

Individualized Monitoring of Drug Bioavailability and Immunogenicity in Rheumatoid Arthritis Patients Treated With the Tumor Necrosis Factor α Inhibitor Infliximab

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Objective. Infliximab, an anti-tumor necrosis factor α (anti-TNF α) antibody, is effective in the treatment of several immunoinflammatory diseases. However, many patients experience primary or secondary response failure, suggesting that individualization of treatment regimens may be beneficial. This study was undertaken to investigate whether serologic monitoring of infliximab bioavailability and immunogenicity in individual patients would be useful in optimizing treatment regimens to improve efficacy and tolerability.

Methods. To avoid the use of solid-phase assays, two radioimmunoassays were developed: one for measurement of levels of anti-infliximab antibody, and a functional one for measurement of TNF α binding due to infliximab. Sera from 106 randomly selected rheumatoid arthritis patients were tested within 6 months of therapy initiation, and associations between findings of serum assays and

disease activity, infusion reactions, and treatment failure occurring within 18 months were assessed.

Results. Trough serum infliximab levels after the first 2 intravenous infusions of infliximab at 3 mg/kg varied considerably between patients (range 0–22 μ g/ml). At this stage, only 13% of the patients were anti-infliximab antibody positive. With subsequent infusions, the frequency of antibody positivity rose to 30% and 44% (at 3 months and 6 months, respectively), accompanied by diminished trough levels of infliximab. Indeed, low infliximab levels at 1.5 months predicted antibody development and later treatment failure. There were highly significant correlations between high levels of antibodies and later dose increases, side effects, and cessation of therapy. High baseline disease activity, judged by C-reactive protein level and Disease Activity Score, was associated with low levels of infliximab at the early stage of treatment and later development of anti-infliximab antibodies. Cotreatment with methotrexate resulted in slightly reduced antibody levels after 6 months; other disease-modifying antirheumatic drugs and prednisolone had no effect.

Conclusion. Development of anti-infliximab antibodies, heralded by low preinfusion serum infliximab levels, is associated with increased risk of infusion reaction and treatment failure. Early monitoring may help optimize dosing regimens for individual patients, diminish side effects, and prevent prolonged use of inadequate infliximab therapy.

Infliximab, a human–murine chimeric monoclonal IgG antibody against tumor necrosis factor α (TNF α), in combination with methotrexate, is approved for the treatment of moderate-to-severe rheumatoid arthritis (RA) in patients who have shown an inadequate response to one or more disease-modifying antirheu-

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matic drugs (DMARDs). In randomized clinical trials, intravenous infusions of infliximab, 3 mg/kg every 4–8 weeks, induce a positive response at 30 weeks in ~55% of patients, and in many patients the response can be maintained with repeated infusions (1–4). With repeated infusions, however, the formation of neutralizing anti-infliximab antibodies becomes a problem, necessitating increased doses or more frequent drug administration and sometimes necessitating discontinuation of therapy because of secondary response failure and/or infusion-related side effects; this has been observed both in RA patients and in patients with other immunoinflammatory diseases (5–13). Our own clinical experience, for example, has shown that the generally recommended dose of 3 mg/kg at weeks 0, 2, and 6 and every 8 weeks thereafter was inadequate in a large proportion of patients. The calculated mean dose of infliximab per week in our patients at 3-year followup was 35 mg (n = 5), 54 mg (n = 35), 44 mg (n = 26), and 38 mg (n = 17) at 3-year followup years 2002, 2003, 2004, and 2005, respectively.

Furthermore, the recommended dosing regimen was originally established on the basis of findings in clinical trials that included relatively large cohorts of RA patients of both sexes, with differences in age, comorbidities, and concurrent therapies (4). In clinical practice, however, patients with RA or any other chronic inflammatory disease treated with infliximab may differ considerably from the average patient in randomized clinical trials (14). Thus, even though the initial bioavailability of infliximab approaches 100% because of the intravenous administration of the drug, differences in pharmacokinetics in individual patients may result in inadequate drug levels for extended periods of time between infusions. This problem can be exacerbated by the development of antibodies.

To address these problems, we have developed two radioimmunoassays (RIAs): one for measuring functional serum infliximab levels as assessed by TNF α binding, and another for measuring antibody to infliximab. We used these assays to study infliximab bioavailability and the relationship between trough levels of infliximab and antibody development in individual RA patients. In addition, we investigated these variables in terms of their relationship to disease activity and methotrexate therapy, and their ability to predict response failure and infusion-related side effects.

PATIENTS AND METHODS

Patients. RA patients who were starting anti-TNF α therapy were followed up according to the protocol of the

Table 1. Characteristics of the 106 rheumatoid arthritis patients at the time of enrollment in the study*

| | |
|---------------------------------------|-----------------|
| Age, years | 57 \pm 13 |
| Female, % | 70 |
| Disease duration, years | 11 \pm 8.7 |
| No. of previous DMARDs | 3.1 \pm 1.7 |
| DAS28 | 5.4 \pm 1.3 |
| Swollen joint count, 0–28 | 9.5 \pm 6.2 |
| Tender joint count, 0–28 | 8.6 \pm 7.3 |
| Patient global assessment, 0–100 VAS | 61.5 \pm 23.9 |
| RF positive (ever), % | 83 |
| ESR, mm/hour | 35.4 \pm 26.7 |
| CRP, mg/liter | 31.6 \pm 35.6 |
| Treatment, % | |
| Monotherapy | 20 |
| Combination therapy (\geq 1 DMARD) | 80 |
| Methotrexate | 63 |
| Sulfasalazine | 17 |
| Azathioprine | 6.6 |
| Cyclosporine | 5.7 |
| Hydroxychloroquine | 4.7 |
| Other DMARD | 8.4 |
| Prednisolone | 76 |

* Except where indicated otherwise, values are the mean \pm SD. DMARDs = disease-modifying antirheumatic drugs; DAS28 = 28-joint Disease Activity Score; VAS = visual analog scale; RF = rheumatoid factor; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.

South Swedish Arthritis Treatment Group (SSATG) (15). The followup of patients in Sweden who are treated with biologic agents is part of a nationwide study mandated by the Medical Products Agency of the Swedish government. All patients are provided with information orally and in writing and must give their consent prior to enrollment. To allow for at least 6 months of followup, we randomly selected RA patients in whom infliximab therapy was initiated between March 1999 and December 2004, and who had not previously received anti-TNF α treatment. All patients of the Department of Rheumatology, Lund University Hospital who were born in the months of February, March, May, June, August, October, or November were selected from the SSATG register; patients not seen at the Lund University Hospital Department of Rheumatology were not included in the study. Twelve of the 118 randomized patients were excluded because of missing blood samples at the 1.5-month, 3-month, or 6-month followup time points. Demographic and clinical characteristics of the remaining 106 patients at the time of enrollment are shown in Table 1.

Data on previous and concomitant DMARD therapy, infliximab and prednisolone dosage, C-reactive protein (CRP), and 28-joint Disease Activity Score (DAS28) (16) were collected from the SSATG. Further information gathered from the database included the time of infliximab discontinuation (if applicable) and its cause (adverse event, primary failure, secondary failure). An adverse event was classified as the main reason for discontinuation, regardless of previous response to treatment. The protocol allowed for only 1 reason for discontinuation. Secondary failure was recorded when a patient initially responding to therapy was withdrawn because of nonresponse despite dose adjustment. Primary treatment fail-

Table 2. Clinical events during infliximab treatment, by duration of followup

| | Treatment period | | | | Total |
|--|------------------|------------|-------------|------------|-------|
| | 0–3 months | 3–6 months | 6–12 months | >12 months | |
| All events | | | | | |
| Dosage increase | 0 | 28 | 29 | 20 | 77 |
| Infusion reactions | 4 | 5 | 7 | 7 | 23 |
| Events leading to treatment withdrawal | | | | | |
| All adverse events | 5 | 7 | 17 | 13 | 42 |
| Infusion reactions | 2 | 4 | 5 | 4 | 15 |
| Treatment failure | 2 | 5 | 5 | 13 | 25 |
| Primary failure | 2 | 5 | 5 | 5 | 17 |
| Secondary failure | 0 | 0 | 0 | 7 | 7 |
| Miscellaneous* | 0 | 0 | 1 | 3 | 4 |

* One patient had a lengthy interruption of treatment while living abroad, 1 patient voluntarily discontinued treatment, and treatment was discontinued in 2 patients because of their desire to become pregnant.

ure was recorded if a patient never responded to therapy and did not experience an adverse event. This classification was made prospectively by the treating physician and prior to analysis of patient sera. To facilitate this, the SSATG protocol includes feedback in which several clinical, laboratory, and drug variables, as well as information on fulfillment of established response criteria, are displayed graphically. Formal levels of responses are not required in the SSATG protocol or in the recommendations from the Swedish government. This is important since such recommendations might lead to reporting bias. Data on infusion reactions occurring within 24 hours of infusion were also collected. These were further classified as type I (anaphylactic/hypotension, bronchospasm, wheezing, and/or urticaria), or other (nonspecific rash, arthralgia, headache, fatigue, myalgia, influenza-like symptoms, etc.). To identify possible late infusion reactions and dose changes not recorded in the database, the medical records of patients still receiving infliximab were further reviewed in December 2005.

Serum testing. All sera were tested at BioMonitor ApS, under blinded conditions.

Infliximab levels (TNF α binding to serum IgG). Binding of TNF α to IgG was measured using 1% patient serum added to 5,000 counts per minute/100 μ l of 125 I-TNF α (Perkin Elmer, Boston, MA). After incubation, free and IgG-bound tracer were separated by addition of a rabbit anti-human Fc γ antibody (Dako, Glostrup, Denmark) and centrifugation. The pellet activity was measured using a 1470 automatic gamma counter (Wallac, Allerød, Denmark). Infliximab (Schering-Plough, Farum, Denmark) was used as reference, and TNF α binding capacity was expressed as infliximab equivalents. The detection limit of the assay was 0.4 μ g infliximab/ml of whole serum; inter- and intraassay variations were <20% and <10%, respectively.

Anti-infliximab antibodies. We took advantage of the fact that infliximab is an IgG construct consisting only of kappa light chains, and hence, that anti-human lambda light chain antibody can be used to distinguish between free infliximab and infliximab in complex with any class of lambda-containing human immunoglobulin. The assay was carried out using 1% serum (unless otherwise indicated) added to 4,000 cpm/100 μ l

of 125 I-infliximab (specific activity $1\text{--}2 \times 10^5$ cpm/ng). After incubation, free and immunoglobulin-bound (any isotype) tracer were separated by affinity chromatography using small columns of matrix-bound anti-human immunoglobulin lambda chain antibody (Dako). The data were expressed as the percent bound cpm/total cpm added; the background was 3%, and inter- and intraassay variations were <20% and <10%, respectively.

RESULTS

Clinical characteristics of the study patients.

There were no significant differences in age or sex distribution, disease duration, rheumatoid factor (RF) status, or previous or current DMARD treatment in the patients randomly selected for this study compared with the total group of 201 patients at the Lund University Hospital Department of Rheumatology who were beginning infliximab treatment and had not previously received a biologic agent. In all cases infliximab was started at the recommended dose of 3 mg/kg, but this had to be increased after 3–12 months because of insufficient efficacy in 57 patients. Of the 34 patients still receiving infliximab in December 2005, only 11 were still receiving the same dose established within the first 3 months, while increased doses and/or shortened intervals between infusions were instituted in 19 patients after the first year of treatment and in 4 patients at 3–12 months. A total of 23 infusion reactions occurred in 22 patients, and infusion reaction was the reason for discontinuation of infliximab treatment in 15 patients (Table 2).

Development of anti-infliximab antibodies and association with trough serum infliximab levels. None of the patient sera tested positive for TNF α binding

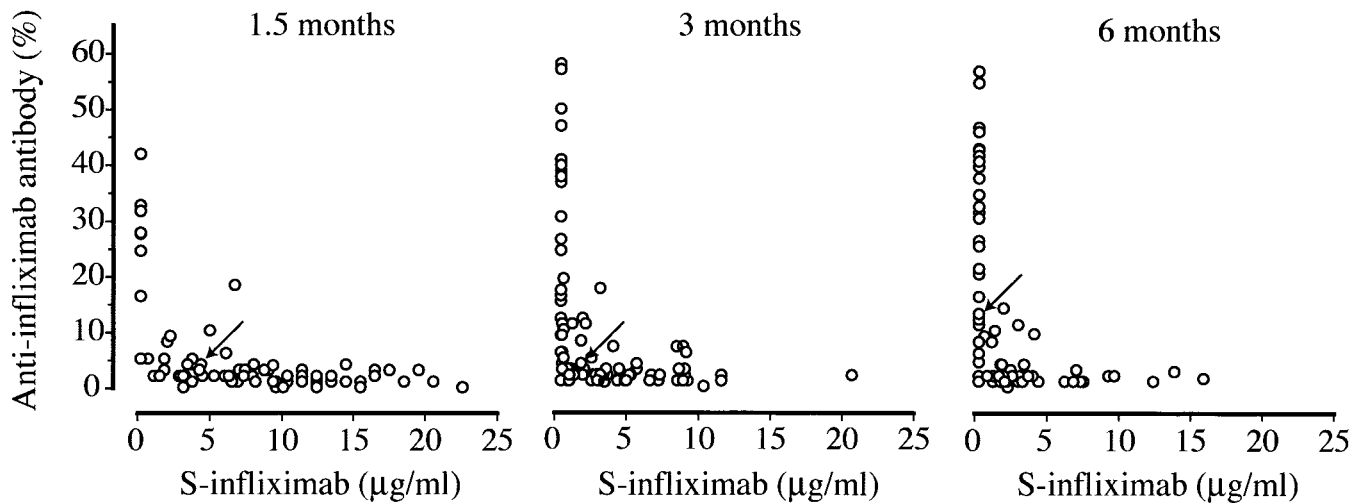


Figure 1. Development of anti-infliximab antibodies and association with trough levels of tumor necrosis factor α (TNF α) binding afforded by infliximab. Levels of infliximab and of anti-infliximab antibody (% bound cpm) were measured in patient sera beginning immediately before the third drug infusion (at 1.5 months) and before the infusions at the 3-month and 6-month time points. TNF α binding was quantified according to the level of infliximab in a serum pool from healthy individuals providing the same TNF α binding as the patient serum tested (S-infliximab). Each circle represents an individual patient; arrows show responses in the patient who tested positive for anti-infliximab antibody before the initiation of therapy.

before the initiation of infliximab therapy. Whereas only 1 patient tested positive (>3 times background binding level) for anti-infliximab antibodies before treatment, 11 of 85 (13%) were positive before the third infusion of infliximab (1.5 months), 28 of 93 (30%) were positive at 3 months, and 33 of 75 (44%) were positive at 6 months (Figure 1). Furthermore, the increased incidence and levels of antibodies over time were accompanied by decreased trough levels of infliximab. Indeed, functional bioavailability of the drug in sera collected immediately before infusions varied considerably between patients, particularly at 1.5 months, when the number of antibody-positive sera was still low (Figure 1).

The known interference by infliximab in assays for anti-infliximab antibodies (7,12) was investigated in separate competition experiments using 12 antibody-positive sera (Svenson M, et al: unpublished observations). While infliximab levels as low as 2 $\mu\text{g/ml}$ reduced the binding of ^{125}I -infliximab by a median of 40% (range 0–76%) in the 12 sera, >200 $\mu\text{g/ml}$ of the drug was needed in order to completely mask the presence of antibodies in our RIA (80–100% reduction).

Interference by RF was tested by cross-titrations with 5 known RF-positive sera without anti-infliximab antibodies. None of these influenced the RIA measurements of anti-infliximab antibodies.

Neutralization of TNF α binding afforded by anti-infliximab antibodies was further quantitated as shown in Figure 2. Infliximab was added in various amounts to

sera from 5 patients who had been treated with infliximab for 6 months, and the resulting ^{125}I -TNF α binding capacity was tested. The 2 antibody-positive sera required 20–80 times more infliximab to achieve the same degree of TNF α binding as a pool of sera from 30

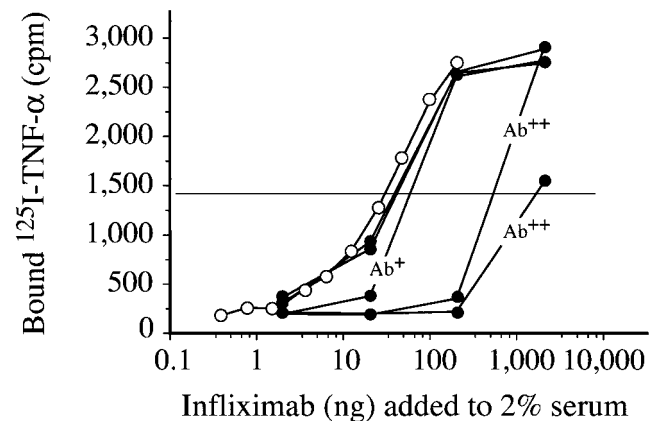


Figure 2. Dose-response curves of tumor necrosis factor α (TNF α) binding capacity in sera to which various amounts of infliximab were added. Sera from 5 patients who had been treated with infliximab for 6 months were assessed (closed circles): 2 had high antibody activity (Ab $^{++}$), 1 had low antibody activity (Ab $^{+}$), and 2 had no detectable antibody activity. The sera were compared with a serum pool from 30 untreated healthy individuals (open circles). The amounts of added infliximab corresponded to the levels observed in patient sera (Figure 1). Horizontal line shows 50% of maximum TNF α binding afforded by added infliximab.

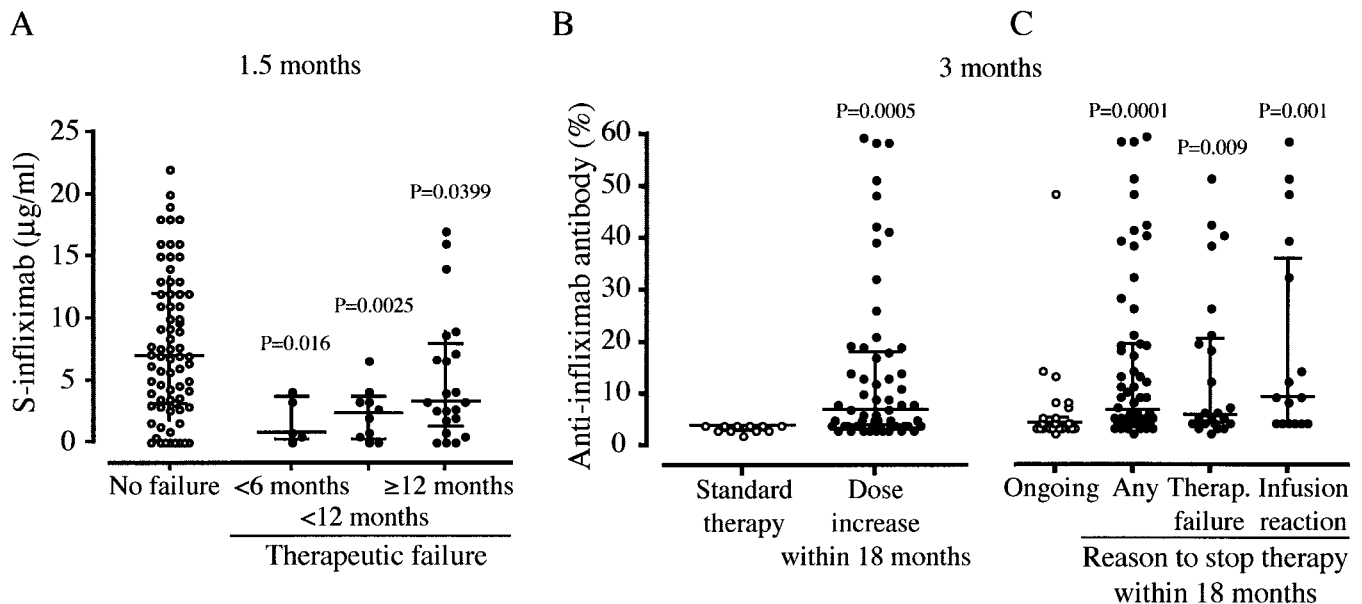


Figure 3. Relationships of infliximab and anti-infliximab antibody levels to clinical outcome. **A**, Trough tumor necrosis factor α binding capacity due to infliximab (S-infliximab; see Figure 1) in sera obtained immediately before the third drug infusion (at 1.5 months). *P* values were determined by Mann-Whitney rank sum test and are versus patients who did not experience treatment failure with infliximab at the standard dose throughout the observation period. **B** and **C**, Anti-infliximab antibody levels (% bound cpm) in sera obtained before the fourth infliximab infusion (at 3 months). *P* values were determined by Mann-Whitney rank sum test and are versus patients who continued to receive infliximab at the standard dose (**B**) and versus patients who did not discontinue infliximab therapy within the first 18 months (**C**). Each circle represents an individual patient; bars show the median and interquartile range.

untreated healthy blood donors. The data also showed that serum TNF α binding attributable to factors other than infliximab, e.g., soluble TNF receptors, was negligible in both the normal and the patient sera.

To further characterize the antibodies, we used RIAs to test 12 antibody-positive sera, obtained after 3 months of infliximab therapy, for cross-binding to 2 other anti-TNF α immunoglobulin constructs, etanercept and adalimumab. The binding of these drugs was <0.001 times that of infliximab.

Associations with clinical outcome. Low infliximab bioavailability predicts treatment failure. Bioavailability of infliximab before the third infusion varied considerably from patient to patient, and a substantial number of patients with low serum levels of infliximab at this early time point (1.5 months) experienced response failure within the observation period (Figure 3A).

High antibody levels predict dose increase and/or discontinuation of therapy. Detection of anti-infliximab antibodies at an early time point (3 months) was associated with later dose increases necessitated by inadequate clinical response (Figure 3B). In fact, antibodies had not been detectable at 3 months in any of the patients who exhibited good response to standard therapy.

Early formation of anti-infliximab antibodies was also associated with subsequent discontinuation of therapy (Figure 3C). This was the case both in general (discontinuation for any reason) and when discontinuation was due to treatment failure or infusion reactions. The same trend was seen when sera were investigated for the presence of antibodies after 6 months of therapy, whereas after 1.5 months too few patients were antibody positive for meaningful comparison.

Initial factors influencing antibody development.

Patients with low trough levels of infliximab after 2 infusions were most prone to subsequent development of anti-infliximab antibodies, whereas patients with high levels were not (Figure 4A). This tendency showed a highly significant linear correlation.

The effect of the degree of inflammation and disease activity at treatment initiation is shown in Figure 4B. Patients with pronounced pretreatment inflammation as judged by plasma CRP levels in the upper quartile exhibited lower trough levels of functional infliximab compared with patients with less inflammation. This tendency disappeared when infliximab levels were tested after 3 months of therapy.

Patients with pronounced disease activity as

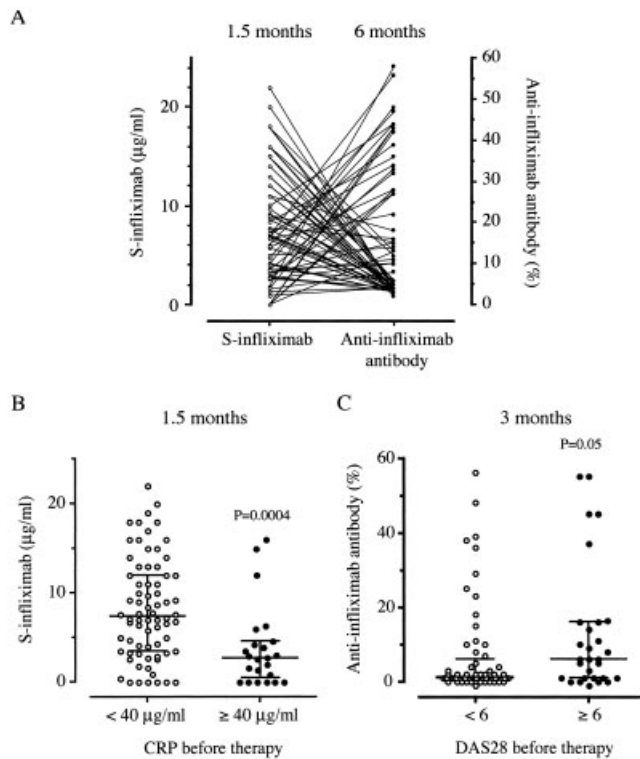


Figure 4. Initial factors influencing antibody development. **A**, Trough tumor necrosis factor α (TNF α) binding capacity due to infliximab (S-infliximab; see Figure 1) in sera obtained immediately before the third infliximab infusion (at 1.5 months), and levels of anti-infliximab antibody in the same sera after 6 months of treatment. A reverse linear correlation was observed ($r = -0.40$, $P = 0.0007$). **B**, Trough TNF α binding capacity due to infliximab in sera obtained immediately before the third infliximab infusion, in patients grouped according to the level of C-reactive protein (CRP) (upper quartile ≥ 40 $\mu\text{g/ml}$) before the initiation of treatment. **C**, Anti-infliximab antibody levels (% bound cpm) in sera obtained before the fourth infliximab infusion (at 3 months) in patients grouped according to the 28-joint Disease Activity Score (DAS28) (upper quartile ≥ 6) before the initiation of treatment. P values were determined by Mann-Whitney rank sum test. Each circle represents an individual patient; bars show the median and interquartile range.

judged by pretreatment DAS28 scores in the upper quartile exhibited higher levels of anti-infliximab antibodies compared with patients with lower disease activity (Figure 4C). A similar tendency was seen when antibodies were tested for at 6 months, although this was not statistically significant.

Among anti-infliximab antibody-positive patients, the percentage who were treated concomitantly with methotrexate was not significantly different from the percentage who were not. At 6 months, 50 patients were receiving concomitant methotrexate, of whom 20 (40%) were antibody positive, and 26 were not receiving

concomitant methotrexate, of whom 13 (50%) were antibody positive. However, among antibody-positive patients, those who were receiving methotrexate had slightly lower antibody levels than those who were not (median percent bound cpm 11% [interquartile range 4–43%] versus 5% [interquartile range 3–20%]; $P = 0.037$). Treatment with other DMARDs (Table 1) or prednisolone did not significantly affect antibody levels.

DISCUSSION

Anti-TNF biologic agents, including infliximab, are increasingly being used to control disease activity in patients with RA and other chronic immunoinflammatory diseases (5–13). The clinical use of these drugs has to a large extent been based on the findings of pivotal clinical trials, in which efficacy, safety, and tolerability with different dosing regimens have been monitored. In clinical practice, however, patients may differ considerably from the average patient enrolled in a randomized clinical trial (14). Another problem with prolonged use of biologic agents, including anti-TNF α agents, is the induction of antibodies against the therapeutic proteins. Although this has been an area of increasing interest, it remains unclear whether anti-TNF antibodies measured with the most commonly used techniques (mostly solid-phase assays such as enzyme-linked immunosorbent assay [ELISA]) are “functional” in that they interfere with the bioactivity of anti-TNF α agents and/or have some association with clinical manifestations such as infusion reactions and reduction of long-term efficacy (12). Monitoring bioavailability and neutralizing antibodies during infliximab therapy may therefore help to optimize dose regimens for individual patients, diminish the risk of serious side effects, and prevent continued and probably futile use of infliximab in patients in whom neutralizing antibodies are present.

We therefore developed a functional RIA for testing infliximab bioavailability in the form of TNF α binding to serum IgG (infliximab) and a fluid-phase RIA for measurement of anti-infliximab antibody levels, and used them to monitor RA patient sera obtained before the initiation of therapy and immediately before readministration of the drug at 1.5, 3, and 6 months after the initiation of therapy (trough levels). The antibody test was based on the fact that infliximab is an antibody constructed solely of kappa light chains and that any radioactive complex extracted by anti-human lambda light chain antibodies would be infliximab bound to antibody (irrespective of heavy chain isotype). We deliberately avoided the use of solid-phase assays because

of the risk of false-positive results due to binding of RF and heterophilic antibodies in sandwich ELISAs, or nonspecific binding to immunoglobulins other than infliximab (17,18).

Monitoring of the RA patients in our study revealed pronounced interindividual differences in trough TNF α binding capacities. This was seen even though all patients were initially treated with the same dose of infliximab, 3 mg/kg, indicating that this generally recommended dose may not be optimal in all patients, as has been suggested before (19).

The reason for the marked differences is obscure. Variable content of natural or disease-associated TNF α binding factors in sera, e.g., soluble TNF receptors, is an unlikely explanation, because prior to addition of infliximab *in vitro*, there was no measurable binding of TNF α in sera from healthy individuals (Figure 2) or from any of the patients before the start of therapy; endogenous binding of TNF α was therefore negligible compared with that of infliximab. However, since infliximab is administered intravenously, with full initial blood bioavailability, a central factor might be differences in consumption of the drug. Thus, the total TNF α "load" in the body (not only serum levels), and therefore the *in vivo* binding of drug, may vary considerably in patients with different levels of disease activity prior to treatment. This is supported by the finding that patients with high initial disease activity, judged both clinically (DAS28) and biochemically (plasma CRP level), were the ones with the lowest trough levels of bioactive infliximab. Similar findings have been reported recently by others (20). Interestingly, these patients were also the most prone to develop antibodies at later time points and, eventually, treatment failure.

The problem of development of antibodies to anti-TNF biologic agents has been investigated in previous studies (5,9,10,21), several of which have shown no clear relationship between the presence of antibodies and treatment failure. In the current study, however, the development of antibodies, heralded by low preinfusion serum infliximab levels, was clearly associated with an increased risk of infusion reaction and subsequent treatment failure. There are several possible explanations for this discrepancy, including the frequent use of ELISAs for antibody measurement in earlier studies; this may have generated false-positive results because nonspecific (low-affinity) immunoglobulins and anti-IgG RF may bind to adsorbed infliximab (17,18). We tried to overcome this problem by using RIAs, which are not influenced by artifacts induced by solid-phase adsorption of proteins and therefore better reflect the *in vivo* situa-

tion, and are not affected by RF positivity. Indeed, our data are consistent with a recent finding of antibodies to the F(ab')₂ fragment of infliximab detected by RIA in 22 of 51 RA patients (43%) (21); in that study, patients without anti-F(ab')₂ antibodies were also more often classified as responders compared with those in whom the antibodies were present (69% versus 36%).

Another issue is the confounding presence of infliximab when testing for anti-infliximab antibodies (12). This is particularly problematic if sera are collected shortly after administration of the drug and if tests that are particularly sensitive to the presence of infliximab are being used. We tried to minimize this by evaluating sera obtained immediately before infusions of infliximab. Even so, the levels of anti-infliximab antibodies may have been underestimated since competition experiments showed that infliximab at concentrations of as little as 2 μ g/ml affected the detection of antibody. Importantly, however, the frequency of false-negative results in the present study is likely to be negligible since >10 times the peak trough level of the drug was needed in order to mask the presence of antibodies in our RIA (Svenson M, et al: unpublished observations). Undercounting of antibody positivity might also occur if anti-infliximab immune complexes consisted solely of kappa light chain antibodies. Although this may occur, it is probably a rare event because size-chromatography of 10 sera with low TNF α binding levels and no detectable anti-infliximab antibodies after 6 months of infliximab treatment did not reveal "hidden" antibody complexes (Svenson M, et al: unpublished observations). Nevertheless, formation of infliximab-containing immune complexes would be expected to radically increase drug clearance in antibody-positive patients.

We did not find that antibodies induced by infliximab therapy reacted significantly with the two other currently approved anti-TNF α immunoglobulin constructs, etanercept and adalimumab. Although this does not rule out the possibility that cross-reacting antibodies may appear after long-term infliximab therapy, it is consistent with previous observations that both etanercept and adalimumab are effective in at least some patients in whom infliximab treatment has been unsuccessful (22,23).

The influence of concomitant therapies on antibody development was marginal, in contrast to findings in some previous studies (12). Thus, we did not find that treatment with prednisolone or DMARDs, apart from methotrexate, had any major effect on incidence or levels of antibody measured after up to 6 months' exposure to the drug. The effect of methotrexate, although not very pronounced in our study, is in accor-

dance with that observed in other studies (1,2,6,24,25). This is usually attributed to the drug's immunosuppressive effect. In view of the lack of effect of prednisolone and the importance of disease activity for antibody development, we would suggest that methotrexate's disease-modifying effect may be important as well. This would be consistent with recent findings that this agent significantly reduces antibody responses to polysaccharides but not to polypeptide antigens (ref. 26 and Kapetanovic MC, et al: unpublished observations).

In conclusion, the findings of the present study suggest that early monitoring of individual RA patients may help to optimize infliximab treatment. In particular, patients with low trough levels of infliximab may benefit from early institution of dose increases or shorter intervals between drug administration, and early development of anti-infliximab antibodies should raise doubts regarding the efficacy, safety, and economy of continued treatment.

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