Anti-TNF-α treatment in rheumatoid arthritis with anti-Ro/SSA antibodies. Analysis of 17 cases among a cohort of 322 treated patients

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Abstract

Objective

To compare the efficacy and safety of anti-TNF-α treatment in RA patients with and without anti-Ro antibodies, in order to detect any change in their immunological or clinical profile.

Methods

Autoantibodies in 322 patients being treated with anti-TNF-α drugs were studied; 17 were found to be anti-Ro positive, while 305 were anti-Ro negative.

Results

Two groups, comparable in terms of sex distribution, RA duration and anti-TNF-α drug employed, showed symmetrical, erosive polyarticular RA with high disease activity. Anti-TNF-α led to significant improvement in both groups. At baseline rheumatoid factor and ANA, globally positive in 68.6% and 40%, were more frequent in anti-Ro positive sera. ANA showed a rising trend beginning in the 6th month of treatment in both groups, which was always statistically significant compared to baseline. Anti-dsDNA antibodies, measured using either CLIFT and ELISA or the Farr assay, remained stable in the first 6 months, then increased at 12th and 18th month, and subsequently declined. No difference was detected between the two groups regarding the number or cause of dropouts, but lupus-like disease was more frequent in anti-Ro positive subjects ($p = 0.012$). In addition, two cases of NHL were detected.

Conclusion

Anti-TNF-α treatment was shown to be effective in patients with anti-Ro antibodies. Although anti-dsDNA and lupus-like disease were more frequent in anti-Ro positive patients, severe manifestations of systemic involvement were not observed. A longer follow-up is warranted to evaluate the risk of NHL in these patients.

Key words

Anti-TNF-α, anti-Ro/SSA, rheumatoid arthritis, infliximab, etanercept.

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Introduction

Anti-TNF-α drugs are widely used in the ordinary treatment of patients affected by severe rheumatoid arthritis (RA), in order to reduce disease activity and functional disability (1-5). However, selective blocking of TNF-α can result in a modulation of the immune system, inducing new circulating autoantibodies (6-17) and, more rarely, lupus-like disease (6, 10, 15-18). Several authors have reported the production of antinuclear and anti-dsDNA antibodies in 7-85% of cases treated with infliximab and etanercept (6-12), frequently detected within the first weeks of treatment (8, 10, 11). In addition, some reports describe the onset of anti-phospholipids (7, 11, 13, 14) or, exceptionally, anti-ENA antibodies, detected by very sensitive assays such as ELISA (8, 9). Nevertheless, clinical features of lupus-like disease have rarely been reported; they are usually linked to IgG anti-dsDNA and usually remitted after the discontinuation of anti-TNF-α (6, 10, 15-17). Furthermore, anti-TNF-α treatment has been tested on small groups of patients affected by systemic lupus erythematosus (SLE) or Sjögren's syndrome (SS), with no reports of significant worsening in the clinical features or autoantibody profile of the patients (19-25).

Anti-Ro/SSA antibodies have been detected in 3-15% of RA patients (26, 27), a subgroup frequently characterized by more extra-articular features (sicca, skin vasculitis, leukopenia), broad immunological activation (hypergammaglobulinemia, high titer rheumatoid factor and ANA) and different immunogenetic features (26-31).

In the present study, we compared the clinical response and safety of anti-TNF-α treatment in RA patients with anti-Ro/SSA antibodies, in order to determine whether there was any difference in their immunological profile or clinical evolution with respect to anti-Ro/SSA negative patients.

Patients and methods

We studied a cohort of 322 patients affected by RA according to ACR criteria (32), who were attending two northern Italian rheumatologic outpatient centres and were being treated with anti-TNF-α drugs (infliximab or etanercept). They were prospectively evaluated every 8 weeks by the same medical staff from January 2000 to September 2005. During each visit, the following clinical outcomes were measured: swollen and tender joint counts, duration of morning stiffness, signs of infection, health status using the Health Assessment Questionnaire (HAQ), and acute phase reactants. In addition, examinations were conducted for all lupus-like features, such as malar rash, photosensitivity, oral ulcers, urinary or haematological alterations, peripheral neuropathies, serositis, according to the SLE classification criteria (33).

Before starting treatment and then every 6-12 months, circulating autoantibodies were measured using the routine methods of the two participating labs. Specifically, antinuclear antibodies (ANA) were tested by indirect immunofluorescence (IIF) with HEp2 cells as substrate (Kallestad, Chaska, MN, USA or Immunoconcept, Sacramento, CA, USA) and were considered positive at a titer of 1:80 or more. Anti-dsDNA antibodies were tested by the Farr assay in 212 sera (Kodak Clinical Diagnostics, Amersham, UK), considering values higher than 4.2 U/ml as positive (low positive = 4.3 to 20 U/ml, medium positive = 20 U/ml to 60 U/ml, and high positive > 60 U/ml). The 110 remaining sera were analysed by a commercial ELISA (Axis-Shield, Dundee, UK) as the first line test; positive results were subsequently confirmed by indirect immunofluorescence test on Crithidia luciliae (CLIFT) (INOVA, San Diego, CA, USA) using an anti-human IgG antiserum according to the manufacturer's recommendations. Anti-cardiolipin (aCL) and anti-$\beta_2$glycoprotein I (β_2GPI) were detected using a home-made ELISA, as previously described (34, 35). Rheumatoid factor and anti-CCP antibodies were detected at baseline using commercial ELISA kits.

Anti-ENA antibody determinations were performed by counterimmunoelectrophoresis (CIE), according to Bernstein *et al.* (36) using rabbit thymus extract (Peel-Freeze, Rogers, AR, USA) and human spleen extract as antigen sources, prepared according to Clark *et al.*

(37) and Venables *et al*. (38). In these sera, the presence of anti-Ro/SSA antibodies was then confirmed by ELISA (Diastat™, Axis Shield, Dundee, UK). Seventeen patients were found to be anti-Ro/SSA positive, while 305 were anti-Ro/SSA negative at baseline. During the follow-up we lost subjects at the same rate in the two groups (difference not significant). In fact, the numbers of anti-Ro/SSA positive and anti-Ro/SSA negative subjects were 16 and 247 at 6 months, 13 and 186 at 12 months, 11 and 131 at 18 months, 9 and 104 at 24 months, 7 and 80 at 30 months, 6 and 54 at 36 months, respectively.

Statistical analysis

All parameters were studied by the χ^2 test, with Yates' correction where indicated. The Student's *t*-test was performed to compare the DAS values of the two groups at any observation point. A longitudinal comparison between the linear regression curves of the DAS was performed by ANOVA using the Statview PC program.

Results

A cohort of 322 patients (female:male ratio 5:1) affected by longstanding RA were treated with infliximab (200 subjects) or etanercept (122 subjects) for a mean period of 24 months (SD: 16.6 months). Seventeen anti-Ro/SSA positive patients with longstanding RA (11.5 years) not responsive to different DMARDs showed a female to male ratio of 3.25. Six of them were treated with etanercept and 11 with infliximab for a mean period of 29.6 months (SD: 17.8).

Clinical and immunological features at baseline

Anti-Ro/SSA positive and negative patients were comparable for sex distribution, RA duration, past treatment with DMARDs, and the anti-TNF-alpha drug employed, as shown in Table I. Before starting anti-TNF-α treatment, all the patients showed symmetrical polyarticular RA with high disease activity (DAS); anti-Ro/SSA positive subjects showed a significant higher DAS index (p: 0.006), comparing to anti-Ro/SSA negative group (Table I). Radiological erosions and anti-CCP antibodies were globally detected in 86.6% and 80% of the patients, respectively, without significant differences between the anti-Ro/SSA positive and anti-Ro/SSA negative groups. Extra-articular features were low and comparable in the two groups, except for xerostomia and xerophthalmia, which were primarily observed in the anti-Ro/SSA positive subjects ($p = 0.0022$ and $p = 0.0001$, respectively), while photosensitivity, and pulmonary and cutaneous involvement were rarely reported, as shown in Table II. Only 5 patients with anti-Ro/SSA antibodies could be classified as having Sjögren's syndrome according to European/American criteria (39). During the treatment no patients reported a worsening in sicca symptoms, although no dacriologic or salivary tests were performed during follow-up. One patient affected by recurrent episcleritis showed a complete resolution of symptoms during infliximab therapy.

At baseline, rheumatoid factor and ANA were globally positive in 68.6% and 40% of the patients, being more frequently detected in anti-Ro/SSA positive sera ($p = 0.02$ and $p = 0.0008$). Anti-dsDNA and anti-phospholipid (aCL and/or anti-beta2GPI) antibodies were detected in 5.5% and 4.5% of patients, with no difference between the two groups (Table II).

Clinical and immunological features during anti-TNF-α treatment

Anti-TNF-α treatment led to a significant clinical improvement in articular involvement in all the subjects, who were examined every 6 months for a period of 36 months ($p < 0.000001$). Analysing the variation in DAS values compared to baseline, we found a significant difference between anti-Ro/SSA positive and negative patients at the 24th and 36th months of treatment, with a better response in the anti-Ro/SSA positive patients ($p = 0.026$ and $p = 0.02$, respectively) (Fig. 1). In contrast, comparison of the raw DAS values between the two groups showed

Table I. Demographic data and articular features before starting anti-TNF-α treatment in 322 patients affected by RA.

	Total	Anti-Ro neg.	Anti-Ro pos.	*p*
No. of patients	322	305	17	
F:M	266/56	253/52	13/4	NS
Mean age, years (SD)	58.9 (13.5)	59.01 (13.7)	56.8 (8.32)	NS
Mean RA duration, years (SD)	11.8 (7.5)	11.81 (7.5)	11.5 (5.9)	NS
No. of DMARDs, mean (SD)	3.46 (1.6)	3.42 (1.57)	4.23 (2.1)	NS
Anti-TNFα duration, months (SD)	24.1 (16.6)	23.8 (16.5)	29.6 (17.8)	NS
Etanercept	122 (37.8%)	116 (37.9%)	6 (35.3%)	NS
Infliximab	200 (62.1%)	189 (62%)	11 (64.7%)	NS
DAS	4.39 (0.96)	4.19 (1.3)	5.017 (1.02)	0.006

Table II. Extra-articular features and autoantibodies before starting anti-TNF-α treatment in 322 RA patients.

	Total n = 322 (%)	Anti-Ro negative n = 305 (%)	Anti-Ro positive n = 17 (%)	*p*
Xerostomia	57 (17.6)	50 (16.4)	7 (41.1)	0.0022
Xerophthalmia	34 (10.5)	27 (8.8)	7 (41.1)	0.0001
Photosensitivity	7 (2.1)	6 (1.9)	1 (5.8)	NS
Pulmonary involvement	14 (4.3)	14 (4.5)	0	NS
Skin vasculitis	2 (0.6)	2 (0.6)	0	NS
Raynaud's phenomenon	6 (1.8)	6 (1.9)	0	NS
Rheumatoid factor	221 (68.6)	205 (67.2)	16 (94.1)	0.02
ANA	108/268 (40)	94/251 (37.4)	14/17 (82.3)	0.0007
Anti-dsDNA antibodies	14/254 (5.5)	12/237 (5)	2/17 (11.7)	NS
Anti-cardiolipin and/or β_2GPI	7/155 (4.5)	6/148 (4)	1/7 (14)	NS

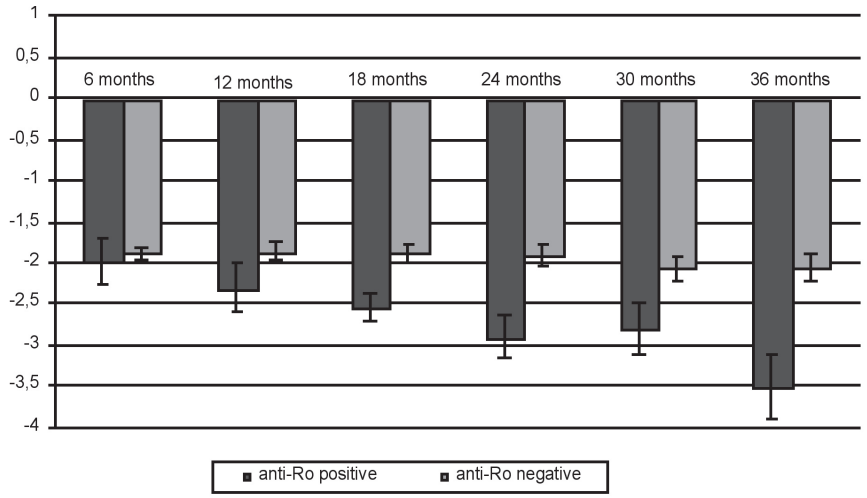

Fig. 1. Variation in the DAS (delta) during anti-TNF-α treatment in patients with and without anti-Ro antibodies.
The anti-Ro positive group appears to show a more impressive reduction in DAS, with a significant difference at the 24th (p = 0.026) and 36th months (p = 0.02) of treatment compared to the anti-Ro negative group.

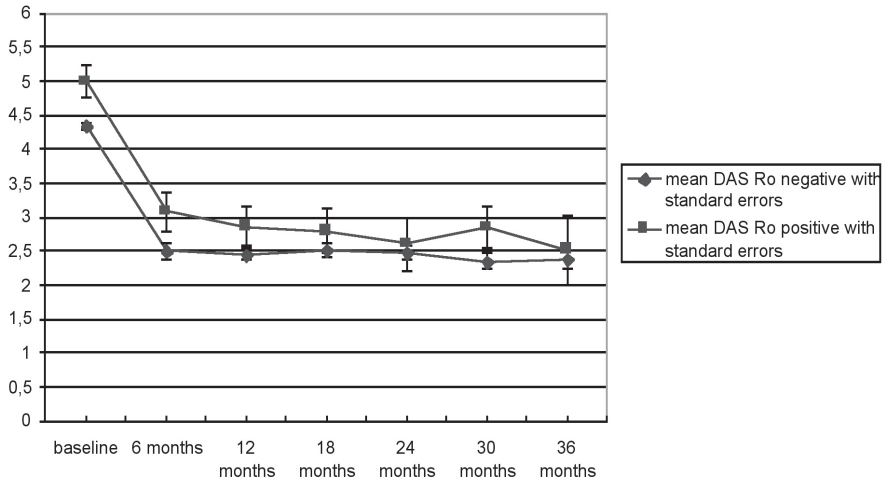

Fig. 2. Mean DAS values (with bars of standard errors) in the anti-Ro/SSA positive and anti-Ro/SSA negative patients during anti-TNF-α treatment.
All the patients showed a reduction in DAS during treatment. The anti-Ro/SSA positive group showed a higher mean DAS at baseline (p = 0.006) and at the 6th month of treatment (p = 0.05), but there was no difference between the two groups at 12, 18, 24, 30, 36 months of treatment (Student's t-test). An ANOVA test comparing the slopes of the two regression curves showed a parallel trend (p = 0.52).

significantly higher scores in the anti-Ro/SSA positive group only at baseline (p = 0.006) and at 6 months (p = 0.05), while during the remaining follow-up the same rate of improvement was seen at every point from the 12th to the 36th month. In addition, a longitudinal analysis comparing the slopes of the two regression curves showed a parallel trend from baseline to the end of the observation period (Fig. 2), thus confirming the same clinical behaviour in anti-Ro/SSA positive and negative subjects.

Autoantibody production in all 322 patients during treatment is shown in Figure 3. The rate of ANA positive sera showed a rising trend since the 6th month of treatment was statistically significant compared to baseline (p < 0.00001 at 6, 12, 18, 24, 30, and 36 months), reaching 72.7% at 36th month of treatment. Antibodies to dsDNA were measured by different methods. The CLIFT and ELISA assays combined detected anti-dsDNA in 2.7% of sera at baseline, with an increasing trend until the 12th month (5.8%) followed by a decrease to 2.2%, 2.4%, 3.4% and 0% at 18, 24, 30 and 36 months, respectively. A higher frequency of anti-dsDNA antibodies was detected by the Farr assay, with 7.5% positive sera at baseline, increasing to 21%, 40% and 44% at 6, 12 and 18 months, respectively, and then decreasing to 23% from the 24th month of observation. Analysing these positive sera, we found that most of them (80-100%) showed anti-dsDNA antibodies at low titres (<20 U/ml) at every point during the follow up.

The autoantibody response to anti-TNF-α therapy was different in anti-Ro/SSA positive and anti-Ro/SSA negative patients. As expected, ANA were more frequently detected in the anti-Ro/SSA positive group at baseline (82.3% *vs.* 37.4%; p = 0.0007) and at the 6th and 12th months (p = 0.004 and 0.005, respectively). This difference was significantly reduced over time due to a progressive increase in ANA positivity in the anti-Ro/SSA negative group (Fig. 4). It is worth noting that these differences were not due to a progressive reduction in the number of treated patients during follow up, because in fact we observed the same dropout rate in both groups.

We did not observe any increase in anti-phospholipids (aCL and/or anti-beta2GPI) or anti-ENA antibodies over time in either group.

Side effects

Withdrawal from anti-TNF-α treatment was globally recorded in 105 cases, mainly due to drug reactions (12%), inefficacy (9.6%), recurrent infections (2.5%) or the new onset of solid tumors (2.8%), as shown in Table III. Among the drug reactions considered we included the new onset of anti-dsDNA antibodies associated with complement reduction but without clinical symptoms; this was observed in 8 cases (2 in the anti-Ro/SSA positive group and 6 in the anti-Ro/SSA negative group). Among the malignancies we observed 2 cases of breast cancer, 5 colon cancers and one non-Hodgkin's (NH) lymphoma in the anti-Ro negative patients, while one case of NH lymphoma was detected in the anti-Ro/SSA positive group. Lupus-like disease was more frequently detected in anti-Ro/SSA

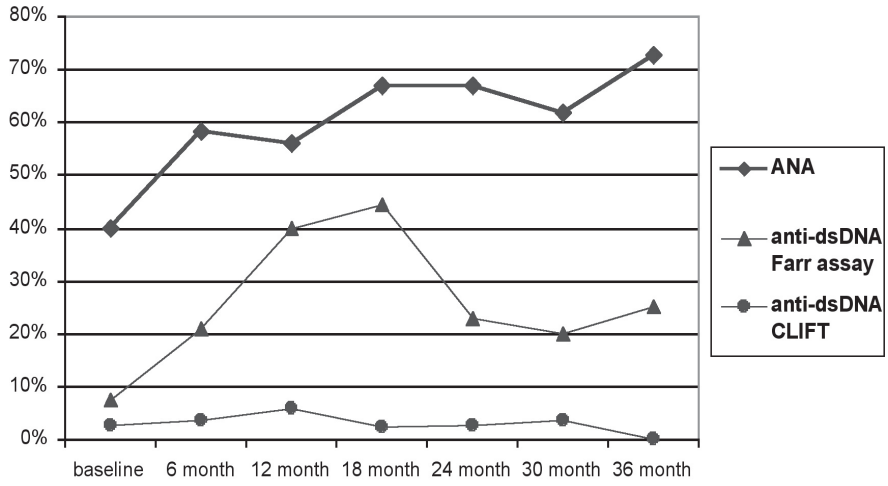

Fig. 3. Variations in ANA and anti-dsDNA in 322 patients treated with anti-TNF-α drugs.
ANA showed a rising trend beginning in the 6th month of treatment. Anti-dsDNA detected by CLIFT (●) remained stable during the first 6 months, then increased at the 12th month, and subsequently decreased until the 36th month. Anti-dsDNA antibodies detected by Farr (▲) significantly increased from baseline to the 12th month ($p = 0.006$), then decreased from the 18th to the 24th month, and remained stable until the 36th month of observation.

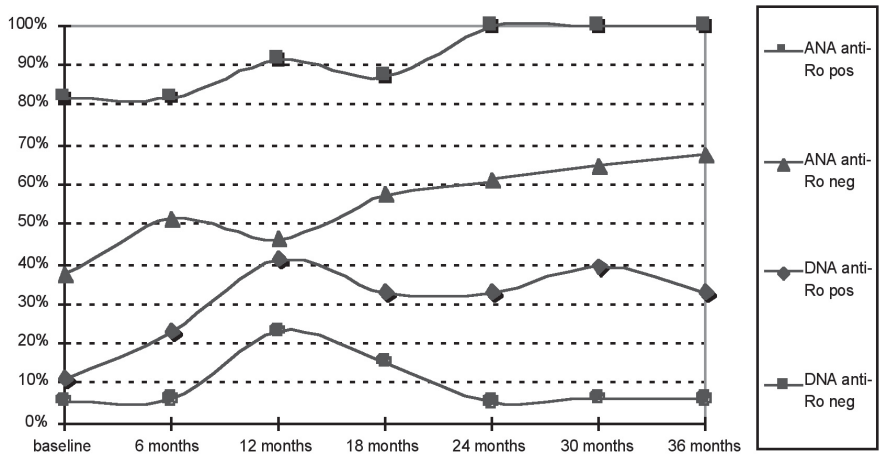

Fig. 4. ANA and anti-dsDNA variations in anti-Ro positive and anti-Ro negative patients during treatment with anti-TNF-α agents.
ANA were more frequently detected in the anti-Ro positive group at baseline ($p = 0.0008$), and at the 6th and 12th month ($p = 0.035$ and 0.008, respectively); this difference appeared to decline over time due to a progressive increase in ANA positivity in the anti-Ro negative group. Anti-dsDNA antibodies significantly increased from the 12th to the 18th month, and then remained stable (positive in 33-41%) in the anti-Ro positive subjects, while decreasing to 6% in the anti-Ro negative group.

positive subjects ($p = 0.012$); it was observed in 5 patients, all of whom exhibited skin involvement (photosensitive skin lesions histologically characterised as LE) and/or alopecia, with oral ulcers in one case. None had anti-dsDNA antibodies at baseline, but between the 6th and the 18th month of treatment developed low titer (3 cases) or medium titer (2 cases) anti-dsDNA, detected by the Farr assay.

In addition, anti-TNF-α drugs were interrupted in 3 patients due to a progressive elevation in liver enzymes – one subject with anti-Ro/SSA antibodies and chronic HCV-related hepatitis who was being treated with etanercept and two being treated with infliximab for acute cholangitis.

Discussion

Our study evaluated the clinical response to anti-TNF-α drugs in a group of anti-Ro/SSA positive RA patients compared to a group of anti-Ro/SSA negative RA patients, and confirmed the relative safety of these treatments in subjects with circulating autoantibodies.

Anti-Ro/SSA antibodies are found in 3-15% of patients affected by rheumatoid arthritis (26-31, 40); therefore, the frequency of 5.3% detected in our study is in line with previous findings and indicates that the presence of this autoantibody does not influence the course of RA. In fact, patients affected by RA, independently of the presence of circulating anti-Ro/SSA, are indistinguishable in terms of their demographic features and clinical course. Furthermore, in our study the group of patients with anti-Ro/SSA had active disease resistant to DMARDs and a similar frequency of erosive progression at baseline compared to the anti-Ro/SSA negative patients. The only clinical difference was in the sicca manifestations, which were prominent in the anti-Ro/SSA positive group, and the higher frequency of ANA and IgM-RF positivity in the same patients. Taken together, these findings can be linked to the anti-Ro/SSA antibodies rather than to the disease, where anti-Ro/SSA are detected (26-31, 41-44).

Furthermore, the presence of anti-Ro/SSA antibodies did not influence the clinical response to anti-TNF-α agents, which was comparable in the two groups during the course of our study. In any case it should also be recalled that arthritis in other anti-Ro/SSA-related diseases, such as SLE and SS, appears to be responsive to anti-TNF inhibition (19, 21, 23, 25) whether or not the other clinical manifestations [such as nephritis in SLE (19) or sicca symptoms in SS (23)] remitted. On the other hand, in SLE as in RA, arthritis recurred when anti-TNF-α treatment was stopped (19).

It is well known that anti-TNF-α agents induce autoantibodies in a high proportion of the patients treated (6-17), even if very little is known about the mechanisms involved in this change in immune system behaviour (6, 45, 46). Therefore it is not surprising that ANA increased from 40% to 73% in our cohort and their frequency did not significantly differ between the anti-Ro negative and the anti-Ro positive groups from the 18th month of treatment and

Table III. Discontinuation of anti-TNF-α among 322 patients affected by RA and treated with anti-TNF-α drugs.

Reason for discontinuation of treatment	Total n = 322	Anti-Ro negative n = 305	Anti-Ro positive n = 17	*p*
Inefficacy	31 (9.6)	31 (10.1)	0	NS
Drug reactions	39 (12.1)	36 (11.8)	3 (17.6)	NS
Malignancies	9 (2.8)	8 (2.6)	1 (5.8)	NS
Recurrent infections	8 (2.5)	8 (2.6)	0	NS
Lupus-like disease	5 (1.5)	3 (0.9)	2 (11.7)	0.012
Voluntary interruption	8 (2.5)	8 (2.6)	0	NS
Cardiopathy (ischemic or arythmic)	3 (0.9)	2 (0.6)	1 (5.8)	NS
Stroke	2 (0.6)	2 (0.6)	0	NS

thereafter. On the contrary, anti-dsDNA antibodies showed a different behaviour, both compared to ANA and in the two groups of anti-Ro/SSA negative and positive patients. Although the trend to develop anti-dsDNA during TNF-α blocker treatment in the entire cohort of patients was the same, the rate of detection of the autoantibodies was very different depending on the assay used to measure them. In fact, the frequency of positivity increased during the first 12 months as measured by CLIFT, but during the first 18 months when tested by the Farr assay. In both cases the methods detected a progressive decline in anti-dsDNA positive sera until the 36th month of observation. These discrepancies could be due either to a differing sensitivity of the two assays, especially as far as concerns the low titer antibodies, or to the different antibody isotypes detected: IgG anti-dsDNA by CLIFT, IgG and IgM anti-dsDNA by Farr. The pattern of anti-dsDNA antibody production has been already reported – either an early increase in the appearance of autoantibodies in the first week of treatment (8, 10, 11), or a subsequent reduction to baseline levels (11). Nevertheless, while in the anti-Ro/SSA negative group a return to the baseline frequency was observed, in the anti-Ro/SSA positive group the frequency of anti-dsDNA was raised until the 12th month and then decreased only slightly.

About one-third of our patients interrupted the anti-TNF treatment; the proportion was similar in the anti-Ro/SSA negative and positive groups, except for those with lupus-like disease (LLD), among whom 2/17 patients with and 3/305 patients without anti-Ro/SSA antibodies stopped treatment. With regard to the clinical manifestations of these LLD patients, only mucocutaneous features were observed, namely photosensitivity skin reactions, alopecia and oral ulcers, rather than systemic involvement, which also have been rarely described by others (6, 14, 18, 47). Such features can easily complicate the course of different anti-Ro/SSA-associated diseases, independently of the associated clinical diagnoses (26, 28-31, 40, 44, 48-51). In addition, the clinical manifestations of LLD rapidly disappeared after discontinuation of the drug, as already described by others (15-17). Interestingly, the appearance of the clinical manifestations of LLD was not correlated to the anti-dsDNA titer. In fact, in all LLD cases the anti-dsDNA titer was rather low (< 20 U/ml in 3 sera, < 30 U/ml in 2 sera).

The development of malignancies during anti-TNF treatment represents a general concern and must be carefully monitored. The frequency that we observed was rather low, confirming previous reports (52, 53). NH lymphoma developed in 2 patients, one of them in the anti-Ro/SSA positive group. The occurrence of lymphoma in patients with anti-Ro/SSA antibodies deserves particular attention considering the increased risk reported in patients with SS (54-57) and active RA (52, 58, 59), especially when treated with some immunosuppressants (60, 61).

In conclusion, anti-TNF-α treatment has been shown to be effective in patients with anti-Ro/SSA antibodies. Even if anti-dsDNA and lupus-like disease are more frequently detected in anti-Ro/SSA positive patients, severe clinical manifestations of systemic involvement were not observed. Further studies and a longer follow-up are warranted to evaluate the risk of NHL in these patients.

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