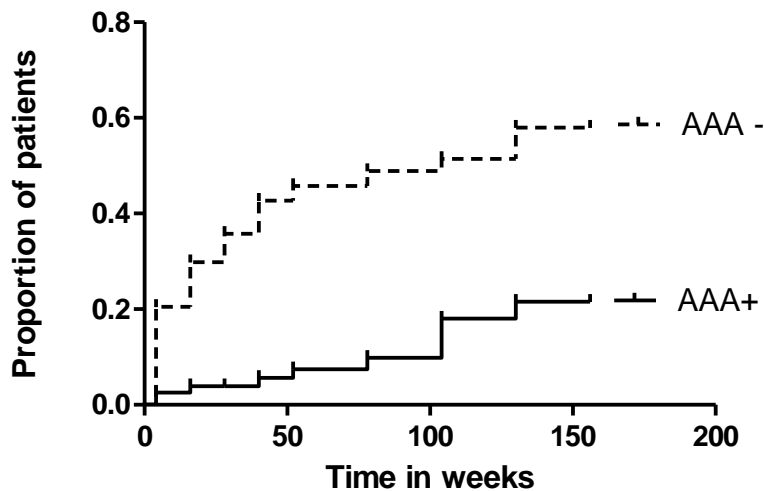


Figure 5A. Proportion of patients who reached sustained minimal disease activity (DAS28<3.2) for patients with and without AAA ($p < 0.0001$).

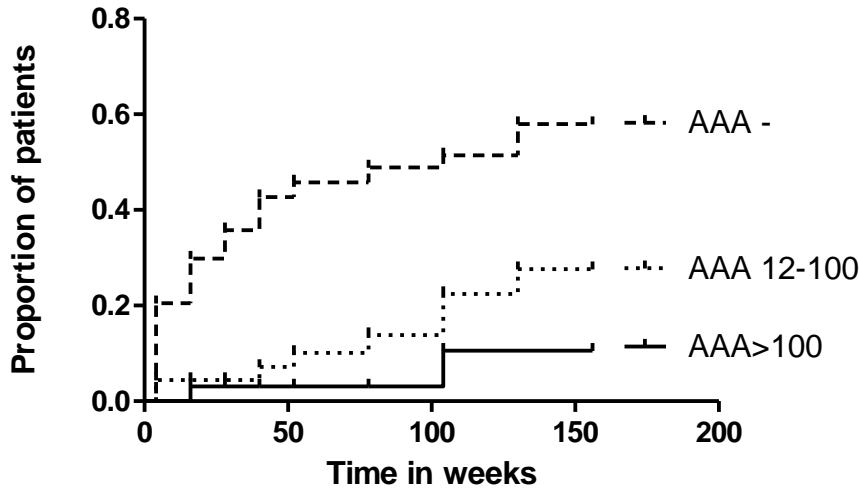


Figure 5B. Proportion of patients who reached sustained minimal disease activity (DAS28<3.2) for patients without AAA, AAA titres ranging from 13 to 100 AU/ml and AAA titres above 100 AU/ml. Both curves with AAA differ significantly from the curve without AAA ($p<0.0001$).

Remission. Three of 76 patients with AAA achieved sustained remission (DAS28 < 2.6) compared to 67 of 196 patients without AAA (figure 5C; $P<0.0001$) in univariate analysis and after adjustment for confounding variables MTX dosage, ESR and CRP (HR:3.6; 95%CI:1.8-7.2, $p<0.0001$). Two of the AAA positive patients developed AAA soon after they had achieved remission and discontinued treatment shortly thereafter owing to adverse events. One AAA positive patients achieved remission at 130 weeks despite the fact that he had already developed AAA before that time point. His adalimumab concentrations during AAA positivity varied from 0 to 2.8 mg/L.

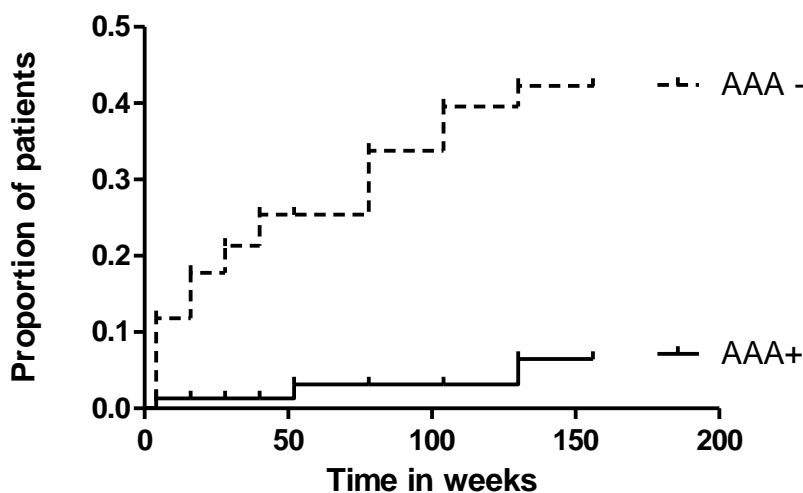


Figure 5C. Proportion of patients who reached sustained remission (DAS28<2.6) for patients with and without AAA ($p<0.0001$).

Increased dosing frequency of adalimumab.

In 51 patients (19%) the dosing frequency of adalimumab was increased to 40 mg weekly in a period ranging from 4 to 144 weeks after start. Median adalimumab concentrations were 5.6 (1.9-8.8) mg/L and 11.8 (5.4-21.1) mg/L before and after dose increase and anti-adalimumab titres in patients positive for anti-adalimumab antibodies ranged from 14-54200 AU/ml and 13-46600 AU/ml respectively. Four of 51 patients had reached sustained minimal disease activity of a DAS28 that stayed below 3.2 before increasing the dosing frequency, however, they subjectively perceived inefficacy. Nine more patients achieved sustained minimal disease activity after dose increase. Of all 13 patients who achieved minimal disease activity, only one patient had anti-adalimumab antibodies (low titres ranging from 14 to 20 AU/ml, detected before and after dose increase together with adalimumab levels of 0.9 to 3.8 mg/L). Twenty-two of 51 patients had developed anti-adalimumab antibodies (43%), of whom 20 had developed AAA before increased dosing. Anti-adalimumab antibodies became undetectable after dose increase in 6 of 20 patients however none of them achieved minimal disease activity. Of the 16 patients in whom AAA were detected at least once after dose increase, 2 achieved minimal disease activity.

DISCUSSION

The results of this study show that development of anti-drug antibodies has a large and clinically relevant negative impact on the treatment-effect of adalimumab in RA patients. Not only discontinued patients with AAA treatment more often and earlier than patients without AAA, they also had a higher disease activity during treatment and only rarely came into remission. In addition, our data show that two thirds of the AAA positive patients developed these antibodies in the first 28 weeks of treatment, and that the presence of AAA substantially influenced serum adalimumab concentrations.

Certain issues have to be taken into account when interpreting the results. In patients with high AAA-titres and without detectable serum adalimumab it is likely that the effect of adalimumab is impaired. We observed a continuously high disease activity in some of these patients, and fluctuating disease activity in others (data not shown). The fluctuating disease activity could have been caused by natural fluctuations in RA disease activity rather than by an effect of (undetectable) adalimumab. With GEE analysis we were able to investigate DAS28 scores over time. The regression coefficient of 0.4 could be interpreted as the average difference in DAS28 between patients with and without AAA at each time point. Nevertheless one should keep in mind that with GEE missing data is imputed based on the data that are still available for analysis at that time point. Since patients with AAA discontinue treatment sooner and more frequently than patients without AAA (figure 4AB), the imputed data in the AAA positive group will be based on the DAS28 of the AAA positive patients that are still on treatment, who are most likely the best responding AAA positives. Therefore, the regression coefficient of 0.4 is probably an underestimation of the real DAS28 difference between patients with and without AAA over time. This is underscored by the substantial difference between the proportion of patients with and without AAA who achieved minimal disease activity and remission.

These results should have implications for clinical practice. In figure 4B we observe that patients with AAA discontinue treatment grossly after 52 weeks of therapy, however the majority of the patients already had detectable AAA within 28 weeks (figure 2). We recommend an immunogenicity assessment after 28 weeks of treatment in all patients because adjusting policy based on the results could lead to more (cost)-effective treatment since patients with AAA had a higher disease activity and

hardly ever achieved remission. Even in patients with low disease activity, but high anti-drug antibody titres and undetectable serum drug levels it is questionable whether the low disease activity is attributable to the drug. After 28 weeks the number of AAA positive patients still increases, so the assessment of an antibody response appears useful subsequent to 28 weeks in non-responding patients. Furthermore, our data showed that approximately 80% of the patients who had not achieved minimal disease activity before increasing the dosing frequency did not achieve minimal disease activity after increased dosing. None of the patients in whom anti-adalimumab antibodies became undetectable after increased dosing achieved minimal disease activity. Although the patient numbers were too small to undertake statistical analyses, these data are in accordance with recently published data that showed that the effectiveness of dose increase of TNF inhibitors was very small or lacking. (14) Figure 2 shows that almost 10% of the patients already developed AAA after only 4 weeks of treatment. This finding sheds new light on the perspective of primary and secondary non-response. From a clinical perspective solely, primary and secondary non-response (or loss of response) is usually defined by time; i.e. whether response to treatment was not observed from treatment start (primary non-response) or an initial response was lost over time (secondary non-response). For clarity reasons, we argue to define primary and secondary non-response from a mechanistic point of view based on objective measurements instead of from a clinical view. Primary non-response can then be defined as non-response despite adequate serum drug levels (without anti-drug antibodies), and secondary non-response as non-response owing to diminished serum drug levels (with or without anti-drug antibodies).

In previous studies in two different cohorts of adalimumab and etanercept patients we showed that the reason for non-response to a first TNF-inhibitor has implications for the response to a second TNF-inhibitor after switching. (15;16) Patients who had developed anti-drug antibodies against their first TNF-inhibitor (infliximab or adalimumab) had a clinical response to their second TNF-inhibitor (adalimumab or etanercept) that did not differ from TNF naive patients. In contrast, patients who did not respond to their first TNF inhibitor despite adequate serum drug levels and the absence of anti-drug antibodies, had a significantly worse response to their second TNF inhibitor compared with both TNF naive patients and patients with anti-drug antibodies to their first TNF inhibitor. This suggests that patients who do not respond to a TNF inhibitor despite adequate serum drug levels and the absence of anti-drug antibodies, are likely to benefit more from a therapy based on another mechanism of action than from another TNF inhibitor yet again. It is possible that in these patients TNF is not the main cytokine instigating disease activity.

Another point of interest is why some patients develop an anti-drug antibody response while others do not. The use of concomitant immunosuppressants has shown to be associated with a lower frequency of anti-drug antibodies. (17;18) This is supported by the baseline differences for patients with and without AAA in this study; patients who later developed AAA less often had concomitant methotrexate in a lower dose, and more often had no concomitant DMARD at all. Genetic differences between individuals might also be of influence in the development of anti-drug antibodies, as we showed previously that patients with certain IL-10 polymorphisms more often developed AAA. (19) Differences in baseline characteristics between AAA positive and negative patients in the present study show that patients with AAA had a higher baseline disease activity and C reactive protein levels, longer disease duration, and more often erosive disease. Why and how these characteristics of more serious disease are associated with the development of anti-drug antibodies is currently unknown.

An association between the occurrence of anti-drug antibodies and diminished serum drug concentrations and short-term treatment response has been described for several biological drugs in a variety of diseases. (7;20-22) Most studies show results after a follow-up period up till one year, but given the negative impact on short-term treatment response described in these studies, it is likely that the effect of immunogenicity on the drug's long-term efficacy will be similar for all conditions in which biological drugs are used. One long-term study on adalimumab treatment for Crohn's disease described a negative effect on serum drug concentration and, in a limited sized subgroup, on sustained clinical benefit. (9) It is time that the impact of immunogenicity on biological treatment is acknowledged in the broad range of medical practice in which these drugs are used. Consensus on issues like use of definitions, optimization regimes (dose increase, co-treatment) and standardization of assays could help develop the best possible way of dealing with immunogenicity. (8)

Current results are supported by a growing body of evidence on immunogenicity, however, this is the first study that showed associations between anti-drug antibodies and long-term clinical endpoints such as discontinuation of treatment, minimal disease activity and remission. These outcomes support a case for the monitoring of serum drug levels and anti-drug antibody titres in daily routine practice. Instead of not knowing why a patient does not respond to a certain drug, the measurement of drug levels and anti-drug antibodies helps in understanding why an individual patient does not respond to treatment which could assist in developing a personalized treatment regimen.

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Reference List

- (1) Baert F, Noman M, Vermeire S, Van Assche G, D' Haens G, 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.
- (2) Wolbink GJ, Vis M, Lems W, Voskuyl AE, de Groot E, Nurmohamed MT et al. Development of anti-infliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum* 2006; 54(3):711-715.
- (3) Bartelds GM, Wijbrandts CA, Nurmohamed MT, Stapel S, Lems WF, Aarden L et al. Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. *Ann Rheum Dis* 2007; 66(7):921-926.
- (4) van Kuijk AW, de Groot M, Stapel SO, Dijkmans BA, Wolbink GJ, Tak PP. Relationship between the clinical response to adalimumab treatment and serum levels of adalimumab and anti-adalimumab antibodies in patients with psoriatic arthritis. *Ann Rheum Dis* 2010; 69(3):624-625.
- (5) Lecluse LL, Driessen RJ, Spuls PI, de Jong EM, Stapel SO, van Doorn MB et al. Extent and clinical consequences of antibody formation against adalimumab in patients with plaque psoriasis. *Arch Dermatol* 2010; 146(2):127-132.
- (6) Calabresi PA, Giovannoni G, Confavreux C, Galetta SL, Havrdova E, Hutchinson M et al. The incidence and significance of anti-natalizumab antibodies: results from AFFIRM and SENTINEL. *Neurology* 2007; 69(14):1391-1403.
- (7) West RL, Zelinkova Z, Wolbink GJ, Kuipers EJ, Stokkers PC, van der Woude CJ. Immunogenicity negatively influences the outcome of adalimumab treatment in Crohn's disease. *Aliment Pharmacol Ther* 2008; 28(9):1122-1126.
- (8) Allez M, Karmiris K, Louis E, Van Assche G, Ben-Horin S, Klein A et al. Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: Definitions, frequency and pharmacological aspects. *Journal of Crohn's and Colitis* 2010.
- (9) Karmiris K, Paintaud G, Noman M, Magdelaine-Beuzelin C, Ferrante M, Degenne D et al. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. *Gastroenterology* 2009; 137(5):1628-1640.
- (10) Furst DE, Breedveld FC, Kalden JR, Smolen JS, Burmester GR, Sieper J et al. Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2007. *Ann Rheum Dis* 2007; 66 Suppl 3:iii2-22.
- (11) Prevoo ML, '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.
- (12) Wolbink GJ, Voskuyl AE, Lems WF, de Groot E, 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) van Schouwenburg PA, Bartelds GM, Hart MH, Aarden L, Wolbink GJ, Wouters D. A novel method for the detection of antibodies to adalimumab in the presence of drug reveals "hidden" immunogenicity in rheumatoid arthritis patients. *J Immunol Methods* 2010; 362(1-2):82-88.

- (14) Blom M, Kievit W, Kuper HH, Jansen TL, Visser H, den Broeder AA et al. Frequency and effectiveness of dose increase of adalimumab, etanercept, and infliximab in daily clinical practice. *Arthritis Care Res (Hoboken)* 2010; 62(9):1335-1341.
- (15) Bartelds GM, Wijbrandts CA, Nurmohamed MT, Stapel SO, Lems WF, Aarden L et al. Anti-infliximab and anti-adalimumab antibodies in relation to response to adalimumab in infliximab switchers and anti-TNF naive patients: a cohort study. *Ann Rheum Dis* 2009.
- (16) Jamnitski A, Bartelds GM, Nurmohamed MT, van Schouwenburg PA, van Schaardenburg D, Stapel SO et al. The presence or absence of antibodies to infliximab or adalimumab determines the outcome of switching to etanercept. *Ann Rheum Dis* 2010.
- (17) Vermeire S, Noman M, Van Assche G, Baert F, D'Haens G, Rutgeerts P. Effectiveness of concomitant immunosuppressive therapy in suppressing the formation of antibodies to infliximab in Crohn's disease. *Gut* 2007; 56(9):1226-1231.
- (18) Kriekaert CL, Bartelds GM, Lems WF, Wolbink GJ. The effect of immunomodulators on the immunogenicity of TNF-blocking therapeutic monoclonal antibodies: a review. *Arthritis Res Ther* 2010; 12(5):217.
- (19) Bartelds GM, Wijbrandts CA, Nurmohamed MT, Wolbink GJ, de Vries N, Tak PP et al. Anti-adalimumab antibodies in rheumatoid arthritis patients are associated with interleukin-10 gene polymorphisms. *Arthritis Rheum* 2009; 60(8):2541-2542.
- (20) Miyasaka N. Clinical investigation in highly disease-affected rheumatoid arthritis patients in Japan with adalimumab applying standard and general evaluation: the CHANGE study. *Mod Rheumatol* 2008; 18(3):252-262.
- (21) Radstake TR, Svenson M, Eijsbouts AM, van den Hoogen FH, Enevold C, van Riel PL et al. Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. *Ann Rheum Dis* 2008.
- (22) Bendtzen K, Geborek P, Svenson M, Larsson L, Kapetanovic MC, Saxne T. Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor alpha inhibitor infliximab. *Arthritis Rheum* 2006; 54(12):3782-3789.