Clinical Significance of Autoantibodies Induced by Infliximab Treatment: Two-Year Follow-up after Infliximab Discontinuation

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Received: December 20, 2010
Accepted: July 14, 2011

SUMMARY Infliximab is anti-TNFα monoclonal antibody, which is widely used in the treatment of rheumatoid arthritis (RA) and other inflammatory diseases. Anti-TNFα treatment can induce the occurrence of autoantibodies but in the majority of treated patients, they have no clinical significance although several cases of drug induced lupus have been described. In our cohort of refractory RA patients treated with infliximab for one year, we found a very high number of patients who developed antinuclear autoantibodies (16 of 24 (66.6%) at the time of infliximab discontinuation) and anti-ds-DNA autoantibodies (12 of 24 (50%) at the time of infliximab discontinuation). However, in most of these patients they had no clinical significance. One patient developed clinical and laboratory signs of systemic lupus erythematosus (SLE), which over time became overt as SLE-RA overlapping unmasked by infliximab.

KEY WORDS: anti-TNFα drugs, autoantibodies, drug induced lupus, infliximab

INTRODUCTION

In the last decade, anti-TNFα drugs have been used in the treatment of rheumatoid arthritis (RA), ankylosing spondylitis, psoriatic arthritis, psoriasis and inflammatory bowel diseases. Monoclonal antibodies to TNFα such as infliximab, a chimeric one, or adalimumab, which is fully humanized, and soluble TNFα receptors such as etanercept, have been on the market for several years now. New generations of anti-TNFα drugs that have recently been registered in most European countries are certolizumab and golimumab. The efficacy of this treatment modality is unquestionable and it usually has to be continued for a long time. Considering safety, infections, particularly tuberculosis (TB), are an important issue, although screening for latent TB infection prior to anti TNF therapy significantly reduces the number of TB cases.

It is also well known that anti-TNFα treatment can induce the occurrence of autoantibodies (AA), particularly in RA patients, although they are described in other diseases where anti-TNF drugs are used (1-6). In the majority of treated patients, they have no clinical significance, although cases of drug induced lupus (DIL) have been described (7-11).

We evaluated the frequency and clinical significance of autoantibodies in RA patients treated with

Acta Dermatovenerol Croat
2011;10(3):156-160

infliximab and methotrexate (MTX) for one-year period, and the presence and clinical significance of autoantibodies in the same RA patients after TNF therapy discontinuation.

PATIENTS AND METHODS

Twenty-four patients (18 women and 6 men) with active RA despite therapy with MTX were treated with MTX and infliximab for one year. The dosage regimen of infliximab was 3 mg/kg given at week 0, 2, 6 and every 8th week thereafter until week 54. After this period, infliximab was stopped in all patients. The dosage of MTX was 7.5-17.5 mg weekly (mean dose MTX 10.72±3.25 mg). All patients were on steroids as well. The mean dose of prednisolone was 10.475±5.26 mg (range, 2-20 mg). All our patients had a long-standing disease. The mean duration of disease was 12.4±4.15 (range, 4-20) years. All of them had been previously treated with at least two disease modifying antirheumatic drugs (DMARDs) including MTX. The most often DMARD after MTX was sulfasalzine in 18, leflunomide in 6 and chloroquine and gold salts in 4 patients. Eight patients were treated with 3 different DMARDs and two with four. Analyses for antinuclear antibodies (ANA), anti-double-stranded DNA antibodies (anti-ds-DNA), antibodies to extractable nuclear antigen (ENA) and anticardiolipin antibodies (ACL) were performed at the baseline, at week 2, 6, 14, 22, 30, 38, 46 and 54, and then, after therapy discontinuation, every 6 months for two years. ANA was done by indirect immunofluorescence; anti-ds-DNA, ENA and ACL were done by ELISA.

RESULTS

Seventeen patients received nine infusions of infliximab, whereas in another 7 patients infliximab was discontinued earlier. The reasons for exclusion of four patients were infusion reactions. In two patients (in one in week 2, in the other in week 6) there were severe allergic reactions. In another two, moderate allergic reactions that worsened after another infusion were the reason for discontinuation of treatment in week 6 and 22. One patient stopped the treatment due to the lack of efficacy in week 22, and another one because of poor compliance in week 22. One of previously ANA negative RA patients developed clinical (worsening of arthritis, pleural effusion and photosensitive rash) and laboratory (ANA 1:320, anti-ds-DNA 64.6 and low C3) signs of systemic lupus erythematosus (SLE) after 4th infusion. Although infections are often reported as the main reason for stopping the treatment, no infection developed in our group of patients.

All 24 patients were evaluated for AA in the period of treatment and in the two-year follow up of after infliximab discontinuation, being stopped either earlier due to adverse events or after week 54 as expected (Table 1).

At baseline visit, 3/24 (12.5%) patients had positive ANA. After nine infusions, at week 54, 14/17 (82.35%) patients who continued receiving infliximab had positive ANA (Fig. 1). Maximum ANA titer was 1:1024. The increase in ANA titers was noticed at week 14 and with every infusion of infliximab the number of ANA positive patients was higher. At the time when infliximab was stopped, 16/24 (66.6%) patients were ANA positive. Twenty-four months after the last infusion, 6/24 (25%) patients were still ANA positive (Fig. 2).

Before treatment, none of the patients had anti-ds-DNA. At week 54, 8/17 (47.05%) patients who continued receiving infliximab throughout 54 weeks were anti-ds-DNA positive (Table 1, Fig. 3). The highest percentage of positive anti-ds-DNA findings was noticed in week 38. At the time of infliximab discontinuation (due to adverse event or at the expected time), 12/24 (50%) patients were anti-ds-DNA positive. Twenty-four months after the last dose of infliximab, 3/24 (12.5%) patients had still slightly positive anti-ds-DNA (Fig. 4).

Table 1. Frequency of occurrence of autoantibodies during and after infliximab treatment

	Week 0	Time of infliximab discontinuation	Month 6	Month 12	Month 18	Month 24
ANA	3/24	16/24	10/24	8/24	6/24	6/24
ANTI-ds-DNA	0/24	12/24	8/24	6/24	4/24	3/24
ENA	0/24	6/24	5/24	4/24	3/24	1/24
ACL	3/24	3/24	3/24	3/24	3/24	3/24

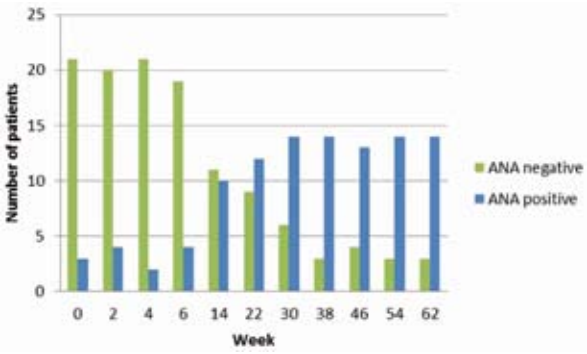

Figure 1. Number of ANA positive and ANA negative patients during treatment with infliximab and methotrexate.

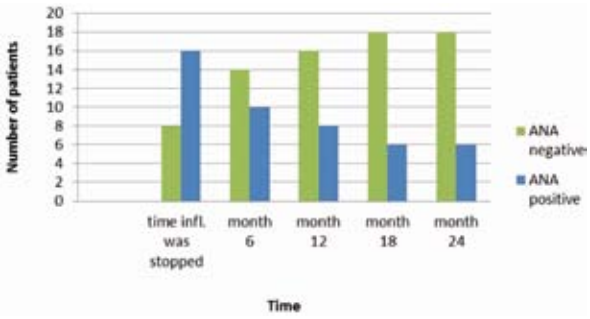

Figure 2. Frequency of ANA after infliximab treatment.

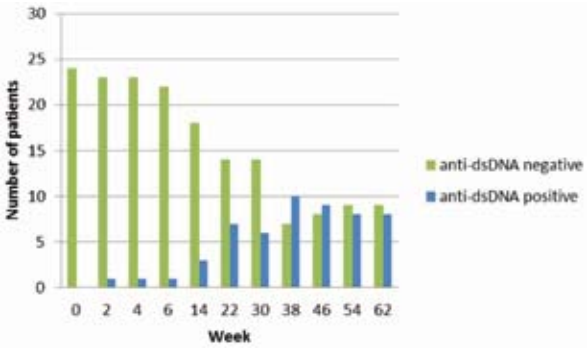

Figure 3. Number of anti-ds-DNA positive and anti-ds-DNA negative patients during treatment with infliximab and methotrexate.

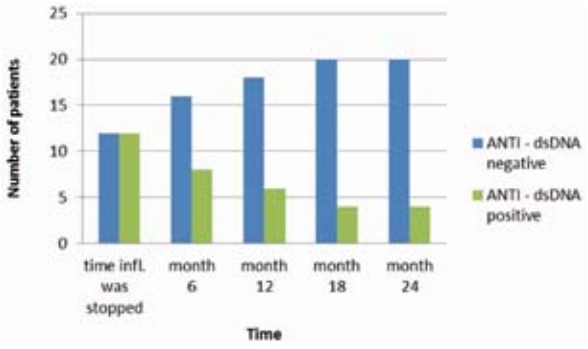

Figure 4. Frequency of anti-ds-DNA after infliximab treatment.

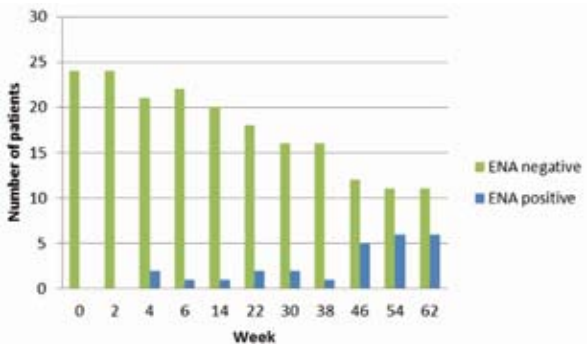

Figure 5. Number of ENA positive and ENA negative patients during treatment with infliximab and methotrexate.

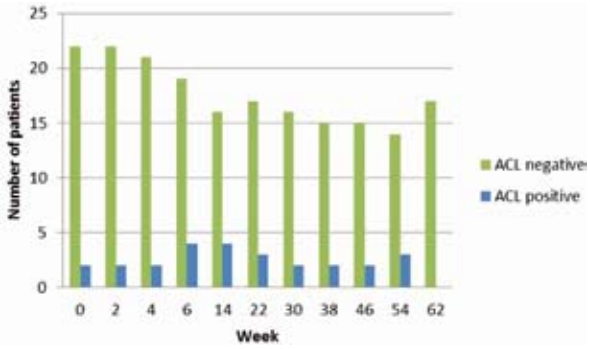

Figure 6. Number of ACLA positive and ACLA negative patients during treatment with infliximab and methotrexate.

Before treatment, none of the patients had antibodies to ENA. At week 54, 6/17 (35.29%) patients were ENA positive (Table 1, Fig. 5). Four of these patients had SS-A and two had Sm/RNP. Before treatment, two of our patients had ACL (IgG or IgM, and during the treatment positive ACL was noticed in another two patients (Table 1, Fig. 6).

In all patients except the one with a lupus-like clinical picture, these AA had no clinical significance and were not connected with allergic reactions or therapeutic response.

DISCUSSION

TNF-α is a proinflammatory cytokine pivotal in the pathogenesis of a variety of inflammatory diseases; it is also an immunoregulatory cytokine with immunosuppressive properties. In (NZB X NZW)F1 mice, genetic deficiency in TNF plays a significant role in the

onset of autoimmunity (12). Induction of apoptosis after anti TNF treatment could constitute a pathway to the induction of new autoantibodies under TNF blockade. Significant down-regulation of CRP that occurs following infliximab therapy may potentiate autoimmunity by reducing the clearance of nuclear material by CRP (1).

Various studies report on a variable incidence of developing ANA (53%-82%) and anti-ds-DNA (11.3%-20.9 %) in patients during anti-TNF-α treatment (1-3). ANA is often positive in RA patients. In combined results of three studies, ANA were positive in 23.7%-51.6%, while anti-ds-DNA was not found before treatment (6). The number of our patients who developed antinuclear antibodies during one-year treatment with infliximab corresponds to that in the literature.

Detection of anti-ds-DNA depends on the tests used. It is well known that ELISA tests are more sensitive and less specific, while IIF to *Crithidia luciliae* or by Farr assay is more specific and less sensitive. The vast majority of antibodies to ds-DNA are of the IgM isotype and are usually nonpathogenic (13). Caramashi *et al*. report that even in 93% patients ANA positivity was found at least at one determination, but only one patient became anti-ds-DNA (IgG by *Crithidia luciliae*) positive after 12 months (13). Relatively rare formation of IgG anti-ds-DNA may be more closely associated with the development of a syndrome like SLE (1).

A high number of our patients developed anti-ds-DNA antibodies. The reasons for that were probably the less specific anti-ds-DNA ELISA tests and longer observation than in other studies. We had no possibility to differentiate isotypes of anti-ds-DNA. Some other studies (14) also found the higher occurrence of anti-ds-DNA to be possibly due to a longer period of treatment, and the latest induction was observed after 24 months.

It seems logical that the presence of AA decreased with the time elapsed from stopping the treatment. Data on this are scarce in the literature, mainly because anti-TNF treatment is a long-lasting therapy. Since we had an opportunity to check AA after stopping the treatment, we found that, in a number of patients, AA induced by infliximab continued to remain positive for up to 2 years of therapy discontinuation. This is somewhat surprising as most of the authors state that the occurrence of AA is largely restricted to the induction of short-term IgM anti-dsDNA antibodies (5).

In some of our patients, anti-ENA antibodies and ACL antibodies occurred, yet we did not find them to be of clinical significance. In the literature, ENA antibodies induced by anti-TNF treatment are sporadically described, while ACL were more often found but they had no clinical significance either (14-16).

One of our previously ANA negative RA patients developed clinical and laboratory signs of SLE after 4th infusion. Since her condition improved after infliximab discontinuation, the diagnosis of DIL was reasonable. However, two years later, this patient had still positive ANA and anti-ds-DNA; the photosensitive malar rash developed suggesting SLE-RA overlapping unmasked by infliximab. We reported this patient earlier (17). The possibility of DIL during infliximab treatment is rare but well-known and lupus induced by TNF blockers is usually benign with resolution upon cessation of TNF blocker therapy. Recently, a case of anti-TNF induced lupus even after seven years of therapy with etanercept has been described (11). In almost half of the cases published, ANA were positive before treatment (9-11) or unavailable (8). In some of the reports, patients had previous symptoms suggesting incomplete lupus or a preexisting overlap between RA and SLE prior to anti-TNF therapy (18,19). Considering our case over time unmasked as SLE-RA overlapping, further and careful monitoring is needed in such patients.

One of the still controversial questions is whether there is any difference in the induction of autoantibodies between soluble TNF receptors such as etanercept and mononoclonal antibodies. The induction of ANA and anti-ds-DNA was observed sporadically in etanercept treated patients (16). Because AA occur more often during treatment with monoclonal antibodies than with etanercept, the prominent ANA and anti-ds-DNA autoantibody response is not a pure class effect of TNFα blockers. On the other hand, the number of cases of anti-TNF induced lupus by infliximab and etanercept is similar (11,20) which implies additional mechanisms in its occurrence.

CONCLUSION

The one-year treatment with infliximab induced antinuclear and anti-ds-DNA antibodies in a high number of our patients but in most of them, there was no clinical impact, which is consistent with literature data. There is a well-known possibility of rare drug induced lupus during infliximab treatment; however, considering our case, the disease overlapping could be unmasked by anti-TNFα treatment. In the minority of patients, ANA and anti-ds-DNA were still positive after two years of therapy discontinuation, without any clinical significance. Nevertheless, careful follow-up of patients treated with TNFα blockers remains mandatory, including monitoring for lupus-like characteristics.

Acknowledgment. This clinical trial was carried out before anti-TNFα drugs were approved for the treatment of rheumatoid arthritis in Croatia. The study was enabled by Schering-Plough, Croatia.

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