Trough Infliximab Concentrations Predict Efficacy and Sustained Control of Disease Activity in Rheumatoid Arthritis

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Abstract: Infliximab is a chimeric monoclonal antibody that binds to human tumor necrosis factor alpha and is approved for refractory rheumatoid arthritis. We studied the association between infliximab concentration and long-term control of disease activity in patients with rheumatoid arthritis treated on a routine basis both in cross-sectional analysis and over the long term. Trough serum infliximab concentrations were measured in patients with rheumatoid arthritis receiving infliximab infusions during the period August to October 2006. Disease activity was assessed by the Disease Activity Score for 28 Joints (DAS28) and usual biologic markers. During a 42-week follow-up period, patients were classified into two groups: those continuing with the same or lower doses of infliximab (Group A = treatment success) and those who switched to another biopharmaceutical or required an increase in infliximab dose (Group B = treatment failure). Treatment maintenance for Group A was analyzed by categories of infliximab concentration at baseline and compared by the log rank test. In 28 patients, C-reactive protein and infliximab concentrations were inversely related. Infliximab concentration in patients with low disease activity (DAS28 3.2 or less) was higher than in those with persistent active disease (DAS28 greater than 3.2); median values were 3.26 and 0.16 mg/L, respectively (P < 0.01). Analysis after 42 weeks showed that patients in Group A had higher infliximab concentrations at baseline than those with treatment failure (P < 0.01). In rheumatoid arthritis, infliximab concentration is predictive of sustained efficacy with the same infliximab regimen and should be considered on a routine basis.

Key Words: infliximab, rheumatoid arthritis, pharmacokinetics, therapeutic drug monitoring

INTRODUCTION

Infliximab is a chimeric monoclonal antibody that binds to human tumor necrosis factor alpha and is approved for refractory rheumatoid arthritis (RA) and several other inflammatory diseases. The dose–response relationship varies between individuals, some patients achieving remission and others being nonresponders. The difference in dose regimen observed in patients under routine treatment and the improvement in control of disease activity after dose increase illustrate this variability. Exposure to infliximab has been suggested to account for this individual variability in the dose–response relationship. Indeed, in Crohn’s disease, patients with an infliximab concentration higher than the median value of 12 mg/L 4 weeks after infusion showed a longer response time than patients with a concentration lower than the median. Studies in RA have shown a relationship between clinical response and trough infliximab concentration. However, these studies provided no precise quantitative information and no definite threshold can be used for individual dose adjustment. The primary objective of the present study was to analyze the relationship between infliximab concentration and clinical or biologic markers of disease activity in RA both at the time of concentration monitoring and over the long term. The secondary objective was to define the infliximab target concentration predictive of low disease activity.

MATERIALS AND METHODS

In this cross-sectional study, we examined data for patients with RA fulfilling the American College of Rheumatology criteria for RA and hospitalized to receive a routine infliximab infusion during the period August to October 2006. The following items were recorded: demographic characteristics, presence of rheumatoid factor and anticyclic citrullinated peptides, duration of the disease, concomitant use of disease-modifying antirheumatic drugs, and time and dose of previous infusions of infliximab. Before proceeding with the infusion, patients were asked about any adverse event since the last infusion; they also underwent physical examination and urine analysis to rule out any concomitant infection. On the day of the infusion (baseline), patients underwent measurement of the total number of swollen and tender joints in 28 joints by a trained rheumatologist, pain intensity on a visual analog scale (0–100 mm), and clinical or biologic markers of disease activity.
morning stiffness intensity during the previous 2 days on a visual analog scale, and self-reported disease activity during the previous week on a visual analog scale. During a 42-week follow-up, current dose and infusion intervals were recorded. Patients were considered to have low disease activity with Disease Activity Score on 28 Joints (DAS28) 3.2 or less and persistent active disease with DAS28 greater than 3.2.6 During the study period, patients were repeatedly admitted to receive their routine infusion and assessed for disease activity. At each infusion and at the end of the 42-week follow up, current infliximab treatment was classified by comparing infusion dose and dosing interval with those at the baseline infusion. Dose alteration, or decision to switch to another biopharmaceutical, was based on clinical assessment of disease activity and tolerance at each visit. Treatment was considered stable if both the dose and interval (±4 days) were equivalent at baseline and the end of follow up. Current treatment was first categorized in the following subgroups: a1 = decreased dose or stable dose with a shorter interval than at baseline, a2 = discontinuation of infliximab, and sw = switch to another biopharmaceutical. Patients were then classified by current treatment into Group A if the infliximab dose was stable or decreased (a1, a2) or into Group B in the other cases (a3, b, and sw). All charts were analyzed from baseline to the last visit, and the time when patients converted to Group B was recorded.

Blood samples were taken just before infusion for routine measurement of erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Serum infliximab concentration was measured from these samples by enzyme-linked immunosorbent assay as described previously.7 Briefly, microtiter plates were sensitized with tumor necrosis factor alpha and saturated with phosphate-buffered saline containing 1% bovine serum albumin. Samples diluted 1:100 in phosphate-buffered saline–1% bovine serum albumin were added, and bound infliximab was detected with horseradish peroxidase-conjugated goat anti-human IgG specific for Fc fragment. The limit of detection was 0.014 mg/L. Lower and upper limits of quantitation were 0.04 mg/L and 4.5 mg/L, respectively. The technique has been validated in sera from patients with various inflammatory diseases (Crohn’s disease, ankylosing spondylitis, and RA). Patienst were categorized according to their infliximab concentration at baseline as low or high exposure (ie, below or above the median value, respectively). Serum concentration of antibodies toward infliximab (ATI) was measured by a double-antigen enzyme-linked immunosorbent assay on the basis of capture by infliximab-coated microplates and detection by peroxidase-coupled infliximab. Enzyme-linked immunosorbent assay was standardized by use of a mouse monoclonal antibody against all subclasses of human IgG. The positive threshold of detection was 0.20 mg/L. The technique has been tested in 195 sera from healthy blood donors (37%), patients with autoimmune disease (59.5%), and patients with hyperglibulinemia (3.5%) and did not give false-positive results even in the presence of rheumatoid factor. Because of the interference with circulating infliximab, results were conclusive if infliximab concentration was less than 1.7 mg/L in the sample.

We obtained a graphic display of the association of infliximab concentration with the level of biomarkers. Linear and nonlinear models were tested to characterize this association. Differences in continuous variables between groups were assessed by a Mann-Whitney nonparametric test. The sensitivity and specificity of infliximab concentration in predicting low disease activity were obtained by receiver operator characteristic curve analysis. Kaplan-Meier curves were used to study the persistence of patients in Group A, and the influence of infliximab exposure at baseline was studied by the log rank test. Statistical analyses involved use of WinNonLin (Pharsight Corp., Mountain View, CA), Stata 9 (StataCorp LP, College Station, TX), and “R” software (http://www.R-project.org/). A P value <0.05 was considered statistically significant.

RESULTS

Twenty-eight patients with RA hospitalized for infliximab infusions were seen during the 3-month period of the study. Table 1 presents patient demographic, clinical, and biologic characteristics. Patients were heterogeneous, especially in terms of duration of treatment, infliximab dose and dosing interval, and markers of disease activity. Methotrexate was the only concomitant disease-modifying antirheumatic drug. We found an inverse relationship between infliximab concentration and markers of disease activity (Fig. 1). The relationship between infliximab concentration and CRP concentration was well described by use of the inhibition Emax model:

\[
E = E_{\text{max}} \times \left(1 - \frac{C}{C + EC_{50}}\right),
\]

where E is the marker of activity, C is infliximab concentration, Emax is the value of E corresponding to C = 0, and EC50 is the concentration of infliximab leading to a 50% decrease in E. Estimated values of Emax and EC50 were 25.33 mg/L and 0.89 mg/L, respectively (Fig. 1A). The relationships between infliximab concentration and ESR and between infliximab concentration and DAS28 were less satisfactorily described by the model (Fig. 1B–C). Infliximab concentration was

<table>
<thead>
<tr>
<th>TABLE 1. Characteristics of Patients at Baseline</th>
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<tr>
<td>Sex (female/male)</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Positivity for RF or anti-CCP antibodies</td>
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<tr>
<td>Duration of infliximab treatment (months)</td>
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<td>Dose of infliximab infusion (mg/kg)</td>
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<td>Time elapsed since previous infusion (weeks)</td>
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<td>ESR (mm/hr)</td>
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<tr>
<td>CRP concentration (mg/L)</td>
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<tr>
<td>DAS28 ≤ 3.2</td>
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<tr>
<td>DAS28 &gt; 3.2</td>
</tr>
<tr>
<td>Infliximab concentration (mg/L)</td>
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Clinical and biologic markers were measured just before baseline infliximab infusion. Continuous variables are reported as median (range). RF, rheumatoid factor; anti-CCP, anticyclic citrullinated peptide; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; DAS28, Disease Activity Score on 28 Joints.
significantly higher for patients with low disease activity than for those with persistent active disease, median values being 3.26 mg/L and 0.16 mg/L, respectively ($P < 0.01$).

Kaplan-Meier analysis revealed that patients with high baseline exposure to infliximab had significantly better treatment maintenance ($P < 0.01$) (Fig. 2).

Among the three patients with no detectable serum infliximab concentration at baseline, two were positive for ATI. The first patient had an ATI concentration of 0.51 mg/L, had high scores for markers of disease activity (DAS28 = 7.6; CRP level = 28 mg/L, and ESR = 52 mm/hr), discontinued infliximab, and switched to another biopharmaceutical. The second patient had an ATI concentration of 0.32 mg/L, had low scores for markers of disease activity (DAS28 = 2.9; CRP level = 3.6 mg/L, and ESR = 11 mm/hr), and was still in treatment Group A 46 weeks after baseline.

There were few alterations of methotrexate dose during the study. Among the 14 patients who maintained infliximab during the study period and who received methotrexate at baseline, 11 were kept at the same dose of methotrexate, whereas three reduced their dose. Among the nine patients who discontinued infliximab and who received methotrexate at baseline, eight were kept at the same dose and only one had his dose reduced.

As shown in Figure 3, an infliximab concentration greater than 1.037 mg/L predicted low disease activity with 84% sensitivity, 78% specificity, and an area under the receiver operator characteristic curve 0.83 ($P < 0.01$).

**DISCUSSION**

In this study of daily practice of treatment for RA, we found that disease activity was strongly related to trough serum infliximab concentration. Previous studies of patients with RA have reported an association of rate and magnitude of clinical response and infliximab concentration measured during the first months of treatment. In the present study of patients with RA under treatment for 10 to 64 months on a routine basis, we also observed a significant difference in infliximab concentrations by level of disease activity.

We found that trough serum infliximab concentration greater than 1 mg/L predicted low disease activity. This cutoff is an estimation of the therapeutic target corresponding to low disease activity status. Our results concur with those from the ATTRACT study, which showed that patients with a concentration between 1 and 10 mg/L achieved a better response than patients with a concentration below 1 mg/L. In addition, Mori et al found that most patients with a good or moderate response had a trough infliximab concentrations greater than 1 mg/L. Wolbink et al found that patients with low infliximab concentration (0–1.2 mg/L) had a lower probability of being responders than those with an intermediate/high concentration (1.3–25.8 mg/L). Rahman et al measured infliximab concentrations in a dose-escalation trial in RA 22 weeks after initiation of infliximab and found that patients who did not require dose escalation (ie, responders and those without disease flare) had a trough infliximab concentration greater than 1 mg/L. Thus, differences in control of disease activity depend at least in part on infliximab concentration, and

![FIGURE 1. Observed (●) and model-predicted (line) relationship between trough infliximab concentrations (mg/L) and CRP concentration (A), ESR (B), and DAS28 (C). CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DAS28, Disease Activity Score on 28 Joints.](image-url)
a trough infliximab concentration greater than 1 mg/L may be a suitable target to achieve DAS28 3.2 or less.

All markers of disease activity measured in our patients were inversely related to trough serum infliximab concentration. An inhibition Emax model described the relationship between infliximab concentration and markers of disease activity, but the relationship was stronger for CRP, an indirect marker of tumor necrosis factor alpha production, than for ESR or DAS28. A previous report of RA described a relationship between infliximab concentration at Week 54 and a decrease in CRP concentration from baseline, but the type of regression analysis (linear or nonlinear) was not described. In our study, CRP concentration was clearly related to infliximab concentration, an observation that suggests that measurement of infliximab concentration in RA is useful and is applicable in routine practice to assess the control of inflammation in individual patients.

In our study, patients with high exposure to infliximab at baseline were more likely to continue their treatment at the same or a lower dose during the next 42 weeks. Despite the limited sample size, the difference between individuals according to baseline infliximab concentration reached statistical significance in favor of high exposure. Thus, individual exposure to infliximab may be associated with prolonged clinical control and treatment maintenance.

Recently, Pavelka et al reported a study involving patients with RA treated with infliximab at 3 mg/kg every 8 weeks for 1 year and who failed to achieve remission. These patients were randomized either to a continuation of infliximab at the same dose or to an increase of the dose to 5 mg/kg, both doses given every 8 weeks. These authors found no difference between the two groups at 12 months in terms of disease activity. However, because infliximab concentration was not studied, exposure to infliximab could not be compared between patients in the escalation arm and patients who were kept at the same dose.

Our study has some limitations. First, trough infliximab concentration cannot solely account for the control of disease activity. Concomitant use of disease-modifying antirheumatic drugs could have influenced patient outcomes and biased our results. However, such a bias is unlikely because methotrexate was kept at the same dose in the majority of patients. ATI could have altered the response to infliximab as was reported for nonresponders to treatment in RA and pediatric Crohn’s disease. However, in our study, ATI could be detected in only two patients and had no clear influence on disease activity or maintenance of treatment. Because detection is hampered by the persistence of infliximab in the circulation, the positivity we found in only two patients may be an underestimate. Further studies are needed to analyze the influence of ATI on infliximab pharmacokinetics. A second limitation is the lack of blood sampling during the infusion interval, which did not allow us to estimate accurately individual exposure to infliximab. Disease activity was measured at a single time point to define the patient category but could also fluctuate over time. Third, because of the study design, patients were heterogeneous, particularly in terms of infliximab dose regimen. This finding is in accordance with our guidelines, which allow dose alterations according to disease activity independent of any economic constraints. Also, our patients had received long-term treatment, and none showed intolerance to infliximab, which possibly introduced bias in selection.

We showed that exposure to infliximab influences the clinical status and long-term maintenance of treatment in patients with RA. Monitoring of infliximab concentration may be useful, particularly for patients with persistent active disease. Those with low exposure should benefit from dose escalation or interval shortening, and those with active disease despite high exposure might be considered for treatment discontinuation and switch to another biopharmaceutical. Systematic monitoring may also be useful to avoid overexposure and dose-dependent adverse effects, although such a concentration–effect relationship has not been shown yet.
CONCLUSION

Dose in RA, like in other rheumatic diseases, of infliximab should be tailored using trough infliximab concentration to improve clinical status and maintenance of treatment. The measurement of infliximab concentration in a given patient may help the clinician to decide whether to intensify, reduce the dose, or switch to another biopharmaceutical.

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REFERENCES


