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Original article

Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis

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Abstract

Objective. To analyse the clinical relevance of the production of anti-infliximab antibodies (anti-infliximab Abs) in patients with RA undergoing infliximab treatment over a prolonged period of time.

Methods. Clinical characteristics, serum trough infliximab and antibody levels were evaluated in 85 RA patients treated with infliximab for a median of 4.42 (interval 0.4–10.2) years. DAS in 28 joints (DAS-28), EULAR response criteria and survival of treatment were assessed at 3 time points (6 months, 12 months and >4 years).

Results. Antibodies against infliximab were detected in 28 (32.9%) patients and were present in all EULAR non-responder patients. Antibody levels were higher in EULAR non-responders throughout the study period ($P = 0.05$ at 6 months, $P = 0.02$ at 1 year, $P = 0.003$ at >4 years) compared with EULAR (good and moderate) responders. Nine (10.5%) patients, all of them with high-serum anti-infliximab Ab levels, developed infusion-related reactions. Patients with anti-infliximab Abs more often required increased infliximab doses (51.7%) ($P = 0.032$) and median survival time on treatment was shorter (4.15 *vs* 8.89 years) ($P = 0.0006$). MTX co-therapy was not associated with lower proportion of anti-infliximab Ab-positive patients, but those receiving both infliximab and MTX had lower levels of anti-infliximab Abs ($P = 0.073$) and longer survival ($P = 0.015$) on treatment.

Conclusion. The formation of anti-infliximab Abs during treatment with infliximab is associated with a loss of clinical response, the appearance of infusion reactions and discontinuation of treatment.

Key words: Rheumatoid arthritis, Infliximab therapy, Immunogenicity, Efficacy, Long-term treatment.

CLINICAL SCIENCE

Introduction

Since the approval of the first therapeutic mAb against TNF 15 years ago, the use of biological drugs in clinical practice has grown constantly [1]. The treatment of RA, Crohn's disease, psoriasis and other inflammatory diseases, which are usually refractory to conventional treatments, has improved considerably since combination regimens of these new biological drugs and the classical DMARDS were introduced [2].

Infliximab is a chimeric (mouse–human) mAb antagonist to TNF, and was the first antibody-based therapy to be introduced to treat patients with RA. Today its use has become more generalized and it is being administered to a growing number of patients at an early stage of disease, mainly because of its clinical efficacy and retarding effects on joint destruction [2]. Although the efficacy of this drug as a treatment for patients with active RA has been widely demonstrated [3, 4], some RA patients initially respond to treatment but subsequently their responsiveness decreases [1]. One of the alleged reasons for this phenomenon is immunogenicity associated with the drug itself. Infliximab can induce the formation of neutralizing

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antibodies [5], resulting in loss of efficacy and appearance of side effects such as infusion-related reactions [6, 7]. The induction of antibodies against the drug has been described in about half of the patients receiving repeated infliximab monotherapy; as a consequence, immune suppression by concomitant administration of MTX is recommended [4, 8, 9].

An antibody response to the drug often appears between the third and sixth month [10]. As long as the relative amount of the anti-drug antibodies is lower than the serum trough level of infliximab, the drug can provide a clinical benefit [11]. However, when the endogenous production of antibodies exceeds the amount of drug in the serum, the latter is cleared from the circulation [11, 12], the therapy is rendered ineffective and free antibodies to the drug can be measured in the patient's serum. The accelerated clearance of infliximab complexed to antibodies may result in decreased pharmacological availability [13, 14] and ultimately the loss of therapeutic effectiveness of infliximab [15]. Therefore, it is very likely that the equilibrium between infliximab and antibody response will regulate the overall effectiveness of the drug [11].

Most publications on the relationship between the presence of anti-infliximab antibodies (anti-infliximab Abs) and clinical response in RA patients focus on the first period of administration, with a maximum of 3 years follow-up [13]. Mulleman *et al*. [14] studied patients for >6 years, but only monitored the infliximab concentration. However, Wolbink and co-workers [11, 15] reported that development of the immune response against infliximab is a gradual process that may change over time because continuation of treatment may either induce immune tolerance or stimulate further antibody formation.

In this study, we present data on 85 RA patients undergoing infliximab treatment at the Rheumatology Unit of La Paz University Hospital since the end of 1999 (>10 years). Infliximab and anti-infliximab Ab levels were measured in order to assess the clinical relevance of infliximab immunogenicity throughout the course of the therapy.

Methods

Patients and sera

A total of 85 consecutive patients with RA, without previous biological treatment were included. Patients were enrolled at the Department of Rheumatology of La Paz University Hospital to receive infliximab therapy. This was a retrospective observational study, approved by the Hospital La Paz Ethics Committee and patients signed an informed consent form according to the Declaration of Helsinki. Serum samples (a total of 1451) were collected at the time of infusion, stored frozen and only thawed for the purpose of this study. The retrospective study period covers the years 1999 until 2010. All patients fulfilled the ACR 1987 revised criteria for RA and all of them had evidence of active disease, as indicated by a 28-joint DAS (DAS-28) at inclusion of 5.49 (1.2) [mean(S.D.)]. At first all patients were given i.v. infusions of 3 mg/kg infliximab at 0, 2, 6 and every 8 weeks thereafter. After 14 weeks of treatment, the rheumatologist was allowed to increase the infliximab dosage to 5 mg/kg depending on the observed clinical response. Every 6 months, disease activity using the DAS-28 and European League Against Rheumatism (EULAR) response criteria [16] was measured to assess clinical response. Six months, 1 year and >4 years [mean (S.D.) 5.9 (2) years] were chosen from the study as representative time points for patients' clinical response. Infusion reactions were defined as any event appearing during infusion requiring either arrest of drug infusion or the administration of parenteral medication.

Blood samples were collected at baseline and just before each infusion at 2, 6 and every 8 weeks thereafter, so that a maximum of 6–8 samples per year were obtained from each patient. Precise timing is required to compare results, because with a longer time interval serum infliximab may become undetectable due to normal drug pharmacokinetics, and not as a consequence of IC formation with anti-infliximab Ab. Sera were stored at −80°C until infliximab and anti-infliximab Abs were measured. At baseline, infliximab and anti-infliximab Ab concentrations in all patients were <10 ng/ml and 50 AU/ml, respectively.

Serum infliximab assay

Serum infliximab levels were determined by a sandwich ELISA, as described by Wolbink *et al*. [17] using a polyclonal anti-infliximab Ab [18]. Briefly, microtitre plates were coated with 2 μg/ml mouse monoclonal anti-TNF antibody (CLB/7) (Sanquin, Amsterdam, The Netherlands) and then incubated with 0.01 μg/ml recombinant human TNF-α (Peprotech, Rocky Hill, USA). Serial dilutions of serum samples and standard curve (0.1–50 ng/ml infliximab) were made in high performance ELISA (HPE) buffer (Sanquin). Bound infliximab was detected with biotinylated affinity purified rabbit immunoglobulin G (IgG) to Fab regions of infliximab, and the reaction was developed with streptavidin–polyperoxidase (polyHRP) (Sanquin). The detection limit of the assay was 1 ng/ml infliximab. Cut-off values were established with sera from 150 healthy blood donors and 100 RA patients who had never received infliximab (of whom 70% were RF positive) to exclude any background signal that might have been caused by RF or other auto-antibodies present in RA patient sera. Serum infliximab levels >10 ng/ml (mean + 6 S.D. control group) were considered positive.

Anti-infliximab Ab assay

Anti-infliximab Abs were detected by a two-site (bridging) ELISA, which takes advantage of the monovalency of the two arms of IgG subclasses 1, 2 and 3, to crosslink the infliximab coated on plates to biotinylated infliximab [11, 19]. Polystyrene plates (Nunc A/S, Roskilde, Denmark) were coated with infliximab (0.5 μg/ml) overnight. The following day, serial dilutions of samples (starting at 1/10) and a standard curve (0.48–250 AU/ml) diluted in HPE were incubated for 1 h with shaking. A standard curve was constructed using a patient serum that showed a high titre of anti-infliximab Ab (mainly IgG1) previously titrated in arbitrary units per millilitre by one of the authors

of this study (L.A., data not shown). After washing, 10 ng/ml infliximab biotinylated by standard procedures (Pierce, Rockford, IL, USA), was added. Bound labelled infliximab was detected by incubation with polyHRP (1 : 10 000) in PBS. The reaction was developed with tetramethylbenzidine (TMB)/H_2O_2 in 0.11 M acetic acid buffer pH = 5.5 and stopped with 2 M H_2SO_4. Washing steps were made in 0.01 M PBS 0.02% Tween 20. The assay detection limit was 2 AU/ml and the cut-off for the presence of anti-infliximab Ab in patient sera was established at 50 AU/ml (mean + 6 S.D.) with the same control group used for the measurement of free infliximab. A linear dose-response curve for inhibition was obtained when positive samples for anti-infliximab Abs were pre-incubated with infliximab.

Other autoantibodies

Antibodies to CCP (aCCP) were measured by ELISA (Eurodiagnostica, Malmö, Sweden), and RF was measured by nephelometry (Siemens, Marburg, Germany) with cut-off values of 25 and 9 UI/ml, respectively.

Statistical analysis

Descriptive statistics were provided using the mean, S.D., median (Mdn) and interquartile range (IQR). Statistical analysis was performed using the Statistical Package for the Social Sciences version 10.0 (SPSS, Chicago, IL, USA). Frequency data were compared by the Pearson's chi-square and Fisher's exact tests. Differences in quantitative values between groups were analysed using Mann–Whitney U and Wilcoxon non-parametric tests. Time course data were analysed using the Kaplan–Meier method. Statistical significance was calculated using the log-rank test and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

A total of 85 RA patients were enrolled in the study, of whom 69 were women, with a mean (S.D.) age of 53.8 (14.2) years at the beginning of infliximab treatment. Demographic and clinical characteristics are shown in Table 1. All patients received 3 mg/kg infliximab at baseline; however, 44 (51.8%) patients needed a gradual infliximab dose escalation by either increasing the dose to 5 mg/kg and/or shortening the interval between infusions, due to an inadequate response.

Clinical response and association with levels of infliximab and anti-infliximab Ab

At baseline, all patients had active disease as indicated by a mean (S.D.) DAS-28 of 5.49 (1.26) with no differences in DAS-28 values between patients that subsequently did [5.75 (1.28)], or did not [5.37 (1.25)] develop anti-infliximab Ab ($P = 0.204$). Anti-infliximab Abs were detected in serum samples from 28 (32.9%) patients, in all cases with undetectable serum trough infliximab levels. These antibodies appeared most frequently after the fourth infusion [Mdn 16 (range 14–79) weeks]; although in four patients

Table 1 Demographic and clinical characteristics of 85 RA patients

Variable	value
At study inclusion	
Age at onset, mean (S.D.), years	53.8 (14.2)
Gender: female, *n* (%)	69 (81)
aCCP positive, *n* (%)	69 (81)
RF positive, *n* (%)	67 (78)
During the study	
Concomitant anti-rheumatic therapy	
MTX alone, *n* (%)	29 (34)
MTX + other DMARDs, *n* (%)	40 (47)
Other DMARDs,[a] *n* (%)	15 (18)
None, *n* (%)	1 (1)
Concomitant use of glucocorticoids, *n* (%)	63 (74)
Time under infliximab, mean (interval), years	4.42 (0.4–10.2)
Infliximab discontinued, *n*/total (%)	45/84[b] (53.5)
Patients with acquired drug resistance, *n* (%)	44 (51.8)

[a]Other DMARDs: LEF, SSZ, HCQ and AZA. [b]The evolution of one patient was missed because she moved to another country.

the appearance of anti-infliximab Ab was delayed for >1 year. In most patients, antibody titres did not disappear, but increased during treatment and were only modulated by an increase in the dose of infliximab. Patients with antibodies against infliximab had higher DAS-28 values at 6-month follow-up (16 out of 49 patients), 1 year (7 out of 31 patients) and >4 years (11 out of 47 patients) [4.85 (1.24) *vs* 3.67 (1.12), $P = 0.004$; 4.95 (1.24) *vs* 3.13 (1.17), $P = 0.002$; 4.00 (1.35) *vs* 3.46 (1.22), $P = 0.004$, respectively]. Similar results were found for ΔDAS-28 from baseline [1.10 (0.93) *vs* 1.73 (1.03), $P = 0.044$; 1.24 (0.86) *vs* 1.92 (0.72), $P = 0.061$; 0.57 (1.86) *vs* 1.98 (1.26), $P = 0.025$] at the three time points, respectively.

Patients classified as responders were mainly patients with no detectable anti-infliximab Ab levels (Fig. 1). Only 24% of EULAR (good and moderate) responders ($n = 75$) showed anti-infliximab Ab *vs* 100% of non-responder patients ($n = 10$) ($P < 0.001$). Serum trough infliximab levels (Mdn, IQR) were higher in EULAR responders (good and moderate) than in EULAR non-responder patients at 6 months (992, 46–2960 *vs* 0, 0–60 ng/ml, $P = 0.005$), 1 year (1792, 384–3904 *vs* 0, 0–555 ng/ml, $P = 0.021$) and >4 years (1536, 220–3456 *vs* 0, 0–2672 ng/ml, $P = 0.101$), respectively (Fig. 2A). Serum anti-infliximab Ab concentration (Mdn, IQR) was higher in non-responders than in responders at 6 months (208, 0–1087 AU/ml *vs* 0, 0–100 AU/ml, $P = 0.054$), 1 year (60, 12–8924 *vs* 0, 0–0 AU/ml, $P = 0.018$) and >4 years (791, 0–6303 *vs* 0, 0–0, $P = 0.003$), respectively (Fig. 2B).

Survival of infliximab treatment

A total of 45 (53.5%) out of 84 patients interrupted infliximab therapy, with a median survival rate of 5.75 (95%

Fig. 1 Relationship between the presence of anti-infliximab Ab and EULAR response in RA patients treated with infliximab. Good: DAS-28 decrease >1.2 with an attained DAS-28 <3.2 Moderate: DAS-28 decrease ⩽1.2 and ⩾0.6 with an attained DAS-28 ⩾3.2 and ⩽5.1. No response: DAS-28 decrease <0.6 with an attained DAS-28 >5.1.

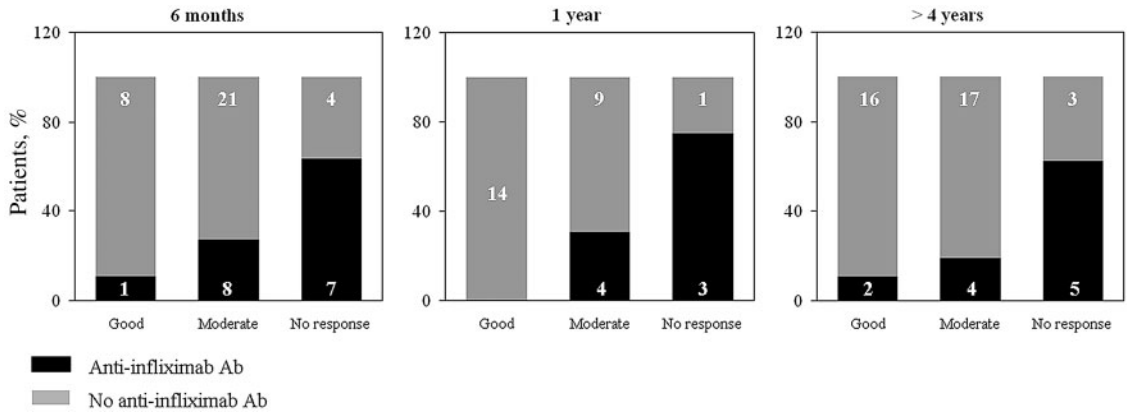
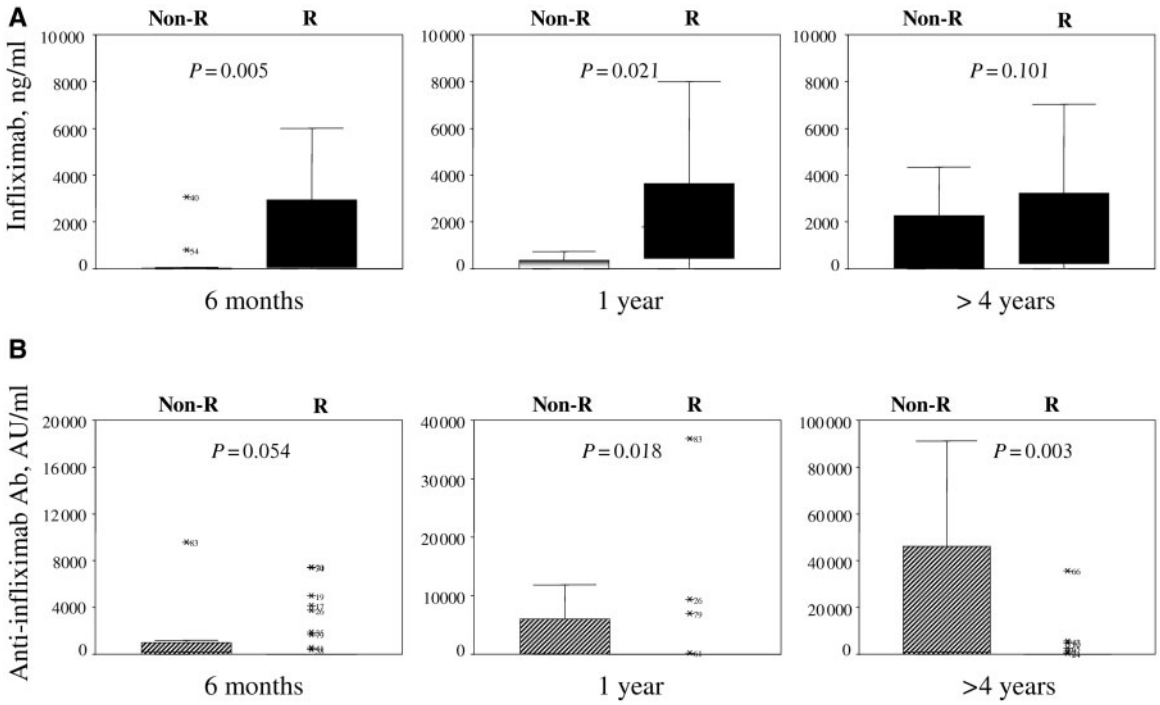


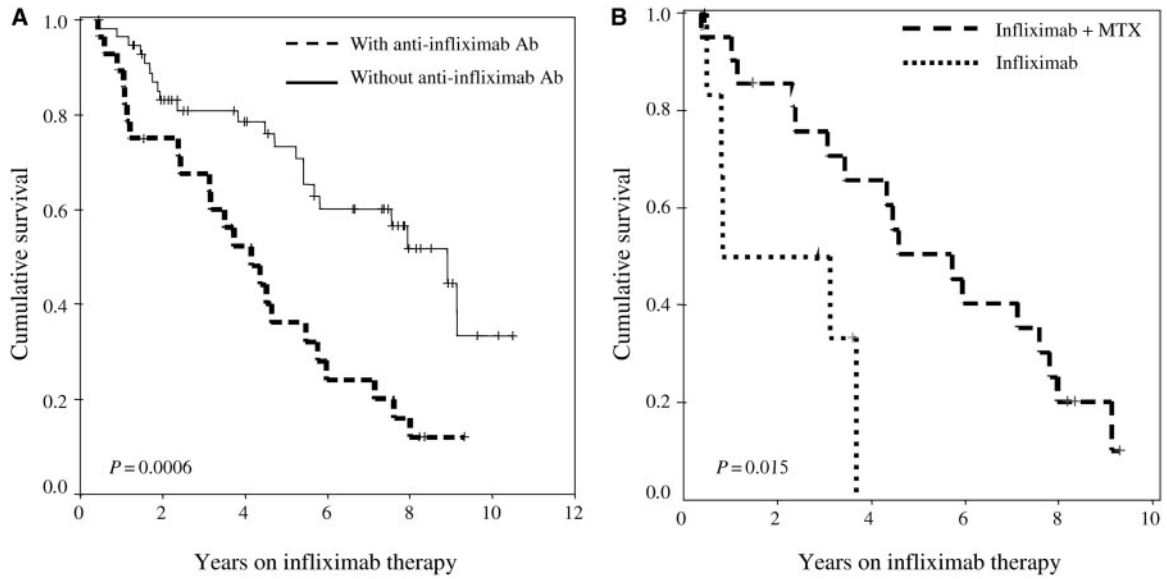
Fig. 2 Serum trough infliximab (**A**) and anti-infliximab Ab levels (**B**) in RA patients responding (R) (good and moderate) ($n = 38$ at 6 months, $n = 27$ at 1 year, $n = 39$ at >4years) and not responding (non-R) ($n = 11$ at 6 months, $n = 4$ at 1 year, $n = 8$ at >4 years) by EULAR criteria, to infliximab treatment. Data are shown as box plots, where the boxes represent the 25th to 75th percentiles, and the lines outside the boxes represent the 10th and 90th percentiles.



CI 4–7.5) years on the drug. The number of patients that discontinued infliximab therapy ($n = 45$) was significantly higher among those who developed anti-infliximab Ab [23 (82.1%) out of 28 *vs* 22 (39.3%) out of 57; $P < 0.001$]. Median survival time on infliximab treatment was 4.15 (95% CI 2.78–5.53) years in patients with anti-infliximab Ab *vs* 8.89 (95% CI 6.7–11) years for those without antibodies ($P = 0.0006$; Fig. 3A). Among patients who developed anti-infliximab Ab, median survival time on infliximab treatment was longer ($P = 0.022$) in patients receiving concomitant MTX ($n = 22$; 4.52 years, 95% CI 3.8–5.23 years) than in those not receiving MTX ($n = 6$; 1.06 years, 95% CI 0–3.79 years) (Fig. 3B).

Twenty-three (82.1%) of 28 patients who developed anti-infliximab Ab discontinued infliximab treatment. In four out of the five remaining patients, anti-infliximab

FIG. 3 Kaplan–Meier curves for survival on infliximab therapy of RA patients. (**A**) Patients who either developed (— –) or did not develop (——) anti-infliximab Ab. (**B**) Patients who developed anti-infliximab Ab and are treated with (— –) or without MTX (. . .).



Ab concentration decreased below detection levels after dosage escalation to 5 mg/kg, with a consequent clinical improvement, and the fifth patient continued treatment because her clinical response was good, despite remaining antibodies in circulation.

Modulation of anti-infliximab Ab levels by drug dose escalation

In 44 (51.7%) of the 85 patients, an acquired resistance to the drug was observed necessitating either an increased dosage of infliximab or a reduced time interval between infusions to achieve a clinical improvement. This drug resistance was higher in patients with anti-infliximab Ab [19 (67.9%) out of 28] than in those without anti-infliximab Ab [25 (43.9%) out of 57] ($P = 0.032$). Two kinds of response on dose escalation were observed in our cohort. Type I: anti-infliximab Ab disappeared after dose increase to 5 mg/kg coinciding with measurable infliximab serum trough levels and a DAS-28 decrease. In these cases, anti-infliximab Ab could be detected again if the dose of infliximab was subsequently reduced, with a simultaneous clinical worsening (Fig. 4A). Type II: anti-infliximab Ab did not disappear after drug escalation (Fig. 4B), reaching high levels, which in some patients were associated with development of infusion-related reactions (three patients).

Relation between infusion-related reactions and anti-infliximab Ab

Infusion-related reactions were recorded in nine patients, all of whom had detectable anti-infliximab Ab. Anti-infliximab Ab levels [Mdn (IQR)] at the time of infusion reaction were higher in the patients who developed reactions [20 565 (5000–30 625) AU/ml] than in those patients with detectable anti-drug antibodies, but without infusion-related reactions [10 152 (491–8162) AU/ml] ($P = 0.041$; Fig. 5).

Influence of combined therapy with MTX on anti-infliximab Ab presence

Sixty-nine (81.1%) patients received MTX [7.5–25 mg/weekly, mean (S.D.) 15 (4.96) mg/weekly] concomitantly with infliximab. MTX was subcutaneously and orally administered in 19 and 50 patients, respectively. We did not find a lower proportion of patients developing anti-infliximab Ab in association with the use of MTX (32% with MTX *vs* 37% without MTX, $P = 0.77$). However, in patients receiving MTX who did make antibodies ($n = 22$), maximal levels [Mdn (IQR)] tended to be lower than in those with antibodies on infliximab monotherapy ($n = 6$) [3414 (808–7426) AU/ml with MTX *vs* 21 250 (7049–47 656) AU/ml without MTX; $P = 0.07$].

Discussion

It is widely accepted that immunogenicity of biological drugs such as infliximab is the main cause of loss of clinical response in the treatment of RA [10, 13, 18, 20]. In this study, we have analysed the clinical significance of free infliximab and anti-infliximab Ab concentration in serum in a cohort of 85 Spanish RA patients treated for >4 years. Our findings indicate that one-third of RA patients develop antibodies and this is correlated with clinical response.

The variable incidence of anti-drug antibodies reported in earlier literature was mainly methodological and related to the method used to measure the antibodies [19]. Radioimmunoassay seems to be the most reliable and sensitive method to detect all antibody isotypes [5, 10, 18, 19, 21], but has the drawback of the use of radioactivity. The

FIG. 4 Modulation of serum infliximab and anti-infliximab Ab levels, as well as clinical response, with infliximab dose changes. Infliximab dose ranges between 3 and 5 mg/kg. (**A**) A 'type I' representative patient in whom anti-infliximab Ab levels are inhibited only by a high infliximab concentration. Lowering infliximab dose results in the appearance of antibodies and DAS-28 increase. (**B**) A 'type II' representative patient in whom anti-infliximab Abs do not disappear after infliximab dose increase, with a poor clinical response (DAS-28).

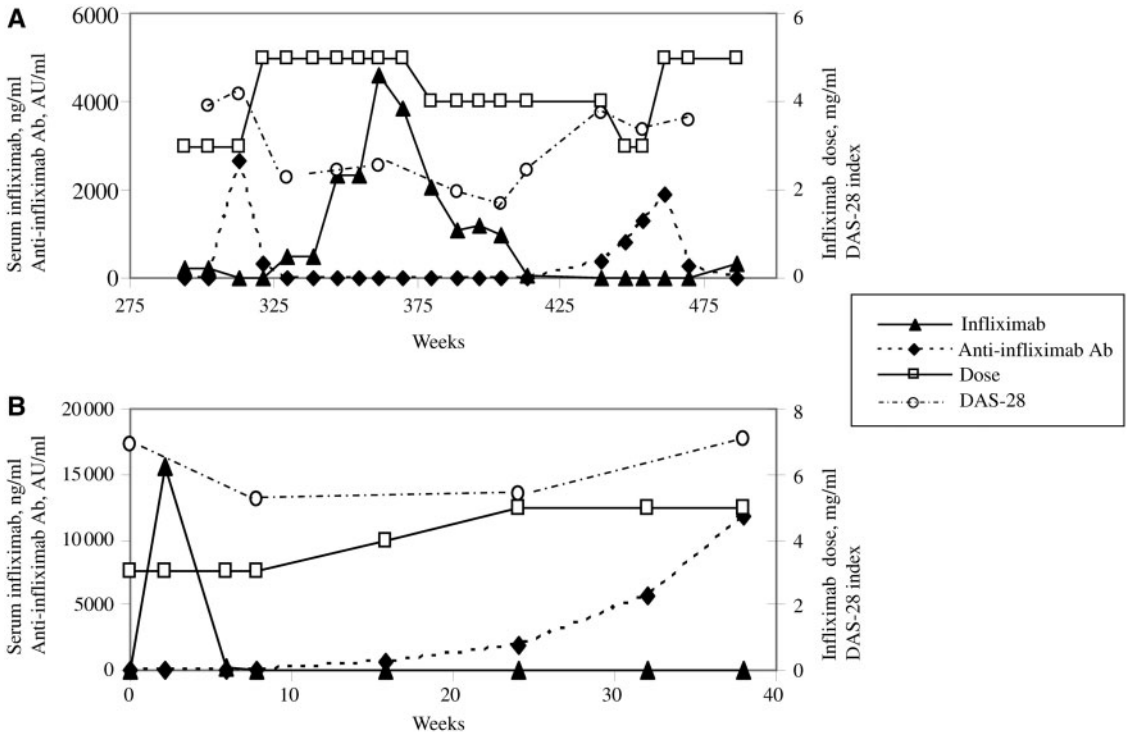
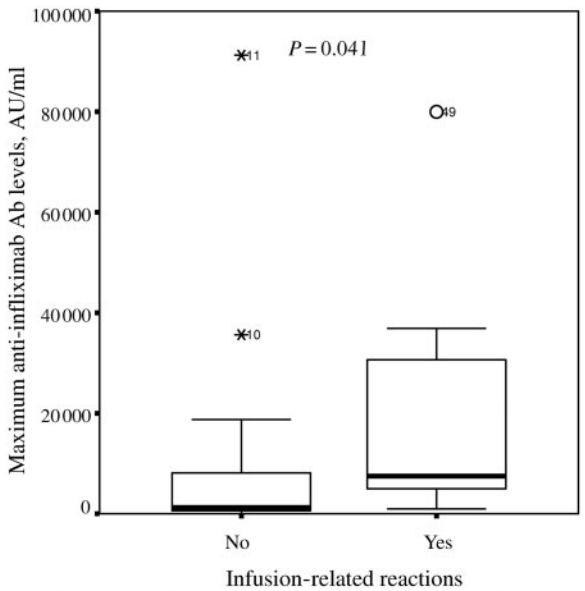


FIG. 5 Maximum antibody levels in patients with anti-infliximab Ab who developed or did not develop an infusion-related reaction, defined as in 'Material and methods' section.



bridging ELISA employed in this work suffers the disadvantage that it fails to measure IgG4 antibodies. However, this is not believed to represent a major problem as isolated IgG4 antibodies usually do not occur in the absence of other IgG isotypes [11] and the bridging ELISA approach for measuring anti-drug antibodies has been validated by its use in several other studies [11, 13, 20].

Serum levels of anti-infliximab Ab strongly correlate with the clinical response as DAS-28 was significantly lower in those patients without anti-infliximab Ab at all time points. According to the EULAR response criteria, 100% of non-responder patients at any studied time point showed anti-infliximab Ab *vs* only 24% of responders. Whereas most previously published studies were performed over relatively short periods of time ($\leqslant$1 year) [5–7], we have extended the analysis over $>$4 years, since we believe that immunogenicity rates can be underestimated if studies are restricted to $<$1 year. Moreover, during our long-term follow up, we have seen that patients with detectable levels of anti-infliximab Ab had to discontinue treatment earlier than those who did not develop anti-infliximab Ab.

As reported in previous literature, we have found that serum trough infliximab levels inversely correlate with the presence of antibodies against the drug and with the clinical response [10, 14, 17]. One could, therefore, argue

that there is no need to monitor both the drug and anti-drug antibodies, as complexes are formed between antibodies and infliximab [11]. However, undetectable or low levels of infliximab before the appearance of antibodies may indicate that the patient will develop a high titre of antibodies following the subsequent infusion. In fact, in our cohort one patient developed an infusion-related reaction during the fourth infusion, after a sharp decrease in circulating infliximab, but before antibodies could be detected.

The development of antibodies to infliximab occurred mainly in the first 4 months of treatment, although it can be delayed in patients with an early drug escalation, because only when the immune system makes sufficient antibodies to overcome the infliximab concentration is antibody detection possible. Detection of infliximab and anti-infliximab Ab levels can be used to customize treatment and help to avoid unnecessary therapy. As we have shown, patients differ in their clinical response to an increased dose of infliximab. In some patients, an improvement in DAS-28 was seen to coincide with measurable serum trough infliximab levels and loss of anti-infliximab Ab, which can reappear after dose decrease because of clinical improvement. In another group of patients, the antibodies remained in the circulation despite a drug dosage increase to maximum levels. These patients did not show clinical improvement and had a higher risk of developing infusion-related reactions. Another important utility of biopharmaceuticals monitoring by means of drug and anti-drug Ab determinations has been clearly exposed in a recent publication by Jamnitski *et al.* [22]. The authors show that among patients who discontinued treatment with a first TNF-α inhibitor, those who had developed antibodies against the drug achieved a significantly better clinical response after switching to another anti-TNF (etanercept) than patients without antibodies. Authors argue that immunogenicity monitoring is needed in order to differentiate patients who will benefit from a change in anti-TNF therapy from those who show no primary response.

Patients receiving infliximab treatment show a high rate of infusion-related reactions [5, 20]. In our cohort, all patients with infusion-related reactions had anti-infliximab Ab at high titres. These data support the view that elevated titres of anti-infliximab Abs are associated with increased risk of infusion reactions, probably because of the formation of large antibody complexes. These complexes are removed with difficulty by the liver and spleen, and are associated with the occurrence of serious adverse reactions [6].

It has been reported that combined therapy with infliximab and MTX is inversely associated with the formation of anti-infliximab Ab [23]. In our study, 81% of patients received concomitant MTX with infliximab and 32% of them developed anti-infliximab Ab. A similar number of patients developed antibodies when they were treated with infliximab alone or together with other DMARDs. These results, although similar to the findings reported by Haraoui *et al.* [13], were different from those observed in previous studies, which suggested that combined therapy reduced the number of patients developing anti-drug antibodies [4], probably due to differences in administration guidelines. However, in our study we observed that patients who developed anti-infliximab Ab continued on anti-TNF treatment significantly longer if they were receiving concomitant therapy with MTX, probably because the immunosuppression is associated with the production of lower antibody levels and the effectiveness of MTX itself on disease activity. This fact encourages us to recommend the routine use of MTX concomitantly with infliximab administration.

In conclusion, the formation of anti-infliximab Ab is associated with a poor clinical response and with the appearance of infusion reactions. Long-term follow-up shows that levels of these antibodies may be modulated by increasing drug concentration, which suggests that they may be used to monitor the appropriate therapeutic regime. Moreover, they are associated with the discontinuation of treatment over time.

Rheumatology key messages

- Immunogenicity of infliximab is associated with loss of clinical response and appearance of infusion reactions.
- Detection of anti-infliximab Ab can be used to customize treatment and help to avoid unnecessary therapy.
- Patients with anti-infliximab Ab discontinue infliximab treatment earlier than those who did not develop antibodies.

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References

1 Weaver AL. The impact of new biologicals in the treatment of rheumatoid arthritis. Rheumatology 2004;43(Suppl. 3): iii23.

2 Bressler B, Haraoui B, Keystone EC, Sette A. Optimizing use of tumor necrosis factor inhibitors in the management of immune-mediated inflammatory diseases. J Rheumatol 2010;37:40–52.

3 Maini RN, St Clair EW, Breedveld F *et al*., for the ATTRACT Study Group. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. Lancet 1999; 354:1932–9.

4 Maini RN, Breedveld FC, Kalden JR *et al*. Therapeutic efficacy of multiple intravenous infusions of anti-tumour necrosis factor α monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum 1998;41:1552–63.

5 Wolbink GJ, Vis M, Lems WF *et al*. Development of anti-infliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. Arthritis Rheum 2006;54:711–5.

6 van der Laken CJ, Voskuyl AE, Roos JC *et al*. Imaging and serum analysis of immune complex formation of radiolabelled infliximab and anti-infliximab in responders and non-responders to therapy for rheumatoid arthritis. Ann Rheum Dis 2007;66:253–6.

7 Bendtzen K, Geborek P, Svenson M, Larsson L, Kapetanovic MC, Saxne T. Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumour necrosis factor alpha inhibitor infliximab. Arthritis Rheum 2006;54:3782–9.

8 Lipsky P, van der Heijde D, St Clair EW *et al*. Infliximab and methotrexate in the treatment of rheumatoid arthritis. New Engl J Med 2000;343:1594–602.

9 St Clair EW, Wagner CL, Fasanmade AA, Wang B, Schaible TF, Kavanaugh A. The relationship of serum infliximab concentrations to clinical improvement in rheumatoid arthritis. Arthritis Rheum 2002;46: 1451–9.

10 Radstake T, Svenson M, Eijsbouts A *et al*. Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. Ann Rheum Dis 2009;68: 1739–45.

11 Wolbink GJ, Aarden LA, Dijkmans BAC. Dealing with immunogenicity of biologicals: assessment and clinical relevance. Curr Opin Rheumatol 2009;21:211–5.

12 Rojas JR, Taylor P, Cunningham MR *et al*. Formation, distribution and elimination of infliximab and anti-infliximab immune complexes in cynomolgus monkeys. J Pharmacol Exp Ther 2005;313:578–85.

13 Haraoui B, Cameron L, Ouellet M, White B. Anti-infliximab antibodies in patients with rheumatoid arthritis who require higher doses of infliximab to achieve or maintain a clinical response. J Rheumatol 2006;33:31–6.

14 Mulleman D, Méric J-C, Paintaud G *et al*. Infliximab concentration monitoring improves the control of disease activity in rheumatoid arthritis. Arthritis Res Ther 2009;11: R178.

15 Bartelds GM, Wijbrandts CA, Nurmohamed MT *et al*. Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. Ann Rheum Dis 2007; 66:921–6.

16 van Gestel A, Prevoo M, van't Hof M, van Rijswijk M, van de Putte L, van Riel P. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996;39:34–40.

17 Wolbink GJ, Voskuyl AE, Lems WF *et al*. Relationship between serum trough infliximab levels, pretreatment C reactive protein levels, and clinical response to infliximab treatment in patients with rheumatoid arthritis. Ann Rheum Dis 2005;64:704–7.

18 de Vries MK, Wolbink GJ, Stapel S *et al*. Decreased clinical response to infliximab in ankylosing spondylitis is correlated with anti-infliximab formation. Ann Rheum Dis 2007;66:1252–4.

19 Aarden AL, Ruuls SR, Wolbink GJ. Immunogenicity of anti-tumour necrosis factor antibodies—toward improved methods of anti-antibody measurement. Curr Opin Immunol 2008;20:431–5.

20 Finckh A, Dudler J, Wermelinger F *et al*. Influence of anti-infliximab antibodies and residual infliximab concentrations on the occurrence of acquired drug resistance to infliximab in rheumatoid arthritis patients. Joint Bone Spine 2010;77:313–8.

21 Svenson M, Geborek P, Saxne T, Bendtzen K. Monitoring patients treated with anti-TNFa biopharmaceuticals: assessing serum infliximab and anti-infliximab antibodies. Rheumatology 2007;46:1828–34.

22 Jamnitski A, Bartelds GM, Nurmohamed MT *et al*. The presence or absence of antibodies to infliximab or adalimumab determines the outcome of switching to etanercept. Ann Rheum Dis 2011;70:284–8.

23 Baert F, Noma M, Vermeire S *et al*. Influence of immunogenicity on long-term efficacy of infliximab in Crohn's disease. New Engl J Med 2003;348:601–8.