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The formation of autoantibodies and antibodies to TNF- α blocking agents in relation to clinical response in patients with ankylosing spondylitis

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Abstract

Objective

To investigate the influence of antibody formation to TNF- α blocking agents on the clinical response in AS patients treated with infliximab (IFX), etanercept (ETA), or adalimumab (ADA), and to investigate the development of ANA, ANCA, and anti-dsDNA antibodies in association with the formation of antibodies to TNF- α blocking agents.

Methods

Consecutive AS outpatients with active disease who started treatment with IFX (n=20), ETA (n=20), or ADA (n=20) were included in this longitudinal observational study. Clinical data were collected prospectively at baseline and after 3, 6, and 12 months of anti-TNF- α treatment. At the same time points, serum samples were collected. In these samples, antibodies to TNF- α blocking agents, serum TNF- α blocker levels, and ANA, ANCA, and anti-dsDNA antibodies were measured retrospectively.

Results

Anti-IFX, anti-ETA, and anti-ADA antibodies were induced in 20%, 0%, and 30% of patients, respectively. Although ANA, ANCA, and anti-dsDNA antibodies were detected during anti-TNF- α treatment, no significant association was found between the presence of these autoantibodies and the formation of antibodies to TNF- α blocking agents. Patients with anti-IFX or anti-ADA antibodies had significantly lower serum TNF- α blocker levels compared to patients without these antibodies. Furthermore, significant negative correlations were found between serum TNF- α blocker levels and assessments of disease activity.

Conclusion

This study indicates that antibody formation to IFX or ADA is related to a decrease in efficacy and early discontinuation of anti-TNF- α treatment in AS patients. Furthermore, autoantibody formation does not seem to be associated with antibody formation to TNF- α blocking agents.

Key words

Ankylosing spondylitis, TNF- α blocking therapy, antibody formation, autoantibodies, clinical response

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Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory rheumatic disease that primarily affects the axial skeleton. Peripheral joints and extra-articular structures may also be involved. The availability of the tumour necrosis factor alpha (TNF- α) blocking agents infliximab (IFX), etanercept (ETA), and adalimumab (ADA) has significantly improved outcome in AS (1). Although the majority of patients respond very well to these TNF- α blocking agents, approximately 30% fail to reach efficacy (2-4). One possible explanation for this failure could be that the formation of antibodies to TNF- α blocking agents may decrease serum TNF- α blocker levels. Previous studies in rheumatoid arthritis (RA) have shown that the development of neutralising antibodies is associated with allergic reactions and reduced response to treatment (5-7). Recently, de Vries *et al.* published 3 studies on antibody formation to TNF- α blocking agents in different AS cohorts. They detected antibodies in 29% and 31% of the patients treated with IFX and ADA, respectively, and no antibodies in patients treated with ETA (8-10). Unlike several other inflammatory auto-immune diseases, there are no disease specific autoantibodies found in AS. However, the presence of well-known autoantibodies such as antinuclear antibodies (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), and anti-double-stranded DNA (anti-dsDNA) antibodies has been described in AS patients (11, 12). Furthermore, previous studies have shown that ANA and anti-dsDNA antibodies can be induced during anti-TNF- α treatment (12-15). Since patients who produce autoantibodies have a more activated immune system, it may be suggested that these patients are more prone to the production of antibodies to TNF- α blocking agents.

The objective of the present study was to investigate the influence of antibody formation to TNF- α blocking agents on the clinical response in AS patients treated with IFX, ETA, or ADA in daily clinical practice. The second goal was to investigate the development of ANA, ANCA, and anti-dsDNA antibodies in

association with the formation of antibodies to TNF- α blocking agents.

Patients and methods

Patients

Consecutive AS outpatients with active disease who started treatment with infliximab (IFX, n=20), etanercept (ETA, n=20), or adalimumab (ADA, n=20) at the Medical Center Leeuwarden (MCL) or the University Medical Center Groningen (UMCG) were included in this longitudinal observational study. All patients were aged ≥ 18 years, fulfilled the modified New York criteria for AS (16), and started anti-TNF- α treatment because of active disease according to the Assessments in Ankylosing Spondylitis (ASAS) consensus statement (17). Patients were excluded if they had previously received anti-TNF- α treatment. IFX (5mg/kg) was given intravenously at 0, 2 and 6 weeks and then every 8 weeks. ETA was administered as a subcutaneous injection once (50 mg) or twice (25 mg) a week. ADA (40 mg) was administered as a subcutaneous injection every 2 weeks. The choice of the TNF- α blocking agent was based on the judgment of the treating rheumatologist and/or the specific preference of the patient. Patients were allowed to receive concomitant medication as usual in daily clinical practice. The study was approved by the local ethics committees of the MCL and UMCG and all patients provided written informed consent to participate in this study.

Clinical assessments

Clinical data were collected prospectively at baseline and after 3, 6, and 12 months of anti-TNF- α treatment. Continuation of anti-TNF- α treatment was based on decrease in Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), amounting to 50% or 2 units compared with baseline, and/or expert opinion in favour of treatment continuation. Response to anti-TNF- α treatment was defined using BASDAI and ASAS20 response criteria. ASAS20 response was defined as an improvement of at least 20% and absolute improvement of at least 1 unit (on a scale of 0-10) compared with baseline

Competing interests: none declared.

in 3 or more of the 4 domains: physical function (Bath Ankylosing Spondylitis Functional Index (BASFI)), pain, patient's global assessment of disease activity, and inflammation (mean from BASDAI questions 5 and 6), with no worsening by more than 20% in the remaining domain (18). Disease activity was assessed using BASDAI, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and the ASAS-endorsed disease activity score (ASDAS) calculated from BASDAI questions 2, 3 and 6, patient's global assessment of disease activity, and CRP (19, 20).

Laboratory assessments

Antibodies to TNF- α blocking agents, serum TNF- α blocker levels, and ANA, ANCA, and anti-dsDNA antibodies were measured retrospectively at baseline and after 3, 6, and 12 months of anti-TNF- α treatment. Analyses in samples from one patient were always performed in one assay to avoid inter-assay variability. Samples were stored at -20°C until analysis.

Anti-IFX and anti-ADA antibodies were detected by radioimmunoassay (RIA) as described in detail previously (5, 6). The assay measures specific high-avidity IgG antibodies to IFX or ADA by an antigen-binding test. In short, 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 ¹²⁵I-radiolabeled F(ab)'2 fragment of IFX or ADA was added. After overnight incubation, non-bound radiolabel was washed away and Sepharose-bound radioactivity was measured. Test results were converted into arbitrary units per milliliter (AU/ml) by comparison with dilutions of a reference serum. The reference value was set at 12 AU/ml, as derived from 100 healthy donors. Anti-ETA antibodies were detected using a two-site assay RIA using Sepharose-immobilised ETA as solid phase for capturing ETA-specific antibody and ¹²⁵I-radiolabelled ETA for detection (9). It should be noted that antibodies to the TNF- α blocking agent may be

underestimated or undetectable in patients with high serum concentrations of TNF- α blocker since the presence of TNF- α blocking agent interferes with the assay.

Serum IFX, ETA, and ADA levels were measured by enzyme-linked immunosorbent assay (ELISA; Sanquin, Amsterdam, The Netherlands) as described previously (8, 9). The ELISA is based on the principle that the TNF- α blocking agent is captured through its ability to bind TNF- α . The sensitivity of detection for IFX, ETA and ADA was 0.0003 μ g/ml, 0.001 μ g/ml, and 0.001 μ g/ml, respectively.

Serum samples were tested for ANA by indirect immunofluorescence using fixed Hep-2000 cells (ImmunoConcepts, Biomedical Diagnostics, Antwerp, Belgium) as recommended by the manufacturer. ANA titer and pattern were reported. An ANA titer \geq 1:40 was considered positive. Detection of ANCA by indirect immunofluorescence was performed on ethanol-fixed granulocytes as described before (21, 22) with minor modifications (23). An ANCA titer \geq 1:40 was considered positive. Anti-dsDNA antibodies were measured by Farr RIA (Siemens Healthcare Diagnostics, Breda, The Netherlands) and

by anti-dsDNA IgG and IgM ELISA on the Alegria (ORGENTEC, supplied by Siemens Healthcare Diagnostics). The Farr assay detects both IgG and IgM antibodies against dsDNA using ¹²⁵I-labelled recombinant dsDNA as a substrate. A result \geq 10 IU/ml was considered positive. To be able to distinguish between IgG and IgM responses, all samples positive for anti-dsDNA in the Farr assay were also measured in the separate IgG and IgM assays on the Alegria. A result \geq 20 IU/ml was considered positive.

Statistical analysis

Statistical analysis was performed with SPSS 16.0 software (SPSS, Chicago, IL, USA). Results were expressed as mean \pm SD or median (range) for parametric and nonparametric data, respectively. The Independent Samples *t*-test and Mann-Whitney U-test were used to compare differences between groups. The Chi-Square test and Fisher Exact test were used to compare percentages between groups. The Paired Samples *t*-test and Wilcoxon Signed Rank test were used to compare differences within groups. The Fisher Exact test was used to analyse the relationship between the presence or absence

Table 1. Baseline characteristics of the ankylosing spondylitis study population.

N	Total 60	IFX 20	ETA 20	ADA 20
Gender (male) (%)	38 (63)	12 (60)	13 (65)	13 (65)
Age (years)	42.7 \pm 11.4	46.6 \pm 11.0	39.4 \pm 9.0	42.2 \pm 13.1
Duration of symptoms (years)	18 (1-49)	24 (3-49)*	15 (4-30)	8 (1-34)
Time since diagnosis (years)	8 (0-35)	19 (1-35) [†]	8 (1-25)	5 (0-34)
HLA B27 positive (%)	49 (84)	18 (90)	17 (85)	16 (80)
History of IBD (%)	7 (12)	5 (25) [‡]	0 (0)	2 (10)
History of uveitis (%)	17 (28)	10 (50) [†]	5 (25)	2 (10)
History of psoriasis (%)	6 (10)	3 (15)	1 (5)	2 (10)
History of peripheral arthritis (%)	17 (28)	6 (30)	6 (30)	5 (25)
Concomitant NSAID use (%)	53 (88)	19 (95)	18 (90)	16 (80)
Concomitant DMARD use (%)	14 (23)	6 (30)	4 (20)	4 (20)
BASDAI (range 0-10)	5.9 (2.1-9.8)	5.9 (3.6-8.4)	5.8 (3.5-9.0)	5.5 (2.1-9.8)
CRP (mg/l)	17 (2-92)	18 (3-70)	14 (3-55)	18 (2-92)
ESR (mm/h)	25 (2-90)	24 (4-90)	24 (3-80)	27 (2-74)
ASDAS	3.8 (1.4-5.8)	3.8 (2.4-5.1)	3.8 (2.6-5.1)	3.8 (1.4-5.8)

Values are mean \pm SD or median (range) unless otherwise indicated. IFX: infliximab; ETA: etanercept; ADA: adalimumab; HLA B27: human leukocyte antigen B27; IBD: inflammatory bowel disease; NSAID: non-steroidal anti-inflammatory drug; DMARD: disease-modifying antirheumatic drug; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; ASDAS: ASAS-endorsed disease activity score.

*Statistical difference ($p < 0.05$) calculated with respect to values of the ETA and ADA groups.

[†]Statistical difference ($p < 0.05$) calculated with respect to values of the ADA group.

[‡]Statistical difference ($p < 0.05$) calculated with respect to values of the ETA group.

Table II. Antibodies to TNF- α blocking agents and autoantibodies in the IFX (n=20), ETA (n=20), and ADA (n=20) groups.

	Present at baseline	Induction during treatment
<i>IFX</i>		
Anti-IFX (%)	0 (0)	4 (20)
ANA (%)	10 (50)	6 (30)
ANA \geq 1:80 (%)	4 (20)	3 (15)
ANCA (%) [†]	3 (15)	3 (15)
Anti-dsDNA Farr (%)	0 (0)	6 (30)
Only IgM	–	4
Only IgG	–	–
Both IgM and IgG	–	1
IgM and IgG negative [‡]	–	1
<i>ETA</i>		
Anti-ETA (%)	0 (0)	0 (0)
ANA (%)	12 (60)	3 (15)
ANA \geq 1:80 (%)	4 (20)	1 (5)
ANCA (%) [†]	1 (5)	3 (15)
Anti-dsDNA Farr (%)	0 (0)	4 (20)
Only IgM	–	1
Only IgG	–	–
Both IgM and IgG	–	1
IgM and IgG negative [‡]	–	2
<i>ADA</i>		
Anti-ADA (%)	0 (0)	6 (30)
ANA (%)	6 (30)	4 (20)
ANA \geq 1:80 (%)	4 (20)	3 (15)
ANCA (%) [†]	1 (5)	3 (15)
Anti-dsDNA Farr (%)	0 (0)	6 (30)
Only IgM	–	–
Only IgG	–	1
Both IgM and IgG	–	3
IgM and IgG negative [‡]	–	2

ANA: antinuclear antibodies; ANCA: anti-neutrophil cytoplasmic antibodies; anti-dsDNA Farr: anti-double-stranded DNA measured by Farr. See Table I for other definitions.

[†]The majority of positive ANCA showed a *p*-ANCA pattern of fluorescence.

[‡]No positive IgG or IgM anti-dsDNA response was seen in the Alegria test, mostly due to responses just below the reference value of the assays.

of antibodies to TNF- α blocking agent and the presence or absence of ANA, ANCA, and anti-dsDNA antibodies. The Spearman's correlation coefficient was used to analyse the relationship between serum levels of the TNF- α blocking agent and BASDAI, CRP, ESR, and ASDAS. Patients who had temporarily stopped their anti-TNF- α treatment in the period just before their 3, 6, or 12 month visit were excluded from the corresponding analysis. *P*-values <0.05 were considered statistically significant.

Results

Mean age of all patients (n=60) was 42.7 years (SD \pm 11.4), median disease duration was 18 years (range 1-49), and 63% were male. The 3 treatment groups were comparable for age, gen-

der, HLA-B27 status, and baseline BASDAI, CRP, ESR, ASDAS, and concomitant medication. Disease duration was significantly longer in the IFX group. Patients with a history of inflammatory bowel disease (IBD) were absent in the ETA group as a results of a distinct preference of IFX and ADA for these patients. The percentage of patients with a history of uveitis was significantly higher in the IFX group compared to the ADA group (Table I).

Antibody formation to TNF- α blocking agents

At baseline, 1 patient tested positive for anti-ADA antibodies (24 AU/ml) and these antibodies were still found after 3 months of treatment (18 AU/ml). In the treatment period of one year, antibodies to TNF- α blocking agents were in-

duced in 4 (20%) patients treated with IFX and in 6 (30%) patients treated with ADA. No antibodies to the TNF- α blocking agent were detected in patients treated with ETA (Table II). In 6 patients (IFX, n=1; ADA, n=5), the antibodies were detected for the first time at 3 months. In the remaining 4 patients (IFX, n=3; ADA, n=1), the antibodies were detected for the first time at 6 months. Serum concentrations of antibodies were between 12 and 5154 AU/ml. The formation of antibodies to TNF- α blocking agents was not related to the use of concomitant non-steroidal anti-inflammatory drugs (NSAIDs) or disease-modifying antirheumatic drugs (DMARDs) (data not shown).

Autoantibody formation

No statistically significant differences were found in ANA, ANCA, and anti-dsDNA formation between the 3 treatment groups. ANA and ANCA were present at baseline and new ANA and ANCA were induced during anti-TNF- α treatment in all 3 groups (Table II). ANA patterns varied between homogeneous, coarse speckled, and nucleolar. The majority of ANCA showed a *p*-ANCA fluorescence pattern. No anti-dsDNA antibodies were detected in the Farr assay at baseline. In all treatment groups, anti-dsDNA antibodies were induced during anti-TNF- α treatment in 20% to 30% of patients (Table II). In 33% to 50% of these patients, the anti-dsDNA antibodies disappeared at 12 months. Serum concentrations of anti-dsDNA autoantibodies were low, ranging from 10 to 23 IU/ml. The anti-dsDNA autoantibodies detected in the Farr assay were of IgM isotype in 5 patients, of IgG isotype in 1 patient, and of both isotypes in 5 patients, as determined by separate IgG and IgM assays on the Alegria. In the remaining 5 patients, no positive IgG or IgM anti-dsDNA response was seen in the Alegria test, mostly due to responses just below the reference value of the assays. The seroconversion to IgG anti-dsDNA antibodies was not associated with the appearance of clinically relevant lupus-like symptoms. No statistically significant association was found between the presence of

Table III. Serum TNF- α blocker levels, treatment response and reasons for treatment discontinuation of the IFX (n=20), ETA (n=20), and ADA (n=20) groups at 3, 6 and 12 months of anti-TNF- α treatment.

	At 3 months	At 6 months	At 12 months	Total
IFX				
Serum IFX levels ($\mu\text{g/ml}$)	30.6 \pm 30.0	36.3 \pm 43.2	23.4 \pm 26.9	
BASDAI response (%)	17 (85)	14 (70)	12 (60)	
ASAS20 response (%)	17 (85)	15 (75)	13 (65)	
Treatment discontinuation (%)	1 (5)	0 (0)	4 (20)	5 (25)
Allergic reaction, inefficacy	1			1
Inefficacy		3		3
Pregnancy wish		1		1
ETA				
Serum ETA levels ($\mu\text{g/ml}$)	3.1 \pm 1.2	2.8 \pm 1.6*	2.9 \pm 1.0	
BASDAI response (%)	15 (75)	12 (60)	15 (75)	
ASAS20 response (%)	15 (75)	15 (75)	14 (70)	
Treatment discontinuation (%)	2 (10)	0 (0)	0 (0)	2 (10)
Inefficacy	1			1
Cardiac complaints	1			1
ADA				
Serum ADA levels ($\mu\text{g/ml}$)	5.7 \pm 4.0	5.5 \pm 3.8	8.0 \pm 5.1	
BASDAI response (%)	11 (55) [†]	11 (55)	9 (45) ^{†††}	
ASAS20 response (%)	13 (65)	9 (45) ^{††}	10 (50)	
Treatment discontinuation (%)	3 (15)	4 (20)	1 (5)	8 (40)
Inefficacy	3	3	1	7
Diarrhea		1		1

Values are mean \pm SD unless otherwise indicated. See Table I for definitions.

*Statistical difference ($p < 0.05$) calculated with respect to values at 3 months.

[†]Statistical difference ($p < 0.05$) calculated with respect to values of the IFX group.

^{††}Trend to statistical difference ($p = 0.053$) calculated with respect to values of the IFX and ETA groups.

^{†††}Trend to statistical difference ($p = 0.053$) calculated with respect to values of the ETA group.

Table IV. Serum TNF- α blocker levels of patients with and without anti-IFX or anti-ADA antibodies at 3, 6, and 12 months of treatment.

	At 3 months	At 6 months	At 12 months
Serum IFX levels ($\mu\text{g/ml}$)			
Patients with anti-IFX antibodies	0.1 [†]	0.0 (0.0-1.1)*	0.6 (0.0-1.2)*
Patient without anti-IFX antibodies	20.3 (0.0-99.9)	32.7 (3.7-153.9)	15.1 (2.4-93.2)
Serum ADA levels ($\mu\text{g/ml}$)			
Patient with anti-ADA antibodies	1.5 (0.0-4.0)*	1.6 (0.0-2.4)*	0.0 [†]
Patient without anti-ADA antibodies	6.4 (3.5-16.6)	6.8 (5.9-11.4)	8.0 (3.2-16.3)

Values are median (range). See Table I for definitions.

*Statistical difference ($p < 0.05$) calculated with respect to values of patients without anti-IFX or anti-ADA antibodies.

[†]Anti-IFX or anti-ADA antibodies were detected in only 1 patient.

ANA, ANCA, or anti-dsDNA antibodies and the formation of antibodies to TNF- α blocking agents in both the IFX and ADA group.

Antibody formation to TNF- α blocking agents and ASAS20 response

In all 3 groups, BASDAI, CRP, ESR, and ASDAS significantly improved after 3, 6, and 12 months of anti-TNF- α treatment compared with baseline, except for ESR in the ADA group after 12 months of treatment (data not shown).

The percentage of patients that reached BASDAI response ranged from 60% to 85% for IFX, from 60% to 75% for ETA, and from 45% to 55% for ADA over time. At 3 months, the percentage of BASDAI responders was significantly lower for ADA compared to IFX. Furthermore, there was a trend suggesting a lower percentage of BASDAI responders to ADA compared to IFX at 12 months ($p = 0.053$). The percentage of patients that reached ASAS20 response ranged from 65% to 85% for

IFX, from 70% to 75% for ETA, and from 45% to 65% for ADA over time. At 6 months, there was a trend suggesting a lower percentage of ASAS20 responders to ADA compared to both IFX and ETA ($p = 0.053$) (Table III).

During the first year, 15 patients discontinued anti-TNF- α treatment because of inefficacy (IFX, n=3; ETA, n=1; ADA, n=7), adverse events (ETA, n=1; ADA, n=1), inefficacy and adverse events (IFX, n=1), or other reasons (IFX, n=1). There was a trend suggesting a higher percentage of patients that discontinued ADA treatment compared to ETA treatment ($p = 0.065$) (Table III). Of patients who discontinued anti-TNF- α treatment because of inefficacy, antibodies to TNF- α blocking agents were detected in 3 of 4 patients treated with IFX and in 4 of 7 patients treated with ADA. No antibodies were detected in the patient treated with ETA.

Antibodies to TNF- α blocking agents were also detected in 3 patients who continued anti-TNF- α treatment. One patient was BASDAI and ASAS20 responder at all time points, although anti-IFX antibodies were detected at 6 and 12 months (24 and 20 AU/ml, respectively), resulting in very low serum IFX levels (1.05 and 1.22 $\mu\text{g/ml}$, respectively). Also an increased ESR level was found in this patient at 12 months. In the second patient, anti-ADA antibodies were detected at 3 and 6 months (60 and 18 AU/ml, respectively), resulting in low serum ADA levels (1.81 and 1.64 $\mu\text{g/ml}$, respectively) and non-response. At 12 months, this patient reached BASDAI and ASAS20 response and antibodies were not found anymore. In the final patient, anti-ADA antibodies were detected at 3, 6 and 12 months (12, 5154 and 1120 AU/ml, respectively), resulting in low and undetectable serum ADA levels (2.77, 0.02 and 0.02 $\mu\text{g/ml}$, respectively) and increased ESR levels. However, the patient reached BASDAI and ASAS20 response at 12 months.

Serum TNF- α blocker levels and disease activity

No significant differences were found in serum IFX or ADA levels over time. Serum ETA levels were significantly

lower at 6 months compared to levels at 3 months (Table III). Patients with antibody formation to IFX or ADA had significantly lower serum TNF- α blocker levels compared to patients without these antibodies (Table IV).

Spearman correlations between serum levels of the TNF- α blocking agent and disease activity measured by BASDAI, CRP, ESR, and ASDAS are presented in Table V. No significant correlations were found between serum IFX levels and BASDAI, CRP, ESR, or ASDAS. Serum ETA levels were negatively correlated with CRP after 12 months and with ESR after 3, 6, and 12 months ($p < 0.05$). Serum ADA levels were negatively correlated with BASDAI after 6 months, with CRP after 12 months, with ESR after 6 months, and with ASDAS after 3 months ($p < 0.05$).

Discussion

The present study indicates that antibody formation to IFX or ADA is related to a decrease in efficacy and early discontinuation of anti-TNF- α treatment in daily clinical practice, since antibodies were detected in the majority of patients who discontinued IFX or ADA treatment due to inefficacy. Furthermore, patients with anti-IFX or anti-ADA antibodies had significantly lower serum TNF- α blocker levels compared to patients without these antibodies. Significant negative correlations were observed between serum ADA levels and BASDAI, CRP, ESR, or ASDAS, which suggests the importance of sufficiently high serum TNF- α blocker levels to obtain clinical response.

The percentage of patients that developed anti-IFX and anti-ADA antibodies was comparable. No anti-ETA antibodies were detected in this study, which is consistent with the report of de Vries *et al.* (9). The absence of antibodies to ETA may be explained by the fact that the dimeric fusion protein ETA has a less immunogenic structure in comparison with the 2 monoclonal antibodies IFX and ADA. Significant negative correlations between serum ETA levels and CRP or ESR were found, which again seems to reflect the importance of sufficiently high serum anti-TNF- α levels to obtain response

Table V. Spearman correlations between serum TNF- α blocker levels and BASDAI, CRP, ESR, and ASDAS of the IFX (n=20), ETA (n=20), and ADA (n=20) groups at 3, 6, and 12 months of anti-TNF- α treatment.

	BASDAI	CRP	ESR	ASDAS
<i>IFX</i>				
Serum IFX levels at 3 months	NS	NS	NS	NS
Serum IFX levels at 6 months	NS	NS	NS	NS
Serum IFX levels at 12 months	NS	NS	NS	NS
<i>ETA</i>				
Serum ETA levels at 3 months	NS	NS	-.561*	NS
Serum ETA levels at 6 months	NS	NS	-.519*	NS
Serum ETA levels at 12 months	NS	-.599*	-.808*	NS
<i>ADA</i>				
Serum ADA levels at 3 months	NS	NS	NS	-0.552*
Serum ADA levels at 6 months	-.570*	NS	-.604*	NS
Serum ADA levels at 12 months	NS	-.848*	NS	NS

NS: not significant. See Table I for other definitions.

*Statistical significant correlation ($p < 0.05$).

in terms of decreased inflammation. However, no significant correlation between serum ETA levels and BASDAI was found. BASDAI is a disease activity score solely based on the opinion of the patient. A possible explanation for these findings may thus be that patients with fewer subjective complaints are less compliant to therapy, which results in low serum ETA levels, high CRP and ESR levels, and normal BASDAI scores.

Interestingly, anti-ADA antibodies first appeared and then disappeared in one patient. This finding is in accordance with Wolbink *et al.*, who found that treatment continuation with IFX resulted in a decrease of anti-IFX antibodies in RA patients (6). However, they continued IFX treatment with increased dosages after the detection of anti-IFX antibodies and in the present study we did not change the dose of ADA. Our patient had relatively low anti-ADA antibody titers and low, but detectable serum ADA levels. Therefore, continuation of treatment may be effective in patients with low antibody titers, whereas switching to another TNF- α blocking agent may be a better therapeutic option in patients with high antibody titers.

When we compare clinical data of the IFX, ETA, and ADA groups, the response to ADA treatment seems to be somewhat lower in comparison with the other two agents, and the percentage of patients that discontinued ADA treatment seems to be somewhat higher

in comparison with ETA. However, the response rates to IFX and ETA found in this observational study were comparable to those reported in randomised clinical trials (24, 25), while the response rate to ADA was somewhat inferior to that reported in the literature (4). The small-sized treatment groups may have contributed to the differences in response rates in this study.

There were two unexpected findings in our study. First, one baseline sample behaved in an atypical manner, showing a slightly positive test result for antibodies against ADA. This is remarkable, since this patient had not yet received the drug at the time when the blood sample was drawn. Inhibition experiments did not lead to conclusive results, which means that we have to consider this sample as false-positive. This is a rare phenomenon which we have not encountered previously, neither during test validation using 100 supposedly negative samples, nor during extensive ADA testing which we perform routinely. Second, we found that 2 patients were responders to treatment according to the BASDAI and ASAS20 criteria, despite the presence of antibodies to the TNF- α blocking agent and low or undetectable serum TNF- α blocker levels. Moreover, ESR levels were increased in these patients. This indicates that it may be useful to consider more objective measures such as acute phase reactants as well as patient-related outcome measures when assessing clinical response to anti-TNF- α treatment in AS.

ANA and ANCA antibodies were present at baseline and additionally developed during anti-TNF- α treatment in all 3 groups. Anti-dsDNA antibodies were not present at baseline, but were induced during anti-TNF- α treatment in all 3 groups. Interestingly, the anti-dsDNA antibodies disappeared in approximately half of the patients after 12 months of treatment. The presence of anti-dsDNA antibodies did not seem to be clinically relevant in this study since serum concentrations were low and seroconversion to the more pathogenic IgG class anti-dsDNA antibodies was not associated with the appearance of lupus-like symptoms. These findings are in agreement with previous studies (12, 13, 26). To our knowledge, this is the first study that investigates the relation between autoantibodies and antibodies to TNF- α blocking agents. Although ANA, ANCA, and anti-dsDNA antibodies were detected in AS patients treated with TNF- α blocking agents, no significant association was found between the presence of these autoantibodies and the induction of antibodies to TNF- α blocking agents.

There are some limitations to this study. First, the 3 treatment groups were small and differed significantly in disease duration as well as in the percentage of patients with extra-articular manifestations. Second, the collection of blood samples was not linked to the timing of administration of the TNF- α blocking agent. The administration of anti-TNF- α treatment to patients with antibodies to TNF- α blocking agents results in the formation of immune complexes, which may accelerate the clearance of the applied agent and the antibodies (7). Therefore, the frequency of antibody formation to TNF- α blocking agents may have been underestimated in our study. In addition, the lack of standardisation of the collection of blood samples increases the variation in measured serum TNF- α blocker levels. Despite this variation, we still found significant negative correlations between serum anti-TNF- α levels and assessments of disease activity. Only in the IFX group, no significant correlation was found. This may be explained by the fact that the lack of standardisation of the col-

lection of blood samples influences the results of the IFX group most since IFX is administered every 8 weeks.

In conclusion, this longitudinal observational study in daily clinical practice indicates that the presence of ANA, ANCA, or anti-dsDNA antibodies is not associated with the formation of antibodies to TNF- α blocking agents. The percentage of patients that developed anti-IFX and anti-ADA antibodies was comparable in this study, while no anti-ETA antibodies were detected. The formation of anti-IFX and anti-ADA antibodies seems to be related to a decrease in efficacy and early discontinuation of anti-TNF- α treatment in AS patients, since anti-IFX or anti-ADA antibodies were detected in the majority patients who discontinued treatment due to inefficacy. Furthermore, patients with antibodies had significantly lower serum TNF- α blocker levels compared to patients without antibodies. Finally, significant negative correlations were found between serum levels of TNF- α blocking agents and assessments of disease activity. Further studies in larger groups of AS patients are needed to confirm the relation between antibody formation to TNF- α blocking agents, serum TNF- α blocker levels, and assessments of disease activity. In these studies, blood samples should be taken immediately before administration of the TNF- α blocking agent. However, based on the results of this study it seems useful to determine antibody formation to TNF- α blocking agents in non-responsive AS patients.

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