Antinuclear antibodies and anti-cyclic citrullinated peptide antibodies in patients treated with adalimumab

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Abstract

Introduction: *Adalimumab is a fully human monoclonal anti-TNF-α antibody used in the treatment of rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis. Other available TNF-α inhibitors, such as infliximab (chimeric monoclonal antibody) and etanercept (soluble anti-TNF-α receptor), can induce the synthesis of autoantibodies and even cause drug-induced lupus. Anti-cyclic citrullinated (anti-CCP) antibodies are highly specific to rheumatoid arthritis and are predictive for rapid progression and erosive disease.*

Objective: *The aim of the study was to assess the presence of antinuclear antibodies (ANAs) and anti-CCP antibodies in patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS) before treatment with adalimumab and after 16 weeks of treatment.*

Material and Methods: *The study group consisted of 10 patients with RA, 7 patients with PsA, and 5 patients with AS. Autoantibodies were measured in their sera before the treatment and after 16 weeks. Antinuclear antibodies were tested by immunofluorescence. Anti-CCP antibodies were detected by a commercial second-generation ELISA kit. The data analysis included clinical and laboratory response indices.*

Results: *Antinuclear antibodies were detected in 68% of the patients before treatment and in 54% after 16 weeks of therapy. Although the number of ANA-positive patients was reduced, there was no statistically significant change in their titers. Anti-CCP antibodies were detected in 41% (8 with RA, 1 with AS) of the patients before and in 36% after treatment (all patients with RA). Increases in anti-CCP antibody concentration were observed in 87.5% of the RA patients (P=0.069, Wilcoxon's test). The mean increase was 77%.*

Key words: *antinuclear antibodies (ANAs), anti-cyclic citrullinated peptide (anti-CCP) antibodies, adalimumab, rheumatoid arthritis (RA), psoriatic arthritis (PsA), ankylosing spondylitis (AS).*

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Introduction

Adalimumab is a human monoclonal anti-TNF-α antibody that is successfully used in the treatment of rheumatoid arthritis (RA) and other chronic arthritides, including ankylosing spondylitis (AS) and psoriatic arthritis (PsA). Regardless of their beneficial therapeutic effect, anti-TNF-α therapies have various impact on the synthesis of autoantibodies. All currently used TNF-α inhibitors induce the synthesis of non-organ-specific antibodies, which include antinuclear antibodies in general, and specific anti-dsDNA and anti-nucleosome antibodies [1]. Non-organ-specific autoantibodies are most often induced during infliximab treatment, but have also been described, with lower fre-

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Table 1. Inclusion and exclusion criteria

Inclusion criteria:
1) patient's written consent was obtained
2) established diagnosis of RA, PsA, or AS
3) high disease activity
a) RA and PsA patients
i) 6 swollen or 6 tender joints
ii) ESR >28 mm/h and or CRP >1.5 mg/dl
iii) VAS patient's disease activity >4 cm (VAS 0-10 cm)
b) AS patients
i) Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) ≥4
ii) total back pain ≥4 (VAS 0-10 cm)
iii) morning stiffness duration ≥1 hour
4) for RA and PsA patients: inefficacy of at least one disease-modifying antirheumatic drug (DMARD) (including methotrexate if no contraindications were known) taken for at least three months or shorter if side effects were observed
5) for AS patients: inefficacy of at least two non-steroidal anti-inflammatory drugs (NSAIDs) taken for at least three months in maximal well-tolerated doses unless contraindicated
6) female in child-bearing age use contraceptives
7) patients are allowed to take stable doses of one NSAID, one glucocorticoid in a dose not higher than the equivalent of 10 mg of prednisone, and one DMARD
Exclusion criteria:
1) medical history of treatment with adalimumab
2) hypersensivity to adalimumab
3) renal or hepatic failure of clinical significance
4) unstable chronic diseases, especially heart failure, ischemic heart disease, hypertension, diabetes, chronic obstructive pulmonary disease, or asthma
5) hepatitis B or hepatitis C
6) HIV (human immunodeficiency virus) infection
7) coincidence of other systemic inflammatory diseases
8) demyelinization disease or multiple sclerosis in anamnesis
9) medical history of neoplasm
10) tuberculosis in anamnesis
11) positive PPD (purified protein derivative) test (≥12 mm)
12) abnormal chest X-ray, esp. if suggesting tuberculosis
13) active infection
14) patients with high risk of infection, esp. with chronic lower limb ulceration or medical history of bacterial arthritis in the last 12 months
15) males: elevated serum PSA level (>4 ng/ml)
16) females:
a) abnormal mammography
b) abnormal cytology of uterine cervix, esp. if suggesting a higher risk of neoplasm
c) pregnancy or breast-feeding

quency, in patients treated with etanercept and adalimumab [2-5]. In the majority of cases there were no associated clinical symptoms of autoimmune diseases, although some reports of drug-induced lupus or lupus-like syndrome have been described [1, 2, 6, 7]. Some authors suggest that ANA positivity may be related to worse treatment response. Induction of the antibodies to antiphospholipids has been described as well and is associated with poorer therapeutic effect or concomitant infections. Induction of autoantibodies is not limited to patients with rheumatoid arthritis, and this phenomenon has also been observed in patients with ankylosing spondylitis and Crohn's disease [6].

Interestingly, titers of arthritis-associated antibodies in rheumatoid arthritis patients, such as rheumatoid factor (RF) and antibodies against cyclic citrullinated peptide, can decrease significantly during therapy with TNF-α inhibitors [4, 8]. Antibodies against cyclic citrullinated peptide (CCP) are highly specific to RA and are associated with its subtype in patients who are carriers of certain HLA DRB1 alleles, which contain the so-called "shared epitope" [9]. They can be detected in patients' sera long before the clinical diagnosis and are markers of rapidly progressing erosive disease. Their presence predicts more severe disease, but they are not directly correlated to the markers of disease activity [10, 11]. Rheumatoid factors are less specific to RA, but their high titer is a marker of poor prognosis; IgA RF positivity, for example, is associated with rapidly progressing erosive disease.

The aim of our study was to assess the presence and titers of antinuclear antibodies and antibodies to cyclic

Table 2. Demographic and clinical characteristic of the study group

Characteristic	Results (n=22)
female (%)	45
mean (SD) age [years]	48.8 (11.1)
mean (SD) disease duration* [years]	14.7 (10.5)
diagnosis:	
– RA (%)	45
– PsA (%)	32
– AS (%)	23
concomitant diseases (%):	59
– hypertension (%)	41
– gastroesophageal reflux, chronic gastritis or peptic ulcer in anamnesis (%)	13
– amyloidosis (%)	5
– obesitas (%)	5
– iridocyclitis in anamnesis (%)	5
– other (%)	18
concomitant treatment:	
– DMARDs:	
• MTX (%) (mean dose)	45 (15.25 mg weekly)
• SSZ (%) (mean dose)	5 (2 g daily)
• CsA (%) (mean dose)	14 (167 mg daily)
• No DMARDs (%)	36
– NSAIDs:	
• NSAIDS (%)	64
• No NSAIDs (%)	36
– glucocorticoids:	
• prednisone (%) (mean dose)	14 (9 mg daily)
• methylprednisone (%) (mean dose)	36 (7 mg daily)
• no glucocorticoids (%)	50

*SD – standard deviation, * – since the diagnosis was established, RA – rheumatoid arthritis, PsA – psoriatic arthritis, AS – ankylosing spondylitis, DMARDs – disease-modifying antirheumatic drugs, MTX – methotrexat, SSZ – sulfasalazine, CsA – cyclosporine, NSAIDs – non-steroid anti-inflammatory drugs.*

citrullinated peptide in three groups of patients treated with adalimumab: subjects with rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis. We also evaluated the relationship between autoantibody production and treatment efficacy or its complications.

Material and Methods

Patients

Twenty-two patients (12 males, 10 females, mean age: 48.3 years, range: 26-67 years, mean disease duration: 14.7 years, range: 2-34) with chronic arthritis entered the study. The study group comprised 10 patients with rheumatoid arthritis, 7 patients with psoriatic arthritis, and 5 patients with ankylosing spondylitis who fulfilled the respective classification criteria of the American College of Rheumatology. All patients who entered the study fulfilled the inclusion and exclusion criteria (Table 1). The study was approved by the Wroclaw University of Medicine Local Bioethical Committee and all the subjects gave their written informed consent before they entered the study.

Study protocol

Sixteen weeks of therapy with adalimumab was planned. Four visits were scheduled during the study: visit 0 (screening) prior to the treatment with adalimumab and visits 1, 2, and 3 at weeks 0, 6, and 16 of treatment to assess its safety and efficacy. To evaluate the efficacy of adalimumab therapy at each visit, the number of tender and swollen joints were obtained, ESR (erythrocyte sedimentation rate) and CRP (C-reactive protein) levels were measured, and DAS28 for RA patients and BASDAI for SpA patients were assessed. Blood samples for ANA and anti-CCP assays were obtained during visits 0 and 3.

Dosage and route of adalimumab

Adalimumab in the dose of 40 mg was given subcutaneously every other week. The very first dose of the drug was given in the hospital and further injections were done by the patients themselves after adequate training.

Detection of anti-CCP autoantibodies

Tests for anti-CCP autoantibodies were performed at baseline and after 16 weeks of adalimumab treatment. Anti-CCP antibodies were tested using a second-generation commercially available ELISA kit (EUROIMMUN Laboratories, Luebeck, Germany) as recommended by the manufacturer. All samples were tested in duplicate. Serum samples were diluted to 1:100 and distributed in separate wells of an antigen-coated microplate together with standard samples to evaluate a five-point standard curve and positive and negative controls. The samples were incubated for 60 min at room temperature and washed three times with washing buffer. In the next step, enzyme conjugate was incubated in the microplate wells for 30 min and then the samples were washed three times. Afterwards the chromogen/substrate solution was incubated for 30 min. Incubation was stopped by a stop solution. The absorbance was measured at a wavelength of 450 nm and a reference wavelength of between 620 and 650 nm within 30 min of adding the stop solution. Anti-CCP concentrations of 5 RU/ml or more were considered positive.

Detection of antinuclear antibodies

Antinuclear antibodies were tested in the patients' sera at baseline and after 16 weeks of adalimumab treatment using an indirect immunofluorescence method. Commercially available Hep2 cell substrates were used (EUROIMMUN, Luebeck, Germany). The serum samples were tested with different dilutions, and a titer of 1:160 or more was considered positive. The titer and staining type were assessed by

two qualified examiners. The laboratory data analysis included clinical and laboratory response indices.

Statistical analysis

The parametric distribution of the variables was verified by the Shapiro-Wilk and Liliefors tests. When the continuous variables met the assumptions for parametric distribution, Student's *t*-test was used to compare groups. Variables not meeting the distribution assumptions were analyzed using the Mann-Whitney *U* test, Wilcoxon test, Kruskal-Wallis test, and Friedman test. Correlations were established by Spearman's correlation test, and when a linear correlation was detected the Pearson's coefficient was determined. Two-sided P values less than 0.05 were considered significant.

Results

Demographic characteristic

The study was performed at the Department of Rheumatology and Internal Diseases of Wroclaw University of Medicine between June 2006 and April 2007. Table 2 shows the demographic and clinical characteristic of the study group.

Efficacy of the therapy

In the study group we observed clinical and laboratory improvement after 16 weeks of therapy with adalimumab (Table 3). The laboratory tests revealed statistically significant decreases in ESR and CRP, which are the most commonly used laboratory parameters of inflammation. We observed simultaneous reduction of disease activity indices (DAS28 in RA and PsA and BASDAI in AS and PsA) and functional improvement assessed by the HAQ (RA and PsA) and BATH (AS and PsA) questionnaires.

Influence of adalimumab therapy on autoantibody levels

The influence of 16 weeks of therapy with adalimumab on the presence of autoantibodies in the sera of patients with chronic arthritis is shown in Table 4.

Antinuclear antibodies were detected in 68.2% of the patients before treatment and in 54.5% after 16 weeks of therapy. Although the number of ANA-positive patients decreased (among the patients with PsA and AS), there was no statistically significant change in their titers. Anti-CCP antibodies were detected in 40.9% (8 patients with RA, 1 with AS) of patients before treatment and in 36.4% after the treatment (8 with RA). Eighty percent of the RA patients were positive for anti-CCP antibodies at baseline. In RA patients the concentration of anti-CCP antibodies tended to increase, albeit not significantly (the concentration of anti-CCP antibodies decreased only in one patient in this subgroup). Increased anti-CCP antibody concentrations were observed in 87.5% of RA patients (P=0.069, Wilcoxon's test), the mean increase being 77%. The only RA patient with a decreased anti-CCP antibody concentration received adalimumab in monotherapy. The other RA patients with positive anti-CCP tests received adalimumab in combination with DMARDs. An AS patient with a positive anti-CCP test at visit 0 suffered from AS accompanied by peripheral joint inflammation. In the other AS patients, no peripheral

Table 3. Selected laboratory and clinical results at visit 0 and visit 3

Parametres	Results	
	Visit 0	Visit 3
Mean (SD) ESR [mm/h]#	45.7 (23.5)	26.0 (27.4)
Mean (SD) CRP [mg/dl]#	3.2 (2.4)	1.22 (1.65)
Mean (SD) Hb [g/dl]	13.1 (1.9)	13.7 (1.8)
Mean (SD) leucocytosis [G/l]	10.4 (3.2)	9.7 (3.6)
Mean (SD) thrombocytosis [G/l]	319.8 (99.9)	268.4 (80.2)
Mean (SD) morning stiffness [min]#	111 (86)	39 (92)
Mean (SD) number of tender joints (RA and PsA)#	9.4 (6.3)	3.0 (4.0)
Mean (SD) number of swollen joints (RA and PsA)#	6.1 (4.5)	1.3 (2.5)
Mean (SD) DAS28 (RA and PsA)#	5.85 (1.09)	3.67 (1.33)
Mean (SD) HAQ (RA and PsA)#	1.93 (0.59)	1.39 (0.67)
Mean (SD) BASDAI (AS and PsA)#	6.02 (2.64)	3.23 (2.96)
Mean (SD) BATH (AS and PsA)#	60.2 (26.8)	39.8 (28.4)

*SD – standard deviation, ESR – erythrocyte sedimentation rate, CRP – C-reactive protein concentration, Hb – hemoglobin concentration, DAS28 – Disease Activity Score 28, BASDAI – Bath Ankylosing Spondylitis Disease Activity Index, *HAQ – Health Assessment Questionnaire, # – statistically significant difference between visits 0 and 3 was observed with P<0.05.*

Table 4. The influence of therapy with adalimumab on autoantibodies

Parametres	Results	
	Visit 0	Visit 3
ANA positive:*	15 (68.2%)	12 (54.5%)
– RA	7	7
– PsA	4	3
– AS	4	2
anti-CCP positive (%):#	9 (40.9%)	8 (36.4%)
– RA	8	8
– PsA	0	0
– AS	1	0
mean (SD) anti-CCP [RU/ml]	80.1 (179.6)	107.9 (245.8)

*ANA – antinuclear antibodies, * – ANAs were considered positive if their titers were >1:160, anti-CCP – anti-cyclic citrulinated protein antibodies, # – anti-CCP were considered positive if their concentrations were >5 RU/ml.*

joints were inflamed. In the patient with AS parallel with decreased anti-CCP antibody concentration we observed resolution of peripheral joint inflammation.

Discussion

The treatment of different types of arthritis with anti-TNF-α agents has been associated with increased synthesis of the non-organ-specific autoantibodies, such as antinuclear antibodies, anti-dsDNA antibodies, anti-nucleosome antibodies, and others. Enhanced synthesis of antiphospholipid antibodies has been reported as well and was generally associated with poor response to treatment. Autoantibody induction is very rarely accompanied by clinically overt autoimmune diseases and their symptoms usually disappear with treatment withdrawal [5]. However, the latency between autoantibody induction and autoimmune disease manifestation is long, so a longer follow-up time is needed to draw final conclusions.

Antinuclear antibodies can be detected in about 25% of RA patients in general. They are usually present in low titers and are not of any known specificity. The higher frequency of positive ANA antibodies in our group of RA patients at baseline can be due to short-lasting treatment with anti-TNF-α agents in the past which some of them had had. According to previous reports, antinuclear antibodies were detected in 34 to 95% of RA patients treated with infliximab, in 11-36% treated with etanercept, and in 11-36% of patients on adalimumab. Anti-dsDNA antibodies, which are considered specific to systemic lupus and are usually not detected in RA patients' sera, were induced in up to 36% of RA patients on infliximab therapy, up to 10% on etanercept, and up to 13% on adalimumab [2-4]. The anti-dsDNA antibodies induced are mainly of low avidity and in most cases belong to the IgM class (while anti-dsDNA of IgG class are considered pathogenic) [2]. A relatively high percentage of our study group had detectable antinuclear antibodies, and about 45% revealed a homogenous staining pattern, which could correspond to the presence of antibodies against chromatine antigens, but we did not test for antibodies against dsDNA. None of our patients developed any features of lupus, but the follow-up time was limited. According to the studies reported to date, this complication of anti-TNF-α therapy affects less than 1% of patients [2]. These autoantibodies were induced as well in patients with spondyloarthropathies receiving various anti-TNF-α therapies, and according to some authors the increase in autoantibodies is SpA patients is even more significant. Many SpA patients, however, have previously been treated with sulfasalazine, which is known for inducing ANA and anti-dsDNA antibodies.

Antibodies against cyclic citrullinated peptide are highly specific to rheumatoid arthritis and are associated with a shared epitope. They can be detected in patients' sera long before diagnosis, they are present in sera of about 40% of seronegative rheumatoid arthritis patients, and are associated with progressive erosive disease. Treatment with classical disease-modifying anti-rheumatic drugs can induce significant decreases in anti-CCP antibody levels, but this effect seems limited to early disease. The impact of anti-TNF-α therapy on anti-CCP levels is less elucidated. Some authors report a significant decrease in anti-CCP concentration, which reflects good treatment response, and propose that these antibodies can have a role as a serological marker of response. Ahmed et al. reported a significant decrease, which was not sustained, in the serum levels of anti-CCP and IgA RF early in the course of therapy of rheumatoid arthritis with infliximab. Only the levels of IgM RF decreased steadily during treatment [12]. Significant decreases in anti-CCP antibodies and RF after 6 months of infliximab treatment was reported as well by Alessandri et al., but only in the patients showing clinical improvement [13].

Atzeni et al. investigated in a prospective study the relationship between the treatment efficacy of adalimumab in 56 RA patients and the incidence and titers of non-organ-specific and disease-associated autoantibodies. They reported significant decreases in RF and anti-CCP antibodies after 24 weeks which correlated with the clinical response to therapy; however, they did not observe complete disappearance of disease-associated antibodies in any of the subjects. No significant decreases in these antibodies were detected in patients treated with methotrexate alone. The authors also detected the induction or titer increase of antinuclear antibodies (in 28% of subjects) and de novo induction of anti-dsDNA antibodies (in 14.6% of subjects) which was not associated with clinical manifestations [14].

There are many reports on rheumatoid factor titer decreases in the serum of RA patients on anti-TNF-α therapy, and IgM RF decrease seems to parallel clinical response. Pre-treatment presence of IgM RF, however, does not influence the therapeutic effect, while high pre-treatment levels of IgA RF seem to be a marker of poor prognosis. Changes in anti-CCP antibodies induced by anti-TNF-α therapy remain a controversial issue [8].

Our study did not show a significant decrease in anti-CCP antibody titers in RA patients treated with adalimumab. Anti-CCP levels tested at 16 weeks of treatment showed even a non-significant trend to increase. All patients responded well to the therapy and there was no correlation between antibody titers and clinical response to treatment. Our findings can be explained by the shorter time of observation than that reported in other studies (16 weeks vs. 24 or more weeks), as it is possible that the synthesis of anti-CCP antibodies is inhibited later in the course of treatment. The other factor that may influence changes in anti-CCP antibody titer can be related to disease duration. Studies with classic disease-modifying drugs have shown that anti-CCP antibodies fall significantly during treatment in patients with relatively early disease, and the patients in our group had an average disease duration of 13 years. The other reason can be the small group of patients investigated in comparison with other studies.

Yazdani et al. observed similar effects of anti-TNF-α therapy in patients with RA. They analyzed autoantibody levels in patients treated with etanercept or adalimumab at baseline and after a follow up of 9 months. The only change they found was an increase, and not the decrease reported by others, in IgA and IgG RF in etanercept therapy, while the serum levels of ANA, anti-dsDNA, and anti-nucleosome antibodies remained unchanged after 9 months of treatment with etanercept or adalimumab. Treatment efficacy was confirmed by a decrease in DAS 28 and did not correlate with autoantibody levels [15].

No effect of anti-TNF-α therapy on anti-CCP levels in RA patients was also reported by Caramaschi et al. [16]. They tested serum levels of various autoantibodies in a group of RA patients treated with infliximab at monthly intervals up to 22 weeks. Anti-CCP levels remained stable in all but one patient, in whom the anti-CCP antibodies were in fact induced by treatment. In contrast, IgM RF decreased significantly in parallel to CRP. ANA antibodies were induced in over 30% of the previously negative subjects.

The mechanism underlying the decreased synthesis of autoantibodies related to rheumatoid arthritis by TNF-α inhibitors is not fully elucidated. It is possible that an anti-inflammatory effect or the modulation of apoptosis can influence the citrullination processes and autoantibody synthesis. The induction of non-organ-specific antibodies may have a different background. There are many hypotheses explaining this phenomenon. Antibodies induced by anti-TNF-α therapy, such as antibodies against chromatin and antiphospholipids, are aimed at antigens exposed and modified in cells undergoing apoptosis. It is likely that autoimmunity induction by this therapy is associated with its interference with programmed cell death. Aringer et al. have shown that anti-TNF-α treatment withdrawal in SLE patients correlated with decreases in autoantibodies against chromatin antigens and phospholipids, and an *in vitro* experiment with complete blocking of TNF-α was associated with an increase in the apoptosis rate of CD4+ cells from SLE patients [17]. Another possible explanation is defective clearance of apoptotic cells and increased tissue and systemic levels of nucleosomal autoantigens. D'Auria et al. have shown that infliximab treatment is associated with the accumulation of nucleosomes in patients' plasma and that infliximab induces cell death and nucleosome release from apoptotic cells *in vitro* [18]. The rapid decrease in CRP concentration induced by anti-TNF-α treatment may cause defective clearance of apoptotic material, which may become immunogenic [2].

The impact of anti-TNF-α therapy on the immune system is very complex. The influence of this therapy on the induction of autoimmune processes will be better understood with longer follow-up of the treated patients. The utility of RF decrease as a serological marker of treatment response seems warranted; however, the relationship between the synthesis of anti-CCP antibodies and TNF-α inhibition deserves further investigations.

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