Autoantibodies in patients with psoriatic arthritis on anti-TNFα therapy

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ABSTRACT

Introduction: Anti-TNFα therapy has been effective in the treatment of patients with refractory psoriatic arthritis (PSA). However, the risk of developing autoantibodies commonly found in rheumatic diseases in PSA patients undergoing this therapy is not clear. **Objective:** To evaluate the induction of specific autoantibodies after anti-TNFα therapy in PSA patients. **Patients and methods:** Serum samples from 23 PSA patients (women: 61%, age: 45.04 ± 12.68 years, polyarticular: 69.6%, disease duration: 13.3 ± 7.7 years, infliximab: 82.60%) obtained immediately before (baseline) and approximately one year after the introduction of anti-TNF therapy (last sample) (385 ± 131.45 days), were analyzed. The analysis included detection of antinuclear antibodies (ANA) and anti-dsDNA antibodies (indirect immunofluorescence on Hep-2 cells and *Crithidia luciliae*, respectively); anti-RNP and anti-Sm (passive hemagglutination); and anti-Ro/SS-A and/or anti-La/SS-B, anti-chromatin, anti-histones, anti-citrullinated peptide (CCP), and anti-cardiolipin (ELISA) antibodies. **Results:** At baseline, ANA was positive in 47.8% of patients, with predominance of homogeneous nuclear pattern (81.8%). All baseline serum samples were negative for rheumatoid factor and antibodies to cardiolipin, RNP, Sm, Ro/SS-A, anti-La/SS-B, anti-histone, and anti-dsDNA antibodies, while two patients were positive for anti-chromatin and one for anti-CCP. All ANA-positive samples at baseline, except for one, remained positive after the introduction of anti-TNF therapy; however, *de novo* ANA reactivity was observed in four originally negative patients (33.3%). Anti-Ro/SS-A, La/SS-B, cardiolipin, histones, dsDNA, and rheumatoid factor antibodies remained negative in all final serum samples tested, and anti-chromatin positivity was detected in three other patients. **Conclusion:** Our findings have shown that anti-TNF therapy induced ANA positivity in one third of PSA patients. The concomitant use of methotrexate did not interfere with this finding. In addition, all serum samples were systematically negative for specific rheumatic autoantibodies tested after the introduction of the biological treatment.

Keywords: psoriatic arthritis, anti-TNFα therapy, autoantibodies.

INTRODUCTION

Psoriatic arthritis (PSA) is characterized by peripheral, asymmetrical joint involvement of the lower extremities (oligo or polyarticular) and/or spine (axial) secondary to a chronic inflammatory process. This manifestation is associated with cutaneous psoriasis, which, in the majority of the cases (70% to 85%), precedes the articular manifestation. It equally affects men and women at the third to fifth decade of life (30-50 years), is more common in Caucasians and has a clear genetic predisposition. It has a prevalence of 0.04% to 0.2%, in the general population and 25%-34%, in patients with psoriasis.[1]

Laboratory findings frequently (63%) show changes in indicators of inflammatory activity, such as increase in serum levels of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Other non-specific indicators of the disease include anemia, hypoalbuminemia, polyclonal hypergammaglobulinemia and the presence of circulating immune complexes. As for autoantibodies, the presence of

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antinuclear (10%) and anti-citrullinated peptide (CCP) (13%-17.5%) antibodies has been reported.[2,3] On the other hand, among the classification criteria for PSA is the absence of serological positivity for rheumatoid factor.[4]

Evidence shows that immunologic mechanisms mediated by activated T lymphocytes (CD8+) contribute for the increased production of proinflammatory cytokines (IL-1β and TNF-α) that participate in the genesis of PSA, which make it vulnerable to therapeutic intervention.[5,6] Thus, among the new therapies available, the introduction of systemic biological agents, selective TNF-α antagonists, have resulted in a marked improvement in the quality of life of PSA patients refractory to conventional therapy.[6]

However, the expansion in the use of those biological agents in inflammatory arthritis has provided the observation of adverse clinical and biological events, including the development of autoantibodies, which, in rare cases, are accompanied by disease manifestations.[7] More than 60% of patients with rheumatoid arthritis treated with infliximab developed antinuclear antibodies.[8] Similarly, a high incidence of those antibodies was observed in patients with Crohn's disease treated with anti-TNF.[9] On the other hand, the findings in patients with juvenile idiopathic arthritis show that the induction of this altered humoral reactivity by immunobiologicals is rare,[10] suggesting that this phenomenon might be associated with the underlying disease. Regarding psoriatic arthritis, few studies on the development of anti-TNF-associated autoantibodies are available and most are short-term studies.[7,11]This latter aspect is relevant, as the development of autoantibodies is associated with longer exposure to those compounds.[12]

The present study evaluated the production of autoantibodies commonly associated with rheumatic diseases in PSA patients on long-term therapy with anti-TNFα.

PATIENTS AND METHODS

Patients

Twenty-three patients who met the diagnostic criteria for psoriatic arthritis (PSA)[13] followed at the High-Cost Drug Dispensation Center (CEDEMAC, from the Portuguese) of FMUSP participated in this study. Anti-TNF therapy consisted of recommended doses of adalimumab, etanercept, and infliximab. Blood samples were obtained before the administration of the first dose (baseline) and at least five months after the introduction of the treatment with the immunobiological agent. Serum samples obtained from all patients were immediately distributed in aliquots and stored at -70°C until analysis. All patients signed the informed consent form, and the study was approved by the local Ethics Committee (Protocol number 1298/06).

Autoantibodies

Analysis of autoantibodies was carried out in serum samples before the first (baseline) and after the last dose (final) of the immunobiological agent during the study period. Blood samples from each patient were stored and underwent paired testing (baseline and final), always in the same experiment, to avoid interassay reactivity differences.

Antinuclear antibodies (ANA) and anti-native or double stranded DNA (dsDNA) antibodies were detected by in-house indirect immunofluorescence (IIF) using Hep-2 cells and *Crithidia luciliae* as substrates, respectively. Titers ≥ 1:80 and ≥ 1:10, respectively, were considered positive for those antibodies.

Determination of the presence of saline-extractable antigens , anti-RNP and anti-Sm, was performed by using the in-house passive hemagglutination using chicken erythrocytes sensitized with rabbit thymus extract as the source of antigen.[14] Samples with titers ≥ 1:100 were considered positive.

The presence of anti-Ro/SS-A antibodies of 52 and 60 kDa and anti-La/SS-B antibodies was determined by in-house ELISA using purified recombinant proteins.[15] Results were expressed in optical density (OD). Serum samples with reactivity greater than the mean value of 15 serum samples of healthy individuals by three standard deviations were considered positive (cutoff values: 0.31, for anti-Ro of 53 kDa, 0.30, for anti-Ro of 60 kDa, and 0.32, for anti-La/SS-B). The presence of rheumatoid factor was determined by latex particle agglutination using a commercially available reagent (Laborclin, PR, Brazil). Positivity was defined as equal to or greater than 16 IU/mL.

Anti-chromatin IgG (INOVA, USA), anti-citrullinated peptide (CCP) (INOVA, USA), and anti-histones (Euroimmun, Germany) antibodies were detected by ELISA using commercial kits. Sera were tested in duplicate, according to the protocol recommended by each manufacturer. Levels higher than 20 U/mL were considered positive for all three antibodies, according to the recommendation of each manufacturer.

The presence of anti-cardiolipin IgG and IgM antibodies was detected by in-house ELISA, and levels > 10 U were considered positive. Calibrators (Louisville APL Diagnostics, Inc., TX, USA) were used to plot the curve of optical density *versus* units of IgG (GPL) and IgM (MPL).

RESULTS

The majority of patients were females (61%) with a mean age of 45.04 ± 12.68 years. Polyarticular disease predominated (6.6%), followed by isolated oligoarticular (21.73%) and axial involvement (8.7%) (Table 1). Disease duration was 13.3 ± 7.7 years. Twenty-two (95.6%) patients had exacerbated psoriasis at the time of study enrollment.

The mean time of analysis of the immunobiological therapy was 385 ± 131.45 days, with a median of 425 days. Nineteen (82.60%) patients with PSA were treated with infliximab, three (12%) with etanercept, and one (4%) with adalimumab. Twelve of nineteen (63.15%) patients treated with infliximab underwent combined therapy with methotrexate (MTX), whereas it was used in 2/3 (66.7%) of the patients treated with etanercept (Table 2).

Antinuclear antibodies in baseline serum samples of PSA patients were positive in 47.82% of the cases (11/23) with a predominance of homogenous nuclear pattern (81.8%), of which 6/11 (54.5%) had high titers (arbitrarily defined as ≥ 1/160). Characterization of the antigenic specificity of this reactivity showed an absence of antibodies against Ro/SS-A, La/SS-B, histones, and dsDNA (Table 3). Two serum samples showed moderate positivity for anti-chromatin antibodies, with a mean titer of 47 U, and one for anti-CCP, with a low titer (23 U).

Evaluation of the blood samples obtained at the time of the last dose of anti-TNF showed that 10/11 (90.9%) originally ANA-positive serum samples remained positive, without changes in the immunofluorescence pattern, but showing an increase in the mean titer (167 ± 104 *versus* 208 ± 101). *De novo* ANA reactivity was observed in four originally negative patients (33.3%), two of which had speckled nuclear pattern (1/80), whereas the other two showed homogenous nuclear pattern with

Table 1
Clinical-demographic characteristics of patients with psoriatic arthritis undergoing anti-TNFα therapy

Demographic and clinical data	Prevalence (N = 23)
Female gender	14 (61%)
Age (mean ± SD, years)	45.04 ± 12.68
Duration of the disease (mean ± SD, years)	13.3 ± 7.7
Clinical type	
Polyarticular	16 (69.6%)
Isolated oligoarticular	5 (21.73%)
Isolated axial	2 (8.7%)

Table 2
Type and duration of immunobiological therapy and concomitant pharmacological agent

Therapy	Prevalence (N = 23)
Adalimumab	1 (4.3%)
Number of doses*	24
Etanercept	3 (13%)
Number of doses*	48
Infliximab	19 (82.6%)
Number of doses*	8
Current use of methotrexate Dose: 7.5-20 mg/week	14 (61%)
Other concomitant DMARDS	
Prior	19 (82.6%)
Current	5 (21.7%)
Duration of immunobiological therapy (mean ± SD, days)	385 ± 131.45
Duration of immunobiological therapy ≥ 1 year	17 (73.9%)

* During the study period.

Table 3
Frequency of autoantibodies in patients with psoriatic arthritis undergoing anti-TNFα therapy

	Frequency (N = 23) n (%)	
Autoantibody	BASAL	FINAL
ANA (Results)		
Positive	11 (47.8)	14 (60.9)
Became negative	—	1
Became positive	—	4
Titer, mean ± SD	131.4 ± 115.8	183 ± 115
Titer ≥ 1/160	6 (54.5)	9 (64.2)
Immunofluorescence pattern		
Homogenous	9/11 (81.8)	10/14 (71.4)
Fine dense speckled	1/11 (0.9)	1/14 (0.7)
Nucleolar	1/11 (0.9)	1/14 (0.7)
Speckled	0	2/14 (14.3)
Anti-CCP	1 (4.3)	0
Anti-chromatin	2 (8.7)	5 (21.7)
Anti-dsDNA	0	0
Anti-Ro/SS-A	0	0
Anti-La/SSB	0	0
Rheumatoid factor	0	0

titers of 1/80 and ≥ 1/320, respectively. The mean duration of the immunobiological therapy of these four patients was 281 ± 152 days (median: 266.5 days). No significant differences in mean age (P = 0.89), disease duration (P = 0.85), and polyarticular disease (P = 1.00) were observed when the patients who became ANA-positive during the treatment were compared to patients who remained persistently ANA-negative.

The positivity of antinuclear antibodies in 14 patients under concomitant treatment with MTX and in nine patients who did not receive this drug was similar (P = 0.15). Similarly, differences in anti-chromatin antibodies were not observed between the two groups (P = 1.00). Regarding the prior use of disease-modifying drugs, 5/23 (22%) patients had been treated with cyclosporine, five (22%) with leflunomide, and four (17.4%) with acitretin. Two patients who had been treated with leflunomide and two with acitretin were ANA-positive.

Anti-dsDNA antibodies were uniformly negative (basal/final samples), while anti-chromatin reactivity was found in basal samples of two patients (8.7%), with mean titer of 47 ± 9.90 U, which is equivalent to the moderate positivity range established by the manufacturer (20 to 60 U). At the end of the evaluation period, these patients maintained this level of reactivity (39 ± 14.14 U), while three other patients became positive (31.33 ± 11.01).

On the other hand, anti-Ro/SS-A, anti-La/SS-B, anticardiolipin IgG/IgM, and anti-histone antibodies, as well as rheumatoid factor, were systematically negative in all tested blood samples. Regarding anti-CCP antibodies, the patient with low reactivity (23 U) at the beginning of the study showed subsequent negativity.

DISCUSSION

Our data suggest that anti-TNF-induced production of autoantibodies commonly associated with autoimmune rheumatologic diseases is rare in PSA.

The present study describes the largest systematic evaluation of autoantibody development in patients with PSA treated with anti-TNF drugs.[7,11]

It is unlikely that the low rate of autoantibody production induced by anti-TNF therapy observed in the present study could be explained by a restriction in the duration of the evaluation, as the design of the study included an observation period that was longer than the 12-month therapy. The temporal importance of immunological changes induced by immunobiologicals has been reported in the literature.[12] A significant rise in the frequency of ANA has been observed during treatment in rheumatoid arthritis and spondyloarthropathies.[16,17]

The frequency of ANA antibodies observed in PSA patients, of approximately 50%, is similar to that described in the literature.[18] The analysis of the baseline profile is fundamental for the interpretation of eventual immunological changes induced by anti-TNFa therapy. Thus, the more prevalent finding of homogenous pattern at immunofluorescence before treatment might suggest a higher risk of developing autoantibodies that are specific for autoimmune diseases, such as anti-dsDNA. In fact, a study in our country with 394 ANA-positive samples showed that the homogenous pattern is associated, almost exclusively, with autoimmune diseases.[19] In this context, the finding of the present study becomes more relevant, as it demonstrates that this baseline reactivity does not indicate a greater predisposition for the development of autoantibodies specific for autoimmune rheumatologic diseases in PSA. Moreover, the analysis of patients treated concomitantly with MTX, compared to those that did not receive this drug, did not show any difference in ANA positivity between the groups. The data also showed that disease duration does not seem to influence the development of ANA in these patients.

As for specific autoantibodies produced as a consequence of this type of treatment, the majority of the reports have focused on those with specificity for the double-stranded DNA, a known serological marker of systemic lupus erythematosus.[12,20] The frequency of this antibody reported in the literature varies extremely, possibly due to different methods of detection used and encompassing immunoglobulin isotypes.[12] These parameters seem to justify the clinical-serological dissociation observed in patients treated with anti-TNFa. Indeed, the antibodies produced in these conditions, subtypes IgA and IgM, usually have low affinity and are present at low titers, in contrast with the pathogenic reactivity that it is usually identified in systemic lupus erythematosus, which is characterized by high-affinity antibodies, subtype IgG, present at high titers.[8] Therefore, in the present study, a highly specific technique (IIF), restricted to IgG antibody detection, demonstrated that the immunobiological therapy does not induce the production of anti-dsDNA antibodies in PSA. However, positivity for anti-chromatin antibodies, which has higher sensitivity and involves reactivity against the DNA-histone complex, was detected by ELISA in some patients treated with immunobiologicals. The clinical importance of this finding should be evaluated in future prospective studies.

On the other hand, two studies showed a reduction in the titers of rheumatoid factor and anti-CCP antibodies in RA patients treated with infliximab.[21,22] This is in agreement with the present study, in which only one patient presented low

positivity for anti-CCP antibodies prior to the infusion of the anti-TNF drug, which became negative during the study.

The findings of the present study demonstrate that anti-TNF therapy induced ANA positivity in one third of originally negative PSA patients and the concomitant use of MTX did not affect this finding. Moreover, all serum samples were systematically negative for autoantibodies specific for rheumatologic diseases after the introduction of biologicals.

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