Anti-Ro/SSA Antibodies Are an Independent Factor Associated with an Insufficient Response to Tumor Necrosis Factor Inhibitors in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To study the significance of anti-Ro/SSA antibodies (anti-Ro) in the clinical response to tumor necrosis factor (TNF) inhibitors in patients with rheumatoid arthritis (RA).

Methods. The clinical responses of a cohort of 190 patients with RA who were treated with infliximab, etanercept, or adalimumab (n = 112, 64, and 14, respectively) as the first biologics were examined using the Disease Activity Score in 28 joints (DAS28) at 24 weeks and the discontinuation rate at 56 weeks. The baseline characteristics of responders and the nonresponders were compared. The clinical response was compared between anti-Ro-negative and -positive patients. The factors associated with the inefficiency of TNF inhibitors were estimated with a multivariable logistic regression analysis.

Results. The positive rate of anti-Ro was significantly higher in patients with no European League Against Rheumatism (EULAR) response at 24 weeks (OR 3.64, 95% CI 1.45–9.01, p = 0.003). In anti-Ro-positive patients, a moderate or good EULAR response rate was significantly lower with a sustaining higher median DAS28 (p = 0.006), and this difference was greater among infliximab-treated patients. The discontinuation rate for TNF inhibitors due to inefficacy at 56 weeks was also higher in anti-Ro-positive patients (OR 4.68, 95% CI 1.82–11.99, p = 0.0005), and 75% of these patients received infliximab. The presence of anti-Ro was strongly associated with no EULAR response at 24 weeks and a higher discontinuation rate of TNF inhibitors by 56 weeks (OR 5.22, 95% CI 1.75–15.57, p = 0.003 and OR 10.18, 95% CI 2.18–49.56, p = 0.003).

Conclusion. The presence of anti-Ro might be related to the lesser clinical response to infliximab compared to other TNF inhibitors, suggesting that the presence of anti-Ro should be considered when choosing the appropriate biologics for patients with RA. (First Release Oct 1 2011; J Rheumatol 2011;38:2346–54; doi:10.3899/jrheum.101295)

Key Indexing Terms:
ANTI-RO/SSA ANTIBODIES TUMOR NECROSIS FACTOR INHIBITORS
RHEUMATOID ARTHRITIS AUTOANTIBODIES INFLIXIMAB

One of the crucial factors to consider when treating patients with rheumatoid arthritis (RA) is the presence of autoantibodies. It is well accepted that the anticyclic citrullinated peptide antibody (ACPA) is a prognostic factor for disease severity and radiographic progression in patients with RA[1,2,3,4]. Further, the production of autoantibodies, such as antinuclear antibodies (ANA) and anti-double stranded DNA antibodies (anti-dsDNA), is commonly observed in patients who have been treated with tumor necrosis factor (TNF) inhibitors, although these autoantibodies are induced at different rates for each TNF inhibitor[5,6,7].

Anti-Ro/SSA antibodies (anti-Ro) are frequently detected in rheumatic diseases such as Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), lupus-like condition, neonatal lupus erythematosus (NLE) and RA. The target antigen of anti-Ro consists of 2 different Ro proteins, 60 kDa and 52 kDa; and tissue injury in patients with NLE depends on the transplacental passage of these autoantibodies[8,9]. Anti-Ro is detected in 3% to 15% of patients with RA[10,11], and is associated with secondary SS, which is thought to be a clinically poor prognostic condition of RA[12]. However, anti-Ro also exists independently of SS, and the

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relationship between anti-Ro and the clinical features of RA have not been well studied.

We investigated the significance of anti-Ro in relation to the clinical response to TNF inhibitors in patients with RA. TNF inhibitors that were used as the first biologic disease-modifying antirheumatic drugs (DMARD) were less effective in anti-Ro-positive patients than in anti-Ro-negative patients. Moreover, multivariable logistic regression analysis demonstrated that anti-Ro was strongly associated with the inefficacy of TNF inhibitors in patients with RA.

MATERIALS AND METHODS

Patients. We examined a cohort of 190 Japanese patients with RA who visited Juntendo University Hospital, Tokyo, from October 2003 to May 2009 and were treated with one of the following TNF inhibitors as the first biologic DMARD: infliximab (IFX), etanercept (ETN), or adalimumab (ADA). All patients fulfilled the 1987 American College of Rheumatology classification criteria for RA[13]. Patients were diagnosed with secondary SS if they satisfied the following American-European consensus criteria for SS: the presence of ocular symptoms or oral symptoms plus any 2 from ocular signs, histopathology of the minor salivary gland, and salivary gland involvement[14]. Disease activity of RA was assessed by calculating the Disease Activity Score in 28 joints/C-reactive protein (DAS28/CRP). The clinical response rates at 24 and 56 weeks were compared between anti-Ro-positive and anti-Ro-negative patients with RA based on the DAS28 European League Against Rheumatism (EULAR) response criteria.

Antibody measurements. Anti-Ro and anti-La/SSB antibodies (anti-La) were measured using a double immunodiffusion test (DID) and precipitin reactions without serum dilutions were considered positive. Titers were determined by precipitin reactions with dilutions of serum (1:1 to 1:32). If the titer by DID was 1:32, a second assay was run, with serum diluted 1:64 to 1:2048. The prevalence of anti-Ro in healthy individuals as well as patients with SS, SLE, and scleroderma based on the DID assay was 0/100 (0%), 44/68 (64.7%), 28/57 (49.1%), and 4/22 (18.2%), respectively, which was comparable with previous reports[15,16,17,18]. Rheumatoid factor (RF) was measured by immunonephelometry, and levels > 20 IU/ml were considered positive. ACPA was detected using a second-generation ELISA (Mesacup; Medical & Biological Laboratories, Tokyo, Japan). The cutoff level for ACPA positivity was set at 4.5 arbitrary U/ml, and serum samples with ACPA levels above 200 arbitrary units were diluted further. ANA was tested using an indirect immunofluorescence assay on a fixed HEp-2 cell substrate, and levels ×20 were considered positive. Anti-dsDNA was measured using a radioimmunoassay, and levels > 6 IU/ml were considered positive. Serum samples were obtained from all patients before and 24 and 56 weeks after treatment and then stored at –20°C until used.

This study was approved by the Institutional Review Board at Juntendo University, and all patients provided written informed consent.

Statistical analysis. Continuous and categorical data are presented as the median and 25th–75th percentiles and counts or percentages, respectively. At the end of the study, differences in the following variables at baseline were compared between responders and nonresponders at 24 weeks and between patients with continuation and discontinuation of the TNF inhibitors at 56 weeks: sex, age, disease duration, methotrexate dose, steroid dose, previous DMARD, tender joint count, swollen joint count, global health using a 0–100 mm horizontal visual analog scale, modified Health Assessment Questionnaire (mHAQ), CRP levels, DAS28/CRP, IgG levels, ANA, anti-dsDNA, ACPA, RF levels, anti-Ro, anti-La, presence of secondary SS, and types of TNF inhibitors. The differences in these variables were also compared between the anti-Ro-negative and -positive groups. Categorical variables were analyzed using Fisher's exact test, while continuous variables were analyzed with the Mann-Whitney U test, and p values < 0.05 were considered statistically significant. The factors that were associated with a clinical response to the TNF inhibitors were assessed using a multivariable logistic regression analysis, and were used to estimate OR and their 95% CI. All the variables listed above were used to select the appropriate variables for the multivariable analysis using a backward stepwise method under the Akaike Information Criteria[19]. The goodness-of-fit of the model for the response variable vs the explanatory variables was evaluated based on the r-square value. Statistical analyses were performed using R version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org; 2008).

RESULTS

Baseline characteristics related to the clinical response to TNF inhibitors in patients with RA. Data from 188 patients who were treated with TNF inhibitors as a first biologic DMARD were analyzed. Two patients were withdrawn from the study because they had discontinued the TNF inhibitor by 24 weeks because of an infection. IFX, ETN, or ADA was administered to 112, 64, and 14 patients, respectively. Among these patients, 149 (79.3%) showed a moderate or good response at 24 weeks based on the DAS28 score. The baseline characteristics were compared between the responders and nonresponders at 24 weeks and between patients with continuation and discontinuation of the TNF inhibitors at 56 weeks (Table 1).

It was notable that the positive rate of anti-Ro and the prevalence of secondary SS at baseline were significantly higher in the nonresponders than the responders at 24 weeks (Table 1; OR 3.64, 95% CI 1.45–9.01, p = 0.003, and OR 2.68, 95% CI 0.99–6.98, p = 0.037, respectively). These measures were also significantly higher in patients with discontinuation of the TNF inhibitors at 56 weeks (Table 1; OR 4.68, 95% CI 1.82–11.99, p = 0.0005, and OR 3.35, 95% CI 1.21–8.94, p = 0.012, respectively). CRP and IgG levels were also higher in the nonresponders at 24 weeks (p = 0.008 and p = 0.006, respectively), but these were not statistically different between the responders and nonresponders at 56 weeks. Patients who had discontinued the TNF inhibitors at 56 weeks had a longer disease duration (p = 0.045).

Inefficiency of TNF inhibitors in anti-Ro-positive patients. We focused on the presence of anti-Ro and compared the baseline characteristics between anti-Ro-positive and anti-Ro-negative patients. There were no significant differences in sex, age, disease duration, or disease activity between these patient groups (Table 2). The prevalence of secondary SS was significantly higher in anti-Ro-positive patients than anti-Ro-negative patients (OR 30.09, 95% CI 10.29–98.00, p < 0.0001). Regarding serological factors, serum IgG was higher (p = 0.003) and anti-dsDNA and anti-La were detected more frequently in anti-Ro-positive patients (OR 9.27, 95% CI 1.69–63.23, p = 0.004, and OR infinity, 95% CI 2.12–infinity, p = 0.004, respectively), while the RF levels and the positive rate of ACPA were not significantly different between the 2 patient groups. When the clinical efficacy of the TNF inhibitors was compared at

Table 1. Baseline characteristics and TNF inhibitors according to clinical response at 24 and 56 weeks. The values of continuous variables are expressed as median and 25th-75th percentile.

Characteristic	24 Weeks				56 Weeks			
	Responders, n = 149	Nonresponders, n = 39	OR (95% CI)	p	Continuation, n = 149	Discontinuation, n = 33	OR (95% CI)	p
Female/male	127/22	31/8	1.49 (0.53–3.92)	0.459	127/22	25/8	1.87 (0.67–5.23)	0.119
Age, yrs	51.5 (39–61)	52 (37.5–61.5)	NE	0.997	53 (39–61)	42 (34–60)	NE	0.113
Disease duration, yrs	7.4 (2.1–13.4)	5 (1.8–11)	NE	0.431	7.8 (2.5–14.3)	5 (1–9)	NE	0.045
MTX dose, mg/week	8 (4–8)	6 (4–8)	NE	0.143	7.5 (4–8)	8 (4–8)	NE	0.146
Steroid dose, mg/day	5 (2–7)	5 (0–7.5)	NE	0.937	5 (2–7)	5 (0–8)	NE	0.609
Previous DMARD (no/yes)	49/100	10/29	1.45 (0.62–3.60)	0.442	45/104	13/20	0.67 (0.29–1.59)	0.309
TJC	4 (3–8)	6 (1.3–12.5)	NE	0.125	5 (3–9)	6 (3–11)	NE	0.241
SJC	5 (2–8)	6 (1.3–9.8)	NE	0.661	5 (2–8)	4 (1–8)	NE	0.279
VAS global	52 (36–73.8)	53 (38.5–70)	NE	0.889	53 (36–73)	51 (46–75)	NE	0.703
mHAQ	0.5 (0.3–0.9)	0.6 (0.3–1.3)	NE	0.081	0.5 (0.25–1)	0.63 (0.4–1.2)	NE	0.106
CRP, mg/dl	1.3 (0.4–3.6)	2.7 (1.1–5.6)	NE	0.008	1.4 (0.5–3.9)	1.5 (0.4–6.3)	NE	0.677
DAS28/CRP	4.6 (3.9–5.3)	4.9 (4.2–5.6)	NE	0.147	4.7 (4.0–5.3)	4.9 (3.9–5.6)	NE	0.515
IgG, mg/dl	1360 (1192–1565)	1592 (1315–1845)	NE	0.006	1360 (1191–1600)	1583 (1280–1890)	NE	0.053
ANA (negative/positive)	9/140	5/34	0.39 (0.10–1.59)	0.147	8/141	3/30	0.57 (0.13–3.53)	0.142
Anti-dsDNA (negative/positive)	145/3	35/4	4.07 (0.72–23.02)	0.063	143/6	32/1	0.76 (0.02–6.66)	1.000
ACPA (negative/positive)	16/133	2/37	NE	0.908	16/133	7/26	NE	0.799
RF levels, IU/ml	57 (24–210)	67 (23.5–260)	NE	0.738	59.5 (25–229)	43 (12–209)	NE	0.326
Anti-Ro (negative/positive)	131/18	26/13	3.64 (1.45–9.01)	0.003	131/18	20/13	4.68 (1.82–11.99)	0.0005
Anti-La (negative/positive)	146/3	39/0	0.00 (0.00–9.40)	1.0	146/3	33/0	0.00 (0.00–11.07)	1.0
Secondary SS (no/yes)	132/17	29/10	2.68 (0.99–6.98)	0.037	132/17	23/10	3.35 (1.21–8.94)	0.012
TNF inhibitors: IFX/ETN/ADA	80/58/11	32/4/3	NE	0.0012	82/58/9	24/5/4	NE	0.016

DMARD: disease-modifying antirheumatic drugs; MTX: methotrexate; TJC: tender joint count; SJC: swollen joint count; VAS global: global health assessed visual analog scale; mHAQ: modified Health Assessment Questionnaire; CRP: C-reactive protein; DAS28: Disease Activity Score 28; ANA: antinuclear antibody; anti-dsDNA: anti-double stranded DNA antibody; ACPA: anticyclic citrullinated peptide antibody; RF: rheumatoid factor; anti-Ro: anti-Ro/SSA antibodies; anti-La: anti-La/SSB antibodies; SS: Sjögren's syndrome; TNF: tumor necrosis factor; IFX: infliximab; ETN: etanercept; ADA: adalimumab; NE: not estimated.

24 weeks, the percentage of patients who achieved a moderate or good EULAR response was lower (p = 0.006; Figure 1) and the median DAS28 score was higher in anti-Ro-positive patients (p = 0.047; Figure 2). It is noteworthy that the differences in the clinical response between anti-Ro-positive and -negative patients varied among the TNF inhibitors and was most pronounced in patients treated with IFX [9/20 (45.0%) and 70/91 (76.9%), respectively (p = 0.002; Figure 1)]. Further, there was a significant difference in the median DAS28 score between anti-Ro-positive and -negative patients at 24 weeks among IFX-treated patients (p = 0.026; Figure 2), while there was no statistical difference among patients treated with ETN (p = 0.432; Figure 2). Moreover, the median DAS28 score in the total patient population was higher at 56 weeks in anti-Ro-positive patients (p = 0.038; Figure 2).

The TNF inhibitors were discontinued at 56 weeks in 41 patients (21.6%), including 33 patients who discontinued TNF inhibitor treatment because of inefficacy, and the discontinuation rate was higher in the anti-Ro-positive patients than anti-Ro-negative patients (OR 3.74, 95% CI 1.53–9.11, p = 0.002, and OR 4.09, 95% CI 1.58–10.48, p = 0.002, respectively; Table 3). These differences were also more prominent in IFX-treated patients, compared to ETN- and ADA-treated patients (OR 3.69, 95% CI 1.27–10.33, p = 0.008; OR 1.24, 95% CI 0.02–13.10, p = 1.000; and OR 5.13, 95% CI 0.36–73.21, p = 0.133, respectively; Table 3).

Anti-Ro is an independent factor associated with a poor response to the first TNF inhibitor. To further investigate the relationship between the clinical characteristics and the response to TNF inhibitors, we used a multivariable logistic regression analysis and adjusted for all the confounding factors examined in Table 2, including anti-Ro and the type of TNF inhibitors (Table 4). This analysis revealed that the presence of anti-Ro was strongly associated with the inefficacy of TNF inhibitors at 24 weeks (OR 5.22, 95% CI 1.75–15.57, p = 0.003) and with an increased discontinuation rate at 56 weeks (OR 10.18, 95% CI 2.18–49.56, p = 0.003). IgG levels were also associated with treatment inefficacy in a multivariable logistic regression analysis, and IFX or ADA treatment was also associated with the greater discontinuation of the TNF inhibitors (OR 6.113, 95% CI 1.451–25.758, p = 0.014). The r-square values for the multivariable models at 24 and 56 weeks were 0.300 and 0.275, respectively, and they were sufficient for accurate predictions. We focused on other variables that were significantly

Table 2. Comparison of the baseline characteristics between anti-Ro-negative and -positive patients before commencement of first TNF inhibitors. The values of continuous variables are expressed as median and 25th-75th percentile.

Characteristic	Total, n = 190	Anti-Ro-negative, n = 158 (83.2%)	Anti-Ro-positive, n = 32 (16.8%)	OR (95% CI)	p
Female/male	160/30	130/28	30/2	0.31 (0.03–1.35)	0.119
Age, yrs	52 (38.5–61)	53 (39–62)	45 (37–58)	NE	0.152
Disease duration, yrs	6.75 (2–13.5)	7.5 (2–13.8)	6 (3.8–11.3)	NE	0.575
MTX dose, mg/wk	8 (4–8)	8 (4–8)	6 (4–8)	NE	0.154
Steroid dose, mg/day	5 (2–7)	5 (3–6.8)	4 (2–7.5)	NE	0.490
Previous DMARD (no/yes)	60/130	53/105	7/25	1.79 (0.69–5.25)	0.218
TJC	5 (3–9.8)	4.5 (3–8.8)	6 (3–12)	NE	0.212
SJC	5 (2–8)	5 (2–8)	2.5 (1–7)	NE	0.130
VAS global	52 (36–73)	52 (36–74)	50 (36.8–70.5)	NE	0.798
mHAQ	0.5 (0.1–0.9)	0.63 (0.3–1)	0.5 (0.2–1.1)	NE	0.855
CRP, mg/dl	1.5 (0.5–4)	1.5 (0.7–4.2)	1.1 (0.3–3.3)	NE	0.119
DAS28/CRP	4.68 (3.95–5.42)	4.66 (3.99–5.38)	4.79 (3.93–5.46)	NE	0.547
IgG, mg/dl	1382 (1206–1662)	1352 (1190–1610)	1553 (1352–1846)	NE	0.003
ANA-positive, %	178 (93.7)	145 (91.8)	32 (100)	Inf (0.63–Inf)	0.130
Anti-dsDNA-positive, %	7 (3.7)	2 (1.3)	5 (15.6)	9.27 (1.69–63.23)	0.004
Anti-La-positive, %	3 (1.6)	0 (0.0)	3 (9.4)	Inf (2.12–Inf)	0.004
ACPA-positive, %	167 (87.9)	136 (86.1)	31 (96.9)	NE	0.168
RF levels, IU/ml	59 (24–215)	57 (25–200)	76.5 (18.8–284.5)	NE	0.628
Secondary SS (yes, %)	28 (14.7)	8 (5.1)	20 (62.5)	30.09 (10.29–98.00)	< 0.0001
TNF inhibitors					
IFX positive, %	112	92 (82.1)	20 (17.9)	NE	NS
ETN positive, %	64	55 (85.9)	9 (14.1)	NE	NS
ADA positive, %	14	11 (78.6)	3 (21.4)	NE	NS

Anti-Ro: anti-Ro/SSA antibodies; DMARD: disease-modifying antirheumatic drugs; MTX: methotrexate; TJC: tender joint count; SJC: swollen joint count; VAS global: global health assessed on visual analog scale; mHAQ: modified Health Assessment Questionnaire; CRP: C-reactive protein; DAS28: Disease Activity Score 28; ANA: antinuclear antibody; anti-dsDNA: anti-double stranded DNA antibody; anti-La: anti-La/SSB antibodies; ACPA: anticyclic citrullinated peptide antibody; RF: rheumatoid factor; SS: Sjögren's syndrome; TNF: tumor necrosis factor; IFX: infliximab; ETN: etanercept; ADA: adalimumab; NE: not estimated; Inf: infinity; NS: not significant.

different in a univariable analysis, such as CRP and IgG at 24 weeks and disease duration at 56 weeks. However, these variables were not associated with the inefficacy of the TNF inhibitors in a multivariable analysis.

Changes in autoantibody profiles after TNF inhibitor treatment. The positive rate and titers of anti-Ro did not change at 24 and 56 weeks, while the ANA and anti-dsDNA titers were increased from baseline at 56 weeks (93.7% to 99.5% and 3.7% to 29.5%, respectively). Anti-La remained positive during the treatment period in 3 patients with secondary SS. Moreover, the positive rate of anti-dsDNA was notably increased in anti-Ro-positive patients compared to anti-Ro-negative patients (56.7% and 26.0%, respectively; p = 0.003). The TNF inhibitors were inefficacious in 8 of 14 of these patients, including 7 who were treated with IFX. None of the patients with increased anti-dsDNA titers developed clinical signs of SLE. ACPA and RF decreased frequently, with no correlation with the clinical response.

DISCUSSION

In this study, we showed that TNF inhibitors as the first biologics to treat RA were less efficacious in anti-Ro-positive patients compared to anti-Ro-negative patients. It was previously reported that RA patients with anti-Ro are more likely to have more severe disease and require more aggressive therapy[20,21]. Simmons-O'Brien, *et al* also reported that anti-Ro persisted for years in patients with RA and that these patients had chronic progressive disease[22]. In our patient population, the anti-Ro titers did not change from baseline during the treatment period with TNF inhibitors.

On the other hand, Cavazzana, *et al* reported that TNF inhibitors were equally effective in anti-Ro-positive patients[11,23]. In our study, the clinical response was lower in anti-Ro-positive patients with a sustained high DAS28 score at 24 weeks. At 56 weeks, this difference in clinical response was reduced, but this might be due to the discontinuation of TNF inhibitors in patients with inefficacy. The contrast between our results and those of the previous study[11,23] could be partially explained by differences in patient backgrounds, such as race, prevalence of secondary SS, history of other RA medications, and the assay used to detect anti-Ro. In fact, the frequency of anti-Ro in our study (16.8%) was higher than in other studies. However, Moutsopoulos, *et al* reported that 14.3% of Greek patients with RA were anti-Ro-positive by DID[24], which was similar to our results. Moreover, they reported that the prevalence of anti-Ro-pos-

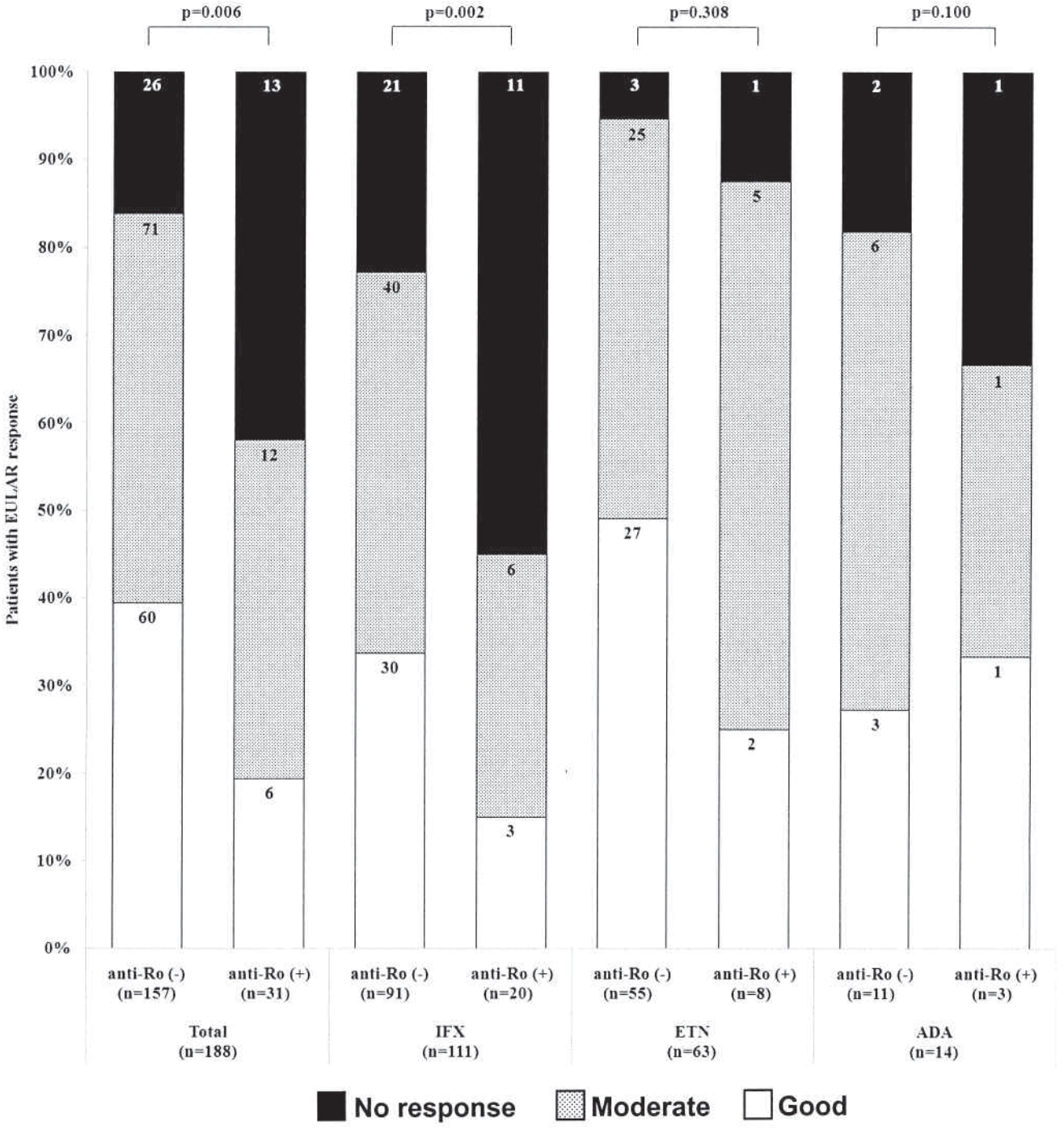

Figure 1. Comparison of the European League Against Rheumatism (EULAR) response rate at 24 weeks between anti-Ro/SSA antibody (anti-Ro) -negative and -positive patients treated with TNF inhibitors. For each TNF inhibitor, the percentage of patients who achieved a moderate or good EULAR response at 24 weeks was compared between the anti-Ro-positive and -negative patients. Numbers inside the bars represent the number of patients with a good, moderate, or no EULAR response. IFX: infliximab; ETN: etanercept; ADA: adalimumab.

itive individuals was higher in Greek than in British populations[25]. In terms of assays for detecting anti-Ro, an ELISA was used in the studies by Cavazzana, *et al*[11,23], while we used DID in this study. In our patients who had negative titer of anti-Ro with DID, 9 patients showed low or equivocal positive titers with ELISA and none of them had SS (data not shown). Morozzi, *et al* has also reported that low or equivocal positive titers by ELISA were obtained among patients who were anti-Ro-negative by DID, and sensitivity was different among the assays[26]. High sensitivity with

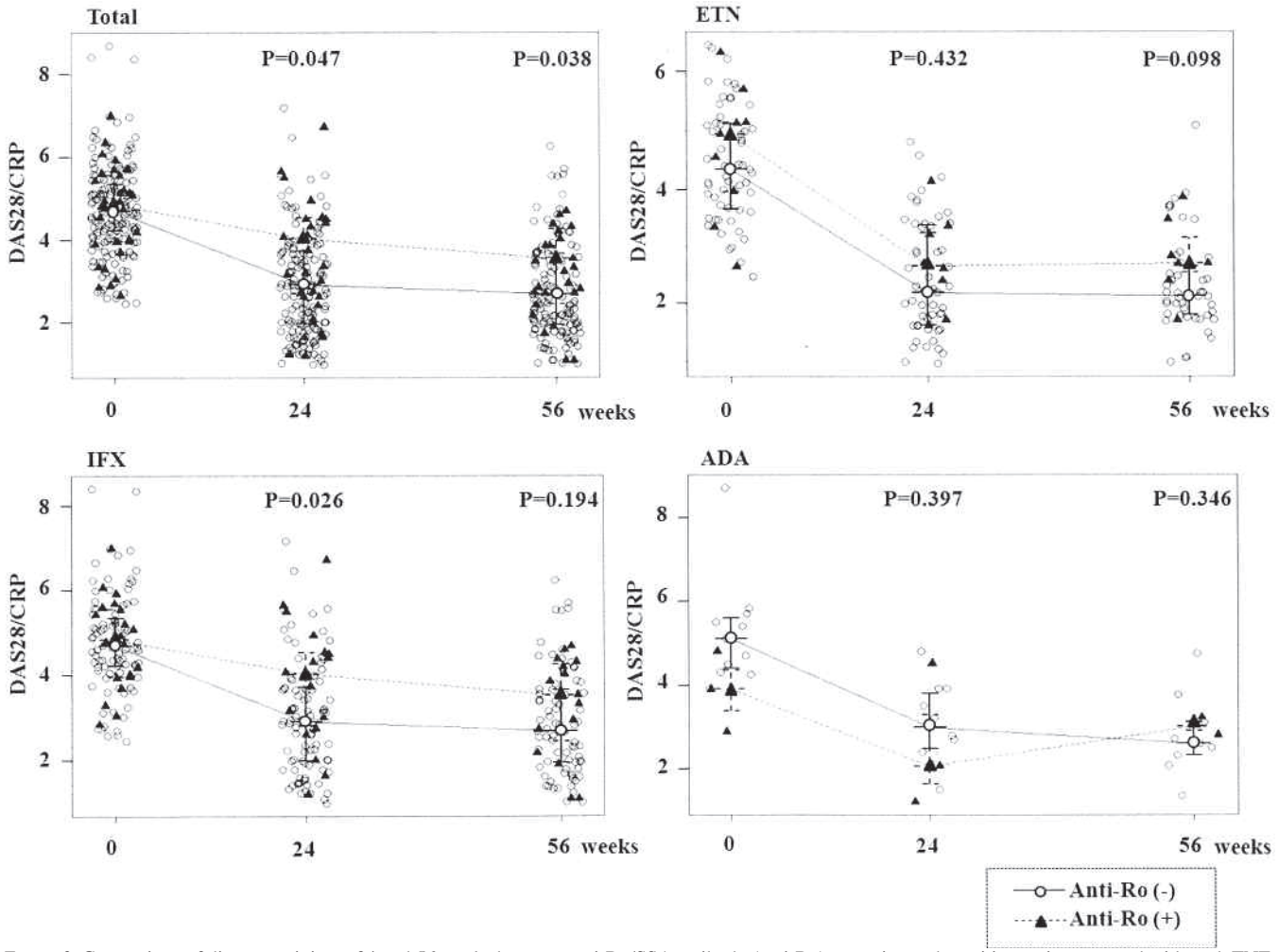

Figure 2. Comparison of disease activity at 24 and 56 weeks between anti-Ro/SSA antibody (anti-Ro) -negative and -positive patients treated with each TNF inhibitor. Values are expressed as the median, 25th–75th percentiles, and range. IFX: infliximab; ETN: etanercept; ADA: adalimumab; DAS28/CRP: Disease Activity Score 28/C-reactive protein.

ELISA might be related if low or equivocal titers were considered as a positive, and the assay might affect the association with clinical manifestations. Additionally, as noted in Materials and Methods, the positive rates of anti-Ro in healthy individuals and patients with other diseases that were determined by DID in our laboratory were comparable with previous reports.

Finally, a multivariable logistic regression analysis indicated that there was a significant association between anti-Ro and the inefficacy of IFX compared to the other TNF inhibitors in our patients. It is unclear why the presence of anti-Ro was strongly associated with the inefficacy of TNF inhibitors. Among the anti-Ro-positive patients in our study, the prevalence of secondary SS was 62.5%. It was previously reported that SS is a poor prognostic factor for RA[12], and indeed, RA patients with secondary SS were more prevalent among nonresponders. However, secondary SS was not independently associated with the inefficacy of TNF inhibitors in our study by a multivariable logistic regression analysis.

Some investigators have reported that anti-Ro-positive patients with RA have a higher incidence of DMARD-induced toxicity, and B cell activation was commonly observed in these patients[20,21,27,28,29]. TNF inhibitors are also known to induce the production of non-organ-specific autoantibodies, such as ANA, anti-dsDNA, and antiphospholipid antibodies[5,6,30,31,32]. The mechanism of ANA and anti-dsDNA might be produced after treatment with TNF inhibitors, especially IFX, because of increased release of autoantigens from apoptotic lymphocytes in the lamina propria[33,34,35,36]. Indeed, in the patients in this study the positive rate of anti-dsDNA frequently increased after they were treated with TNF inhibitors. Moreover, accelerated anti-dsDNA production was predominantly observed in patients treated with IFX, and this occurred more commonly in anti-Ro-positive patients. In addition, we examined the

Table 3. Discontinuation of TNF inhibitor treatment at 56 weeks.

Cause of Discontinuation	Total, n = 190	Anti-Ro-negative, n = 158	Anti-Ro-positive, n = 32	OR (95% CI)	p
All causes					
Total	41	27	14	3.74 (1.53–9.11)	0.002
IFX	30	20	10	3.11 (1.145–8.13)	0.015
ETN	6	4	2	2.55 (0.22–18.73)	0.266
ADA	5	3	2	3.41 (0.27–31.14)	0.198
Inefficacy					
Total	33	20	13	4.09 (1.58–10.48)	0.002
IFX	25	15	10	3.69 (1.27–10.33)	0.008
ETN	5	4	1	1.24 (0.02–13.10)	1.000
ADA	4	2	2	5.13 (0.36–73.21)	0.133
Infusion reaction					
Total	4	4	0	0.00 (0.00–7.59)	1.000
IFX	4	4	0	0.00 (0.0–7.59)	1.000
ETN	0	0	0	0.00 (0.00–Inf)	NA
ADA	0	0	0	0.00 (0.00–Inf)	NA
Infection					
Total	3	2	1	2.50 (0.00–49.41)	0.472
IFX	2	2	0	0.00 (0.00–26.55)	1.000
ETN	1	0	1	0.00 (0.13–Inf)	0.168
ADA	0	0	0	0.00 (0.00–Inf)	NA
Malignancy					
Total	1	1	0	0.00 (0.00–191.99)	1.000
IFX	0	0	0	0.00 (0.00–Inf)	NA
ETN	0	0	0	0.00 (0.00–Inf)	NA
ADA	1	1	0	0.00 (0.00–191.99)	1.000

TNF: tumor necrosis factor; Anti-Ro: anti-Ro/SSA antibodies; IFX: infliximab; ETN: etanercept; ADA: adalimumab; Inf: infinity; NA: not available.

Table 4. Association of anti-Ro with clinical response of TNF inhibitors.

Variable	24 Weeks* Coefficients	OR	(95% CI)	p	56 Weeks† Coefficients	OR	(95% CI)	p
Anti-Ro	1.65	5.22	(1.75–15.57)	0.003	2.32	10.18	(2.18–49.56)	0.003
Gender male	1.35	3.89	(1.23–12.26)	0.021	0.96	2.61	(0.74–9.22)	0.138
CRP	–1.56	0.211	(0.01–3.37)	0.271				
IgG	1.40	4.06	(1.47–11.19)	0.007				
IFX or ADA	–1.88	0.15	(0.00–29.87)	0.485	1.81	6.11	(1.45–25.76)	0.014
Duration					0.13	0.14	(0.96–1.35)	0.129
Secondary SS					1.26	3.54	(0.71–17.59)	0.123

* Association with no EULAR response at 24 weeks. † Association with discontinuation rate at 56 weeks. r-square values for 24 weeks and 56 weeks were 0.300 and 0.275, respectively. Anti-Ro: anti-Ro/SSA antibodies; TNF: tumor necrosis factor; CRP: C-reactive protein; IFX: infliximab; ADA: adalimumab; SS: Sjögren's syndrome.

immunoglobulin classes of anti-dsDNA in several patients, with the result that IgM anti-dsDNA was detected in most of the patients, while IgG or IgA anti-dsDNA was positive in few patients (data not shown).

It is possible that anti-Ro-positive patients are more likely to induce immune responses and produce autoantibodies in response to IFX treatment. We measured the trough concentration of IFX and examined human antichimeric antibodies (HACA) in several anti-Ro-positive patients who did not respond to IFX. This revealed that the IFX concentration was lower than 1 μg/ml in most patients. However, HACA was detected in only half of these patients (data not shown). Further, infusion reactions were not seen in anti-Ro-positive patients (Table 3), and the anti-Ro titers did not correlate with the clinical response (data not shown), suggesting that this correlation could not be explained simply by production of HACA.

Our data suggested that use of the anti-TNF-α antibodies IFX or ADA might be related to a lower clinical response, as shown in Table 4. We also analyzed whether each TNF

inhibitor influenced the clinical response in a multivariable logistic regression model, but this analysis was difficult because the number of patients treated with ETN or ADA was very small. In addition, all the anti-Ro-positive patients who did not respond to IFX or ADA improved clinically when they switched to ETN or tocilizumab as the second biologic DMARD.

The presence of anti-Ro in patients with RA might be related to the inefficacy of IFX compared to the other TNF inhibitors. Further studies are needed to confirm the relationship between anti-Ro and clinical response, because of the limited number of the patients in our study treated with ETN or ADA. Although the mechanisms contributing to this association should be examined further, our results indicate that the presence of anti-Ro should be considered when choosing appropriate biologic DMARD for patients with RA.

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