Changes in Anti-Citrullinated Protein Antibody Titers Following Treatment with Infliximab for Rheumatoid Arthritis

Joao L.P. Vaz MD[1], Mirhelen M. Abreu MD[2] and Roger A. Levy MD PhD[3,4]

[1]Department of Rheumatology, Federal State University Rio de Janeiro, Rio de Janeiro, Brazil
[2]Department of Rheumatology, State University of Sao Carlos, Sao Carlos, Brazil
[3]Faculty of Medical Sciences, State University of Rio de Janeiro, Rio de Janeiro, Brazil
[4]Federico Foundation, Liechtenstein

ABSTRACT: **Background:** The presence of anti-citrullinated peptide/protein antibody (ACPA) has a high specificity and predictive value for the development of rheumatoid arthritis (RA). Some studies have shown decreased titers of this antibody after treatment with infliximab.

Objectives: To assess the changes in ACPA titers in patients with RA after treatment with infliximab as a first biological agent, and to correlate these variations with non-infusion-related adverse effects.

Methods: In a prospective multicenter observational study involving 48 research centers, we assessed 139 patients with established moderate-to-severe RA diagnosed according to American College of Rheumatology criteria. Samples were collected before and 6–12 months after treatment.

Results: The mean age of the study patients was 50.6 years and 118 were female (84.9%). Statistically significant variations in ACPA titers were noted in 47 patients (before and after treatment) ($P = 0.012$). Overall, ACPA titers were decreased in 32 (65.3%) and increased in 15 (34.7%). No correlation was found between severe or mild adverse effects in patients presenting variations in ACPA titers.

Conclusions: The present study showed that infliximab affected ACPA titers, promoting mainly a decrease; however, this was not related to the occurrence of non-infusion-related adverse effects.

IMAJ 2014; 16: 17–19

KEY WORDS: anti-citrullinated peptide/protein antibody (ACPA), autoantibodies, infliximab, anti-tumor necrosis factor (TNF), rheumatoid arthritis (RA)

The anti-cyclic citrullinated peptide/protein (ACPA, formerly termed anti-CCP) antibody belongs to a group of autoantibodies capable of reacting with many citrullinated peptides present in numerous proteins (filaggrin, vimentin, fibrin, alpha-enolase) [1]. In 2004 Nielen et al. [2] suggested that high levels of ACPA in the serum of healthy individuals implied a high risk of developing rheumatoid arthritis. Additionally, the presence of this autoantibody could predict the occurrence of the disease, given that 49% of their patients (n=79) presented elevated serum levels of this antibody a few years before the onset of RA symptoms. Raza et al. [3] showed that the combination of ACPA and rheumatoid factor in patients with very early synovitis (≤ 3 months) (n=97) had high specificity and predictive value for development of RA.

Some studies have shown decreased titers of this antibody after treatment with infliximab. In 2004 Alessandri and co-authors [4] showed a significant reduction in the levels of rheumatoid factor and ACPA in patients with RA after 6 months of treatment with infliximab (n=43), and correlated this decrease with patients who achieved clinical improvement. In their 2006 study on 33 patients with RA, Ahmed et al. [5] showed a significant reduction in ACPA levels after 30 weeks of infliximab treatment ($P = 0.002$). However, this decrease did not persist after 54 weeks ($P = 0.147$). In 2008 Vis et al. [6] reported a significant reduction in the levels of rheumatoid factor and ACPA after one year of infliximab treatment in RA patients (n=62), although no relationship was noted between DAS-28 (Disease Activity Score, evaluating 28 joints) and autoantibody titers. A recent study in 101 RA patients treated with infliximab also failed to correlate the reduction in DAS-28 with the reduced titers of several ACPA isotypes, suggesting that this autoantibody may not be a reliable marker for predicting response to infliximab [7].

The primary objective of the present study was to evaluate the changes in ACPA titers in patients with RA before and after treatment with infliximab as the first biological agent. In addition, we attempted to correlate this variation with the adverse events observed during the study (non-infusion related).

PATIENTS AND METHODS

We performed a multicenter prospective open-label phase IV observational study. Forty-eight research centers were involved and 209 patients were selected. These patients presented estab-

ACPA = anti-citrullinated peptide/protein antibody

RA = rheumatoid arthritis

lished moderate-to-severe RA diagnosed according to American College of Rheumatology criteria, and with an indication for treatment with anti-tumor necrosis factor due to therapeutic failure or intolerance of disease-modifying anti-rheumatic drugs. Patients aged 18 and over were included. Pregnant or lactating women, and patients who presented uncontrolled comorbidities or suspected coronary artery disease, heart failure, demyelinating disease, cancer, severe infections (tuberculosis, human immunodeficiency virus, hepatitis C virus), and other autoimmune diseases (except for Sjögren's syndrome) were excluded. Also excluded were patients with a history of alcohol or drug abuse in the previous 12 months, or who received an investigational drug within 30 days prior to the study.

A protocol based on an international format was used to record the data and all patients were asked to sign an informed consent form. Both documents had been previously approved by the research ethics committees of all study centers and by the National Health Surveillance Agency (ANVISA, for the regulation of human studies in Brazil).

Patients continued to take a DMARD: usually methotrexate at a maximum dose of 20 mg/week, and infliximab was prescribed at a dose of 3 mg/kg in the first infusion, 2 and 6 weeks later, and every 2 months thereafter for a total of 6 to 12 months.

Laboratory analyses were performed at a central laboratory. Samples of peripheral blood were collected and processed in the respective study centers and tested at a central laboratory at BioRad facilities (Benicia, CA, USA). This process was under the supervision of one of the authors (R.A.L.) using the Bioplex 2200. A cutoff point ≥ 3.0 IU/ml was used to determine reactive samples. Only in patients who completed the treatment with infliximab for 6 to 12 months were antibody titers (before and after the medication) measured.

STATISTICAL ANALYSIS

Data are expressed as mean values ± SD, median or range. Comparisons between groups were performed by unpaired *t*-test or Wilcoxon test as appropriate. The level of statistical significance was established as $P < 0.05$. Statistical analysis was performed using SPSS 12 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Of the 209 patients, 139 completed the study and gave two blood samples (before and after treatment). The mean age of the 139 patients was 50.6 years (50.7 for women and 50 for men); 118 were female (84.9%). Forty-seven patients (33.8%) had significant variations in ACPA titers (before and after treatment) ($P = 0.012$). Overall, 32 (65.3%) had reduced ACPA titers and 15 (34.7%) had increased titers. Among those with decreased titers, the percentage of variation was 9.6% to 99.7% [Table 1]. The variation in antibody titers observed before and after infliximab was highly significant for patients with reduced titers (n=32) ($P = 0.0004$). Additionally, patients with increased titers (n=15) seemed arbitrary ($P = 0.061$) [Figure 1].

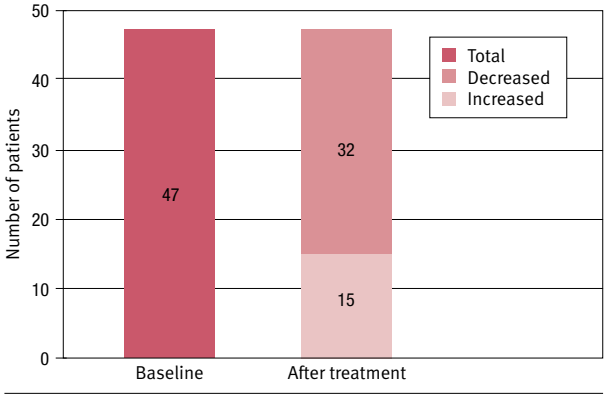
No correlation was found between adverse events and changes in antibody titers. Among those 47 patients, only 6 had an infection, mainly of the upper respiratory tract. None of the patients showed any suggestive signs or symptoms of drug-induced lupus.

Table 1. Percentage of variation of ACPA titers after infliximab treatment in patients who achieved remission

Baseline	After infliximab	Variation (%)
3.1	2.4	- 22.6
4.3	11	+ 74.5
6.5	2	- 69.3
7.8	6	- 23.1
10.4	8	- 23.1
16.7	0.4	- 97.6
17	8.8	- 48.3
34.9	10.1	- 71.1
71.1	54.9	- 22.8
88.3	76.9	- 13.0
96	71.6	- 25.5
185.7	137	- 26.3
221.7	188.7	- 14.9
225.4	93.5	- 58.6
242.2	141	- 41.8
304.2	196.9	- 35.3
306.9	16.8	- 94.6
396	1.3	- 99.7
505.2	121.7	- 76.0
549.4	338.5	- 38.4
572.6	254.5	- 55.6
672	294.8	- 56.2
673.7	609.6	- 9.6
731.7	458.3	- 37.4
771.1	613.3	- 20.4
776.7	641.9	- 17.3
779.8	610.6	- 21.6
826.2	592.5	- 28.1
865.4	709.1	- 18.0
1073.1	636	- 40.7
1105.9	876.6	- 20.7
1317.8	1028.9	- 21.9

DMARD = disease-modifying anti-rheumatic drug

Figure 1. Variation in ACPA titers before and after infliximab treatment



DISCUSSION

This study of ACPA in patients with RA treated with infliximab appears to represent the largest sample size (n=139) to date. We have shown that the reduction in titer was more marked than the increase. Significant results were noted in the total group of patients presenting titer changes (n=47) and even more in those patients whose initial titers were reduced (n=32).

In RA, inflammation of the joint leads to an infiltration of inflammatory cells that will eventually die and release citrullinated proteins and PAD enzymes, which may subsequently increase the citrulline expression by synovial proteins. The formation of immune complexes will stimulate the inflammatory process by up-regulation of pro-inflammatory cytokines. This way, ACPA contributes to the perpetuation of joint inflammation, and thereby to the chronicity and severity of RA. Treatment with anti-TNFα agents such as infliximab may significantly reduce the serum levels of RF and ACPA parallel with a clinical improvement of RA, a phenomenon that may be linked to restored apoptosis. Despite this, clinical studies have yielded controversial findings.

In 2004 Bobbio-Pallavicini et al. [8] showed that reactive ACPA titers in 83% of the 30 patients at the beginning of the treatment had declined significantly after 30 weeks. Nevertheless, ACPA titers returned to baseline at the end of the treatment (78 weeks). In the 2005 study by De Rycke et al. [9] there were no significant differences between ACPA titers before and 30 weeks after the use of infliximab in RA patients (n=62), although antibody titers were reduced in some patients and increased in others. This suggests that the medication had no effect on ACPA titers. In the same year, Caramaschi et al. [10] reported similar results, showing a significant reduction in RF levels, although there were no changes in ACPA before and after treatment with infliximab (n=27).

TNFα antagonists have dramatically changed the treatment of RA in recent years. The diagnostic usefulness of antibodies associated with RA has been extensively studied, with the results establishing ACPA as a better diagnostic marker than RF. However, data on the relationship between antibody titers and response to treatment are conflicting. Our study has shown that infliximab may interfere with ACPA titers, promoting mainly their reduction. Conversely, this was not related to the occurrence of non-infusion-related adverse events in these patients. We demonstrated a significant reduction in ACPA titers after medication. Nonetheless, we did not correlate this with the intensity of adverse events. The literature has shown that this reduction was purely random, while other studies, like ours, show that infliximab reduces ACPA titers. Despite this limitation, to date our study appears to present the largest sample size which, together with the decrease in ACPA titers after infliximab treatment, ensures the quality and reliability of our results.

TNFα = tumor necrosis factor-alpha

Acknowledgments

The authors thank the biological technicians at BioRad (Benicia, CA, USA) for allowing us the use of Bioplex 2200 during our research, as well as Maria Crisostomo and Bruno Larida for facilitating this task. We would also like to thank Dr. Ana Cristina Monteiro for thoughtful discussion, and the AURUS protocol investigators in Brazil.

Corresponding author:
Dr. R.A. Levy
CITIPA. Rua Barao da Torre, 145/ 4° andar. Ipanema, 22411-001
Rio de Janeiro, RJ, Brazil
Phone: (55-21) 2287-2550
email: roger.a.levy@gmail.com

References

1. Alessandri C, Priori R, Modesti M, Mancini R, Valesini G. The role of anti-cyclic cytrullinate antibodies testing in rheumatoid arthritis. *Clin Rev Allerg Immunol* 2008; 34: 45-9.
2. Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004; 50 (2): 380-6.
3. Raza K, Breese M, Nightingale P, et al. Predictive value of antibodies to cyclic cytrullinated peptide in patients with very early inflammatory arthritis. *J Rheumatol* 2005; 32: 231-9.
4. Alessandri C, Bombardieri M, Papa N, et al. Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNFα therapy (infliximab) in rheumatoid arthritis is associated with clinical improvement. *Ann Rheum Dis* 2004; 63: 1218-21.
5. Ahmed MM, Mubashir E, Wolf RE, et al. Impact of treatment with infliximab on anticyclic citrullinated peptide antibody and rheumatoid factor in patients with rheumatoid arthritis. *South Med J* 2006; 99 (11): 1209-15.
6. Vis M, Bos WH, Wolbink G, et al. IgM-rheumatoid factor, anti-cyclic citrullinated peptide, and anti-citrullinated human fibrinogen antibodies decrease during treatment with the tumor necrosis factor blocker infliximab in patients with rheumatoid arthritis. *J Rheumatol* 2008; 35 (3): 425-8.
7. Klaasen R, Cantaert T, Wijbrandts CA, et al. The value of rheumatoid factor and anti-citrullinated protein antibodies as predictors of response to infliximab in rheumatoid arthritis: an exploratory study. *Rheumatology* 2011; 50: 1487-93.
8. Bobbio-Pallavicini F, Alpini C, Caporali R, Avalle S, Bugatti S, Montecucco C. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. *Arthritis Res Ther* 2004; 6: R264-72.
9. De Rycke L, Verhelst X, Kruithof E, et al. Rheumatoid factor, but not anti-cyclic citrullinated peptide antibodies, is modulated by infliximab treatment in rheumatoid arthritis. *Ann Rheum Dis* 2005; 64: 299-302.
10. Caramaschi P, Biasi D, Tonolli E, et al. Antibodies against cyclic citrullinated peptides in patients affected by rheumatoid arthritis before and after infliximab treatment. *Rheumatol Int* 2005; 26: 58-62.