Influence of Trough Serum Levels and Immunogenicity on Long-term Outcome of Adalimumab Therapy in Crohn's Disease

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This article has an accompanying continuing medical education activity on page 1836. Learning Objective: Upon completion of this examination, successful learners will be able to interpret the role of immunogenicity in the use of biologic agents in inflammatory bowel diseases.

See related article, Lewis JD et al, on page 1195 in *CGH*.

BACKGROUND & AIMS: Adalimumab is an efficacious therapy for active Crohn's disease, but long-term data are scarce. We conducted an observational study to assess the long-term clinical benefit of adalimumab in patients who failed to respond to infliximab, specifically focusing on the influence of trough serum concentration and antibodies against adalimumab on clinical outcome. **METHODS:** A total of 168 patients with Crohn's disease treated with adalimumab in a tertiary center were included in a prospective follow-up program. Trough serum concentration and antibodies against adalimumab were measured at predefined time points using enzyme-linked immunosorbent assays. **RESULTS:** A total of 71% and 67% of patients responded by weeks 4 and 12, respectively; among them, 61.5% demonstrated sustained clinical benefit until the end of follow-up (median [interquartile range], 20.4 [11.7–30.0] months). Of the 156 patients receiving maintenance therapy, 102 (65.4%) had to step up to 40 mg weekly and 60 (38.5%) eventually stopped adalimumab therapy mainly due to loss of response. Significantly lower adalimumab trough serum concentrations were measured throughout the follow-up period in patients who discontinued therapy as compared with patients who stayed on adalimumab. Antibodies against adalimumab were present in 9.2% of the patients and affected trough serum concentration. Serious adverse events occurred in 12% of the patients. **CONCLUSIONS: Introduction of adalimumab after failure of infliximab therapy resulted in a sustained clinical benefit in two thirds of patients during a median follow-up period of almost 2 years. Discontinuation was directly related to low adalimumab trough serum concentration, which was observed more frequently in patients who developed antibodies against adalimumab.**

Crohn's disease (CD) is a chronic, relapsing, transmural inflammation of the gastrointestinal tract affecting mainly young patients and resulting in a considerably decreased quality of life.[1] So far, there is no medical or surgical cure for CD and the goal of the existing spectrum of therapeutic modalities is to induce and maintain remission.[2]

Tumor necrosis factor (TNF)-α holds a pivotal role in the pathogenesis of CD.[3] The introduction of infliximab, a chimeric monoclonal immunoglobulin G1 antibody against TNF-α, more than 10 years ago for inducing and maintaining clinical response and remission in patients with moderate to severe luminal and fistulizing CD created new perspectives for the management of this disease.[4–6] Unfortunately, 25%–40% of the patients who respond to this therapy need multiple dose and interval adjustments to maintain clinical response over the long-term and about 10% of patients per year discontinue therapy because of loss of response or side effects.[5–8]

The development of antibodies to infliximab and, as a consequence, low trough serum concentration of the drug have been implicated as predisposing factors for infliximab treatment failure.[9–13]

Adalimumab (Humira, D2E7; Abbott Laboratories, Abbott Park, Chicago, IL) is a recombinant, fully human, subcutaneously delivered immunoglobulin G1 monoclonal antibody. This second anti-TNF agent was found to be effective for treatment of refractory luminal CD, both

Abbreviations used in this paper: CRP, C-reactive protein; IQR, interquartile range; OR, odds ratio; SAE, severe adverse events; TNF, tumor necrosis factor.

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in patients who were naive to infliximab and in those who had already been treated with infliximab.[14–17] Although fully human, adalimumab is not devoid of immunogenicity. Antibodies against adalimumab have been reported in 2.6%–38% of patients treated for CD or rheumatoid arthritis, the rate depending also on the presence of concomitant immunomodulator therapy.[16,18,19] In patients with rheumatoid arthritis, antibodies against adalimumab have been associated with low adalimumab trough serum concentration and decreased clinical response,[18] but data in CD are lacking.

The aims of this study were (1) to assess the long-term outcome of adalimumab therapy, including safety, in a consecutive series of 168 patients with CD who discontinued infliximab therapy because of loss of response or intolerance; (2) to evaluate the influence of adalimumab trough serum concentration on clinical response, adalimumab dose escalation, and discontinuation of therapy; and (3) to investigate the presence of antibodies against adalimumab and their relationship to adalimumab trough serum concentration and clinical outcome.

Patients and Methods

Patients

This was an observational cohort study performed at a single tertiary care center, where a prospectively designed standardized schedule was used for follow-up by experienced clinicians of all patients treated with adalimumab. Eligible patients were male and female patients with CD who had presented an initial response to infliximab and subsequently became intolerant (acute and/or delayed hypersensitivity reactions) or lost response (worsening of clinical status as judged by the treating physician) despite dose adjustment. Thirty percent of the patients received a course of corticosteroids after discontinuation of infliximab therapy, while the others (1) were included in randomized controlled trials with experimental therapies but dropped out due to lack of efficacy, (2) remained under treatment with concomitant immunomodulators until initiation of adalimumab therapy, or (3) received no specific treatment (infliximab washout period before entering adalimumab trials). The modified Vienna classification was used for assessing CD location and behavior.[20] Assessment of CD and extraintestinal manifestation activity was based on endoscopic, histopathologic, radiologic, and clinical criteria. In all patients, latent tuberculosis was again excluded before initiation of adalimumab therapy via a protein purified derivative skin test and a chest x-ray. Patients with evidence of tuberculosis exposure at initiation of adalimumab therapy who had not received prophylaxis while being treated with infliximab received anti-tuberculosis prophylactic treatment for at least 6 months. Patients were seen at the IBD outpatient clinic every 2 weeks for the first 12 weeks of adalimumab treatment and every 1–3 months afterwards, depending on the patient's clinical status.

Primary and Secondary Analyses

The primary analysis concerned the proportion of patients with an initial response to adalimumab who demonstrated sustained clinical benefit of this treatment throughout their entire follow-up. Second, we examined the influence of immunogenicity on the efficacy of adalimumab by measuring adalimumab trough serum concentration and antibodies against adalimumab at predetermined time points and relating the data to loss of response, need for dose escalation, and discontinuation rate.

In the secondary analysis, we assessed the efficacy of adalimumab on fistula closure and on extraintestinal manifestations, analyzed the rate of severe adverse events (SAE), and looked at clinical factors influencing treatment outcome with special focus on concomitant medications.

Definitions

Short-term clinical response was defined as an improvement of symptoms of the disease at 4 and 12 weeks after the first injection as judged by the treating physician. Patients who became completely symptom-free were considered full responders. Patients who had clear clinical improvement with an obvious decrease of disease activity but who still had symptoms were considered partial responders. Patients who discontinued therapy by week 4 due to absence of clinical benefit were considered primary nonresponders. Improvement in fistulizing CD was defined as a $\geq$50% decrease in the number of draining perianal fistulas from baseline during at least 2 consecutive treatment visits and remission as complete closure of all draining fistulas according to the treating physician's assessment.

Patients who reported lasting control of disease activity by the end of follow-up, regardless of the need for dose escalation, were considered to have sustained clinical benefit.

Patients with recurrent symptoms necessitating adalimumab dose escalation (standard intervention was adalimumab 40 mg every other week to 40 mg every week) were considered to have lost response; however, if they regained response and sustained it until the end of follow-up, they were considered to have sustained clinical benefit. The adalimumab dosing interval was decreased to once weekly in patients who presented with symptoms of active luminal disease that were dominant at baseline and were accompanied by an increase in C-reactive protein (CRP) level or endoscopic lesions. In patients predominantly treated for perianal fistulizing CD or for extraintestinal manifestations, these clinical conditions were used as indications for escalating from every other week to weekly therapy.

Biologic activity was assessed at baseline and 4 and 12 weeks after initiation of therapy, and biologic response was defined as a decrease in CRP level of at least 50% from baseline or normalization of CRP level (<3 mg/L).

SAE was defined as any adverse event that resulted in hospitalization, was fatal or life threatening, or led to significant disability.

All patients gave informed consent to participate in the study.

Measurement of Adalimumab Trough Serum Concentration and Antibodies Against Adalimumab

Blood samples were drawn at standardized time points just before injection of adalimumab (trough serum concentration). The trough serum concentration of adalimumab was measured using an enzyme-linked immunosorbent assay derived from the one developed for infliximab.[21] In brief, recombinant human TNF-α was coated on the solid phase and recognized by adalimumab, and the therapeutic monoclonal antibody was detected by an anti-human immunoglobulin G Fcγ-specific antibody conjugated to horseradish peroxidase. The limit of detection was 0.004 μg/mL and the lower and upper limits of quantification were 0.1 and 4.8 μg/mL, respectively. Sera exceeding the upper limit of quantification were diluted 1:10, with the coefficient of variation of this procedure being <10%.

Serum concentrations of antibodies against adalimumab were analyzed using a double-antigen enzyme-linked immunosorbent assay based on their capture by adalimumab-coated plates and their detection by peroxidase-coupled adalimumab. Because of the interference of circulating adalimumab, antibodies against adalimumab could not be measured if the adalimumab concentration was >0.094 μg/mL. The cutoff value (for false-positive antibodies against adalimumab), determined using untreated samples of patients with an autoimmune disease, was 0.128 μg/mL (obtained from the 99th percentile). However, this applies to the first sample in each patient for which the interpretation cannot be based on adalimumab concentration. The trough serum concentration of adalimumab and antibodies against adalimumab were determined after the patients completed follow-up; the treating physician was unaware of these data.

Statistical Analysis

SPSS 15.0 software package (SPSS Inc, Chicago, IL) was used for performing all appropriate statistical analyses. Medians with interquartile range (IQR) or means with SD were calculated for continuous data, and percentages were computed for discrete data. The χ^2 test was used for comparison of categorical data, and odds ratios (OR) were provided where necessary. Differences between independent groups were traced with the use of Student *t* test for normally distributed values and the Mann–Whitney *U* test for nonnormally distributed values. For differences between dependent groups, the Wilcoxon signed rank test was used. The sustained clinical benefit of adalimumab was estimated by Kaplan–Meier analyses. To compare hazard rates in populations defined by one variable at the time, the log-rank test was used. Logistic and Cox proportional hazard models were conducted for the detection of predictors of clinical response to adalimumab treatment. A receiver operating characteristic analysis was performed for tracing an adalimumab trough serum concentration with an influence on sustained clinical benefit. $P < .05$ was considered significant.

Results

Patients and Induction Scheme Schedule

A total of 209 patients with inflammatory bowel disease initiated treatment with adalimumab between April 2003 and December 2007 at the IBD unit of the University Hospital of Leuven. Of these patients, 191 had CD and 168 of 191 had previously failed to respond to infliximab. Seventy-one of these 168 patients (42%) took part in clinical trials with adalimumab, but only time points when active drug was administered (assessed after unblinding) were taken into consideration. Long-term follow-up until the end of March 2008 was completed in all 168 patients with CD.

The main indication for initiation of adalimumab was luminal CD in 160 patients (95.2%), fistulizing CD in 2 patients, and arthritic manifestations in 6 patients. Before starting treatment with adalimumab, these patients had received a median (IQR) of 8.0 (4.0–14.2) infusions of infliximab for 29.3 (8.4–56.5) months. Adalimumab therapy was started within 5.0 (2.0–16.2) months after the last infliximab infusion. During infliximab treatment, 51% of the patients needed either dose escalation or shortening of the interval between infusions to maintain response. Fifty-five percent experienced at least one infusion reaction, either acute or delayed, and 32% stopped infliximab therapy because of intolerance. Loss of response was the cause for discontinuation in 68% of the patients. Patients' baseline characteristics are listed in Table 1.

A total of 126 of 168 patients (75%) received 160 mg adalimumab subcutaneously at week 0 and 80 mg at week 2 as an induction scheme. In 5 of 126 patients, luminal CD was quiescent at baseline and these patients were treated for perianal fistulizing CD (n = 1), persistent postoperative anastomotic enterocutaneous fistulas (n = 1), and arthritic manifestations (n = 3). The remainder (n = 121) had active luminal CD. Twenty-eight patients (16.7%) received 80/40 mg and 14 (8.3%) first received placebo in a clinical trial but were then treated with 40 mg subcutaneously every 2 weeks without a loading dose.

Table 1. Patients' Baseline Characteristics (n = 168)

Female/male (%)	120/48 (71.4/28.6)
Main indication for adalimumab treatment (%)	
Luminal CD	160 (95.2)
Fistulizing CD	2 (1.2)
Arthritic manifestations	6 (3.6)
Median (IQR) age at diagnosis (*y*)	22.8 (17.7–29.8)
Median (IQR) age at initiation of adalimumab therapy (*y*)	36.3 (27.3–47.1)
Median (IQR) disease duration before initiation of adalimumab therapy (*y*)	10.5 (5.7–17.2)
Median (IQR) follow-up of adalimumab treatment (*mo*)	20.3 (11.7–29.9)
Median (IQR) baseline CRP level (*mg/L*, n = 159)	14.1 (3.9–33.3)
Location of disease (%)	
Ileitis	37 (22)
Colitis	43 (26)
Ileocolitis	88 (52)
Upper gastrointestinal tract	7 (4.2)
Smokers (%)	69 (41)
Concomitant medication at initiation of adalimumab therapy (%)	
Corticosteroids	41 (24.4)
Azathioprine/6-mercaptopurine	41 (24.4)
Methotrexate	21 (12.5)
Indication for adalimumab treatment in relation to infliximab failure (%)	
Intolerance of infliximab	54 (32)
No response to infliximab	11 (6.5)
Loss of response to infliximab	97 (58)

Short-term Clinical Response of Luminal CD to Adalimumab

Overall, 105 patients (70.5%) in both induction scheme groups (n = 149) responded by week 4 and 100 (67.1%) by week 12. Forty-nine (40.4%) of the patients who received 160/80 mg showed a complete response, 35 (29%) a partial response, and 37 (30.6%) no clinical response by week 4. In the 80/40 mg group, 12 of 28 patients (43%) showed a complete response, 9 (32%) a partial response, and 7 (25%) no clinical response by week 4. By week 12, in patients who received 160/80 mg, a complete clinical response was present in 37 patients (30.6%), a partial response in 47 patients (38.8%), and no clinical response in 37 patients (30.6%). In the 80/40 mg group, 11 patients (39.3%) showed a complete response, 5 (17.9%) a partial response, and 12 (42.8%) no clinical response by week 12 (Figure 1*A*).

The majority of patients had an elevated CRP level at baseline (>3 mg/L, n = 130, 77.4%). The median CRP level at baseline was 14.1 (3.9–33.3) mg/L and decreased to 4.2 (1.1–11.3) mg/L by week 4 ($P < .0001$) and to 4.4

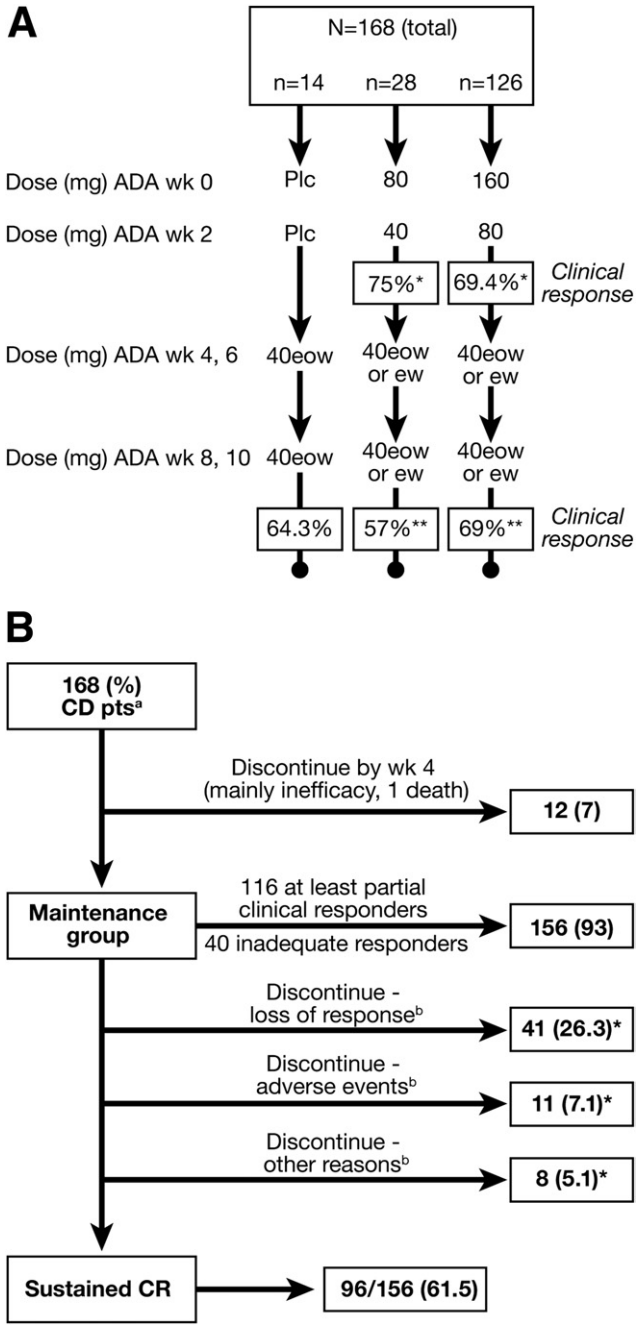


Figure 1. (*A*) Short-term outcome in 168 patients with CD treated with adalimumab who previously failed to respond to infliximab therapy. Fourteen patients received placebo as an induction scheme (weeks 0 and 2), 28 received 80/40 mg adalimumab, and 126 received 160/80 mg. Subsequently, all patients received adalimumab either 40 mg every other week or 40 mg every week. No significant difference was observed in clinical response between the 2 arms that received active drug as induction scheme both at week 4 (*$P = .57$) and week 12 (**$P = .23$). (*B*) Long-term outcome of adalimumab therapy. The initial cohort comprised 168 patients with CD. Twelve patients (7%) discontinued treatment by week 4 mainly due to inefficacy. A total of 156 patients (93%) entered the maintenance phase; from these patients, 60 (38.5%) discontinued adalimumab therapy (*) due to loss of response (26.3%), adverse events (7.1%), and other reasons described in the text (5.1%). Ninety-six patients (61.5%) demonstrated sustained clinical response until the end of follow-up. [a]Median follow-up period, 20.4 (11.7–30.0) months. [b]Median treatment duration if adalimumab stopped, 29.3 [8.7–52.8] weeks. ADA, adalimumab; CR, clinical response; eow, every other week; ew, every week; plc, placebo; pts, patients.

(1.3–18.9) mg/L by week 12 ($P < .0001$). Ninety-one of 121 patients (75.2%) showed a biologic response to adalimumab at week 4, and CRP decreased to normal values in 39 patients (32.2%). At week 12, 64 of 94 patients (68.1%) responded biologically, with 33 patients (35.1%) showing a normalization of CRP level. Clinical and biologic response correlated well at both time points ($P = .006$).

Long-term Clinical Benefit and Discontinuation of Adalimumab Treatment

Of the 168 patients included in this analysis, 12 (7%) discontinued adalimumab by week 4 due to primary nonresponse (n = 10), adverse events (n = 1, intense injection site reaction and acne), and a fatal event (n = 1, cardiac arrest of unknown origin). Half of these patients showing primary nonresponse underwent early bowel resection, and the others were rescued with corticosteroids (Figure 1*B*).

A total of 156 patients (93%) continued adalimumab after week 4, of whom 116 (74.4%) had at least a partial clinical response. In 40 patients (25.6%), therapy was continued beyond week 4, although response was considered unsatisfactory, in an effort to achieve a delayed response. Thirty-two of these inadequate responders (80%) received 40 mg adalimumab weekly after induction and 20 of 32 (62.5%) responded. Of the 116 responders at week 4, 70 (60.3%) needed dose escalation and 53 of 70 (75.7%) regained clinical response. In total, 102 patients (65.4%) had a dose escalation, of whom 73 (71.6%) responded. Median time to dose escalation was 14.0 (10.7–24.0) weeks. In particular, the probability of dose escalation was approximately 15% at 30 weeks, 42% at 60 weeks, and 80% at 120 weeks of adalimumab treatment (Figure 2*A*). No difference was observed in the rate of or time to dose escalation between the patients who received 160/80 mg and those who received 80/40 mg.

At the end of follow-up, 96 of 156 patients (61.5%) showed sustained clinical benefit to adalimumab therapy (Figure 1*B*). The probability of a patient sustaining clinical benefit was approximately 60% at 60 weeks, 50% at 120 weeks, and 38% at 180 weeks (Figure 2*B*). Sixty out of 156 patients (38.5%) discontinued adalimumab because of loss of response (n = 41; 26.3%), adverse events (n = 11; 7.1%), or other reasons (n = 8; 5.1%) (Figure 1*B*). The probability of a patient ceasing treatment was 40% at 60 weeks, 50% at 120 weeks, and 62% at 180 weeks (Figure 2*B*). Discontinuation of adalimumab treatment occurred after a median of 29.4 (8.7–52.8) weeks.

At baseline, 62 patients (37%) were receiving concomitant immunomodulators. The latter was stopped in 29 patients (46.8%) after 10.6 (6.8–18.2) months. Forty-one patients (24.4%) received corticosteroids at baseline and 21 (51.2%) could definitively stop them after 3.0 (0.7–3.6) months.

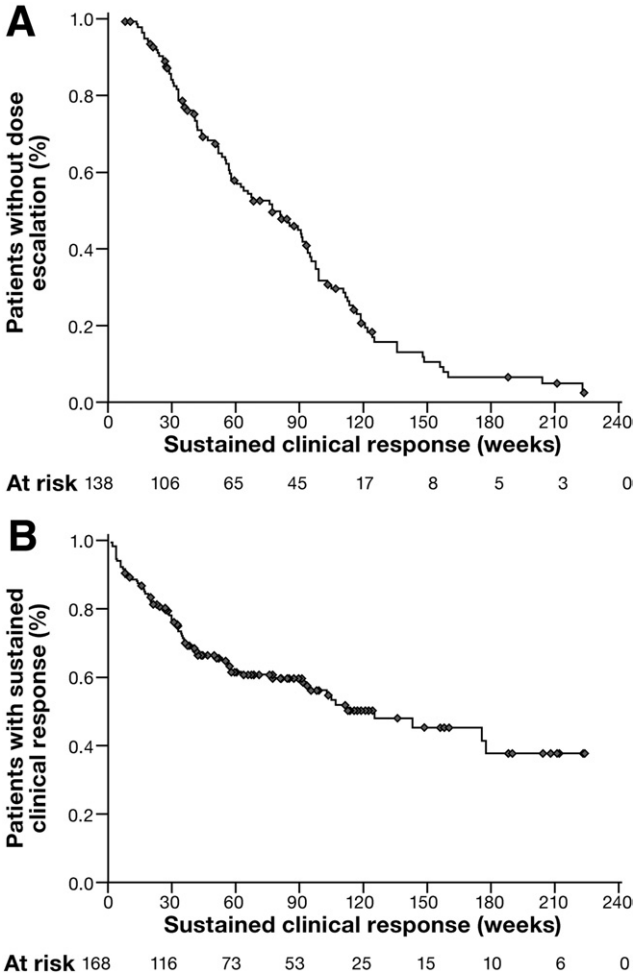
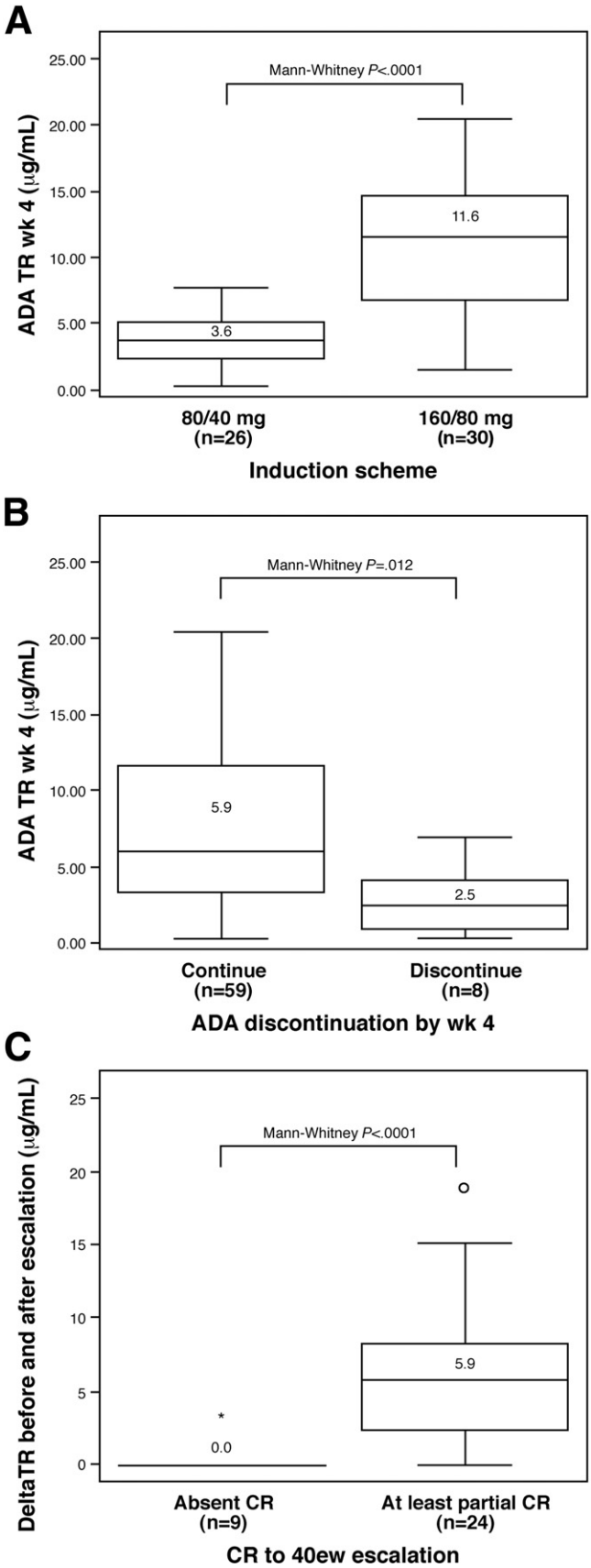


Figure 2. (*A*) Sustained clinical benefit of adalimumab in the patients of the maintenance group according to dose escalation. The curve represents the proportion of patients with sustained clinical benefit who did not experience an escalation of dose during follow-up. *At risk* represents the patients who continued treatment demonstrating a sustained clinical response at given time points, considering those with available data on dose escalation as the initial cohort (n = 138). (*B*) Sustained clinical benefit of adalimumab in the overall cohort. The curve represents the proportion of patients with a sustained clinical response during follow-up. *At risk* represents the patients who continued treatment demonstrating a sustained clinical response at given time points, considering as initial cohort the study population (n = 168).

Relationship Between Trough Serum Concentration and Antibodies Against Adalimumab and Short-term Outcome

Adalimumab trough serum concentration and antibodies against adalimumab were available in 130 patients (77.4% of the total cohort). Seventy-six percent received 160/80 mg as an induction dose, 65% responded on a short-term basis, 65% needed dose escalation, and 37% received immunomodulators at baseline. Median trough serum concentration was 8.6 (6.5–10.8) μg/mL, 5.3 (2.8–10.9) μg/mL, 7.9 (3.2–12.0) μg/mL, 7.8 (1.8–12.2) μg/mL, and 10.7 (6.1–18.1) μg/mL at weeks 2, 4, 12, 24, and 54, respectively. In comparison with patients who



received 80/40 mg, those who received 160/80 mg as a loading dose showed higher adalimumab trough serum concentration at week 4 ($P < .0001$; Figure 3*A*) and a higher rate of CRP normalization (OR, 3.9 [1.1–14.4]; $P = .04$) but also more frequent (OR for discontinuation, 0.3 [0.1–0.7]; $P = .004$) and longer sustained clinical benefit (31.8 [15.3–41.9] vs 12.0 [4.0–38.2] weeks; $P = .04$), less frequent primary nonresponse (OR, 0.02 [0.003–0.2]; $P < .0001$), and faster withdrawal of immunomodulators (8.9 [3.7–12.0] vs 22.8 [10.0–33.2] months; $P = .01$). Patients in the 160/80 mg induction scheme developed numerically less frequently antibodies against adalimumab, but this was not statistically significant (5.7% vs 17.9%; $P = .05$).

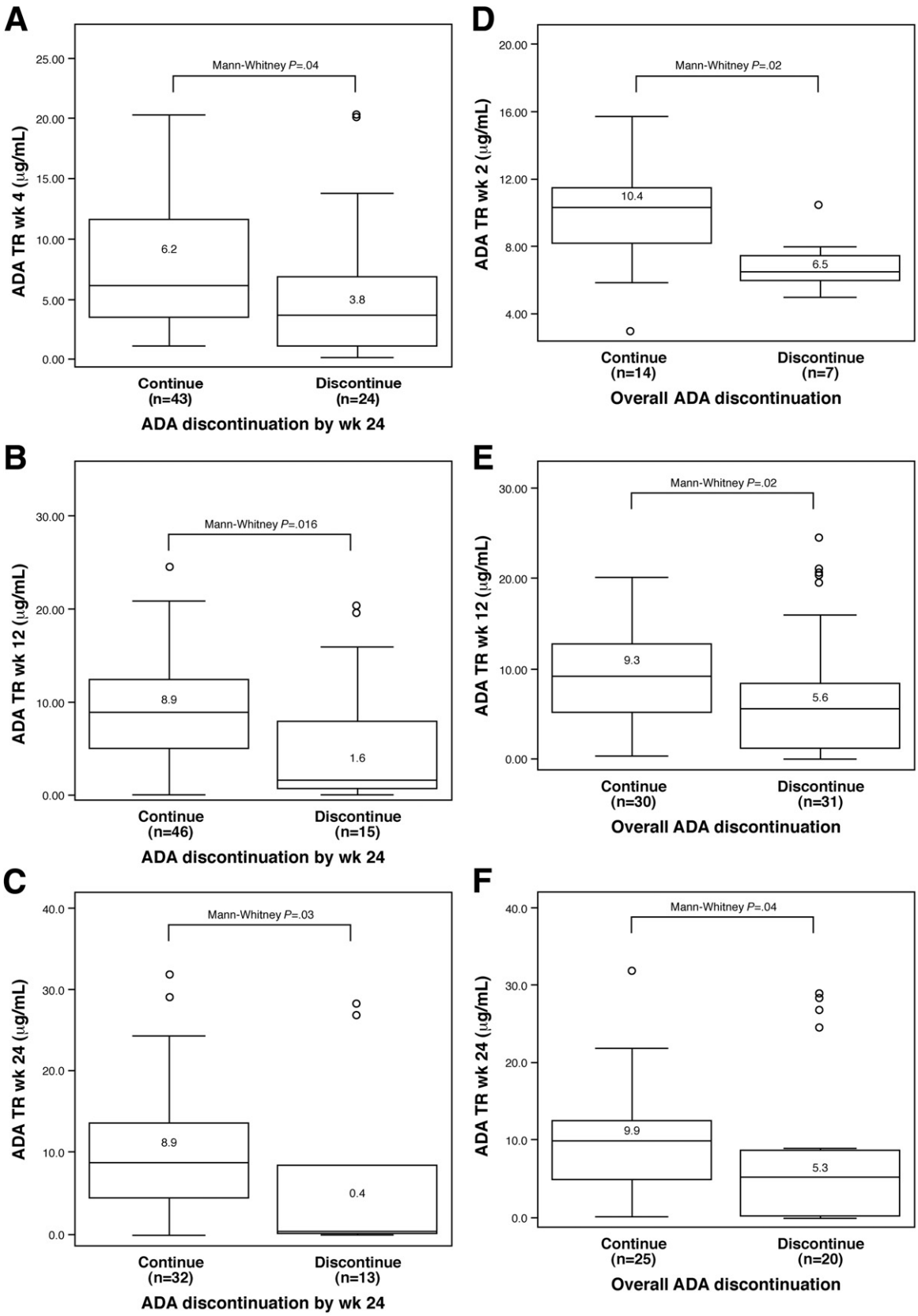
No relationship was found between adalimumab trough serum concentration or antibodies against adalimumab and short-term clinical response, although patients who discontinued adalimumab by week 4 had lower trough serum concentration compared to those who continued throughout maintenance treatment ($P = .012$; Figure 3*B*).

In our multivariate analysis, no predictors for short-term clinical response were detected.

Relationship Between Trough Serum Concentration and Antibodies Against Adalimumab and Long-term Outcome

Patients who lost response received dose escalation. Adalimumab trough serum concentration increased after dose escalation from 4.8 (2.3–8.9) to 9.4 (1.2–16.4) μg/mL ($P = .001$), and this increase correlated well with the clinical response to escalation (5.9 [1.9–8.3] for responders vs 0.0 [0.0–1.7] μg/mL for nonresponders, $P < .0001$, Figure 3*C*). The median trough serum concentration at the time of discontinuation in the 59 patients (45.4%) who stopped therapy was 3.2 (0.3–11.7) μg/mL. The patients who discontinued adalimumab by 6 months of treatment demonstrated lower trough serum concentration throughout this respective period of follow-up compared to those who could continue therapy beyond

Figure 3. Influence of induction schedule on (*A*) median (IQR) adalimumab trough serum concentration (for 160/80 mg: 11.6 [6.7–14.7] vs 3.6 [2.3–5.2] μg/mL for 80/40 mg; $P < .0001$), (*B*) the relationship between therapy discontinuation by week 4 and median adalimumab trough serum concentration (for discontinuation: 2.5 [0.8–4.3] vs 5.9 [3.2–11.9] μg/mL for continuation; $P = .012$), and (*C*) the relationship between increase of median adalimumab trough serum concentration and clinical response to dose escalation (for responders: 5.9 [1.9–8.3] vs 0.0 [0.0–1.7] μg/mL for nonresponders; $P < .0001$). Mann–Whitney tests; the *asterisk* and *small circle* represent mild outliers; the *upper* and *lower whiskers* indicate the distance from the end of the box to the largest and smallest observed values that are less than 1.5 box lengths from either end of the box; the *top* and *bottom of the box* indicate the 75th and 25th percentiles, respectively; and the *band near the middle of the box* indicates the 50th percentile. ADA, adalimumab; CR, clinical response; ew, every week; TR, trough serum concentration.


A
Mann-Whitney P=.04
ADA TR wk 4 (μg/mL)
25.00
20.00
15.00
10.00
5.00
0.00
6.2
3.8
Continue (n=43)
Discontinue (n=24)
ADA discontinuation by wk 24
B
Mann-Whitney P=.016
ADA TR wk 12 (μg/mL)
30.00
20.00
10.00
0.00
8.9
1.6
Continue (n=46)
Discontinue (n=15)
ADA discontinuation by wk 24
C
Mann-Whitney P=.03
ADA TR wk 24 (μg/mL)
40.0
30.0
20.0
10.0
0.0
8.9
0.4
Continue (n=32)
Discontinue (n=13)
ADA discontinuation by wk 24
D
Mann-Whitney P=.02
ADA TR wk 2 (μg/mL)
20.00
16.00
12.00
8.00
4.00
10.4
6.5
Continue (n=14)
Discontinue (n=7)
Overall ADA discontinuation
E
Mann-Whitney P=.02
ADA TR wk 12 (μg/mL)
30.00
20.00
10.00
0.00
9.3
5.6
Continue (n=30)
Discontinue (n=31)
Overall ADA discontinuation
F
Mann-Whitney P=.04
ADA TR wk 24 (μg/mL)
40.0
30.0
20.0
10.0
0.0
9.9
5.3
Continue (n=25)
Discontinue (n=20)
Overall ADA discontinuation

24 weeks (Figure 4*A–C*). A similar difference was also apparent between the patients who stopped adalimumab by the end of follow-up and those who still had a sustained clinical benefit by that time (Figure 4*D–F*). In 67 patients, adalimumab trough serum concentration was available 4 weeks after initiation of therapy. From these patients, those who developed antibodies against adalimumab by the end of follow-up (n = 9) demonstrated a lower median adalimumab trough serum concentration at week 4 than those who never developed measurable antibodies against adalimumab (n = 58) (2.1 [0.8–4.1] vs 6.1 [3.2–11.5] μg/mL; $P < .02$; Figure 5). Patients who displayed an adalimumab trough serum concentration <0.33 μg/mL at least once (sensitivity, 95%; positive predictive value, 81%) demonstrated significantly less sustained clinical benefit than patients never showing such low trough serum concentration (log-rank test; $P = .01$; Figure 6*A*).

Twelve patients had a trough serum concentration <0.094 μg/mL at least once. Eleven of them (91.6%) discontinued adalimumab therapy and all 11 displayed antibodies against adalimumab, while the discontinuation rate in patients with trough serum concentration persistently >0.094 μg/mL was 40.7% (OR, 16.0 [2.0–128.4]; $P = .001$). The patients with antibodies against adalimumab had lower median trough serum concentration throughout the entire follow-up period ($P < .0001$; Figure 5) independently of the time point that antibodies against adalimumab were detected.

Concomitant immunomodulator therapy at baseline did not affect treatment outcome (log-rank test; $P = .45$; Figure 6*B*), did not influence adalimumab trough serum concentration, and did not decrease the development of antibodies against adalimumab. Only time to dose escalation was longer in patients who were treated with immunomodulators (17.0 [12.0–27.5] vs 12.0 [8.0–22.0] weeks; $P = .008$). There was also no correlation between sustained clinical benefit and concomitant corticosteroid therapy at baseline (log-rank test; $P = .2$; Figure 6*C*). The patients who had normalized CRP levels at both week 4 and week 12 discontinued adalimumab less frequently (OR for week 4, 0.2 [0.1–0.6]; $P = .001$) and showed longer sustained clinical benefit (log-rank test for week 4, $P = .008$; Figure 6*D*). The presence of an adverse event or injection site reaction was not related to the presence of antibodies against adalimumab. Clinical response was not influenced by the reason that led to therapy discontinuation. In the Cox regression analysis, no predictors for sustained clinical benefit turned out to be significant.

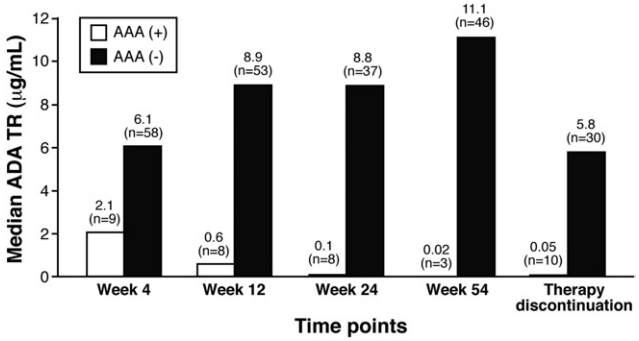


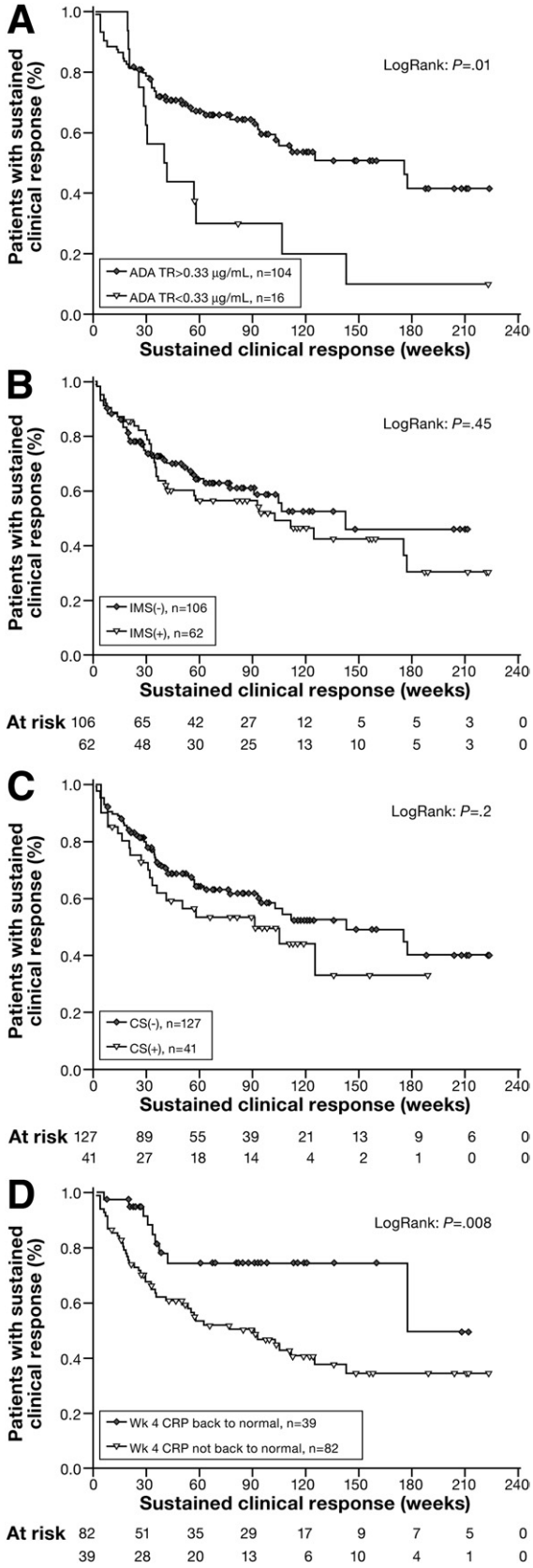
Figure 5. Relationship between antibodies against adalimumab and adalimumab trough serum concentration in different time points. AAA, antibodies against adalimumab; ADA, adalimumab; TR, trough serum concentration.

Antibodies against adalimumab were detected at any time in 12 patients (9.2%). The first positive sample was detected after 22.5 (13.0–39.5) weeks. In the majority of the patients who developed antibodies against adalimumab (10 of 12), these were still present at the time of therapy discontinuation. Finally, we revisited antibodies to infliximab status in 121 of 130 patients (93.1%) for whom antibodies against adalimumab and adalimumab trough serum concentration were measured. Of these 121 patients, 8 were indeterminate for antibodies to infliximab (due to circulating levels of infliximab at the time of assessment). Therefore, 113 patients were analyzed. The presence of antibodies to infliximab before initiation of adalimumab therapy was not associated with a higher incidence of antibodies against adalimumab (6 of 12 antibodies against adalimumab–positive patients were also antibodies to infliximab positive and 6 of 12 were antibodies to infliximab negative), with discontinuation of adalimumab therapy, or with the need for dose escalation (data not shown).

Adalimumab Treatment and Fistula Closure

At baseline, 19 of 32 patients with perianal CD lesions (59.4%) presented with actively draining fistulas. In 12 of 19 patients (63.1%), improvement in fistula

Figure 4. Comparison of median (IQR) adalimumab trough serum concentration at (*A*) week 4 (for discontinuation: 3.8 [1.1–6.9] vs 6.2 [3.5–11.9] μg/mL for continuation; $P = .04$), (*B*) week 12 (for discontinuation: 1.6 [0.3–8.0] vs 8.9 [4.9–12.6] μg/mL for continuation; $P = .016$), and (*C*) week 24 (for discontinuation: 0.4 [0.1–8.4] vs 8.9 [4.4–14.2] μg/mL for continuation; $P = .03$) of the patients who discontinued therapy by month 6 and those who continued treatment beyond that point and at (*D*) week 2 (for discontinuation: 6.5 [5.6–8.0] vs 10.4 [7.9–11.5] μg/mL for continuation; $P = .02$), (*E*) week 12 (for discontinuation: 5.6 [1.1–8.9] vs 9.3 [5.2–12.9] μg/mL for continuation; $P = .02$), and (*F*) week 24 (for discontinuation: 5.3 [0.1–8.8] vs 9.9 [4.8–13.6] μg/mL for continuation; $P = .04$) of the patients who discontinued therapy and those who sustained clinical benefit by the end of follow-up. Mann–Whitney tests; *small circles* represent mild or extreme outliers; the *upper* and *lower whiskers* indicate the distance from the end of the box to the largest and smallest observed values that are less than 1.5 box lengths from either end of the box; the *top* and *bottom of the box* indicate the 75th and 25th percentiles, respectively; and the *band near the middle of the box* indicates the 50th percentile. ADA, adalimumab; TR, trough serum concentration.



drainage was observed with complete response in 52.6% (mean [±SD] time to fistula closure, 16.2 ± 11.7 weeks). Three patients experienced a relapse after 38.0 ± 47.1 weeks. One of these patients escalated to 40 mg every week and responded. From the patients showing initial response, 3 discontinued adalimumab therapy after 58.7 ± 38.3 weeks. In total, 11 patients (58%) were still being treated with adalimumab by the end of follow-up. One patient who was treated for enterocutaneous fistulas did not respond.

Short-term Clinical Response of Extraintestinal Manifestations to Adalimumab

Seventy-three patients (43.5%) presented with at least one extraintestinal manifestation at baseline (81% female patients). The majority (70 of 73 [96%]) had arthritic manifestations (68 peripheral arthralgias, 5 ankylosing spondylitis, and 1 sacroiliitis), 4 (5.5%) had ocular manifestations (3 uveitis, 1 episcleritis), and 3 (4%) had skin extraintestinal manifestations (1 erythema nodosum, 1 pyoderma gangrenosum, and 1 Sweet syndrome). At week 4, 23 patients (31.5%) showed a complete response, 24 (33%) had a partial response, and 26 (35.5%) had no clinical response. At week 12, 30 (41%) showed a

Figure 6. (*A*) Sustained clinical benefit stratified by adalimumab trough serum concentration of 0.33 μg/mL (sensitivity, 95%; positive predictive value, 81%). *Filled diamonds* and *open triangles* indicate patients with adalimumab levels greater than and less than 0.33 μg/mL, respectively, who discontinued treatment throughout follow-up. The *curves* indicate the proportion of patients in each group with a sustained clinical response during follow-up. Log-rank test was used to compare hazard rates between the 2 groups. (*B*) Sustained clinical benefit stratified by concomitant therapy with immunomodulators. *Filled diamonds* and *open triangles* indicate patients without and with immunomodulator therapy at baseline, respectively, who discontinued treatment throughout follow-up. The *curves* indicate the proportion of patients in each group with sustained clinical response during follow-up. Log-rank test was used to compare hazard rates between the 2 groups. *At risk* indicates patients who continued treatment in either group at given time points while demonstrating sustained clinical response. (*C*) Sustained clinical benefit stratified by concomitant use of corticosteroids. *Filled diamonds* and *open triangles* indicate patients without and with corticosteroid therapy at baseline, respectively, who discontinued treatment throughout follow-up. The *curves* indicate the proportion of patients with a sustained clinical response during follow-up. Log-rank test was used to compare hazard rates between the 2 groups. *At risk* indicates patients who continued treatment in either group at given time points while demonstrating a sustained clinical response. (*D*) Sustained clinical benefit stratified by normalization of CRP levels by week 4 in patients with an increased baseline CRP level. *Filled diamonds* and *open triangles* indicate patients with and without CRP normalization, respectively, who discontinued treatment throughout follow-up. The *curves* indicate the proportion of patients with sustained clinical response during follow-up. Log-rank test was used to compare hazard rates between the 2 groups. *At risk* indicates patients who continued treatment in either group at given time points while demonstrating a sustained clinical response. ADA, adalimumab; CS, corticosteroids; IMS; immunomodulator; TR, trough serum concentration.

Table 2. SAEs

Gender/age (*y*)	Duration of adalimumab treatment until event (*wk*)	No. of infliximab infusions (treatment duration, *mo*)	Immunomodulators	Event category	Event
M/44	2	11 (18)	Null	Fatal	Death (cardiac arrest of unknown origin)
F/64[a]	166	1 (0)	Azathioprine	Fatal	Death (lung cancer-chemotherapy-sepsis)
M/30	33	23 (69)	Null	Malignancy	Intraperitoneal B large cell lymphoma
F/41	69	14 (19)	Methotrexate	Malignancy	Ductal breast cancer in situ (cT2N2M0)
M/55	6	30 (57)	Null	Malignancy	Clear cell renal carcinoma (pT1bN0M0)
F/43	178	6 (11)	Null	Malignancy	Papillary renal cell carcinoma (pT1aN0M0)
F/49[b]	91	45 (47)	Null	Malignancy	Basal cell carcinoma
F/51[b]	178	45 (47)	Null	Malignancy	Basal cell carcinoma
F/54	76	4 (25)	Null	Benign tumor	Meningioma (grade I)
F/62[a]	92	1 (0)	Azathioprine	Benign tumor	Bowen skin tumor
F/29	3	Unknown	Null	Infectious	*Campylobacter enteritis/Pseudomonas cystitis*
F/44	21	27 (47)	Azathioprine	Infectious	Pneumonia
F/49	18	8 (25)	6-Mercaptopurine	Infectious	Fever of unknown origin
F/24	31	6 (8)	Null	Infectious	Esophageal moniliasis
F/27	55	37 (66)	Null	Infectious	Streptococcal pneumonia
F/51	58	11 (59)	Methotrexate	Infectious	Upper respiratory tract infection
F/22	25	41 (69)	Azathioprine	Infectious	Pleuritis
M/42	6	4 (4)	Null	Infectious	Multifocal pneumonia
F/56	20	1 (0)	Null	Infectious	Pneumonia
F/38	144	9 (13)	Null	Various	Elective abortion (week 12; trisomy 18)
F/34	29	4 (57)	Null	Various	Empty amniotic sac pregnancy
F/47	88	5 (21)	Null	Various	Suicide attempt

[a]Same patient.
[b]Same patient.

complete response, 15 (20.5%) had a partial response, and 28 (38.5%) had no clinical response. Clinical response did not depend on the induction scheme (160/80 mg or 80/40 mg) used but correlated well with the clinical response to luminal CD at both week 4 and week 12 (OR for week 4, 12.5 [3.7–42.3]; $P < .0001$). From the patients showing an initial response, 36% discontinued adalimumab after a median of 41.7 (29.0–92.0) weeks mainly due to loss of response.

Safety

A total of 22 SAEs occurred in 20 patients (12%) during follow-up of adalimumab treatment (Table 2). Before the initiation of adalimumab therapy, these patients had been treated with a median of 9.0 (4.5–25.0) infusions of infliximab for 25.0 (12.0–57.0) months. The 2 fatal events were considered unrelated to adalimumab therapy. Seven of these 20 patients were being treated with concomitant immunomodulators while SAEs occurred. No difference was detected regarding rate and kind of SAEs between the patients who were and were not being treated with immunomodulators. Thirty-two patients (19%) experienced an injection site reaction. All events occurred while patients were being treated with adalimumab. No cases of opportunistic infections, demyelinating disease, or congestive heart failure occurred.

The adverse events that led to therapy discontinuation were intense injection site reaction (n = 1), delayed hypersensitivity reaction (n = 1), excessive fatigue, nausea, productive cough, pruritus, urticaria, emerged extensive psoriasis and lupus-like syndrome, or SAEs including diagnosis of various tumors (n = 5; Table 2). Other causes for discontinuation were pregnancy in 2 patients, operated inactive stricturing CD in 2, definite inadequate response in 3 (discontinued therapy between week 4 and week 12 from baseline), and self-cessation of treatment while in remission in 1.

Discussion

Adalimumab, a recombinant fully human immunoglobulin G1 monoclonal antibody, was introduced for the treatment of patients with moderate to severe CD who were either naive to infliximab therapy or who previously failed to respond to infliximab therapy.[14–17] The GAIN study showed efficacy in patients who had already been treated with infliximab, although adalimumab seemed less efficacious in these patients than in patients who had never been treated with infliximab.[17] Open-label studies reported a short-term clinical response up to 67% and a discontinuation rate between 25% and 30%. These studies either did not exceed 1 year or comprised small size cohorts. Also, only limited results were presented and not all patients treated were followed up on a long-term basis.[22–25] Although human antibodies like adalimumab are considered less immunogenic, the CLASSIC II study described

2%–4% of antibodies against adalimumab,[16] but their influence on clinical outcome has not yet been studied.

The present study assessed the long-term efficacy and safety of treatment with adalimumab in a large cohort of patients with CD who failed to respond to infliximab therapy and evaluated the influence of adalimumab trough serum concentration and antibodies against adalimumab on clinical response and discontinuation rate. Overall, sustained clinical benefit (defined as having symptom control and ongoing adalimumab maintenance therapy at the end of follow-up) was observed in 61.5% during a median follow-up of 20 months (50% at 120 weeks and 38% at 180 weeks). Escalation to 40 mg every week was needed in 65.4% of patients. Antibodies against adalimumab were detected in 9.2%. Adalimumab trough serum concentration was lower throughout the entire follow-up period in patients who discontinued therapy and was affected by the presence of antibodies against adalimumab. SAEs occurred in 12% of patients.

Sustained clinical benefit was satisfactory not only during 1 year as in the published studies[15,16,22,23] but also during a median follow-up of 20 months, especially taking into account the disease characteristics of our patients. Almost two thirds of the initial responders to adalimumab in our cohort (61.5%) maintained benefit until the end of their follow-up. However, 50% of the patients followed up for 120 weeks or more lost clinical benefit and 80% needed dose escalation. The efficacy of adalimumab therapy was similar for luminal and fistulizing CD and also for the extraintestinal manifestations. A corticosteroid-sparing effect was seen in 51% of our patients. Baseline characteristics such as concomitant immunomodulator therapy, location or duration of the disease, and prior reason for stopping infliximab treatment did not influence outcome. The major limitation of a cohort study including patients treated in clinical practice is the absence of response criteria based on clinical scores such as Crohn's Disease Activity Index. However, patients with ongoing symptoms despite dose escalation were discontinued from further adalimumab therapy, and therefore discontinuation of adalimumab probably most accurately reflects complete loss of response to adalimumab in this study.

Two thirds of the patients entering maintenance therapy escalated to 40 mg every week. The probability of a patient escalating the dose was comparable with what is described in the placebo-controlled trials (27% in CHARM,[15] 46% in CLASSIC II[16]) for the first year of follow-up but increased significantly during the second year, probably because all our patients had previously failed to respond to infliximab. The clinical response to dose escalation was, however, very good (71.6%).

The short-term clinical response rate was higher than that described in placebo-controlled studies (58% in CHARM,[15] 30% in CLASSIC,[14,16] and 38% in GAIN[17]), probably due to differences in definitions used for clinical response (physician's evaluation versus use of the Crohn's Disease Activity Index). In the present study, we investigated specifically the relationship between adalimumab trough serum concentration and treatment outcome and between the formation of antibodies against adalimumab and trough serum concentration. Early discontinuation of therapy correlated with lower adalimumab trough serum concentration. The use of 160/80 mg as an induction scheme was superior in terms of discontinuation rate and time compared with 80/40 mg, and this correlated with higher trough serum concentration. In 25.6% of our patients entering the maintenance phase, clinical response had been unsatisfactory by week 4, but these patients did respond by week 12. In this cohort study, we did not intend to analyze optimal adalimumab induction regimens in patients previously failing to respond to infliximab, but our data do indicate that early dose escalation to 40 mg every week in patients with absence of response at week 4 is a strategy that recruits a significant number of late responders.

Long-term treatment discontinuation of adalimumab was related to lower trough serum concentration, although there was no direct relationship between short-term efficacy and trough serum concentration. We observed that in patients who had a trough serum concentration <0.33 μg/mL at least once, sustained clinical benefit was significantly decreased; however, given the limited size of this specific population (16 patients), these data should be interpreted with caution. In this study, the rate of antibodies against adalimumab positivity was higher than what has been described in previous adalimumab studies.[16] These discrepancies probably reflect differences in the study populations, the assays used for the measurements, and of course the different dose regimens.

The patients who developed antibodies against adalimumab in our cohort had more frequently low trough serum concentration. Interestingly, in 92% of the patients with a trough serum concentration measured below the threshold for detection of antibodies against adalimumab (0.094 μg/mL) at least once, antibodies against adalimumab were detected, showing that antibodies against adalimumab led to more rapid elimination of adalimumab (low trough serum concentration) and to treatment discontinuation. In the majority of patients who lost clinical benefit, adalimumab trough serum concentration was still above the limit of detection of our assay at the time of the last assessment. Therefore, these patients were indeterminate for antibodies against adalimumab and we were unable to ascertain the association between antibodies against adalimumab and loss of clinical benefit. In our study, concomitant immunomodulator therapy did not influence treatment outcome.

The proportion of patients who had to discontinue treatment because of injection site reactions was very low,

and this is most likely due to the "human" nature of adalimumab and the administration route. The presence of concomitant immunomodulators but also of antibodies against adalimumab did not influence these events. Although injection site reactions occurred in one fifth of our patients, only 2 patients discontinued adalimumab treatment. In previous studies reporting long-term follow-up in patients treated at a single center with infliximab, the rate of SAEs ranged from 8.6% to 18.9%, depending on the definitions used for SAEs.[26–28] The rate reported here falls within this range, although all patients had already been exposed for a long time to infliximab.

In conclusion, this long-term observational study showed that up to 70% of patients with CD who present with secondary failure to infliximab demonstrate a response to adalimumab, with 61.5% maintaining clinical benefit during a median follow-up of almost 2 years. Low trough serum concentration of adalimumab is associated with increased early and late discontinuation rates, although there is no direct relationship between trough serum concentration and short-term efficacy of treatment. The added value of tailoring adalimumab maintenance therapy in individual patients based on adalimumab trough serum concentration should be studied in a prospective controlled trial. The great majority of patients with undetectable trough serum concentration also display antibodies against adalimumab. These antibodies were detected in 9.2% of our patients. Concomitant immunomodulator therapy at baseline did not affect treatment outcome, did not influence adalimumab trough serum concentration, did not decrease the development of antibodies against adalimumab, and had no negative impact on SAEs. Hence, we believe that adalimumab can be used as monotherapy, which would increase the safety of this treatment.

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Conflicts of interest

The authors disclose the following: Dr Paintaud is on an advisory committee for Roche, has a research contract with Innate Pharma, is a consultant for LFB, and receives a speaker fee from Pierre Fabre; Dr Van Assche receives a speaker fee/research support from Centocor, Schering-Plough, Abbott, and UCB; Dr Vermeire receives grants/research support from UCB; is a consultant for AstraZeneca, Ferring, and Pfizer; is on the speakers bureau for Schering-Plough, Abbott, Ferring, and UCB; and is on an advisory committee for Shire and Ferring; and Dr Rutgeerts receives research grants, lecture fees, and consultant fees from Abbott, Centocor, Schering-Plough, and UCB. The remaining authors disclose no conflicts.

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