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Original article

The frequency of anti-infliximab antibodies in patients with rheumatoid arthritis treated in routine care and the associations with adverse drug reactions and treatment failure

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Abstract

Objectives. To investigate the frequency of anti-infliximab antibodies in patients with RA and the associations with adverse drug reactions and treatment failure.

Methods. Based on the DANBIO registry, patients with RA who initiated treatment with infliximab at Hvidovre Hospital between 2000 and 2008 and had available serum samples were identified. The patients were followed for 52 weeks. Anti-infliximab antibodies were determined prior to infusion at baseline and during follow-up (weeks 2, 6, 14 and 52 or at withdrawal) using the IMPACT indirect assay (Roche Diagnostics) and merged with clinical data prospectively registered in the DANBIO registry.

Results. A total of 218 patients with RA were included (80% females, median age 56 years, disease duration 10 years, 65% RF positive, median DAS28 = 5.0). During the 52-week follow-up, 28 patients (13%) withdrew due to adverse events and 50 (23%) due to treatment failure. Antibodies were detected in 118 patients (54%) during follow-up. Patients with detectable anti-infliximab antibodies after 6 weeks had an increased risk of adverse drug reactions [hazard ratio (HR) = 5.06, 95% CI 2.36, 10.84; $P < 0.0001$] compared with patients without anti-infliximab antibodies. Similar results were observed in patients with anti-infliximab antibodies after 14 weeks (HR = 3.30, 95% CI 1.56, 6.99; $P = 0.0009$). Patients with detectable anti-infliximab antibodies during the 52-week follow-up were less likely to achieve sustained minimal disease activity and remission.

Conclusion. Early anti-infliximab antibody formation increased the risk of adverse drug reactions, including infusion reactions. Anti-infliximab antibody formation during the 52-week follow-up decreased the likelihood of minimal disease activity and remission in patients with RA treated in routine care.

Key words: adverse drug reactions, anti-TNF therapy, DANBIO registry, drug response, infliximab, neutralizing antibodies, pharmacological biomarkers, rheumatoid arthritis, treatment failure.

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Introduction

TNF inhibitors have dramatically improved the outcome of patients with RA [1–3]. However, response is variable and at least 30% of patients with RA do not respond or lose their initial response over time [4, 5]. Adverse drug reactions, including infusion reactions, are common during treatment with TNF inhibitors [4, 6, 7]. Immunogenicity is a potential risk of protein drugs, and antidrug antibodies (ADAs) against TNF inhibitors have been reported in patients with RA, psoriasis, AS and Crohn's disease (CD) [7–13]. ADAs against TNF inhibitors are associated

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with a low level of active drug and treatment response in patients with RA [8, 10, 14–16]. However, ADAs are not detected in all patients with treatment failure [7, 9]. This suggests that treatment failure is a heterogeneous event and only partly caused by ADAs against TNF inhibitors [16, 17].

Little is known about the role of ADAs in relation to the development of adverse drug reactions and whether the presence of ADAs may predict serious adverse drug reactions. A higher incidence of adverse drug reactions has been reported in patients with RA, AS and CD with ADAs against TNF inhibitors compared with patients without ADAs [7, 12, 18, 19]. However, data are limited and it is still debated whether and how to apply ADA measurements in clinical practice. The frequency of ADAs against TNF inhibitors varies because different assays have different sensitivity, and the need for a sensitive assay has been stressed [15, 17, 20].

In the present study, we measured the development of anti-infliximab antibodies by a new, sensitive assay and investigated the association between the presence of antibodies and the development of negative clinical outcomes, i.e. adverse drug reactions and treatment failure, in patients with RA treated in routine care.

Patients and methods

Patients

Based on data from the Danish nationwide DANBIO registry, we identified 218 patients fulfilling the 1987 ACR criteria for RA [21] who initiated treatment with infliximab at Copenhagen University Hospital, Hvidovre, between October 1999 and August 2008. DANBIO is a Danish nationwide registry that prospectively collects clinical data on patients with inflammatory rheumatic joint diseases [4, 22]. All patients included in the study were TNF naïve and had available serum samples drawn at baseline. Treatment with infliximab was initiated in patients with continuously active disease indicated by a DAS in 28 joints (DAS28) >3.2 or progression of radiographic joint damage despite treatment with at least two different DMARDs including MTX. Serum samples for anti-infliximab antibody analysis were collected prior to infusion on the day of treatment start (baseline) ($n = 218$) and at weeks 2 ($n = 167$), 6 ($n = 172$), 14 ($n = 180$) and 52 ($n = 128$), or when the treatment was terminated ($n = 34$). All patients were treated with infliximab 3 mg/kg at baseline, weeks 2 and 6 and then every 8 weeks. The treating rheumatologist was allowed to change dose according to local guidelines; however, no patients received a dose increase of infliximab before week 14.

Clinical response to infliximab

Clinical assessments were performed at baseline, at weeks 2 and 6 and then every 8 weeks. Clinical evaluation, registered prospectively and independently in DANBIO, included tender and swollen joint counts (28 joints), visual analogue scale scores of pain, patient global and physician global, HAQ, serum CRP and DAS28 based on four variables including serum CRP [23]. Using the EULAR response criteria, patients were allocated to one of the following outcomes: primary responders (continued EULAR good or moderate response), primary non-responders (continued EULAR no response) and secondary non-responders (decrease in EULAR response after initial EULAR good or moderate response at week 14). Sustained minimal disease activity and sustained remission were defined according to Bartelds *et al.* [16] as a DAS28 of <3.2 or 2.6, respectively, at all consecutive visits after a certain time point, with a minimum of two measurements of <3.2 or 2.6 in patients who withdrew prematurely. To define anti-infliximab antibody status at week 52, we used the visit closest to week 52 within the time interval 44–60 weeks. If no visit had occurred, the latest visit during the first 43 weeks was selected. In case of treatment withdrawal, the date and reasons were registered by the treating rheumatologist. This included treatment failure, adverse drug reactions or other reasons (which cover other known or unknown reasons). Withdrawal due to adverse drug reactions was defined as any adverse event leading to withdrawal of infliximab treatment. Infusion reactions were defined as reactions occurring during infusion, including skin rash, respiratory symptoms, decrease in systemic blood pressure and need for close monitoring. If withdrawal was due to a combination of treatment failure and adverse drug reactions, the case was classified as an adverse drug reaction.

The study was performed according to the Declaration of Helsinki and written informed consent was obtained from all patients. The study was approved by the Ethical Committee of the Capital Region (Copenhagen), Denmark.

Multiplex automated assay for measurement of antidrug antibodies

We used the multiplex platform IMPACT (Immunological Multi-Parameter Chip Technology), developed by Roche Professional Diagnostics (Roche Diagnostics GmbH). The IMPACT platform is based on a small polystyrene chip that is coated with a streptavidin layer, onto which biotinylated antibodies, proteins or peptides are spotted. During the assay, the arrayed markers are probed with a small volume (40 μl) of diluted sample and with a digoxigenylated secondary monoclonal antibody. The secondary antibody is then detected by the addition of an anti-digoxigenin antibody conjugated to a fluorescent latex label. This label enables highly sensitive detection of <10 individual binding events in a single spot, down to a fmol/l concentration. After a final incubation, chips are transferred to a detection unit where a charge-coupled device camera creates an image that is converted to signal intensities, and fluorescence intensity of the array features is quantified by image analysis.

In the present study, we developed an assay for the determination of anti-infliximab antibodies. For that purpose, infliximab was used as a biotinylated Fab fragment and spotted onto the streptavidin-coated surface of the chip. Patient samples were diluted 1:50 in a specific dilution buffer and each chip was probed with 40 μl of a

diluted patient sample. In order to minimize interference, the buffer contained interference minimizing substances, among them Fab-poly antibodies. After a washing step, each chip was probed with 40 μl of a digoxigenylated mouse monoclonal IgG antibody. A serial dilution of rabbit polyclonal anti-infliximab antibody was used as a standard. The lower detection limit of the indirect assay was determined at 0.27 ng/ml. A preliminary cut-off for the assay was determined using 100 blood donor samples and 218 baseline samples from the Copenhagen cohort. A sample was considered positive if the signal was at least 2-fold above the highest signal seen in any blood donor or baseline sample, otherwise as negative. This preliminary cut-off was 75 ng/ml.

We determined inter-assay coefficient variation (CV) by measuring three samples with low (4.3 ng/ml), medium (87.4 ng/ml) and high (246.3 ng/ml) anti-infliximab antibody levels, respectively, in six independent runs on different days. Intra-assay CV was determined in samples with low (4.3 ng/ml), medium (87.4 ng/ml) and high (246.3 ng/ml) anti-infliximab antibody levels each measured 21 times. We determined the functional sensitivity, defined as the level corresponding to 20% inter-assay CV in five samples spiked with calibrator material (polyclonal rabbit antibodies fused to human IgG) measured in five independent runs. The accuracy was determined in nine different donor serum samples and horse serum samples by measuring the recoveries of a fixed amount of either strongly positive sample or a fixed amount of calibrator material (polyclonal rabbit antibodies fused to human IgG). The recovery of spiked analyte was calculated considering the intrinsic anti-infliximab antibody level of each sample, which was close to 0 for all samples (range 0–0.1 ng/ml). We determined the functional sensitivity in five samples spiked with calibrator material (polyclonal rabbit antibodies fused to human IgG) measured in five independent runs. Infliximab interference was tested in 30 samples selected according to anti-infliximab antibody level. Samples were grouped into weak positive (<150 ng/ml), medium positive (150–2000 ng/ml) and strongly positive (>2000 ng/ml). Each sample was spiked with 0, 1, 100 and 100 μg/ml infliximab, respectively. IgM and IgG RF interference was tested in 146 IgM RF-positive (IgM > 20 U/ml) baseline samples from the Copenhagen cohort.

Infliximab trough levels

Serum infliximab trough levels were measured at baseline, week 14 and week 52 or when infliximab treatment was terminated. All samples were measured at Biomonitor ApS using RIA (Biomonitor ApS, Denmark), as previously described [8].

Statistical analysis

Descriptive statistics for continuous variables are presented as medians and range. Categorical variables are presented as frequencies and percentages. Patients were dichotomized into patients with detectable anti-infliximab antibodies and patients without detectable anti-infliximab antibodies using the cut-off level of 75 ng/ml. In addition, patients were classified using the EULAR response classification, the sustained low disease activity definition (DAS28 < 3.2) and the sustained remission definition (DAS28 < 2.6), respectively [16]. Differences between groups were analysed using χ^2 or Mann–Whitney *U* statistics as appropriate. The threshold for significance was set at a two-sided *P*-value <0.05. Kaplan–Meier plots were used to estimate the probability of drug survival, sustained low disease activity and sustained remission. Drug survival in patients with detectable anti-infliximab antibodies after 6 weeks and 14 weeks of treatment were compared with drug survival in patients without detectable anti-infliximab antibodies using log-rank statistics and hazard ratio (HR). Sustained low disease activity and sustained remission in patients with detectable anti-infliximab antibodies at week 52 were compared with sustained low disease activity and sustained remission in patients without detectable anti-infliximab antibodies using log-rank statistics and HR. All data were analysed using PASW Statistics 18 (SPSS Inc., Chicago, IL, USA). *P*-values <0.05 were considered significant.

Results

IMPACT assay

Inter- and intra-assay CV were 11.2% and 6.4%, respectively. The functional sensitivity was 2.4 ng/ml. Recoveries were within a range of ±12% with one exception (17%). In spiking experiments of infliximab interference, weakly positive anti-infliximab antibody samples showed borderline/negative assay results starting from 10 μg/ml infliximab, while medium and strongly positive anti-infliximab antibody samples showed borderline/negative assay results starting from 1000 μg/ml infliximab. Spiking with 100 μg/ml infliximab showed a positive assay result in nearly all samples classified as medium or strongly positive. The IgM RF interference test showed slightly elevated unspecific signals between 80 and 194 counts, corresponding to 3.3–11.2 ng/ml anti-infliximab antibodies in 4 of 146 IgM RF-positive baseline samples. All other samples showed signals below 40 counts, corresponding to 1.8 ng/ml anti-infliximab antibodies. The distribution of the signals did not differ between IgM RF-positive patients and IgM RF-negative patients, therefore the elevated signals in the four samples was due to non-specific binding rather than to specific IgM RF interference. Similarly, no IgG RF interference was observed. Supplementary Fig. S1A, available at *Rheumatology* Online, shows a method comparison experiment using 571 random serum samples from the Copenhagen cohort of patients with RA. The figure illustrates (shaded area) that several serum samples with anti-infliximab antibody levels below the detection limit when measured with a commercial assay [8] were positive when measured with the IMPACT assay.

Baseline characteristics

Baseline characteristics of the 218 patients with RA are given in Table 1. The majority of patients (80%) were women with median age 56 years and a median disease

Table 1 Baseline characteristics and clinical responses

Variable	All	Anti-infliximab AB+	Anti-infliximab AB−
No. of patients	218	118	100
Demographics			
Age, years	56 (21–86)	56 (21–86)	57 (25–86)
Women	175 (80)	98 (83)	77 (77)
Disease duration	6 (0–56)	6 (0–56)	5 (0–47)
Ever smokers[a]	132 (65)	64 (68)	64 (62)
Glucocorticoids	53 (24)	25 (21)	28 (28)
MTX	181 (91)	95 (90)	86 (91)
MTX dose, mg/week	20 (0–25)	20 (0–25)	22.5 (0–25)
Laboratory values at baseline			
IgM-RF positive	141 (65)	84 (71)	57 (57)
Anti-CCP positive[b]	59 (53)	38 (58)	21 (46)
Serum CRP, mg/l	13 (3–280)	14 (3–280)	12 (4–76)
Disease activity measures at baseline			
HAQ score (0–3)	1.250 (0–3.0)	1.250 (0–3.0)	1.375 (0–2.8)
Pain score (0–100)	58 (2–100)	61 (2–100)	55 (3–100)
Patient global score (0–100)	62 (0–100)	62 (2–100)	62 (0–100)
Physician's global score (0–100)	47 (0–95)	46 (0–95)	49 (0–95)
DAS28	5.0 (1.6–8.2)	5.0 (1.8–8.2)	5.0 (1.6–7.8)
Clinical response at week 14			
DAS28	3.4 (2.2–4.6)	3.6 (1.7–7.6)	3.2 (1.6–7.3)
EULAR good response[c]	32 (18)	15 (15)	17 (22)
EULAR moderate response[c]	68 (39)	43 (44)	25 (33)
EULAR no response[c]	74 (43)	40 (41)	34 (45)

Anti-infliximab AB+: patients with anti-infliximab antibodies during the 52-week follow-up. Anti-infliximab AB−: patients without anti-inflimixab antibodies during the 52-week follow-up. Values are given as median (range) or as number (percentage of total). [a]Six patients had missing smoking data; [b]107 patients had missing anti-CCP values; [c]21 patients had missing clinical data.

duration 6 years, 65% were IgM RF positive and 56% were anti-CCP antibody positive.

Anti-infiximab antibodies and infliximab trough levels

During the 52-week follow-up, anti-infliximab antibodies were detected in a total of 118 patients (54%). After 6 weeks of treatment 39 of the 118 anti-infliximab antibody-positive patients (33%) had detectable anti-infliximab antibodies. After 14 weeks it was 79 of 118 patients (67%), while 92 of 118 patients (78%) had detectable anti-infliximab antibodies after 28 weeks of treatment (supplementary Fig. S1B, available at *Rheumatology* Online). After 14 weeks of treatment the median infliximab trough level was 0.22 μg/ml (range 0–221.6 μg/ml), while the median infliximab trough level was 0.13 μg/ml (range 0–135.7 μg/ml) after 52 weeks of treatment. Patients with detectable anti-infliximab antibodies after 14 weeks of treatment had lower median infliximab trough levels compared with patients without detectable anti-infliximab antibodies [0 μg/ml (0–70.4 μg/ml) *vs* 0.375 μg/ml (0–221.6 μg/ml), $P < 0.001$]. Similarly, patients with detectable anti-infliximab antibodies after 52 weeks of treatment had lower median infliximab trough levels compared with patients without detectable anti-infliximab antibodies [0 μg/ml (0–7.40 μg/ml) *vs* 0.29 μg/ml (0–135.70 μg/ml), $P < 0.001$] (supplementary Fig. 2, available at *Rheumatology* Online).

Formation of anti-infliximab and risk of withdrawal

The time course of withdrawal is summarized in supplementary Fig. S3, available at *Rheumatology* Online. Overall, 136 patients completed 52 weeks of treatment, while 82 patients withdrew. In the 50 patients (23%) who withdrew due to treatment failure, the median DAS28 at termination was 4.9 (IQR 4.35–5.56). When patients were stratified according to anti-infliximab antibody status, 51 (43%) of the 118 anti-infliximab antibody-positive patients withdrew during the 52-week follow-up due to treatment failure ($n = 30$) and adverse drug reactions ($n = 21$). Of the 100 anti-infliximab antibody-negative patients, 31 (31%) withdrew due to treatment failure ($n = 20$) and adverse drug reactions ($n = 7$). The number of patients and reasons for withdrawal during follow-up are summarized in Table 2 and supplementary Table S1, available at *Rheumatology* Online. Patients with detectable anti-infliximab antibodies during the 52-week follow-up had an increased risk of adverse drug reactions compared with patients without detectable anti-infliximab antibodies [21 (18%) *vs* 7 (7%), $P < 0.018$]. Patients with detectable anti-infliximab antibodies during the 52-week follow-up had an increased risk of infusion reactions [17 (14%) *vs* 0 (0%), $P < 0.001$]. Twelve of 17 patients (71%) who withdrew due to infusion reactions had detectable anti-infliximab antibodies after 6 weeks of treatment. Patients with detectable anti-infliximab antibodies after 6 weeks of treatment had an increased risk of withdrawal due to adverse drug

Table 2 Reason for withdrawal during the 52-week follow-up

	No. (%) of patients		
	Total ($n = 218$)	Anti-infliximab AB+ ($n = 118$)	Anti-infliximab AB− ($n = 100$)
Completed treatment	136 (62)	67 (57)	69 (69)
Withdrawn			
Treatment failure	50 (23)	30 (25)	20 (20)
Adverse drug reaction	28 (13)	21 (18)[a]	7 (7)[a]
Infusion reaction	17 of 218 (8)	17 of 118 (14)[b]	0 of 100 (0)[b]
Urticaria	2 of 218 (1)	1 of 118 (1)	1 of 100 (1)
Infection	2 of 218 (1)	1 of 118 (1)	1 of 100 (1)
Exanthema	2 of 218 (1)	1 of 118 (1)	1 of 100 (1)
Polyneuropathia	1 of 218 (1)	0 of 118 (0)	1 of 100 (1)
Other	4 of 218 (2)	1 of 118 (1)	3 of 100 (3)
Other	4 (2)	0 (0)	4 (4)
Total withdrawn	82 (38)	51 (43)	31 (31)

Anti-infliximab AB+: patients with anti-infliximab antibodies during the 52-week follow-up. Anti-infliximab AB−: patients without anti-infliximab antibodies during the 52-week follow-up. [a]Anti-infliximab AB+ patients had an increased risk of adverse drug reactions during the 52-week follow-up compared with patients without anti-infliximab antibodies ($P < 0.018$). [b]Anti-infliximab AB+ patients had an increased risk of infusion reactions during the 52-week follow-up compared with patients without anti-infliximab antibodies ($P < 0.001$).

reactions during the 52-week follow-up compared with patients without anti-infliximab antibodies (HR = 5.06, 95% CI 2.36, 10.84; $P < 0.0001$) (Fig. 1A). Similar results were found for patients with detectable anti-infliximab antibodies after 14 weeks of treatment (HR = 3.30, 95% CI 1.56, 6.99; $P = 0.0009$) (Fig. 1C). There was no significant association between anti-infliximab antibody status after 6 and 14 weeks of treatment and withdrawal due to treatment failure during the 52-week follow-up (Fig. 1B and D).

Formation of anti-infliximab antibodies and treatment response

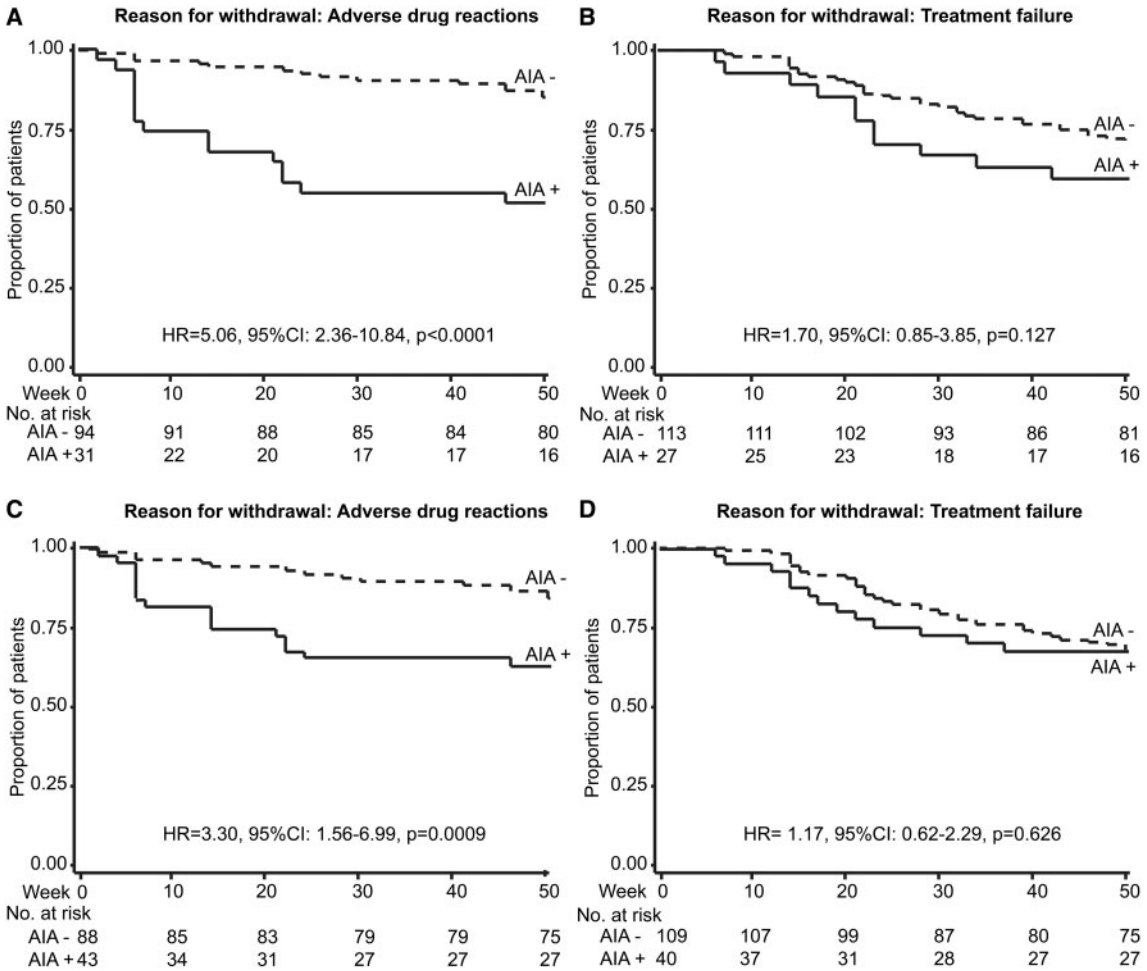
In this analysis, we included only patients with a DAS28 $\geqslant 3.2$ at baseline ($n = 175$ patients). In total, 64 (37%) patients were classified as primary responders, 67 (38%) patients as primary non-responders and 32 (19%) patients as secondary non-responders. Twelve patients (6%) had only one follow-up visit after initiation of infliximab. Secondary non-responders had lower median serum infliximab trough levels than primary non-responders [0 μg/ml (0–91.3 μg/ml) *vs* 0.215 μg/ml (0–221.6 μg/ml), $P = 0.012$]. There was no difference in median serum infliximab levels between primary responders and primary/secondary non-responders [0.155 μg/ml (0–114 μg/ml) *vs* 0.140 μg/ml (0–221.6 μg/ml), $P = 0.548$]. Of the 175 patients, 83 (47%) had detectable anti-infliximab antibodies during the 52-week follow-up. There was no difference in anti-infliximab antibody status between patients classified as primary responders and primary non-responders [27 (42%) *vs* 30 (45%), $P = 0.765$]. Fewer primary responders had detectable anti-infliximab antibodies in serum than secondary non-responders [27 (48%) *vs* 22 (69%), $P = 0.014$]. More patients with secondary non-response had detectable anti-infliximab antibodies in serum than patients with primary non-response [22 (69%) *vs* 30 (45%), $P = 0.025$]. Patients with detectable anti-infliximab were less likely to achieve sustained minimal disease activity (DAS28 <3.2) compared with patients without detectable anti-infliximab antibodies (HR = 0.49, 95% CI 0.27, 0.92, $P = 0.023$) (Fig. 2A). Similarly, patients with detectable anti-infliximab antibodies were less likely to achieve sustained remission (DAS28 < 2.6) compared with patients without (HR = 0.53, 95% CI 0.28, 0.98; $P = 0.04$) (Fig. 2B).

Discussion

To our knowledge, this is the first study demonstrating that early formation of anti-infliximab antibodies increases the risk of serious and potentially life-threatening adverse drug reactions in patients with RA treated with TNF inhibitors in clinical practice. Patients with detectable anti-infliximab antibodies had lower median infliximab trough levels and were less likely to achieve sustained minimal disease activity and remission.

In accordance with previous studies [8, 10, 24], our results showed that early formation of anti-infliximab antibodies was common in patients with RA during infliximab treatment despite concomitant MTX. In our study, only IgM RF and glucocorticoid treatment at baseline differed between patients with and without detectable anti-infliximab antibodies. It is largely unknown why some patients develop ADAs against TNF inhibitors [24]. One study reported that patients with detectable anti-infliximab antibodies that switch to adalimumab are more prone to develop anti-adalimumab antibodies than TNF-naïve patients, suggesting a genetic disposition [25]. This is supported by a study that identified an association between IL-10 polymorphisms and increased formation of anti-adalimumab antibodies in patients with RA treated with adalimumab [26].

Fig. 1 Drug survival in relation to antibody status after 6 weeks and 14 weeks of treatment.



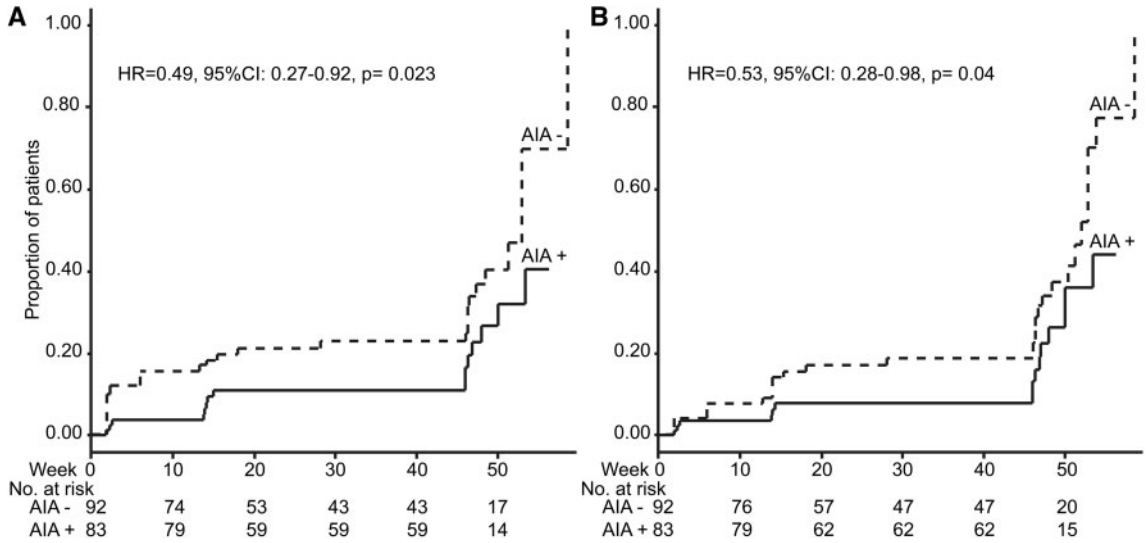
In (**A**) and (**C**), the reason for withdrawal was adverse drug reactions and patients withdrawn due to other reasons were not included. In (**B**) and (**D**), the reason for withdrawal was treatment failure and patients withdrawn due to other reasons were not included. AIA+: patients with detectable anti-infliximab antibodies. AIA−: patients without detectable anti-infliximab antibodies.

ADAs have been suggested to cause adverse drug reactions, including anaphylactic reactions, during TNF inhibitor therapy, but data are limited [7, 27]. We found that patients with detectable anti-infliximab antibodies after either 6 or 14 weeks had an increased risk of adverse drug reactions during the 52-week follow-up.

In a recent study by Bartelds *et al.* [16], patients with detectable anti-adalimumab antibodies during the 156-week follow-up were less likely to achieve sustained minimal disease activity and sustained remission compared with patients without detectable anti-adalimumab antibodies. This was also demonstrated in our study. In contrast, we did not find any association between antibody status and withdrawal due to treatment failure. Differences between adalimumab and infliximab with regard to dosing regimens, pharmacokinetic properties and immunogenicity might explain this lack of association. Intravenous administration of infliximab results in high initial serum levels and large fluctuations, whereas serum levels of adalimumab are relatively constant and reach steady state in 2 weeks. Another reason for lack of association might be differences in outcome measures. In our study, the decision to withdraw treatment was made by the treating physician, whereas sustained low disease activity and remission are more objective criteria. Furthermore, infliximab treatment initiated during the first years of post-marketing was less likely to be withdrawn by the clinicians due to the limited availability of alternative biologic agents. The time of follow-up was longer in our study than in the study by Bartelds *et al.* [16]. One may also hypothesize that differences between the two cohorts with regard to treatment with glucocorticoids and DMARDs, erosive disease and smoking status may, at least in part, explain some of the differences between the two studies.

Patients with detectable anti-infliximab antibodies had lower median infliximab trough levels compared with patients without detectable anti-infliximab antibodies. However, a few patients had high infliximab trough

FIG. 2 Sustained minimal disease activity and sustained remission.



Sustained minimal disease activity (**A**) and sustained remission (**B**) in patients classified according to anti-infliximab status during the 52-week follow-up. AIA+: patients with detectable anti-infliximab antibodies. AIA−: patients without detectable anti-infliximab antibodies.

levels despite being anti-infliximab antibody positive, because they had blood samples drawn after infliximab infusion. Patients with secondary non-response had lower median infliximab levels in serum and more often detectable anti-infliximab antibodies compared with patients with primary non-response. This supports the hypothesis that secondary non-response is caused by ADAs and that patients with primary non-response to one TNF inhibitor are more likely to benefit from another TNF inhibitor than to dosage escalation [17].

Different methods including ELISA and RIA can be used to assess ADA in patients with RA treated with TNF inhibitors [7–10, 15, 18–20, 28–31]. Some assays have poor sensitivity, and interaction with non-specific immunoglobulins or IgG RF and the active drug may generate false-negative results [20, 28–31]. In our study, anti-infliximab antibodies were measured in 218 patients with RA using a newly developed highly sensitive and fast assay. No cross-reactivity or interference with rheumatoid factors (IgG, IgA and IgM subclasses) was observed. In spiking experiments, weakly positive samples showed borderline/negative assay results starting from 10 μg/ml infliximab. Thus the assay may underestimate the level of anti-infliximab antibodies and hence the number of patients with a positive anti-infliximab antibody titre may actually be higher. Several publications report an infliximab trough level of between 5 and 10 μl/ml. In weakly positive serum samples, these levels would lead to false-negative results using the IMPACT assay.

Some strengths of our study are the high sensitivity of the IMPACT assay and the well-characterized patient cohort comprising RA patients treated with infliximab. Furthermore, all clinical variables were registered prospectively in the DANBIO registry at each visit. Some limitations must be taken into account when interpreting the results. The patients in our study were heterogeneous with regard to disease duration and disease severity. Patients initiating infliximab treatment during the first years of post-marketing use often had longer disease duration, more severe disease and more joint destruction compared with patients initiating infliximab treatment in 2008. Patients with severe long-lasting disease might experience less benefit from treatment than RA patients with shorter disease duration.

In conclusion, the present study of patients with RA treated with infliximab in routine care demonstrated that early formation of anti-infliximab antibodies increased the risk of adverse drug reactions. Furthermore, patients with detectable anti-infliximab antibody formation during 52 weeks were less likely to achieve sustained minimal disease activity and remission. Thus assessment of anti-infliximab antibodies may support the identification of patients who are likely to develop adverse drug reactions and patients who are less likely to respond convincingly to infliximab treatment.

Rheumatology key messages

- Early formation of anti-infliximab antibodies is common in patients with RA treated with infliximab.
- Anti-infliximab antibodies increase the risk of adverse drug reactions, including infusion reactions, in RA patients.
- Anti-infliximab antibodies decrease the likelihood of sustained minimal disease activity and remission in RA patients.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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