

Development of Antidrug Antibodies Against Adalimumab and Association With Disease Activity and Treatment Failure During Long-term Follow-up

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SHORT-TERM DATA REGARDING the immunogenicity of monoclonal antibodies and the effect on treatment response have been reported for several conditions such as inflammatory bowel disease, rheumatoid arthritis, psoriatic arthritis, psoriasis, and multiple sclerosis; and for several therapeutics such as infliximab, adalimumab, and natalizumab.¹⁻⁷ Most studies were of 6 to 12 months' duration and showed that the presence of antidrug 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 antidrug antibodies influence treatment response or, in other words, how clinically relevant the development of antidrug antibodies is. In addition, how the presence of antidrug antibodies should direct clinicians' management has been a subject of debate.⁸ These questions can be applied to all diseases in which biologic therapeutics are used.

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

Objective To examine the course of antidrug 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 Prospective cohort study February 2004–September 2008; end of follow-up was September 2010. All 272 patients were diagnosed with RA and started treatment with adalimumab in an outpatient clinic.

Main Outcome Measures Disease activity was monitored and trough serum samples were obtained at baseline and 8 time points to 156 weeks. Serum adalimumab concentrations and antiadalimumab antibody titers were determined after follow-up. Treatment discontinuation, minimal disease activity, and clinical remission were compared for patients with and without antiadalimumab antibodies.

Results After 3 years, 76 of 272 patients (28%) developed antiadalimumab antibodies—51 of these (67%) during the first 28 weeks of treatment. Patients without antiadalimumab antibodies had much higher adalimumab concentrations (median, 12 mg/L; IQR, 9–16 mg/L) compared with patients with antibody titers from 13 to 100 AU/mL (median, 5 mg/L; IQR, 3–9 mg/L; regression coefficient, -4.5 ; 95% CI, -6.0 to -2.9 ; $P < .001$) and also those greater than 100 AU/mL (median, 0 mg/L; IQR, 0–3 mg/L; regression coefficient, -7.1 ; 95% CI, -8.4 to -5.8 ; $P < .001$). Patients with antiadalimumab antibodies more often discontinued participation due to treatment failure ($n = 29$ [38%]; hazard ratio [HR], 3.0; 95% CI, 1.6–5.5; $P < .001$) compared with antiadalimumab antibody–negative ones ($n = 28$ [14%]). Ninety-five of 196 patients (48%) without antiadalimumab antibodies had minimal disease activity vs 10 of 76 patients (13%) with antiadalimumab antibodies; patients with antiadalimumab antibodies less often had sustained minimal disease activity score in 28 joints (DAS28) (< 3.2 ; HR, 3.6; 95% CI, 1.8–7.2; $P < .001$) compared with antiadalimumab antibody–negative ones. Three of 76 patients (4%) with antiadalimumab antibodies achieved sustained remission compared with 67 of 196 (34%) antiadalimumab antibody–negative ones; patients with antiadalimumab antibodies less often achieved remission (DAS28 < 2.6 ; HR, 7.1; 95% CI, 2.1–23.4; $P < .001$) compared with antiadalimumab antibody–negative ones.

Conclusion Among outpatients with RA in whom adalimumab was started over 3 years, the development of antidrug antibodies was associated with lower adalimumab concentration and lower likelihood of minimal disease activity or clinical remission.

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Long-term immunogenicity studies could help elucidate the clinical impact of antidrug antibodies. At the time of this study, long-term data regarding immunogenicity of all therapeutic antibodies are scarce. In Crohn disease, 1 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 (median, 20 months) in patients who discontinued therapy and that it was affected by the presence of antibodies against adalimumab. In patients who displayed an adalimumab trough concentration of less than 0.33 µg/mL at least once, sustained clinical benefit was decreased in comparison with patients never showing such low trough serum concentration. However, the study⁹ also warned that this outcome should be interpreted with caution due to the limited number of patients.

This study, to our knowledge, is the first to investigate the course of antidrug antibody development and its clinical relevance as measured by the effect on treatment discontinuation, disease activity, and remission during long-term follow-up.

METHODS

Patients

This prospective observational cohort study consisted of 272 consecutive rheumatoid arthritis (RA) patients treated with adalimumab therapy at the Department of Rheumatology, Jan van Breemen Institute, Amsterdam, the Netherlands. Some patients included in the current study cohort who were treated at this institute were also included in previous reports. Specifically, 93 patients who were followed up for 28 weeks, from February 2004 through January 2005, were reported in a study of clinical response to adalimumab.³ A total of 180 patients from this cohort who were followed up for 28 weeks, from February 2004 through January 2006, were reported in a study examining response to adalimumab in infliximab switchers and anti-tumor ne-

crisis factor (TNF)-naïve patients.¹⁰ A total of 196 patients from this cohort who were followed up for 28 weeks, from February 2004 through May 2006, were included in a study evaluating IgG1 allotype disparity and antiadalimumab formation.¹¹

For the current study, all patients were enrolled between February 2004 and September 2008, with follow-up ending in September 2010. 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 at least 3.2, despite earlier treatment with 2 disease-modifying antirheumatic drugs (DMARDs) including methotrexate at 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.¹² Patients were treated either with adalimumab and concomitant DMARD therapy or with adalimumab monotherapy. None of the patients had previously received adalimumab. 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 committees of the Slotervaart Hospital, BovenIJ Hospital, and the Jan van Breemen Institute, Amsterdam, the Netherlands. 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.¹³ 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 mm/h), and the patient's general health or global disease activity on a visual analog scale (VAS) of

100 mm. The DAS28 can then be calculated using the formula:

$$DAS28 = 0.56 \times \sqrt{TJC28} + 0.28 \times \sqrt{SJC28} + 0.70 \times \ln ESR + 0.014 \times VAS$$

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 of less than 3.2 at all consecutive measurements after a certain time point, with a minimum of 2 measurements of less than 3.2 for patients who discontinued treatment prematurely. Remission was defined as a DAS28 of less than 2.6 at all consecutive measurements after a certain time point, with a minimum of 2 measurements of less than 2.6 for patients who discontinued treatment prematurely.

Dropout

Reason for and time point of dropout was used as an outcome parameter. Documented reasons for dropout were treatment failure, adverse events, combined treatment failure and adverse events, patient relocation, clinical remission, unwillingness to participate, or loss to follow-up. Treatment failure was defined as judged by the treating rheumatologist. No stringent outcome parameters were used to define treatment failure—as is common in daily practice. When patients withdrew from study participation because of a combination of treatment failure and adverse events, the reason was analyzed as an adverse event. Only pure treatment failure was analyzed as such.

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 1 modification.¹⁴ Adalimumab binding was assessed by incubation with biotinylated rabbit immunoglobulin directed to the adalimumab idiotype. Detection limit of the assay is approximately 0.001 mg/L. The validation procedures of the serum level test for the adalimumab ELISA has been accredited by the RvA/CCKL (Dutch Accreditation Council/Dutch Accreditation Board for Medical Laboratories) according to the International Standardization Organization (ISO) guideline ISO17025.

Measurement of Antibodies Against Adalimumab

Using methods described in previous studies,^{3,12,13} trough serum samples were collected at baseline and after 4, 16, 28, 40, 52, 78, 104, 130, and 156 weeks of treatment with adalimumab. A radio immunoassay (Sanquin) was used to detect the presence of antiadalimumab antibodies.¹⁵ After dilution of 1 μ L of serum in phosphate-buffered saline/0.3% bovine serum albumin (*pro analysi* buffer), overnight incubation followed with 1 mg Sepharose-immobilized protein A (GE Health Care, Giles, England) in a final volume of 800 μ L. Then, the samples were washed with phosphate-buffered saline 0.005% polysorbate. The antiadalimumab binding was determined by overnight incubation with 20 000 disintegrations per minute (dpm [\approx 1 ng]) iodine 125-labeled F(ab)2 adalimumab diluted in Freeze buffer (Sanquin). Unbound label was removed by washing, and protein A-bound radioactivity was measured. Serum samples were further diluted if binding was more than 25% of the input. For determining antibody levels, a standard serum containing antiadalimumab was used for comparison. Antiadalimumab levels were expressed in arbitrary units (AU [1 AU \approx 12 ng]). The mean cutoff value was derived from 100 healthy donors and set at 12 AU/mL. In 25 serum samples containing high titers of anti-infliximab antibodies from patients

not treated with adalimumab, no antiadalimumab was detected, demonstrating assay specificity and the absence of cross reactivity. The specificity and validity of the radio immunoassay have been confirmed in a bioassay. The validation procedures of the assays for determining antidrug antibodies have been accredited (see "Measurement of Adalimumab Concentrations"). All baseline samples before the start of treatment were negative for antiadalimumab antibodies. Patients were defined as positive for antiadalimumab antibodies if titers were greater than 12 AU/mL on at least 1 occasion in combination with serum adalimumab levels of less than 5.0 mg/L.

Statistical Analysis

For differences between groups, analyses were facilitated using the independent samples *t* test, χ^2 , or Mann-Whitney *U* (Wilcoxon) statistic, as appropriate. The threshold for significance was set at *P* value of less than .05 and significance was 2-sided. The generalized estimating equation (GEE) approach was used to analyze the course of serum adalimumab concentrations over time for patients with and without antiadalimumab antibodies. Furthermore, GEE was used to investigate the association between antiadalimumab antibodies and the DAS28 score over time. For estimating the proportion of patients who discontinued follow-up prematurely and the proportion who achieved minimal disease activity or remission, we used a log-rank test and Cox regression analysis to adjust for confounders. Variables considered to be potential confounders were chosen from all available baseline variables and determined for every analysis specifically in a stepwise-forward procedure. Variables were included in the regression model as confounders if the β level changed at least 10% after inclusion of the variable. Statistical analyses were performed using SPSS for Windows version 16.0 (SPSS Inc, Chicago, Illinois).

RESULTS

Of the 272 patients enrolled in the study, 148 (55%) completed follow-up. Median follow-up period was 156 weeks (interquartile range [IQR], 40-156). Patient characteristics are shown in TABLE 1. There were differences between patients who were antiadalimumab antibody-positive vs negative at baseline regarding prior DMARD use, concomitant use of methotrexate and other DMARDs, disease duration, erosive disease, ESR, C-reactive protein, and DAS28 score.

Antibodies Against Adalimumab

During 156-week follow-up, antiadalimumab antibodies were detected in 76 patients (28%). FIGURE 1 shows that 51 of 76 patients (67% of antiadalimumab antibody-positive patients) developed antiadalimumab antibodies 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 titer more than 12 AU/mL, together with an adalimumab concentration of more than 5 mg/L, was detected and was therefore considered a false positive for antiadalimumab. Antiadalimumab titers ranged from 13 to 17 AU/mL in these samples. The serum titers of antiadalimumab antibody-positive patients had 2 clusters that could be separated at a cutoff value of 100 AU/mL. Forty-five of 76 patients had antibody concentrations that remained less than 100 AU/mL at all time points (range, 13-88 AU/mL) and 31 patients had antibody concentrations greater than 100 AU/mL (range, 103-110 000 AU/mL) at 1 or more time points. FIGURE 2 shows the median adalimumab concentrations for patients without antibodies (median, 12 mg/L; IQR, 9-16 mg/L), for patients with antiadalimumab titers from 13 to 100 AU/mL (median, 5 mg/L; IQR, 3-9 mg/L), and for greater than 100 AU/mL

Table 1. Demographic and Clinical Characteristics at Baseline

Characteristics	Total Patient Population (N = 272)	Patients With Antiadalimumab Antibodies (n = 76) ^a	Patients Without Antiadalimumab Antibodies (n = 196) ^a
Age, mean (SD), y	54 (12)	53 (13)	54 (11)
Women, No. (%)	219 (81)	62 (82)	157 (80)
DMARD therapy ^b			
Prior DMARDs, mean (SD)	3.1 (1.4)	3.4 (1.5) ^c	3.0 (1.3) ^c
Prior biologics, No. (%)	75 (28)	25 (33)	50 (26)
Methotrexate use, No. (%)	202 (74)	41 (54) ^c	161 (82) ^c
Methotrexate dose, median (IQR), mg/wk	25 (15-25)	18 (10-25) ^c	25 (15-25) ^c
DMARD use other than methotrexate, No. (%)	19 (7)	7 (9)	12 (6)
Methotrexate plus other DMARD use, No. (%)	55 (20)	8 (11) ^c	47 (24) ^c
No concomitant DMARD, No. (%)	51 (19)	28 (37) ^c	23 (12) ^c
Prednisone use, No. (%)	91 (34)	27 (36)	64 (33)
Prednisone dose, median (IQR), mg/d	7.5 (5-10)	7.5 (5-10)	5 (5-10)
Disease status			
Disease duration, median (IQR), y	8 (3-17)	12 (5-18) ^c	8 (3-16) ^c
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) ^c	138 (70) ^c
ESR, median (IQR), mm/h	23 (11-42)	35 (18-60) ^c	21 (11-39) ^c
C-reactive protein, median (IQR), mg/L	12 (5-29)	19 (7-46) ^c	11 (4-22) ^c
DAS28, mean (SD)	5.2 (1.2)	5.5 (1.1) ^c	5.1 (1.3) ^c

Abbreviations: CCP, cyclic citrullinated peptide; DAS28, disease activity score in 28 joints; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; IQR, interquartile range.

^aFor differences between groups, we used the independent samples *t* test, χ^2 , or Mann-Whitney *U* (Wilcoxon) statistic, as appropriate.

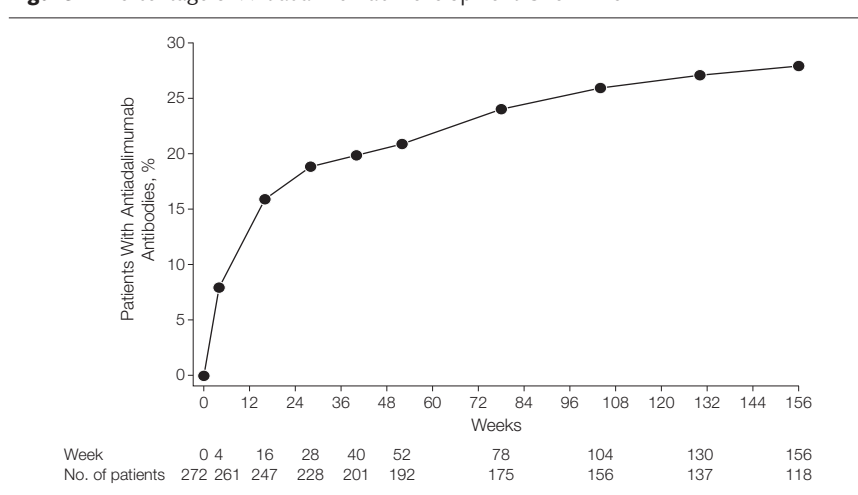
^bDMARDs other than methotrexate were sulfasalazine and/or hydroxychloroquine.

^cThere were significant differences between patients with and without antiadalimumab antibodies for number of prior DMARDs ($P = .01$), methotrexate use ($P < .001$), methotrexate dose ($P = .005$), methotrexate plus other DMARD use ($P < .001$), no concomitant DMARD use ($P < .001$), disease duration ($P = .02$), erosive disease ($P = .04$), ESR ($P < .001$), C-reactive protein ($P = .001$), and DAS28 ($P = .01$).

(median, 0 mg/L; IQR, 0-3 mg/L). Patients without antiadalimumab antibodies had significantly higher adalimumab concentrations compared with patients having both antibody titers from 13 to 100 AU/mL ($P < .001$) and greater than 100 AU/mL ($P < .001$), with regression coefficients of -4.5 (95% confidence interval [CI], -6.0 to -2.9) and -7.1 (95% CI, -8.4 to -5.8), respectively. Although data were not normally distributed, GEE was used without transformation of the data into logarithms for normality because the distribution of adalimumab serum concentrations was similarly skewed in all 3 groups compared, resulting in normally distributed residuals.

Clinical Response and Antiadalimumab Antibodies

Discontinuation of Treatment Overall. Of the 124 patients (45%) who withdrew from study participation, 57 (21%) stopped due to treatment fail-

Figure 1. Percentage of Antiadalimumab Development Over Time

Number of patients with available serum samples are shown.

ure, 30 (11%) because of adverse events, 11 (4%) because of treatment failure and adverse events combined, and 26 (9%) for other reasons such as clinical remission ($n = 2$), relocation ($n = 9$), unwillingness to participate

($n = 6$), and loss to follow-up ($n = 9$) (TABLE 2 and FIGURE 3).

Discontinuation of Treatment by Patient Group. When the cohort was divided between patients with antiadalimumab antibodies and those with-

out, 48 of the antiadalimumab antibody-positive patients (63%) discontinued participation during follow-up due to the following reasons: 29 (38%) for treatment failure, 8 (10%) for adverse events, 2 (3%) because of treatment failure and adverse events combined, and 9 (12%) for other reasons. Of the antiadalimumab antibody-negative patients, 76 (39%) discontinued participation: 28 (14%) for treat-

ment failure, 22 (11%) for adverse events, 9 (5%) because of treatment failure and adverse events combined, and 17 (9%) for other reasons. Patients with detectable antiadalimumab antibodies more often interrupted adalimumab treatment (48 of 76) regardless of the reason for dropout, compared with antiadalimumab antibody-negative patients (76 of 196) in univariate analysis ($P = .002$; FIGURE 4A). However,

after adjustment for confounders, methotrexate dosage, baseline DAS28, and C-reactive protein, the association between antiadalimumab antibodies and dropout was not significant (hazard ratio [HR], 0.7; 95% CI, 0.5-1.0; $P = .08$).

When focusing on dropout because of treatment failure, patients with antiadalimumab antibodies significantly more often discontinued study participation ($n = 29$, 38%) compared with those who were antiadalimumab antibody-negative ($n = 28$, 14%) in univariate analysis ($P < .001$; Figure 4B), and after adjustment for the following confounders: methotrexate use, number of previous DMARDs, and C-reactive protein (HR, 3.0; 95% CI, 1.6-5.5; $P < .001$).

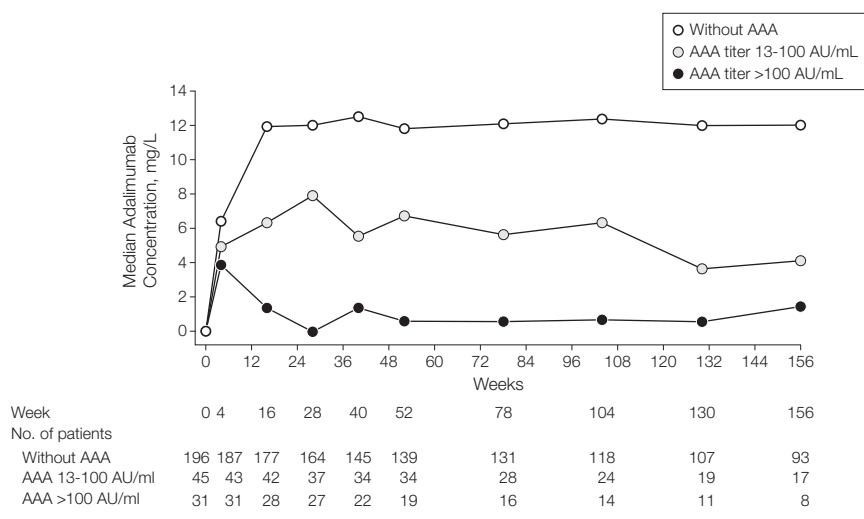
Disease Activity Score Over Time

Analysis by GEE demonstrated a significant association between the presence or absence of antiadalimumab antibodies and DAS28 score over time. Patients with antiadalimumab antibodies had a higher DAS28 score over time (and at all time points) compared with antiadalimumab antibody-negative ones in univariate analysis ($P < .001$; regression coefficient, 0.8; 95% CI, 0.57-1.1). After adjustment for the confounding variables ESR, methotrexate dosage, and age, this association remained significant, but the regression coefficient became smaller ($P = .001$; regression coefficient, 0.4; 95% CI, 0.2-0.6).

Minimal Disease Activity

Patients with antiadalimumab antibodies less often achieved sustained minimal disease activity ($\text{DAS28} < 3.2$) compared with antiadalimumab antibody-negative ones (FIGURE 5A; $P < .001$) in univariate analysis and after adjustment for the confounding variables methotrexate dosage, ESR, and C-reactive protein (HR, 3.6; 95% CI, 1.8-7.2; $P < .001$). Ninety-five of 196 patients without antiadalimumab antibodies (48%) achieved minimal disease activity, compared with 8 of 45 patients (18%) with antiadalimumab antibody titers from 13 to 100 AU/mL

Figure 2. Median Adalimumab Concentrations Over Time



Median adalimumab concentrations (mg/L) per time point are shown for patients without antiadalimumab antibodies (AAA), with low AAA (13-100 AU/mL), and high antiadalimumab titers (>100 AU/mL). Patients who were AAA-negative had significantly higher adalimumab concentrations compared with patients with low AAA ($P < .001$) and high antibody titers ($P < .001$), with regression coefficients of -4.5 (95% confidence interval, -6.0 to -2.9) and -7.1 (95% confidence interval, -8.4 to -5.8), respectively (analysis by generalized estimating equation). The interquartile ranges (25th-75th percentiles) for the adalimumab concentrations ranged from 4.3-8.9 mg/L to 7.5-17.5 mg/L for the AAA-negative patients; from 3.4-7.1 mg/L to 2.2-11.7 mg/L for patients with low AAA; and from 0.0-2.7 mg/L to 0.01-11.2 mg/L for patients with high AAA titers.

Table 2. Discontinuation of Treatment in Patients

	No. (%) of Patients		
	Total (N = 272)	With Antiadalimumab Antibodies (n = 76)	Without Antiadalimumab Antibodies (n = 196)
Completed treatment	148 (55)	28 (37)	120 (61)
Discontinuation			
Treatment failure	57 (21)	29 (38)	28 (14)
Adverse events	30 (11)	8 (10)	22 (11)
Treatment failure and adverse events	11 (4)	2 (3)	9 (5)
Other reasons for discontinuation			
Clinical remission	2 (1)	0	2 (1)
Relocation	9 (3)	4 (5)	5 (3)
Unwillingness to participate	6 (2)	3 (4)	3 (1)
Loss to follow-up	9 (3)	2 (3)	7 (4)
Total discontinuation	124 (45)	48 (63)	76 (39)

and 2 of 31 patients (6%) with antiadalimumab antibody titers greater than 100 AU/mL. Patients with high, as well as those with low antiadalimumab antibody titers achieved sustained minimal disease activity less often compared with antiadalimumab antibody-negative ones (Figure 5B; $P < .001$).

Remission

Three of 76 patients (4%) with antiadalimumab antibodies achieved sustained remission ($\text{DAS28} < 2.6$) compared with 67 of 196 (34%) antiadalimumab antibody-negative ones (Figure 5C; $P < .001$) in univariate analysis and after adjustment for the confounding variables ESR, methotrexate dosage, and C-reactive protein (HR, 7.1; 95% CI, 2.1-23.4; $P < .001$). Two of the antiadalimumab antibody-positive patients developed antiadalimumab antibodies soon after they had achieved remission and discontinued treatment shortly thereafter owing to adverse events. One antiadalimumab antibody-positive patient achieved remission at 130 weeks despite the fact that he had already developed antiadalimumab antibodies before that time point. His adalimumab concentrations during antiadalimumab antibody positivity varied from 0 to 2.8 mg/L.

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 mg/L (IQR, 1.9-8.8) before dose increase and 11.8 mg/L (IQR, 5.4-21.1) after, and antiadalimumab titers in patients positive for antiadalimumab antibodies ranged from 14 to 54 200 AU/mL before dose increase and 13 to 46 600 AU/mL after. Four of 51 patients had reached sustained minimal disease activity of a DAS28 that remained less than 3.2 before increasing the dosing frequency; however, they subjectively perceived inefficacy. Nine more patients achieved sus-

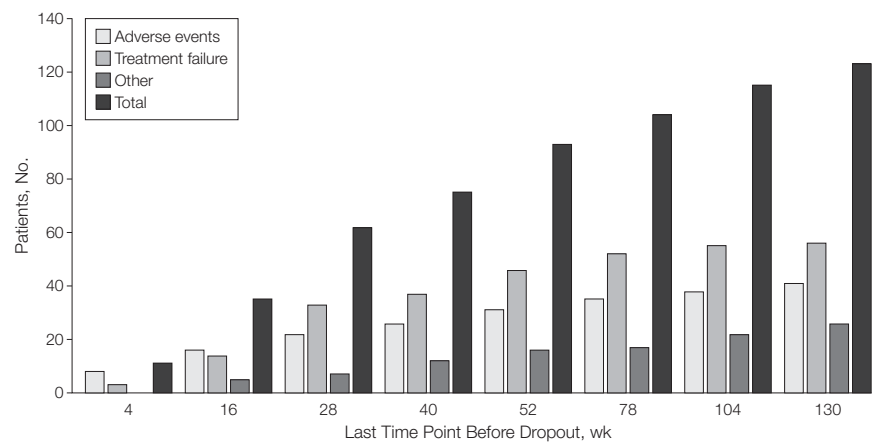
tained minimal disease activity after dose increase. Of all 13 patients who achieved minimal disease activity, only 1 patient had antiadalimumab antibodies (low titers ranging from 14 to 20 AU/mL, detected before and after dose increase together with adalimumab levels of 0.9 to 3.8 mg/L). Twenty-two of 51 patients (43%) had developed antiadalimumab antibodies, of whom 20 had developed antiadalimumab antibodies before increased dosing. Antiadalimumab antibodies became undetectable after dose increase in 6 of 20 patients; however,

none of these patients achieved minimal disease activity. Of the 16 patients in whom antiadalimumab antibodies were detected at least once after dose increase, 2 achieved minimal disease activity.

COMMENT

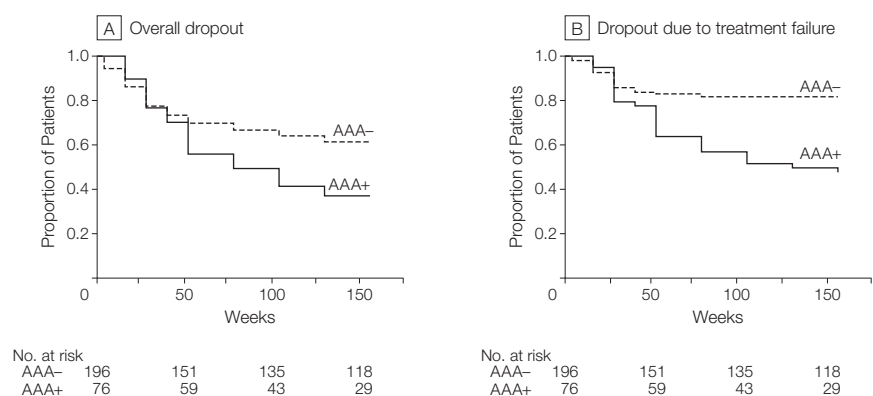
The results of this study show that development of antidrug antibodies is associated with a negative outcome of adalimumab treatment in RA patients. Not only did patients with antiadalimumab antibodies discontinue treatment more often and earlier than pa-

Figure 3. Cumulative Dropout by Time Point and Reason



There were 0 dropouts for the reason indicated as other at the 4-week time point.

Figure 4. Overall Patient Dropout and Dropout Due to Treatment Failure



A, Overall dropout for patients with and without antiadalimumab antibodies (AAA) (survival analysis, $P = .002$) is shown. Forty-eight of 76 AAA-positive patients (63%) and 76 of 196 AAA-negative patients (39%) discontinued treatment. B, Dropout due to treatment failure for patients with and without AAA (survival analysis, $P < .001$). Treatment failure was the reason for dropout in 29 of 48 AAA-positive patients and 28 of 76 AAA-negative patients.

tients without antiadalimumab antibodies, 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 antiadalimumab antibody-positive patients developed these antibodies in the first 28 weeks of treatment and that the presence of antiadalimumab antibodies substantially influenced serum adalimumab concentrations.

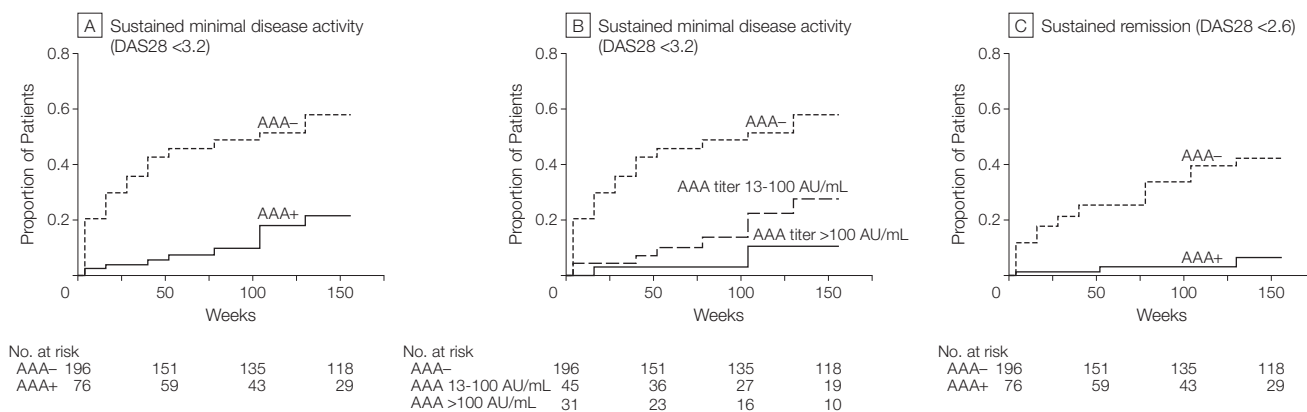
Certain issues must be taken into account when interpreting the results. The level of statistical significance should be interpreted with caution owing to multiple comparisons in this study. Furthermore, in patients with high antiadalimumab antibody titers and without detectable serum adalimumab, it 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 coef-

ficient of 0.4 could be interpreted as the average difference in DAS28 between patients with and without antiadalimumab antibodies at each time point. Nevertheless, one should keep in mind that with GEE missing data are estimated based on the data that are still available for analysis at that time point and based on a quasi-likelihood estimation. Since patients with antiadalimumab antibodies discontinued treatment sooner and more frequently than patients without antiadalimumab antibodies (Figure 4A and Figure 4B), the estimated data in the antiadalimumab antibody-positive group were based on the DAS28 of the antiadalimumab antibody-positive patients who were still in treatment and who were most likely the best responding antiadalimumab antibody-positive patients. Therefore, the regression coefficient of 0.4 is probably an underestimation of the real DAS28 difference between patients with and without antiadalimumab antibodies over time. This is underscored by the substantial difference between the proportion of patients with and without antiadalimumab antibodies who achieved minimal disease activity and remission.

These results could have implications for clinical practice. In Figure 4B, we observed that patients with antiadalimumab antibodies discontinued treatment grossly after 52 weeks of therapy; however, the majority of the patients already had detectable antiadalimumab antibodies within 28 weeks (Figure 1). Hence, there appears to be a time lag between the moment when patients have low serum drug levels owing to antidrug antibodies and the moment when this leads to consequences. Adjusting policy could lead to more (cost)-effective treatment since patients with antiadalimumab antibodies had a higher disease activity and rarely achieved remission.

The merit of increased dosing of biologic therapeutics is questionable. 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 antiadalimumab 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

Figure 5. Sustained Disease Activity and Remission in Patients With and Without Antiadalimumab Antibodies



A, Proportion of patients who reached sustained minimal disease activity score (DAS28 < 3.2) for patients with and without antiadalimumab antibodies (AAA) (survival analysis; $P < .001$). Ninety-five of 196 patients without AAA reached sustained minimal disease activity vs 10 of 76 patients with AAA. B, Proportion of patients who reached sustained DAS28 (< 3.2) for patients without AAA, patients with low AAA titers (13-100 AU/mL), and patients with high AAA titers (> 100 AU/mL). Both curves with AAA differ significantly from the curve without AAA (survival analysis, $P < .001$). Ninety-five of 196 patients without AAA reached sustained minimal disease activity vs 8 of 45 patients with low AAA titers (13-100 AU/mL), and 2 of 31 patients with high AAA titers (> 100 AU/mL). C, Indicates proportion of patients who reached sustained remission (DAS28 < 2.6) for patients with and without AAA (survival analysis, $P < .001$). Sixty-seven of 196 patients without AAA reached remission vs 3 of 76 patients with AAA.

recently published data that showed that the effectiveness of dose increase of TNF inhibitors was very small or lacking.¹⁶

Figure 1 shows that almost 10% of the patients already developed antiadalimumab antibodies after only 4 weeks of treatment. This finding sheds new light on the perspective of primary and secondary nonresponse. From a clinical perspective solely, primary and secondary nonresponse (or loss of response) is usually defined by time (ie, whether response to treatment was not observed from treatment start [primary nonresponse], or an initial response was lost over time [secondary nonresponse]). For clarity reasons, this study argues to define primary and secondary nonresponse from a mechanistic point of view based on objective measurements instead of from a clinical view. Primary nonresponse can then be defined as nonresponse despite adequate serum drug levels (without antidrug antibodies), and secondary nonresponse as nonresponse owing to diminished serum drug levels (with or without antidrug antibodies).

In previous studies in 2 different cohorts of adalimumab and etanercept patients, it was shown that the reason for nonresponse to a first TNF inhibitor has implications for the response to a second TNF inhibitor after switching.^{10,17} Patients who had developed antidrug 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-naïve patients. In contrast, patients who did not respond to their first TNF inhibitor, despite adequate serum drug levels and the absence of antidrug antibodies, had a significantly worse response to their second TNF inhibitor compared with both TNF-naïve patients and patients with antidrug 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 antidrug 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 antidrug antibody response while others do not. The use of concomitant immunosuppressants has shown to be associated with a lower frequency of antidrug antibodies.^{18,19} This is supported by the baseline differences for patients with and without antiadalimumab antibodies in this study; patients who later developed antiadalimumab antibodies 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 antidrug antibodies—as we showed previously that patients with certain IL-10 polymorphisms more often developed antiadalimumab antibodies.²⁰ Differences in baseline characteristics between antiadalimumab antibody-positive and negative patients in the present study show that patients with antiadalimumab antibodies had 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 antidrug antibodies is currently unknown.

Our findings are not applicable to adalimumab treatment in RA alone, but correspond to immunogenicity data published on other biologic therapeutics and on other diseases. An association between the occurrence of antidrug antibodies and diminished serum drug concentrations and short-term treatment response has been described for several biologic drugs in a variety of diseases.^{1,6,7,21-23} Approximately 6% of the patients receiving natalizumab, a humanized monoclonal antibody against cellular adhesion molecule α 4-integrin approved for the treatment of

multiple sclerosis and Crohn disease, developed persistent antibodies to the drug with subsequent loss of efficacy.⁶ Data on infliximab in Crohn disease showed that antibodies against infliximab (during on-demand therapy) developed in 61% of patients and were associated with a reduced duration of response to treatment.¹ Development of anti-infliximab antibodies during infliximab treatment of RA patients was associated with an increased risk of infusion reaction and treatment failure.²³ Most studies show results after a follow-up period of 1 year or less, but given the negative association with 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 biologic drugs are used. One long-term study on adalimumab treatment for Crohn disease described a negative effect on serum drug concentration and, in a limited sized subgroup, on sustained clinical benefit.⁹ Consensus on issues such as the use of definitions, optimization regimens (dose increase, cotreatment), and standardization of assays could help develop the best possible way of dealing with immunogenicity.⁸

In conclusion, this study demonstrated associations between antidrug antibodies and important long-term clinical end points—discontinuation of treatment, minimal disease activity, and remission.

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Study concept and design: Bartelds, Krieckaert, Nurmohamed, Lems, Dijkmans, Aarden, Wolbink.

Acquisition of data: Bartelds, Krieckaert, Nurmohamed, Wolbink.

Analysis and interpretation of data: Bartelds, Krieckaert, Nurmohamed, van Schouwenburg, Twisk, Aarden, Wolbink.

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Study supervision: Nurmohamed, Lems, Twisk, Dijkmans, Aarden, Wolbink.

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