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Antiphospholipid antibodies during 6-month treatment with infliximab: A preliminary report

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Background: The introduction of tumor necrosis factor (TNF) antagonists (adalimumab, infliximab, and etanercept) was a major advance and was highly important and beneficial in most rheumatoid arthritis (RA) patients. The adverse effects of this treatment are infrequent, but include opportunistic intracellular infection (especially the reactivation of latent *Mycobacterium tuberculosis*); exacerbation of demyelinating disorders; and the production of various types of antibodies such as antinuclear antibodies (ANA) or double-stranded DNA autoantibodies (ds-DNA) and antiphospholipid antibodies (aPL) such as anti-cardiolipin antibodies (aCL) and anti-B₂GP-I antibodies (B₂GP-I). The aim of the study was to determine the prevalence of aCL and B₂GP-I in IgM and IgG classes, using ELISA tests, during 6 months of follow-up in patients with refractory RA successfully treated with infliximab.

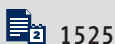
Material/Methods: We determined the prevalence of aCL and B₂GP-I in IgM and IgG classes, using ELISA tests, during 6 months of follow-up in patients with refractory RA successfully treated with infliximab.

Results: We observed a statistically important increase only in the group of B₂GP-I IgM ($p < 0.05$). There are contradictory results concerning the ability of infliximab to induce aPL, but most authors confirm this phenomenon.

Conclusions: Further investigations are needed to determine if the new aPL appears in patients with β 2-GPI gene polymorphisms such as leucine-to-valine substitution at position 247, which can lead to a conformational changes in β 2-GPI protein, leading to aPL synthesis. The role of aPL in pathogenesis of APS is still unclear, but we should remember the immunogenic aspect of TNF antagonist treatment. Therefore, we recommend early detection of aPL and observation of the patient, paying special attention to signs and symptoms of thromboembolism.

MeSH Keywords: **Antibodies, Antiphospholipid • Infliximab • Arthritis, Rheumatoid**

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Background

The introduction of the tumor necrosis factor (TNF) antagonists – adalimumab, infliximab, and etanercept – was a major advance and was highly important and beneficial in treating most rheumatoid arthritis (RA) patients who are refractory to a classic treatment with disease-modifying anti-rheumatic drugs (DMARDs) [1–5].

The adverse effects of this treatment are infrequent, opportunistic, intracellular infections, especially the reactivation of latent *Mycobacterium tuberculosis*, an exacerbation of demyelinating disorders. Moreover, the induction of severe neutropenia and thrombocytopenia can occur [6–8]. It is also possible to induce the production of various types of antibodies, such as antinuclear antibodies (ANA) or double-stranded DNA autoantibodies (dsDNA). Treatment with biological agents like infliximab can additionally induce synthesis of anti-drug antibodies, such as the human anti-mouse antibodies (HAMA) or human anti-chimera antibodies (HACA) [9].

The pathogenetic mechanism that changes the humoral response leading to development of autoimmunity during anti-TNF inhibitors therapy is unknown. A possible mechanism leads through the binding of infliximab to the transmembrane and soluble TNF, rapidly lowering TNF level and enhancing apoptotic cell death, which triggers the development of autoantibodies [10,11]. The other possible mechanisms that may result in autoantibodies production are: a) TNF-alpha inhibition that causes B-cell activation and production of autoantibodies through the upregulation of interleukin-10 [12], b) an increase in Th2 activity [13], and c) an increase in bacterial infections, which leads to the production of antibodies through molecular mimicry [6,14–17].

Only limited data have been published about the induction of antiphospholipid antibodies (aPL) during treatment using TNF inhibitors [18–20]. The stimulation mechanisms of its synthesis and role still remain unclear.

Antiphospholipid antibodies target phospholipid-binding proteins, and may cause a prolongation of phospholipid-dependent coagulation assays, although patients are at risk for thromboembolic rather than bleeding complications. The most often recognized antibodies from this group are now anti-cardiolipin antibodies (aCL) and the recently recognized antiphospholipid syndrome (APS) criteria anti-B₂GP-I antibodies (B₂GP-I). The aCL that are detected in patients with RA and other autoimmune diseases are directed against negatively charged phospholipids associated with B₂-glycoprotein, whereas aCL are associated with infection are directed against negatively charged phospholipids alone [21,22].

In normal populations (healthy blood donors), aCL are found in 2–6% of people, and in an aging population are found in

up to 12% and have been associated with the symptoms of APS such as recurrent thromboembolism and fetal loss [23,24]. In RA patients, the incidence of aCL may be even higher [25]. Their clinical significance in RA is uncertain and their presence has been considered to be a non-specific marker of activation of the immune system [26].

Material and Methods

We enrolled 32 infliximab-treated patients with refractory RA (28 females and 4 males, medium age 45.4 years, range 19–60 years). All of them were RF-positive and 25/32 (78%) were aCCP-positive. Patients were treated at the Department of Rheumatology and Connective Tissue Diseases, Medical University of Lublin, Poland.

All patients had a history of failed treatment with at least 1 DMARD. The patients were allowed to continue DMARDs, steroids, and non-steroid anti-inflammatory drugs before and during infliximab treatment. No patient had an infectious disease, active or latent tuberculosis, neoplastic disease, heart failure, cytopenia, or a demyelinating disorder.

The patients received 3 mg/kg infliximab intravenously at weeks 0, 2, and 6, and every 8 weeks thereafter. Methotrexate was given in a dose of 10 to 20 mg weekly. In addition to methotrexate, chloroquine (250 mg daily) and steroids (maximum daily dose 10 mg of oral prednisone or equivalent) were also permitted.

Written informed consent was obtained from all patients and the study was approved by the Bioethics Committee of the Medical University of Lublin.

Blood serum samples were collected from all patients at baseline and after 3 and 6 months of anti-TNF treatment. The sera were stored at –70°C until further analysis.

The patients were examined clinically at baseline and after 3 and 6 months of the study by the same physician during each visit for infliximab infusion.

The aCL and B₂GP-I antibodies (IgG and IgM classes) were tested using a commercially available enzyme-linked immunosorbent assay (ELISA) (Euroimmun, Germany). All the serum samples of RA patients were analyzed in a single session according to the manufacturer's instructions. The antibodies levels were measured in arbitrary units per milliliter and were considered to be positive at a cut off value of ≥20 U/ml. In further analysis, the sample was described as positive or negative.

In the present study, we investigate the prevalence of such autoantibodies during 6 months of follow-up in patients with

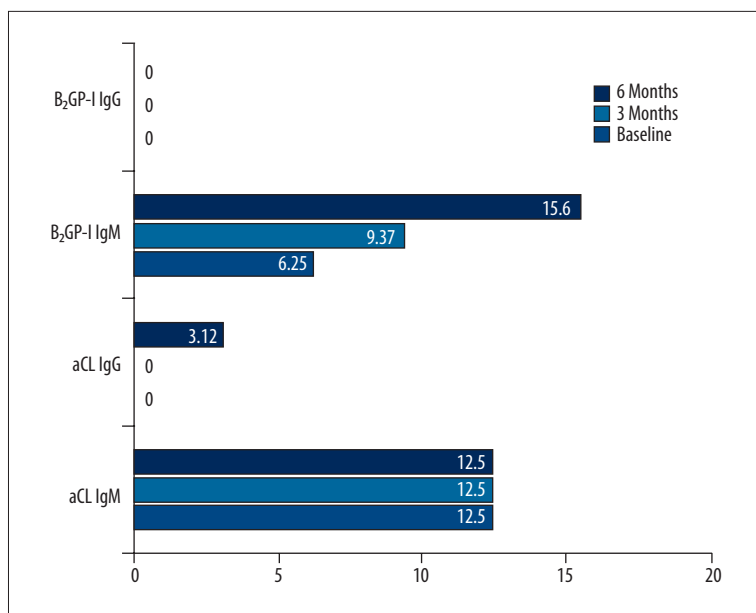


Figure 1. The percentage of aCL and B₂GP-I in IgM and IgG classes at the beginning and after 3 and 6 months of rheumatoid arthritis treatment with infliximab.

RA successfully treated with infliximab. aCL and B₂GP-I auto-antibodies were evaluated at baseline and at 3 and 6 months after the beginning of infliximab treatment.

Statistical analysis

Statistical analysis was performed using Statistica 7.0 PL software. Differences between groups were analyzed using Mann-Whitney U test. A p value less than 0.05 was considered to be statistically significant.

Results

We observed 4 aCL IgM-positive (12.5%) patients before the beginning of infliximab treatment. In these cases, we found no changes after 3 and 6 months of observation. There were no aCL IgG-positive patients at the beginning and after 3 months, but 1 case (3.12%) of seroconversion was observed after 6 months in aCL IgG class. During the whole study, we did not observe any B₂GP-I IgG-positive patients. Otherwise, we noticed a statistically important increase in the group of B₂GP-I IgM-positive patients ($p < 0.05$) (Figure 1). There were 2 (6.25%) seropositive patients before the start of the treatment, 1 (3.33%) after 3 months, and the next 3 (10.3%) after 6 months. Totally, we observed 4 (13.3%) new B₂GP-I IgM-positive patients, which was statistically significant.

Discussion

Elliott et al. found aCL in 1 out of 20 patients with RA treated with anti-TNF (cA2) in the first 8-week open trial on humans

[18]. These preliminary findings were confirmed by subsequent observations [14,17,27,28]. Rankin et al. measured the serological effects of repeated doses of the humanized anti-TNF antibody CDP 571 in patients with RA and found that some patients develop positive aCL (IgG) [29]. Ferraccioli et al. showed variations in aCL titers over time in etanercept-treated patients with concomitant bacterial infection, where lowering of titers was seen after the treatment with antibiotics [17].

Many studies have confirmed induction of ANA and anti-dsDNA in patients treated with infliximab [10,30–33]. Anti-dsDNA antibodies are usually IgM isotype [10] or IgM and IgA isotypes together [32].

Our results showed statistically significant changes only in B₂GP-I IgM class. However, the period of our observation was short and subsequent seroconversions are possible in the near future. We were not able to find an association between new B₂GP-I IgM and thromboembolism risk. A similar observation was presented by Jonsdottir et al., who noted a statistically significant increase in aCL IgM positivity after 3 and 6 months of infliximab treatment. This increase was seen in both aCL IgG and IgM classes. Another important observation was the worse clinical presentation in aCL-positive patients [34].

If aPL are not involved in APS, their role is difficult to explain. They may be nonspecific markers of the immune system activation and may vanish without a trace [25,26]. There are some reports that RA is associated with an increased frequency of aCL positivity [13,35,36]; the *de novo* production of aPL, especially aCL IgM, is associated with APS signs, such as thrombosis [37] or vasculitis [38].

According to Jonsdottir et al., a statistically significant increase in frequency of aCL IgM and IgG was induced in patients with RA at 3 months of treatment with infliximab [34].

The ASPIRE trial reported no statistically significant differences in aCL IgG and IgM classes between infliximab + MTX and MTX + placebo groups before or after treatment. Fewer than 3% of patients who received infliximab plus MTX and who had negative findings for IgG aCL at baseline were found to have positive results at week 30 or 54. For IgM aCL, a slightly higher proportion of patients who received infliximab plus MTX and had negative findings at baseline were found to have positive results at week 30 or week 54 (11.6%), as compared with the proportion of patients who received placebo plus MTX (7.6%) [39].

In the Ferraro-Peyret et al. study, the researchers found 21% of new aCL IgM antibodies [14].

Some authors suggest that the induction of aPL appears usually at the beginning of treatment, but Morris et al. demonstrated

that it is important to check the aCL appearance even between the 30th and 54th weeks of observation [28]. Similarly, Visvanathan et al. emphasize that it is important to obtain serial samples over at least 1 year to ascertain a patient's aPL status [40].

Further investigations are needed to determine if the new aPL appears in patients with β 2-GPI gene polymorphisms, such as leucine-to-valine substitution at position 247, which can lead to conformational changes in β 2-GPI protein, leading to aPL synthesis [41,42].

Conclusions

The role of aPL in the pathogenesis of APS is still unclear but we should remember the immunogenic aspect of TNF antagonist treatment. Therefore, we recommend early detection of aPL and observation of the patient, paying special attention to signs and symptoms of thromboembolism. A possible preventive treatment should be discussed in certain patients.

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