Tumor Necrosis Factor Alpha Antagonists and Occurrence of Autoantibodies in Inflammatory Bowel Disease Patients: A Single Center Experience

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Abstract

Background & Aims: Appearance of auto antibodies have been described during anti-tumor necrosis factor (TNF) alpha therapy; however, their prevalence and clinical relevance are still unclear. We investigated prevalence of autoantibodies in inflammatory bowel diseases (IBD) patients on anti-TNFα treatment and occurrence of clinical symptoms.

Methods: Titers of ANA, anti-dsDNA, SMA, AMA, LKM were evaluated from blood samples in patients receiving anti-TNFα inhibitor (adalimumab, infliximab).

Results: Among 39 patients treated with anti-TNFα therapy, twenty of them developed ANA, mostly induced by infliximab. 55% ANA positive patients developed peripheral polyarthalgias with no need for intervention. No patients with positive autoantibodies developed a drug-induced lupus. The incidence of dsDNA, SMA and AMA was low and was not associated with autoimmune disease.

Conclusions: Immune response induced by anti-TNFα is restricted to ANA, with lower prevalence of dsDNA antibodies, SMA and AMA. Further studies are needed to clarify the role of autoantibodies during anti-TNFα therapy.

INTRODUCTION

Biological therapies including anti-tumor necrosis factor (TNF) alpha inhibitors are increasingly used for a rapidly expanding number of rheumatic and inflammatory bowel diseases (IBD). These agents have been studied extensively and have demonstrated acceptable efficacy and safety profiles [1-3]. The adverse effects of this treatment are infrequent, such as opportunistic, intracellular infections, especially reactivation of latent Mycobacterium tuberculosis, and an exacerbation of demyelinating disorders [4-6]. Autoimmune phenomena, ranging from asymptomatic laboratory changes to presence of systemic autoimmune diseases, were also reported [6-10].

Many studies, most of them conducted on populations of patients affected by rheumatoid arthritis, have confirmed induction of antinuclear antibodies (ANA) or double-stranded DNA autoantibodies (dsDNA) in patients treated with infliximab (IFX) [11,12]. Data on adalimumab (ADA) have shown lower rates, compared to IFX, of ANA and anti-dsDNA antibodies in both rheumatoid arthritis and Crohn’s Disease (CD) [2,13]. No data have been published about the induction of auto-antibodies directed against smooth muscle (SMA), mitochondrial (AMA) and liver-kidney microsomal (LKM) antigens during treatment using tumor necrosis factor-α (TNFα) inhibitors. The stimulation mechanisms of its synthesis and role remain unclear. However, despite more than a decade using anti-TNFα agents, many questions remain. One of the most important is the association between autoantibody induction and certain diseases such as drug-induced lupus (DIL) with presence of arthritis, skin manifestations, and systemic symptoms. Post-marketing data on the two licensed anti-TNFα drugs have suggested an overall estimated incidence of DIL of 0.19-0.22% for IFX and 0.10% for ADA [8,14].

The aim of this study was to report a “real life” clinical experience about the occurrence of these autoantibodies during 12 months of observation in patients with IBD treated with ADA.
or IFX. In addition, we attempted to correlate the appearance of autoantibodies with clinical manifestations discovered during the study.

**METHODS**

This study was performed in accordance with the principles of the Declaration of Helsinki, and its appendices, and with local and national laws. Approval was obtained from the hospital’s Internal Review Board and the Ethical Committee, and written informed consent from all patients.

Thirty-nine IBD patients eligible to anti-TNFα therapy were consecutively recruited into the study. Seventeen were treated with IFX (5 mg/kg IFX intravenously at weeks 0, 2, and 6, and every 8 weeks thereafter) and 22 were treated with ADA (160 mg at week 0 and 80 mg at week 2; after induction treatment, the dose was 40 mg every 2 weeks via subcutaneous injection). Treatment choice, according to European Crohn’s and Colitis Organization (ECCO) Guidelines, was based on severity of disease, and on patient’s preference for the route of administration [15]. Medical records, including data about the presence of major extra intestinal manifestations, previous surgical procedures, the presence of familiar IBD, smoking habits and perianal involvement, were determined by a thorough review of the patient medical charts, which had been collected in uniform format. Previous and concomitant medical therapy was meticulously registered. The patients were allowed to continue steroids and immunosuppressive drugs before and during anti-TNFα treatment. No patient had an infectious disease, active or latent tuberculosis, neoplastic disease, heart failure, cytopenia or a demyelinating disorder. Follow-up appointments have been performed every 3 months with additional extraordinary visits if needed. These visits included clinical assessment, review of patient diaries and laboratory assessment (including C reactive protein). Levels of autoantibodies were determined at induction and after the one-year anti-TNFα period.

Blood serum samples were collected from all patients at baseline and after 12 months of anti-TNFα treatment. The sera were stored at −80 °C until testing. All the serum samples of IBD patients were analyzed in a single session according to the manufacturer’s instructions. ANA titers were measured by an indirect immunofluorescence (IFI) assay using HEp-2 cells as substrates according to the manufacturer’s guidelines. ANA titers ≥ 1:80 were considered clinically relevant and defined as positive titers. Anti-dsDNA antibodies, both IgM and IgG, were analyzed by a semi quantitative *Crithidia luciliae* fluorescent test (CLIFT) according to the manufacturer’s guidelines (Inova Diagnostics Inc). DsDNA values equal to or greater than 1:10 was interpreted as a positive result. ANA, ASMA, AMA, and anti-LKM were determined by indirect immunofluorescence (IFI) using slides of rat liver/kidney/stomach as antigen. We considered an antibodies titer ≥ 1:80 as positive result.

At the time of auto-Ab testing, patients were interviewed for symptoms of autoimmune disorders (DIL, arthralgia, vasculitis, peripheral neuropathies, skin rash, and autoimmune hepatitis). DIL was defined as arthritis including joint swelling, serositis and positive antibodies requiring an immediate stop of anti-TNFα and initiation of steroids and/or immunosuppressive therapy (azathioprine, methotrexate) [16]. According to The Crohn’s & Colitis Foundation of America, we defined arthralgia as “aching or pain in the joints” in one or more joints [17].

**STATISTICAL ANALYSIS**

Statistical analysis was performed using Statistical Package for Social Science (SPSS) version 13.0 for Windows. Data were expressed as median or range. Continuous variables were analyzed by the Mann-Whitney test. A p value less than 0.05 was considered statistically significant.

**RESULTS**

Demographic data of patients are presented in table 1. We observed four ANA-positive (10.2%) patients before the beginning of anti-TNFα treatment (Table 2). In these cases, we found no change after 12 months of observation. After 12 months of therapy, 20 patients (51.2%) developed a positive ANA titer; pattern was *homogeneous* in 13 cases. ANA induction was associated with *de novo* peripheral polyarthralgias in 11 patients (55.0%); eight of these (72.7%) had concomitant immunosuppression. A single patient who developed positive ANA but not anti-dsDNA experienced polyarthralgia and a persistent skin rash, which led to discontinuation of IFX.

There were no dsDNA-positive patients at the beginning, but two cases (5.1%) of seroconversion was observed after 12 months. These patients were also positive for ANA and developed polyarthralgias.

Five patients (12.8%) were SMA positive at baseline (Table 2). On treatment, two of them remained positive (11.7%) while three became negative (17.6%); only one patient treated with ADA developed new SMA positivity (4.5%). These antibodies were not directed against actin and so they were not considered as a biologic marker of autoimmune liver disease. Indeed, none of the patients experienced alterations of liver function tests. All sera were negative at baseline for AMA antibodies; one induction (2.5%) was observed after 12 months of IFX treatment. During the whole study, we did not observe any anti-LKM positive patient.

**DISCUSSION**

Our data confirm that biological agents trigger the induction of ANA in an elevated number of IBD treated patients. The pathogenic mechanism that changes the humoral response leading to development of autoimmunity during anti-TNFα inhibitors therapy is unknown. Many hypothesis have been formulated concerning autoantibodies formation during anti-TNFα treatment. It could be hypothesized that release of intracellular nuclear substances, because of anti-TNFα-induced cytotoxicity, results in a humoral immune response [18]. Indeed, IFX has been shown to increase both the number of apoptotic T lymphocytes in the *lamina propria* and the number of apoptotic monocytes in peripheral blood in CD [19, 20]. Inhibitors of TNFα also lead to a decrease of C-reactive protein, promoting the clearance of cell debris resulting from apoptosis. The presence of cell debris leads to a prolonged stimulation of antibodies [12]. The other possible mechanisms that may result in autoantibodies production are the B-cell activation and production of autoantibodies through the

Table 1: Demographic and clinical data of IBD patients according to anti-TNFα agents received.

<table>
<thead>
<tr>
<th></th>
<th>IFX (n = 17)</th>
<th>ADA (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48 (34-62)</td>
<td>42 (21-65)</td>
</tr>
<tr>
<td>Male/Female</td>
<td>7 (41.1%)/10 (58.9)</td>
<td>10/12</td>
</tr>
<tr>
<td>CD/UC</td>
<td>9 (52.9)/8 (47.1)</td>
<td>22 (100%)/0</td>
</tr>
<tr>
<td>Age at presentation</td>
<td>41 (22-59)</td>
<td>35 (19-57)</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>94 (29-260)</td>
<td>72 (12-230)</td>
</tr>
<tr>
<td>Refractoriness to immunosuppressant</td>
<td>9 (52.9)</td>
<td>5 (22.7)</td>
</tr>
<tr>
<td>Indication for anti-TNFα treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>steroids dependent</td>
<td>10 (58.8)</td>
<td>8 (36.4)</td>
</tr>
<tr>
<td>steroids refractory</td>
<td>2 (11.7)</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>penetrating Crohn’s disease</td>
<td>3 (17.8)</td>
<td>7 (31.8)</td>
</tr>
<tr>
<td>extraintestinal manifestations</td>
<td>2 (11.7)</td>
<td>4 (18.2)</td>
</tr>
<tr>
<td>Immunosuppressant use ever/during</td>
<td>9 (52.9)</td>
<td>4 (18.2)</td>
</tr>
<tr>
<td>anti-TNFα treatment</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are given as median (range) or as number (%).

Abbreviations: IBD: Inflammatory Bowel Diseases; CD: Crohn’s Disease; UC: Ulcerative Colitis; IFX: Infliximab; ADA: Adalimumab.

Table 2: Frequency of autoantibodies in 39 patients treated with anti-TNFα.

<table>
<thead>
<tr>
<th></th>
<th>IFX (n = 17)</th>
<th>ADA (n = 22)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At inclusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA ≥ 80</td>
<td>4 (10.2)</td>
<td>11 (64.7)</td>
<td>9 (40.0)</td>
</tr>
<tr>
<td>dsDNA ≥ 80</td>
<td>0</td>
<td>1 (5.9)</td>
<td>0</td>
</tr>
<tr>
<td>SMA ≥ 80</td>
<td>5 (12.8)</td>
<td>0</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>AMA ≥ 80</td>
<td>0</td>
<td>1 (5.9)</td>
<td>0</td>
</tr>
<tr>
<td>LKM ≥ 80</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA ≥ 80</td>
<td>4 (10.2)</td>
<td>11 (64.7)</td>
<td>9 (40.0)</td>
</tr>
<tr>
<td>dsDNA ≥ 80</td>
<td>0</td>
<td>1 (5.9)</td>
<td>0</td>
</tr>
<tr>
<td>SMA ≥ 80</td>
<td>5 (12.8)</td>
<td>0</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>AMA ≥ 80</td>
<td>0</td>
<td>1 (5.9)</td>
<td>0</td>
</tr>
<tr>
<td>LKM ≥ 80</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Data as number (percentage).

Abbreviations: IBD: Inflammatory bowel diseases; TNFα: Tumor Necrosis Factor; IFX: Infliximab; ADA: Adalimumab; ANA: Antinuclear Antibodies; dsDNA: Double Strand DNA; SMA: Smooth Muscle Antibodies; AMA: Anti-Mitochondrial Antibodies; LKM: Liver-kidney Microsome.

Consistent with data reported in the literature, IFX seems to be the drug most closely related to the development of autoantibodies, probably because it is a chimeric antibody and this may cause a greater immune response. A possible mechanism leads through the binding of IFX to the transmembrane and soluble TNFα, rapidly lowering TNFα level and enhancing apoptotic cell death, which triggers the development of auto-Ab [23-25].

With regard to ADA, it specifically binds to soluble and transmembrane TNFα, but it is not known why it does not give rise to autoantibodies production as frequently as IFX does [26].

The observed rate of ANA induction (51.2%) in our cohort is consistent with data from the literature. On the contrary, the occurrence of anti-dsDNA (5.1%) is lower than that found previously [12,27-29]. Previous studies on the development of auto-Ab during anti-TNFα therapy in IBD patients have shown discrepant results. A French study showed ANA positivity in 14% of 35 CD patients before IFX treatment that increased to 53% after 12 months; dsDNA antibodies were detected in one patient (3%) at baseline and in 35% after 1 year of IFX treatment [27]. In another study [28], 22% of CD patients were ANA-positive at baseline and after 6 weeks of IFX treatment, an additional 16.7% became ANA-positive; however, only three patients (8.3%) developed dsDNA antibodies. In another cohort of 125 CD patients the cumulative ANA prevalence was 56.8% after a follow-up of 24 months, with 32.6% of dsDNA positivity [12]. In a study by Beigel et al. 44.4% of anti-TNFα treated IBD patients had elevated ANA titers, while 15.6% had dsDNA positivity. Among the subgroups (IFX, ADA, ADA after IFX, with and without immunosuppression, respectively) there was no statistically significant probability of developing ANA and dsDNA [29].

Interestingly, 43% of our patients receiving concomitant immunosuppressive therapy were found to have an induction of ANA. This finding is consistent with some reports [12, 30] though the most recent study suggests a protective effect of concomitant immunomodulator both against ANA and DIL development [29]. Whereas these drugs have been shown to reduce the immunogenicity of anti-TNFα, our data suggest that azathioprine or methotrexate may have an additional or synergistic effect on the cell apoptotic process and the release of nuclear antigens. Only age seems related to the development of clinic signs of autoimmunity. Further, we can also speculate that in our cohort ANA induction correlated with older age and longer disease duration; we do not have enough data to correlate autoantibodies appearance during IFX therapy and female gender, as it have been recently reported [31].
In spite of the appearance of ANA and anti-dsDNA positivity, no cases of DIL were observed. Reviewing the literature, DIL is generally rare among patients treated with anti-TNFα antagonists and most of the cases are reported in rheumatoid arthritis, while only a few reports exist for IBD patients [31,32].

Eleven out of 14 patients (78.6%) who developed arthralgia had ANA positivity, but anti-TNFα therapy could be continued without aggravation of symptoms, except in one patient who also developed a skin rash and was successfully treated with steroids after withdrawal from anti-TNFα therapy. We also searched for other auto-Ab, such as SMA and AMA that have poorly been thoroughly investigated in relation to anti-TNFα treatment until now.

However, we are aware that this study has some limitations. First, because of the small number of patients data need to be confirmed in a larger series [33]. Secondly, follow up assessment of autoantibodies after drug discontinuation is not available because patients were refusal or drop-out.

Nevertheless, our data confirm that despite the high frequency of auto-Ab development associated with anti-TNFα agents, relatively few patients develop autoimmune clinical symptoms. The strength of the present study, although the small sample size, is that we included a cohort of well-characterized IBD patients from high-quality referral center in Italy with standardized patient selection and follow-up. We believe that more extensive long-term studies are needed to clarify the influence of auto-Ab on clinical outcomes.

REFERENCES

27. Atzeni F, Turiel M, Capsoni F, Doria A, Meroni P, Sarzi-Puttini P.


