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, psoriasis, and multiple sclerosis; and for several therapeutics such as infliximab, adalimumab, and natalizumab. 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. 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. 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 antidadalimumab antibody titers were determined after follow-up. Treatment discontinuation, minimal disease activity, and clinical remission were compared for patients with and without antidadalimumab antibodies.

Results After 3 years, 76 of 272 patients (28%) developed antidadalimumab antibodies—51 of these (67%) during the first 28 weeks of treatment. Patients without antidadalimumab 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 antialdalimumab 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 antidadalimumab antibody–negative ones (n = 28 [14%]). Ninety-five of 196 patients (48%) without antidadalimumab antibodies had minimal disease activity vs 10 of 76 patients (13%) with antidadalimumab antibodies; patients with antidadalimumab 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 antidadalimumab antibody–negative ones. Three of 76 patients (4%) with antidadalimumab antibodies achieved sustained remission compared with 67 of 196 (34%) antidadalimumab antibody–negative ones; patients with antidadalimumab antibodies less often achieved remission (DAS28 < 2.6; HR, 7.1; 95% CI, 2.1-23.4; P < .001) compared with antidadalimumab 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 concentrations 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 necrosis factor (TNF)–naive 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 antidadalimumab 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:

\[
\text{DAS28} = 0.56 \times \sqrt{\frac{TJC28}{0.70} + 0.28 \times \sqrt{\frac{SJC28}{0.014} + 0.014 \times \text{VAS}}}
\]

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. 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, 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. After dilution of 1 µL of serum in phosphate-buffered saline/0.3% bovine serum albumin (pro analyzer buffer), overnight incubation followed with 1 mg Sepharose-immobilized protein A (GE Health Care, Giles, England) in a final volume of 800 µL. 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 [≈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 = 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 antinfliximab antibodies from patients not treated with adalimumab, no antidalimumab 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, χ², 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. Antidalimumab 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 antidalimumab titers from 13 to 100 AU/mL (median, 5 mg/L; IQR, 3-9 mg/L), and for greater than 100 AU/mL.
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 failure, 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 without (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

**Table 1. Demographic and Clinical Characteristics at Baseline**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total Patient Population ($N=272$)</th>
<th>Patients With Antiadalimumab Antibodies ($n=76$)</th>
<th>Patients Without Antiadalimumab Antibodies ($n=196$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>54 (12)</td>
<td>53 (13)</td>
<td>54 (11)</td>
</tr>
<tr>
<td>Women, No. (%)</td>
<td>219 (81)</td>
<td>62 (82)</td>
<td>157 (83)</td>
</tr>
<tr>
<td>DMARD therapy*</td>
<td>3.1 (1.4)</td>
<td>3.4 (1.5)*</td>
<td>3.0 (1.3)*</td>
</tr>
<tr>
<td>Prior DMARDs, mean (SD)</td>
<td>202 (74)</td>
<td>41 (54)*</td>
<td>161 (82)*</td>
</tr>
<tr>
<td>Methotrexate use, No. (%)</td>
<td>19 (7)</td>
<td>7 (9)</td>
<td>12 (6)</td>
</tr>
<tr>
<td>No concomitant DMARD use, No. (%)</td>
<td>55 (23)</td>
<td>8 (11)*</td>
<td>47 (24)*</td>
</tr>
<tr>
<td>Prednisone use, No. (%)</td>
<td>91 (34)</td>
<td>27 (36)</td>
<td>64 (33)</td>
</tr>
<tr>
<td>Disease status</td>
<td>8 (3-17)</td>
<td>12 (5-18)*</td>
<td>8 (3-16)*</td>
</tr>
<tr>
<td>Disease duration, median (IQR), y</td>
<td>196 (72)</td>
<td>57 (75)</td>
<td>139 (71)</td>
</tr>
<tr>
<td>Anti-CCP positive, No. (%)</td>
<td>196 (72)</td>
<td>55 (72)</td>
<td>141 (72)</td>
</tr>
<tr>
<td>Erosive disease, No. (%)</td>
<td>201 (74)</td>
<td>63 (83)*</td>
<td>138 (70)*</td>
</tr>
<tr>
<td>ESR, median (IQR), mm/h</td>
<td>23 (11-42)</td>
<td>35 (18-60)*</td>
<td>21 (11-39)*</td>
</tr>
<tr>
<td>C-reactive protein, median (IQR), mg/L</td>
<td>12 (5-29)</td>
<td>19 (7-46)*</td>
<td>11 (4-22)*</td>
</tr>
<tr>
<td>DAS28, mean (SD)</td>
<td>5.2 (1.2)</td>
<td>5.5 (1.1)*</td>
<td>5.1 (1.3)*</td>
</tr>
</tbody>
</table>

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

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

*DMARDs other than methotrexate were sulfasalazine and/or hydroxychloroquine.

*There 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 ($P=.01$).

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out, 48 of the antiadalimumab antibo-
dy–positive patients (63%) discon-
tinued participation during fol-
low-up due to the following reasons: 29
(38%) for treatment failure, 8 (10%) for
adverse events, 2 (3%) because of treat-
ment failure and adverse events com-
bined, and 9 (12%) for other reasons.
Of the antiadalimumab antibody–
negative patients, 76 (39%) discon-
tinued participation: 28 (14%) for treat-
ment failure, 22 (11%) for adverse
events, 9 (5%) because of treatment fail-
ure 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 an-
tiadalimumab antibody–negative pa-
tients (76 of 196) in univariate analy-
sis (P = .002; Figure 4A). However,
after adjustment for confounders, me-
thotrexate dosage, baseline DAS28,
and C-reactive protein, the associa-
tion between antiadalimumab antibo-
dies and dropout was not significant
(hazard ratio [HR], 0.7; 95% CI, 0.5-
1.0; P = .08).

When focusing on dropout because
treatment failure, patients with an-
tiadalimumab antibodies significantly
more often discontinued study partici-
pation (n=29, 38%) compared with
those who were antiadalimumab antibo-
dy–negative (n=28, 14%) in univari-
ate analysis (P < .001; Figure 4B), and
after adjustment for the following con-
founders: methotrexate use, number of
previous DMARDs, and C-reactive pro-
tein (HR, 3.0; 95% CI, 1.6-5.5; 
P < .001).

**Disease Activity Score Over Time**

Analysis by GEE demonstrated a sig-
ificant association between the pres-
ence or absence of antiadalimumab an-
tibodies and DAS28 score over time.
Patients with antiadalimumab antibo-
dies had a higher DAS28 score over time
(and at all time points) compared with
antiadalimumab antibody–negative ones
in univariate analysis (P < .001; re-
gression coefficient, 0.8; 95% CI, 0.57-
1.1). After adjustment for the confound-
ing variables ESR, methotrexate dosage,
and age, this association remained sig-
nificant, but the regression coefficient
became smaller (P = .001; regression co-
efficient, 0.4; 95% CI, 0.2-0.6).

**Minimal Disease Activity**

Patients with antiadalimumab antibo-
dies less often achieved sustained
minimal disease activity (DAS28 < 3.2)
compared with antiadalimumab antibo-
dy–negative ones (Figure 5A; 
P < .001) in univariate analysis and af-
ter adjustment for the confounding vari-
bles 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 an-
tibodies (48%) achieved minimal dis-
 ease activity, compared with 8 of 45 pa-
tients (18%) with antiadalimumab antibo-
dy 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 concen-
tration, −6.0 to −2.9) and −7.1 (95% confidence interval, −8.4 to −5.8), respectively (analysis by

generalized estimating equation).

**Table 2. Discontinuation of Treatment in Patients**

<table>
<thead>
<tr>
<th></th>
<th>No. (%) of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total (N = 272)</strong></td>
<td></td>
</tr>
<tr>
<td>Completed treatment</td>
<td>148 (55)</td>
</tr>
<tr>
<td>Discontinuation</td>
<td></td>
</tr>
<tr>
<td>Treatment failure</td>
<td>57 (21)</td>
</tr>
<tr>
<td>Adverse events</td>
<td>30 (11)</td>
</tr>
<tr>
<td>Treatment failure and adverse events</td>
<td>11 (4)</td>
</tr>
<tr>
<td>Other reasons for discontinuation</td>
<td></td>
</tr>
<tr>
<td>Clinical remission</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Relocation</td>
<td>9 (3)</td>
</tr>
<tr>
<td>Unwillingness to participat</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Loss to follow-up</td>
<td>9 (3)</td>
</tr>
<tr>
<td><strong>Without Antiadalimumab Antibodies (N = 76)</strong></td>
<td></td>
</tr>
<tr>
<td>Completed treatment</td>
<td>52 (37)</td>
</tr>
<tr>
<td>Discontinuation</td>
<td></td>
</tr>
<tr>
<td>Treatment failure</td>
<td>29 (38)</td>
</tr>
<tr>
<td>Adverse events</td>
<td>18 (24)</td>
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<td>Treatment failure and adverse events</td>
<td>2 (3)</td>
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<tr>
<td>Other reasons for discontinuation</td>
<td></td>
</tr>
<tr>
<td>Clinical remission</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Relocation</td>
<td>4 (5)</td>
</tr>
<tr>
<td>Unwillingness to participat</td>
<td>3 (4)</td>
</tr>
<tr>
<td>Loss to follow-up</td>
<td>2 (3)</td>
</tr>
<tr>
<td><strong>Without Antiadalimumab Antibodies (N = 196)</strong></td>
<td></td>
</tr>
<tr>
<td>Completed treatment</td>
<td>92 (61)</td>
</tr>
</tbody>
</table>
and 2 of 31 patients (6%) with antidadalimumab antibody titers greater than 100 AU/mL. Patients with high, as well as those with low antidadalimumab antibody titers achieved sustained minimal disease activity less often compared with antidadalimumab antibody–negative ones (Figure 5B; P < .001).

Remission
Three of 76 patients (4%) with antidadalimumab antibodies achieved sustained remission (DAS28 < 2.6) compared with 67 of 196 (34%) antidadalimumab 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 antidadalimumab antibody–positive patients developed antidadalimumab antibodies soon after they had achieved remission and discontinued treatment shortly thereafter owing to adverse events. One antidadalimumab antibody–positive patient achieved remission at 130 weeks despite the fact that he had already developed antidadalimumab antibodies before that time point. His adalimumab concentrations during antidadalimumab 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 antidadalimumab titers in patients positive for antidadalimumab 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, none of these patients achieved minimal disease activity. Of the 16 patients in whom antidadalimumab 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 antidadalimumab 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 antidadalimumab 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.

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patients 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 coefficient 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 biological 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

![Figure 5](https://example.com/figure5.png)
Development of Antidrug Antibodies Against Adalimumab

Recently published data that showed that the effectiveness of dose increase of TNF inhibitors was very small or lacking. Figure 1 shows that almost 10% of the patients already developed antidrug 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. 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-naive 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-naive 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. This is supported by the baseline differences for patients with and without antidadalimumab antibodies in this study; patients who later developed antidadalimumab 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 antidadalimumab antibodies. Differences in baseline characteristics between antidadalimumab antibody-positive and negative patients in the present study show that patients with antidadalimumab 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. Approximately 6% of the patients receiving natalizumab, a humanized monoclonal antibody against cellular adhesion molecule α4-integrin approved for the treatment of multiple sclerosis and Crohn disease, developed persistent antibodies to the drug with subsequent loss of efficacy. 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. Development of anti-infliximab antibodies during infliximab treatment of RA patients was associated with an increased risk of infusion reaction and treatment failure. 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. 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.

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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