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Chapter 5

Anti-infliximab and anti-adalimumab antibodies in relation to response to adalimumab in infliximab switchers and anti-TNF naive patients: a cohort study

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ABSTRACT

Objective. To investigate how antibodies against anti-TNF agents influence response after switching from infliximab to adalimumab in rheumatoid arthritis (RA).

Methods. This cohort study consisted of 235 RA patients, all treated with adalimumab. At baseline fifty-two patients (22%) were previously treated with infliximab ("switchers"), and 183(78%) were anti-TNF naive. Disease activity (using the DAS28score) and presence of antibodies against infliximab and adalimumab was assessed. Clinical response to adalimumab was compared between switchers and anti-TNF naive patients and their anti-infliximab and anti-adalimumab antibody status.

Results. After 28 weeks of adalimumab therapy the decrease in DAS28 (Δ DAS28) for the 235 patients was 1.6 ± 1.5 (mean \pm SD). Anti-adalimumab antibodies were detected in 46 patients (20%). Δ DAS28 was 1.8 ± 1.4 in patients without anti-adalimumab and 0.6 ± 1.3 in patients with anti-adalimumab ($P < 0.0001$). Thirty-three out of the 52 switchers (63%) had anti-infliximab antibodies. Patients with anti-infliximab more often developed anti-adalimumab than anti-TNF naive patients, (14(27%) versus 32(18%); ($P = 0.039$)). Δ DAS28 was greater for anti-TNF naive patients (1.7 ± 1.5) compared to switchers without anti-infliximab antibodies (Δ DAS28 = 0.9 ± 1.4) ($P = 0.009$). Δ DAS28 for switchers with anti-infliximab was 1.2 ± 1.3 and did not differ significantly from anti-TNF naive patients ($P = 0.262$).

Conclusion. Switchers with anti-infliximab antibodies more often develop antibodies against adalimumab than anti-TNF naive patients. Response to adalimumab was limited in switchers without anti-infliximab antibodies, which raises the question whether a second anti-TNF therapy should be offered to RA-patients who fail on initial treatment with anti-TNF, in the absence of anti-biological antibodies.

INTRODUCTION

Biologics directed against tumour necrosis factor (TNF) have greatly improved the treatment of chronic inflammatory diseases, such as RA, ankylosing spondylitis, Crohn's disease and psoriasis. However, in RA, the proportion of patients who do not respond to anti-TNF therapy is substantial and varies between 30 to 40%.¹⁻³ The lack of response can partly be explained by an immunogenic antibody response against these drugs, but there are also non-responding patients in whom an immunogenic reaction cannot be demonstrated.^{4 5} Lack of response to TNF blockade in these patients might to a certain extent be related to mechanisms that are not primarily driven by TNF.^{6 7} Hence, there seem to be different types of non-responders with different underlying pathogenic mechanisms causing non-response. Currently, these mechanisms and their consequences are not completely understood and it is unclear whether patients who fail one TNF blocker should switch to another TNF antagonist or to a drug with a distinct mechanism of action.

At present three TNF antagonists are available for the treatment of RA: infliximab, adalimumab and etanercept; the mechanisms of action have recently been reviewed in great detail.⁸ Personalised treatment regimens in the field of TNF blocking therapy are still far from optimal and most importantly, the factors influencing treatment outcomes in individual patients are unclear.^{6 9} In RA it is common to try another TNF blocker after treatment with the first failed. Previous studies on switching from one TNF blocker to another after non response have focused on whether switching was useful or not.¹⁰⁻¹³ These studies have also shown that there is considerable variation in response after switching; however, the contributing factors influencing this response have not been investigated.

To achieve a better understanding of factors determining response after switching from one TNF blocker to another, we prospectively studied a cohort of consecutive RA patients receiving adalimumab therapy, some of whom had failed prior treatment with infliximab. We compared clinical response as well as anti-antibody formation for infliximab-adalimumab switchers and TNF blockade naive patients. The ultimate goal is to understand the variation in clinical response after switching in different patient groups and to identify these different types of (non-) responders.

PATIENTS AND METHODS

Patients. Between February 2004 and February 2006 255 consecutive RA patients were included in a prospective observational cohort at the outpatient clinics of the Departments of Rheumatology of the Jan van Breemen Institute and the Academic Medical Center in Amsterdam. All patients fulfilled the American College of Rheumatology 1987 revised criteria for RA¹⁴, and had active disease, indicated by a disease activity score in 28 joints (DAS28) of ≥ 3.2 despite earlier treatment with two disease modifying anti-rheumatic drugs (DMARDs) including methotrexate (MTX) at a dosage of 25 mg weekly or at the maximal tolerable dosage, according to the Dutch consensus statement on the initiation and continuation of TNF blocking therapy in RA.¹⁵ Patients were treated with either adalimumab and concomitant DMARD therapy or adalimumab monotherapy. All patients used adalimumab 40 mg subcutaneously every other week. In patients with an inadequate response as judged by the treating rheumatologist, the dosing frequency of adalimumab could be increased to 40 mg per week. Eligible patients for the current study were all patients who had previously been treated with infliximab and anti-TNF naive patients. 235 patients fulfilled these criteria; fifty-two of these patients had previously been treated with infliximab, and are referred to as "switchers", and 183 were anti-TNF naive patients. The study was approved by the Medical Ethics Committee of the Slotervaart Hospital,

BovenIJ Hospital, the Jan van Breemen Institute, and the Academic Medical Centre/University of Amsterdam. All patients gave written informed consent.

Clinical Response to adalimumab. Disease activity was assessed at baseline and after 4, 16 and 28 weeks of therapy using the DAS28 score. Clinical response was assessed by the decrease in DAS28 score (Δ DAS28) and the European League Against Rheumatism (EULAR) response criteria.¹⁶ The fifty-two patients who had previously been treated with infliximab and in whom infliximab was stopped were qualified as non-responders to infliximab.

Measurement of antibodies against infliximab and adalimumab. Serum samples were collected at baseline and just prior to an injection with adalimumab after 4, 16 and 28 weeks. The presence of anti-infliximab antibodies was determined at baseline prior to the start of adalimumab. The presence of anti-adalimumab antibodies was determined at all time points between baseline and 28 weeks. Anti-infliximab and anti-adalimumab antibodies were detected with a radio immunoassay (RIA) as more extensively described previously.^{4 5 17} The assays measure specific high avid IgG antibodies against adalimumab and infliximab by an antigen binding test. Serum (1 μ L/test) was pre-incubated with Sepharose-immobilised protein A (1 mg/test; Pharmacia, Uppsala, Sweden) in Freeze buffer (Sanquin, Amsterdam, the Netherlands). Non-bound serum components were removed by washing before 50 μ l of ¹²⁵I-labeled F(ab)'2 fragment of adalimumab/infliximab was added. ¹²⁵I F(ab)'2 fragment of adalimumab/infliximab was added as two separate incubations. After overnight incubation non-bound radiolabel was washed away and Sepharose-bound radioactivity was measured. Test results were converted into arbitrary units per millilitre (AU/ml) by comparison with dilutions of a reference serum. The mean cut-off value was set at 12 AU/ml which was derived from 100 healthy donors. Assay specificity was demonstrated by the absence of anti-adalimumab in 25 sera containing high-titre anti-infliximab antibodies. In the assays we did not find cross reactivity. Recently, patient sera were tested in a bioassay, which confirmed the specificity and validity of the RIA.¹⁷

Statistical analysis. For statistical analysis SPSS version 16.0 was used. For differences between groups we used the independent samples t- test, Mann-Whitney test or Chi square as appropriate. To investigate the influence of confounders on Δ DAS28 a multiple regression-analysis was used. The threshold for significance was set at P < 0.05.

To analyze clinical response in patients with and without antibodies after 28 weeks of treatment we used last observation carried forward for patients who stopped treatment due to non-response or adverse events, and for patients who had received increased dosing frequency.

Variables considered potential confounders were chosen from all available baseline variables and were determined for every analysis specifically, based on differences between groups included in the analysis (Table 1). Variables were included in the regression model as confounders if the beta changed 10% or more after inclusion of the variable. Additionally, adjustments for possible intermediates were made.

RESULTS

Patient characteristics. Patient characteristics are shown in Table 1. Of the 235 patients 52 patients had received infliximab treatment prior to the start of adalimumab. The median period between the last infliximab administration and the first adalimumab injection was 47 weeks, interquartile range (IQR 11–

102). Of the 235 patients enrolled in the study, 230 (98%) completed 16 weeks of adalimumab treatment, and 217 (92%) were still on adalimumab treatment at week 28. Before 28 weeks eight patients (3%) stopped due to treatment failure, nine (4%) because of adverse events and one lost to follow up. Twelve patients (6%) had an increased dosing frequency before 28 weeks to 40 mg adalimumab per week; in these patients the last DAS28 before dose increase was carried forward to 28 weeks.

Table 1. Demographic and clinical characteristics at baseline.

	Total population n = 235	Anti-TNF naive patients n = 183	IFX Switchers n = 52	IFX switchers with anti-IFX n = 33	IFX switchers without anti-IFX n = 19
Demographics					
Age, years	53 ± 12	53 ± 12	52 ± 12	54 ± 10	49 ± 13
Female, no. (%)	185 (79)	142 (78)	43 (83)	27 (82)	16 (84)
DMARD therapy					
Prior DMARDs	3.5 ± 1.7	3.2 ± 1.4* ♦ §	4.4 ± 2.2*	4.3 ± 2.2♦	4.7 ± 2.1§
Methotrexate use, no. (%)	193 (82)	150 (82)	43 (83)	28 (85)	15 (79)
Methotrexate dose (mg/wk)	23 (15-25)	25 (15-25)	20 (13-25)	18 (11-25)	20 (15-25)
Prednisone use, no. (%)	80 (34)	56 (31)* §	24 (46)*	13 (39)	11 (58) §
Prednisone dose (mg/day)	7.5 (5-10)	7.0 (5-10) ♦ §	7.5 (5-10)	5 (4-6) ♦ †	10(10-10)§ †
Disease status					
Disease duration (years)	9 (4-17)	8 (4-17)	12 (6-18)	13 (6-18)	9 (3-17)
Rheumatoid factor positive, no. (%)	171 (73)	130 (71)	41 (79)	28 (85)	13 (68)
Erosive disease, no. (%)	182 (77)	140 (77)* ♦	42 (81)* ♦	28 (85)	14 (74)
Erythrocyte sedimentation rate (mm/h)	29 ± 23	28 ± 22	36 ± 26	37 ± 27	33 ± 23
C-reactive protein (mg/dl)	11 (5-24)	11 (5-24)	10 (3-35)	8 (2-36)	14 (5-35)
DAS28	5.2 ± 1.2	5.1 ± 1.2	5.4 ± 1.3	5.3 ± 1.2	5.7 ± 1.4

Table 1. Mean values ± SD, Median (IQR), or percentages are shown. IFX = infliximab, anti-IFX = anti-infliximab, DMARDs = disease modifying anti rheumatic drugs, DAS28= disease activity score in 28 joints. * There were significant differences between anti-TNF naive patients and switchers for prior DMARDs (P=0.000), prednisone use (P=0.037) and erythrocyte sedimentation rate (ESR) (P=0.025). ♦ There were significant differences between anti-TNF naive patients and switchers with anti-infliximab for prior DMARDs (P=0.009), prednisone dose (P=0.013) and ESR (P=0.027). § Between anti-TNF naive and switchers without anti-IFX for prior DMARDs (P=0.006) and prednisone use (P=0.016) and dose (P=0.007). † There was a significant difference between infliximab switchers with and without anti-infliximab for prednisone dose (P=0.000).

Clinical response. The mean decrease in DAS28 after 16 weeks of adalimumab therapy for the whole patient population was 1.7 ± 1.4. There were 55 (23%) non-responders, 95 (40%) moderate responders, and 85 (36%) good responders according to the EULAR response criteria. After 28 weeks of adalimumab therapy the mean decrease in DAS28 was 1.6 ± 1.5. There were 57 (24%) non-responders, 100 (43%) moderate responders, and 78 (33%) good responders at that time point (Figure 1).

There was a difference in response between anti-TNF naive patients and patients who were treated with infliximab prior to adalimumab therapy. The improvement in DAS28 was larger for anti-TNF naive patients compared to infliximab switchers (Δ DAS28 1.7 ± 1.5 versus 1.1 ± 1.4 ; $P = 0.007$) in a univariate analysis, and after adjustment for confounding variable DAS28 at baseline in multivariate regression analysis (95%CI -1.166 to -0.351; $P < 0.0001$).

Among the anti-TNF naive patients 38% were good responders, 39% were moderate responders and 23% were non-responders. In the infliximab-switchers group 15% of the patients were good responders, 54% were moderate responders and 31% were non-responders ($P = 0.008$; Figure 1). Post hoc analysis showed that only the percentage of good responders was significantly different between anti-TNF naive patients and infliximab-switchers ($P = 0.002$).

Immunogenicity. Anti-adalimumab antibodies were determined in 233 patients; for 2 patients appropriate serum samples were not available. During 28 weeks follow-up, anti-adalimumab antibodies were detected in 46 patients (20%). Mean Δ DAS28 was 1.8 ± 1.4 in patients without anti-adalimumab antibodies and 0.6 ± 1.3 in patients with anti-adalimumab antibodies ($P < 0.0001$) (Table 2). After adjustment for confounding variable ESR the difference remained significant (95%CI -1.797 to -0.908; $P < 0.0001$).

Figure 1. EULAR response in anti-TNF naive patients and switchers.

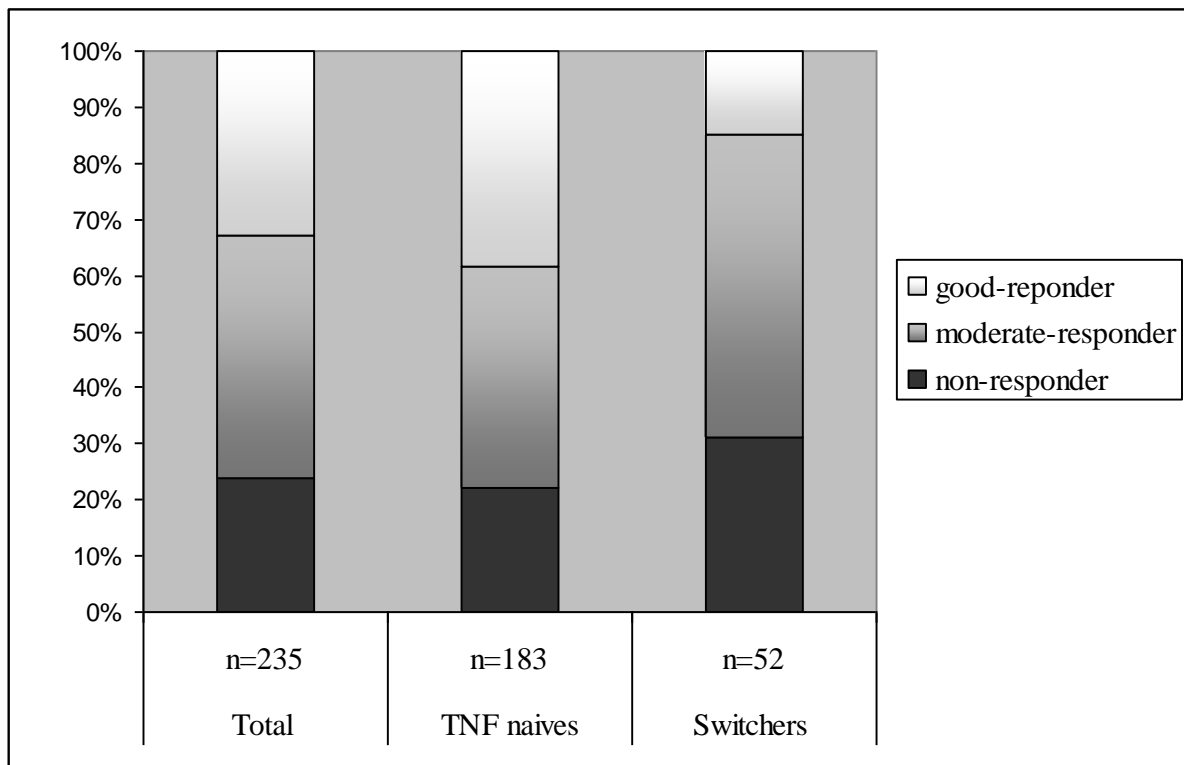


Figure 1. The EULAR response in anti-TNF naive patients and switchers is shown. Of the 235 patients 57 patients (24%) were non-responder, 100 patients were moderate responder (43%) and 78 were good responder (33%). Of the 183 anti-TNF naive patients 41 patients (22%) were non-responder, 72 patients were moderate responder (39%), and 70 were good responder (38%). Of the 52 switchers 16 patients were non-responder (31%), 28 were moderate responder (54%) and 8 were good responder (15%).

Thirty-three of the 52 patients in the infliximab switcher group tested positive (63%) for anti-infliximab antibodies at baseline. Patients with anti-infliximab antibodies significantly more often formed anti-adalimumab antibodies (n = 11, 33%) than anti-TNF naive patients (n = 32, 18%; P = 0.039). Nineteen infliximab switchers did not have anti-infliximab antibodies, 3 out of these 19 (16%) formed antibodies against adalimumab. Switchers with anti-infliximab antibodies more often formed anti-adalimumab antibodies than switchers without anti-infliximab antibodies, but this difference did not reach statistical significance (P = 0.170) (Table 2).

Of all patients without anti-adalimumab antibodies 89% used concomitant MTX therapy versus 54% of the patients with anti-adalimumab antibodies (P < 0.0001).

Table 2. Frequency of anti-ADA antibodies and clinical response (Δ DAS28) after 28 weeks of adalimumab therapy.

	Total population N = 235	Anti-TNF naive patients N=183	IFX switchers N=52	IFX switcher with anti-IFX N=33	IFX switcher without anti-IFX N=19
Anti-ADA status	46 (20%)	32 (18%)	14 (27%)	11 (33%)	3 (16%)
Δ DAS28 all	1.6 \pm 1.5	1.7 \pm 1.5	1.1 \pm 1.4	1.2 \pm 1.3	0.9 \pm 1.4
Δ DAS28 anti-ADA –	1.8 \pm 1.4*	2.0 \pm 1.4*	1.3 \pm 1.3*	1.6 \pm 1.1*	0.9 \pm 1.4 ^{ns}
Δ DAS28 anti-ADA +	0.6 \pm 1.3	0.6 \pm 1.3	0.5 \pm 1.4	0.4 \pm 1.4	0.7 \pm 1.4

Table 2. Anti-adalimumab (anti-ADA) status in number of patients and percentage (%), Δ DAS28 is shown in mean \pm SD. Anti-adalimumab status could not be determined in 2 of the anti-TNF naive patients due to missing serum samples.

The difference in Δ DAS28 between anti-adalimumab negative (anti-ADA –) and positive (anti-ADA +) patients was significant for the total population* (P = 0.000), anti-TNF naive patients*, switchers* and infliximab (IFX) switchers with anti-infliximab (anti-IFX)* (P \leq 0.05). The difference in Δ DAS28 between anti-adalimumab positive and negative patients did not reach significance in infliximab switchers without anti-infliximab (P = 0.809).

Immunogenicity and clinical response. Improvement in DAS28 was significantly greater for anti-TNF naive patients compared to switchers without anti-infliximab antibodies (P = 0.016) and greater compared to switchers with anti-infliximab antibodies, however this difference did not reach statistical significance (P = 0.079; Table 2). After adjusting for confounding variables prednisone dose and DAS28 at baseline, the difference in Δ DAS28 for anti-TNF naive patients compared to switchers without anti-infliximab antibodies remained (95%CI -0.934 to -0.096; P = 0.017), but the trend towards significance disappeared for the difference in Δ DAS28 when anti-TNF naive patients were compared to switchers with anti-infliximab antibodies (95%CI -1.410 to 0.316; P = 0.210). Since the presence of antibodies against infliximab was associated with a higher frequency of anti-adalimumab antibody formation we performed an additional adjustment for the possible intermediate anti-adalimumab; the difference in Δ DAS28 for anti-TNF naive patients compared to switchers without anti-infliximab antibodies remained (95%CI -0.881 to -0.136; P = 0.008) and the p-value for the difference in Δ DAS28 for anti-TNF naive patients compared to switchers with anti-infliximab antibodies remained similar (95%CI -1.155 to 0.374; P = 0.311).

Clinical response to adalimumab did not differ significantly between prior infliximab-treated patients with and without anti-infliximab antibodies (P = 0.356). Adjustment for prednisone dose, DAS28 at

baseline and number of prior DMARDs (95%CI -1.861 to 2.032; P = 0.928) and additional adjustment for anti-adalimumab status ((95%CI -1.972 to 1.900; P = 0.969) did not influence this.

When looking at the EULAR response to subsequent adalimumab therapy for switchers without anti-infliximab antibodies, there were no good responders and 42% of the patients were non responders.

DISCUSSION

The results from this study show that formation of antibodies against adalimumab is associated with a diminished treatment response in patients with active RA. Secondly, our data indicate that switchers with anti-infliximab antibodies more often form antibodies against adalimumab compared to anti-TNF naive patients. Finally, there are different types of non-responders which may be relevant in the context of individualised medicine.

The present data underscore initial data that immunogenicity against monoclonal antibodies is associated with a diminished response.⁵ Several studies have shown a relationship between the development of antibodies against infliximab and a diminished response to treatment in Crohn's disease, ankylosing spondylitis and RA.^{4 18-20}

Data on the immunogenicity of humanised and "fully human" monoclonal antibodies are still limited. However, it is becoming more and more clear that these antibodies can also cause a clinically relevant immune response. Three studies have shown an association between anti-adalimumab antibodies and a diminished clinical response in RA.^{5 20 21} Approximately 6% of the patients receiving natalizumab, a humanised monoclonal antibody against cellular adhesion molecule $\alpha 4$ -integrin approved for the treatment of multiple sclerosis and Crohn's disease, developed persistent antibodies to the drug with subsequent loss of efficacy.²² Our data contribute to the evidence that all biologically active molecules, even being "fully human" or humanised, injected into humans can incite immune reactions, leading to anti-drug antibody formation. This anti-drug immune response leads to immune complex formation (therapeutic drug antibody bound to anti-drug antibody) which may promote the rapid clearance of the drug resulting in low trough levels.²³

An important question is why an immunogenic response is triggered in some patients but not in others. Concomitant immunosuppressive therapy is an important factor in reducing immunogenicity.^{4 5 18} Current data confirm that concomitant MTX reduces the risk of forming anti-adalimumab antibodies. This could be of significance for the treatment of diseases where it is not common to give concomitant MTX, for example in the treatment of psoriasis and ankylosing spondylitis. An important new finding from the current study is that patients who previously formed antibodies against infliximab are more likely to develop antibodies against adalimumab. There are three possible explanations why people develop antibodies against both drugs. Cross reactivity between the assays seems a logical explanation, but this could not be demonstrated. It is more likely that some patients are more prone to develop an immune response, possibly related to the genetic background. Another option is that too low dosing could lead to immunogenicity, and high dosing to the induction of immunotolerance. Initially, every patient receives the same dosage of infliximab or adalimumab, but in some patients with high levels of biologically active TNF the standard dose could be too low. For example, for infliximab there was more anti-infliximab antibody formation in the patient group with 1 mg/kg compared to the 10 mg/kg group.²⁴ High levels of biologically active TNF could lead to patients with a high disease activity at baseline having a greater risk of developing anti-drug-antibodies, however, the baseline characteristics of our patient groups do not support this hypothesis.

The data shows that anti-TNF naive patients had a better response to adalimumab therapy than prior infliximab non-responders. Prior infliximab non-responders without anti-infliximab antibodies had the least improvement with subsequent adalimumab therapy. Previous studies on switching biologicals also identified different patient groups based on their response after switching; primary failures (patients with no response/intolerance, unlike secondary failures/patients with loss of response) to previous infliximab had a poor response to subsequent adalimumab therapy.¹⁰⁻¹² It was suggested by some that there may be a subpopulation of RA patients that does not respond to anti-TNF therapy.^{11 13} Further evidence for the latter is given by a study that showed that high levels of circulating TNF α bioactivity was associated with a good clinical response to infliximab.⁷ A possible explanation is that TNF may not be the crucial cytokine instigating RA in primary non-responders to anti-TNF therapy. Another study showed that responders to infliximab had a significantly higher synovial TNF expression and significantly more infiltration by TNF producing inflammatory cells than non-responders.⁶

Results from our study show that in clinical practice adalimumab is effective in the majority of RA patients who have previously been treated with infliximab. However, effectiveness differs for different types of patient groups. From our results a first step can be made in defining these patient groups based on their immunogenic reaction towards anti-TNF therapy. The clinical response to subsequent adalimumab for patients with anti-infliximab antibodies (who probably had low serum infliximab levels during infliximab therapy) did not differ significantly from TNF naive patients. Prior infliximab non-responders without anti-infliximab antibodies (who did not respond to infliximab despite presumably adequate infliximab levels) had a significantly worse response to subsequent adalimumab than anti-TNF naive responders. We could not demonstrate a difference in response between switchers with and without anti-infliximab in a direct analysis, but this is likely due to a type II error. Most switchers without anti-infliximab antibodies did not respond to subsequent adalimumab despite not having anti-adalimumab antibodies. Since immunogenicity did not cause non-response in these patients, it is possible that these patients are refractory to anti-TNF therapy. Therefore, our study suggests that non-responders to TNF blockers should be treated differently dependent of their anti-drug antibody status. Antibody-positive patients probably benefit most from switching to a less immunogenic drug acting on the same principle, or from optimising concomitant DMARD (MTX) therapy. Furthermore, it is likely that in non-responders without anti-TNF blocker antibodies it is more useful and cost-effective to start treatment based on a mechanism of action other than TNF blockade.

This study is limited by the number of patients and the consequences of the observational cohort study design. The patient population had severe RA: long disease duration and many prior DMARDs; therefore it could be possible that treatment effects are more difficult to be detected. Patients with persistent joint damage may experience less benefit from treatment than patients with early RA. Due to the interval between the last infliximab administration and the first adalimumab injection it might be possible that the frequency of anti-infliximab antibodies may be higher than what we measured. However, we did not find an association between the length of the interval and the presence of anti-infliximab antibodies, data not shown. In addition to these limitations, our study provides unique data demonstrating insight into the role of immunogenicity in treatment with biologicals. In daily practice in RA switching to another biological after non response is often a random decision rather than an evidence-based decision. To our knowledge, this is the first study providing more information on the underlying mechanisms contributing to the possible success of switching. Further studies are necessary to provide more conclusive data regarding this subject. However, we show that determining

the immunogenic status of a non responding patient may be important for further decision making and could assist in developing an optimised treatment for the individual patient.

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