various disease activities at registration, and did not receive standardized treatment in the follow-up period. Third, in most of the cases included in this study, the physical examination, US, and cMRI were not performed on the same day. Fourth, the follow-up period may not have been sufficient. A more organized and standardized study with a large number of RA patients and a sufficiently long follow-up period is needed. Such a study will allow for a more precise assessment of the significance of US-PD in symptom-free joints of RA patients and the observed significant difference in outcomes between the joints with clinical and subclinical synovitis.

In conclusion, cMRI and US-PD are potentially useful for the detection of subclinical synovitis in patients with RA. The presence of subclinical synovitis can be regarded as an early warning sign of future progression to bone destruction, and could potentially be employed in the design of RA treatment strategies.

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Conflict of interest None.

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Discordance in Global Assessments Between Patient and Estimator in Patients with Newly Diagnosed Rheumatoid Arthritis: Associations with Progressive Joint Destruction and Functional Impairment

Yuko Kaneko, Masataka Kuwana, Harumi Kondo, and Tsutomu Takeuchi

ABSTRACT. ***Objective.*** Factors relevant to the discordance between the patient global assessment (PGA) and estimator global assessment (EGA) in patients newly diagnosed with rheumatoid arthritis (RA) were examined.

Methods. Seventy-five consecutive newly diagnosed patients with RA were prospectively enrolled. We used 3 models in which discordance between PGA and EGA at 12 months was set at 5 mm, 10 mm, or 20 mm. We adopted 10 mm as representative and examined time course changes in clinical variables over 12 months.

Results. No significant difference was found between the concordance and the higher PGA groups regarding baseline characteristics and treatment. At 12 months, EGA, swollen joint count, and inflammatory marker values were not different, but pain visual analog scale and tender joint count were significantly higher in the higher PGA group, and the Health Assessment Questionnaire improved less. In the 10 mm and 20 mm models, the structural remission rate was significantly lower in the higher PGA group and the rapid radiological progression rate significantly higher. The discrepancy was already significant at 3 months.

Conclusion. In newly diagnosed RA, PGA at 12 months may be more sensitive for indicating progressive joint destruction and functional impairment when compared with EGA, and there is a discrepancy directed toward a worse assessment by patients. (First Release May 1 2014; J Rheumatol 2014;41:1061–6; doi:10.3899/jrheum.131468)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
ESTIMATOR GLOBAL ASSESSMENT
PATIENT GLOBAL ASSESSMENT
DISCORDANCE

The management of rheumatoid arthritis (RA) involves multiple processes, including discussion and agreement between patients and their physicians. A patient's condition is generally expressed using patient's global assessment (PGA) and the physician's evaluation by estimator global assessment (EGA). The PGA does not necessarily agree with the EGA[1,2,3,4]. The discrepancy between PGA and EGA has been reported to be 24–76%, varying according to the definition of the discrepancy and often directed toward a better assessment by physicians than by patients. Nicolau, *et al*[1] reported that patients with a greater PGA discrepancy presented with higher pain scores and tender joint count (TJC). Barton, *et al*[2] reported that depressive symptoms are associated with greater PGA discordance. Studenic, *et al*[3] described the pain score as the most significant determinant of greater PGA discordance, and Khan, *et al*[4] reported that pain is the most important determinant of the PGA. Although these reports suggest that pain is the most influential factor for elevated PGA, the results were derived from cohorts including patients with long disease duration. Joint tenderness is an important feature of disease activity, but pain is also caused by established joint damage without active inflammation, which physicians may not be willing to take into account in disease activity.

Therefore, we focused on newly diagnosed patients with little joint destruction and examined factors relevant to discordance between the PGA and EGA 12 months after diagnosis.

MATERIALS AND METHODS

Patients. This study was conducted with part of the SAKURA cohort of consecutive patients who were newly diagnosed with RA at Keio

From the Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan.

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Y. Kaneko, Instructor; M. Kuwana, Associate Professor; H. Kondo, Rheumatology Research Nurse; T. Takeuchi, Professor.

Address correspondence to Y. Kaneko, Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, Japan. E-mail: ykaneko@z6.keio.jp

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University Hospital and had never been treated with either disease-modifying antirheumatic drugs or steroids and prospectively observed since September 2007. The diagnosis of RA was made based on the 1987 American College of Rheumatology (ACR) RA criteria[5] or 1994 Japanese College of Rheumatology (JCR) early RA criteria[6]. Our study was approved by the ethics committee, and all patients provided written consent.

Laboratory data included C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Patient pain, PGA, and EGA were measured on a visual analog scale (VAS) ranging from 0 to 100 mm. The questions for the PGA, EGA, and pain were, "How do you estimate your disease activity today?", "How do you estimate the patient's disease activity today?", and "How severe is your pain today?," respectively. A Health Assessment Questionnaire (HAQ) was filled out by each patient. EGA and joint assessment were recorded by 1 of any 5 rheumatologists, all of whom had more than 10 years of experience. Hands and feet radiographs were taken at the time of diagnosis and 12 months later. The radiographs were blinded and read independently by 2 readers (YK and MK) according to van der Heijde/modified total Sharp score (mTSS); the mean values were used in the analysis. The ØmTSS value was the progression over a year by subtracting the mTSS at baseline from the mTSS at 12 months. Structural remission (sREM) and radiological rapid progression (RRP) were defined as ØTSS ≤ 0.5/year and ≥ 5/year, respectively.

Analysis of factors relevant to discrepancies between PGA and EGA 12 months after diagnosis. In previous reports, the definition of discordance between the PGA and EGA was 5 to 30 mm[2,3,4,5]. We used 3 models in which the discordance at 12 months was set at 5 mm, 10 mm, or 20 mm. Each model divided the patients into 3 groups: higher PGA, concordance, and higher EGA.

Time course changes in clinical variables. We examined changes in PGA, EGA, pain VAS, 28-joint Disease Activity Score (DAS28), TJC, swollen joint count (SJC), CRP, and HAQ over 12 months and compared them between groups.

Statistical analysis. The means of continuous variables were compared by Student's t test, and proportions were compared by chi-square test. The level of concordance between PGA and EGA was analyzed using Lin's concordance correlation coefficient. The comparisons of time series data were analyzed by 2-way repeated measures of ANOVA using the posthoc Tukey method. All statistical analyses were performed using SPSS version 20.0.

RESULTS

Patients. A total of 75 consecutive patients were newly diagnosed as having RA in the SAKURA cohort between September 2007 and August 2009 and included in this study. Forty-two patients (56%) fulfilled 1987 ACR classification criteria, and 68 patients (91%) fulfilled 2010 ACR/European League Against Rheumatism (EULAR) criteria[7]. Eighty-six percent were female. At the time of diagnosis, the patients had a mean age of 60.9 years, and the mean duration from symptom onset to the time of diagnosis was 9.1 months. Seventy-nine percent were positive for anticyclic citrullinated peptide antibodies, and a mean DAS28 was 4.5.

Comparison of variables between the concordance group and higher PGA group. When the discordance was defined as 5 mm, 10 mm, or 20 mm, the higher PGA group comprised 38 (51%), 34 (45%), and 24 (32%) patients, the concordance group 29 (39%), 38 (51%), and 48 (64%) patients, and the higher EGA group 8 (10%), 3 (4%), and 3 (4%) patients, respectively. The higher EGA group did not have enough patients to analyze; therefore, we compared the higher PGA group and concordance group.

No significant differences were found between the concordance group and the higher PGA group regarding baseline characteristics and treatment at 12 months (Table 1). The EGA, SJC, CRP, and ESR did not differ between the groups in any model at 12 months. However, in all 3 models at 12 months, the pain and TJC were significantly higher in the higher PGA group than in the concordance group, and HAQ improved less. In the 10 mm and 20 mm model, radiological progression as a proportion of sREM and RRP was significantly worse in the higher PGA group and the RRP higher. In addition, in the 20 mm model, SJC was even higher in the higher PGA group.

Probability plot of yearly radiographic progression with 10 mm discordance. Because a radiological progression and the lesser improvement in HAQ were picked up by defining discordance as 10 and 20 mm, we adopted 10 mm as representative. The probability plot of ΔTSS for 10 mm is shown in Figure 1.

Time course changes in the level of concordance between EGA and PGA and disease activity-related variables. The changes in PGA and EGA over 12 months are presented in Appendix 1. The levels of concordance shown by Lin's concordance correlation coefficient were 0.55, 0.36, 0.37, 0.36, and 0.37 at baseline, 3, 6, 9, and 12 months, respectively. Time course changes in disease activity variables were examined at a discordance of 10 mm (Figure 2). In the concordance group, EGA and PGA decreased in parallel, as well as TJC, SJC, CRP, and HAQ. In the higher PGA group, the PGA did not change over 12 months, but the EGA decreased. The discrepancy between the PGA and EGA was significant at 3 months.

DISCUSSION

Our study shows that about half of newly diagnosed patients with RA exhibit discordance between PGA and EGA 12 months after diagnosis, and the PGA at 12 months might be more sensitive for detecting progressive joint destruction and less improvement of functional impairment when compared with EGA, and there is a discrepancy toward a worse assessment by patients.

There is a growing interest in the use of patient-reported outcomes in RA[8,9]. However, disagreement exists between patients and their physicians, often with PGA showing worse than EGA[1,2,3,4]. We examined patients' clinical characteristics using 3 different definitions and found that, even when defining discordance as 5 mm, a worse PGA reflected more TJC and worse pain. When the discordance was defined as 10 mm, the difference in sREM and RRP rates became significant. These results show that, while we could describe 5 mm as discordance between patients and their physicians, the appropriate definition of discordance

Table 1. Baseline and 12-month characteristics of concordance and higher PGA groups in the 3 models. Data are expressed as mean (SD) unless otherwise indicated. The numbers for current treatments include combination therapy use.

Discordance Model	5 mm			10 mm			20 mm		
	Concordance, n = 29	PGA Higher, n = 38	p	Concordance, n = 38	PGA Higher, n = 34	p	Concordance, n = 48	PGA Higher, n = 24	p
At baseline									
Age, yrs	62.5 (13.3)	57.1 (14.5)	0.12	62.6 (12.5)	57.2 (15.1)	0.10	61.9 (13.6)	56.4 (14.3)	0.13
Duration, mos	9.4 (14.5)	10.2 (20.6)	0.85	9.9 (22.2)	7.7 (9.9)	0.38	9.5 (20.0)	9.5 (11.3)	1.00
Smoking, n (%)	5 (24)	14 (37)	0.38	9 (24)	10 (35)	0.57	14 (29)	7 (29)	1.00
SE, n (%)	19 (66)	24 (63)	0.90	24 (63)	22 (65)	0.93	32 (67)	14 (58)	0.64
Anti-CCP, n (%)	18 (64)	21 (55)	0.40	25 (66)	18 (52)	0.16	30 (61)	13 (56)	0.61
DAS28	4.3 (1.2)	4.6 (1.1)	0.32	4.4 (1.1)	4.7 (1.1)	0.23	4.4 (1.2)	4.6 (1.0)	0.64
SDAI	15.3 (10.1)	16.9 (10.6)	0.54	16.2 (10.3)	17.2 (10.9)	0.68	17.0 (11.4)	16.0 (8.9)	0.66
CDAI	13.3 (8.7)	15.0 (9.2)	0.45	14.4 (9.3)	15.2 (9.5)	0.73	15.1 (10.0)	14.2 (7.8)	0.67
SJC	3.2 (2.8)	3.8 (3.7)	0.43	4.0 (3.5)	3.8 (3.7)	0.9	4.1 (3.9)	3.5 (3.0)	0.50
TJC	2.6 (2.9)	3.1 (3.4)	0.56	3.2 (3.2)	3.2 (3.5)	1.0	3.3 (3.7)	2.9 (3.1)	0.58
PGA, mm	42.6 (33.4)	42.9 (24.2)	0.97	40.7 (31.4)	43.6 (23.4)	0.67	42.4 (30.4)	41.5 (22.0)	0.89
Pain VAS, mm	42.8 (33.3)	43.7 (24.5)	0.90	41.3 (31.9)	44.7 (24.5)	0.61	44.0 (30.0)	40.8 (25.6)	0.65
EGA, mm	32.4 (24.1)	37.9 (21.3)	0.33	32.4 (23.7)	38.2 (20.8)	0.27	34.5 (24.4)	36.3 (18.0)	0.73
CRP, mg/dl	2.0 (2.9)	1.9 (2.1)	0.90	1.8 (2.6)	2.1 (2.2)	0.63	2.0 (2.7)	1.8 (2.0)	0.78
ESR, mm/h	56.7 (36.4)	60.6 (34.0)	0.65	51.4 (34.5)	61.5 (33.7)	0.11	54.2 (34.3)	64.3 (34.8)	0.25
HAQ	0.63 (0.75)	0.84 (0.70)	0.23	0.66 (0.75)	0.84 (0.63)	0.29	0.77 (0.80)	0.69 (0.49)	0.60
TSS	6.6 (7.0)	9.6 (20.5)	0.36	6.3 (7.0)	9.9 (21.4)	0.34	5.4 (6.5)	13.2 (24.9)	0.15
At 12 mos									
DAS28	2.3 (0.8)	3.0 (1.1)	*< 0.01*	2.3 (0.79)	3.1 (1.1)	*< 0.01*	2.3 (0.8)	3.3 (1.0)	*< 0.01*
SDAI	2.8 (5.1)	7.1 (5.1)	*< 0.01*	3.0 (4.6)	7.5 (5.2)	*< 0.01*	3.3 (4.5)	3.3 (4.5)	*< 0.01*
CDAI	2.7 (5.0)	6.8 (5.0)	*< 0.01*	2.85 (4.5)	7.3 (5.1)	*< 0.01*	3.1 (4.4)	8.5 (4.9)	*< 0.01*
SJC	0.8 (2.0)	1.3 (1.8)	0.30	0.8 (1.8)	1.3 (1.9)	0.16	0.7 (1.6)	1.8 (2.0)	*0.04*
TJC	0.2 (0.6)	1.0 (1.8)	*0.01*	0.3 (0.8)	1.0 (1.8)	*0.04*	0.3 (0.8)	1.3 (2.0)	*0.04*
PGA, mm	8.7 (17.0)	37.1 (21.0)	*< 0.01*	8.8 (15.2)	40.1 (20.2)	*< 0.01*	12.1 (16.5)	46.6 (18.9)	*< 0.01*
Pain VAS, mm	8.7 (16.3)	30.0 (23.2)	*< 0.01*	8.5 (14.5)	32.2 (23.6)	*< 0.01*	11.0 (17.1)	37.0 (22.3)	*< 0.01*
EGA, mm	8.2 (17.3)	8.3 (10.0)	0.98	8.7 (15.8)	8.8 (10.4)	0.98	8.7 (15.2)	8.8 (9.0)	0.96
CRP, mg/dl	0.1 (0.2)	0.3 (0.3)	0.06	0.2 (0.2)	0.3 (0.3)	0.16	0.2 (0.2)	0.3 (0.4)	0.23
ESR, mm/h	21.6 (18.7)	24.7 (22.2)	0.54	20.7 (17.5)	26.1 (22.9)	0.28	21.2 (18.7)	27.3 (23.0)	0.27
HAQ	0.26 (0.56)	0.58 (0.46)	*0.01*	0.25 (0.50)	0.61 (0.46)	*< 0.01*	0.29 (0.50)	0.67 (0.45)	*< 0.01*
ØTSS, n (%)	2.4 (6.7)	5.1 (9.2)	0.17	2.4 (6.3)	7.9 (12.8)	0.05	2.6 (6.0)	8.2 (14.8)	0.09
≤ 0.5 (sREM)	17 (59)	15 (39)		24 (63)	12 (35)		29 (60)	7 (29)	
0.5 to 5	7 (25)	10 (27)	0.22	8 (21)	9 (27)	*0.04*	10 (21)	7 (29)	*0.03*
≥ 5 (RRP)	5 (17)	13 (34)		6 (16)	13 (38)		9 (19)	10 (42)	
Current tx, n (%)									
MTX	17 (59)	24 (63)	0.90	24 (63)	21 (62)	0.90	31 (65)	14 (58)	0.80
Steroid	2 (7)	3 (8)	0.88	3 (8)	3 (9)	0.89	3 (6)	3 (13)	0.65
Biologic	5 (17)	10 (26)	0.56	6 (16)	9 (26)	0.41	10 (21)	5 (21)	1.00
Others	14 (48)	16 (42)	0.80	17 (45)	15 (44)	0.96	20 (42)	12 (50)	0.68

P values in italics are considered significant. SE: shared epitope; anti-CCP: anticyclic citrullinated peptide antibody; DAS28: 28-joint Disease Activity Score; SDAI: Simplified Disease Activity Score; CDAI: Clinical Disease Activity Score; TJC: tender joint count; SJC: swollen joint count; PGA: patient global assessment; VAS: visual analog scale; EGA: evaluator global assessment; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; MMP: matrix metalloproteinase; HAQ: Health Assessment Questionnaire; TSS: van der Heijde/modified total Sharp score; sREM: structural remission (ØTSS ≤ 0.5/yr); RRP: rapid radiographic progression (ØTSS ≥ 5/yr); tx: treatment; MTX: methotrexate.

may be 10 mm, which allowed us to detect differences in the progression of structural damage.

Several reports showed that pain is the most influential factor for elevated PGA[1,2,3,4], and our results are compatible with those studies. Although PGA has been shown to be influenced by noninflammatory factors[10,11], our study shows that PGA at 12 months may be more sensitive than the EGA for indicating progressive joint destruction and functional disorder. Studenic, *et al*[3] reported that in patients with average pain a concordance between EGA and PGA is attained at 10 swollen joints, suggesting that physicians weigh SJC heavily. However, 10 swollen joints appears quite many, and some studies have reported that synovitis can be detected by sensitive modalities in joints without swelling[12]. We consider that EGA need to be more reflective of pain in newly diagnosed patients.

When we looked at the time course changes, the discordance was already significant at 3 months and increased at 6 months. This result is presumably due to decreases in SJC leading physicians toward an improved rating, but it is not

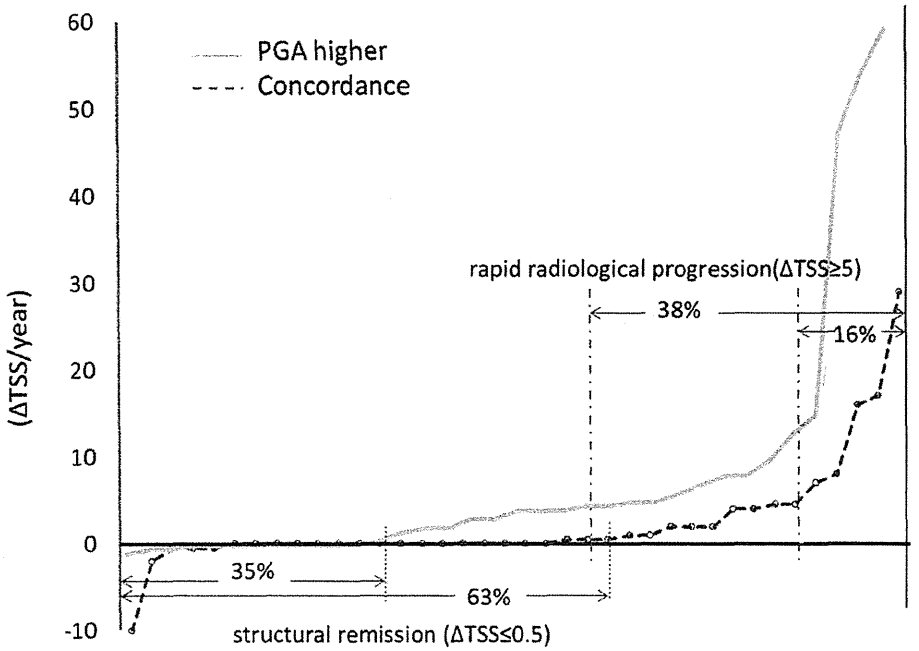

Figure 1. Radiological changes in patients at 12 months expressed by a probability plot with 10 mm discordance. The concordance group is indicated by solid lines, the higher PGA group by dotted lines. The PGA higher group showed worse progression than concordance group. The sREM rate was significantly lower in the higher PGA group and the RRP higher (35 vs 63%, 38 vs 16, respectively). TSS: modified total Sharp score; PGA: patient's global assessment; sREM: structural remission; RRP: rapid radiological progression.

necessarily the same for the perception of patients with persistent pain. Based on our results indicating that a higher level of pain or a modest increase in SJC can be associated with radiological progression, physicians should be more aware of the importance of pain and small changes in SJC in newly diagnosed patients.

Our study has some limitations. It was conducted in a single Japanese center. Because pain is expressed differently among different cultural backgrounds[13], future investigations are encouraged. As a result of the small sample size, very few patients were in the higher EGA group, which forced us to exclude those patients from the analysis. Patients with higher EGA may have different features[4] and need to be investigated. Some characteristics associated with poor prognosis were inclined to be higher in the higher PGA group, including HAQ and mTSS. Although these differences were not statistically significant, it might be partly due to the relatively small number of patients in each group. Moreover, over 12 months, more patients in the higher PGA group started to use biological agents. Hence, the differences in the worse outcomes in HAQ and mTSS may in addition to discordance between PGA and EGA reflect some underlying propensity for worse prognosis. Nonetheless, our findings point to focusing closer attention on the patient's disease experience. We did not examine a Routine Assessment of Patient Index Data 3 (RAPID3) score composed of major patient-reported outcomes: multidimensional HAQ, pain, and patient global estimate. However, our results warrant further research on the importance of patient-reported outcomes. Our patients were diagnosed based on 1987 ACR criteria or 1994 JCR early RA criteria because the SAKURA study was started before 2010 ACR/EULAR classification criteria were announced. However, because more than 90% of our patients fulfilled the new criteria, our results have enough generalizability.

In newly diagnosed patients with RA, PGA at 12 months may be more sensitive for indicating progressive joint destruction and less improvement of functional impairment when compared with EGA, and there is a discrepancy toward a worse assessment by patients.

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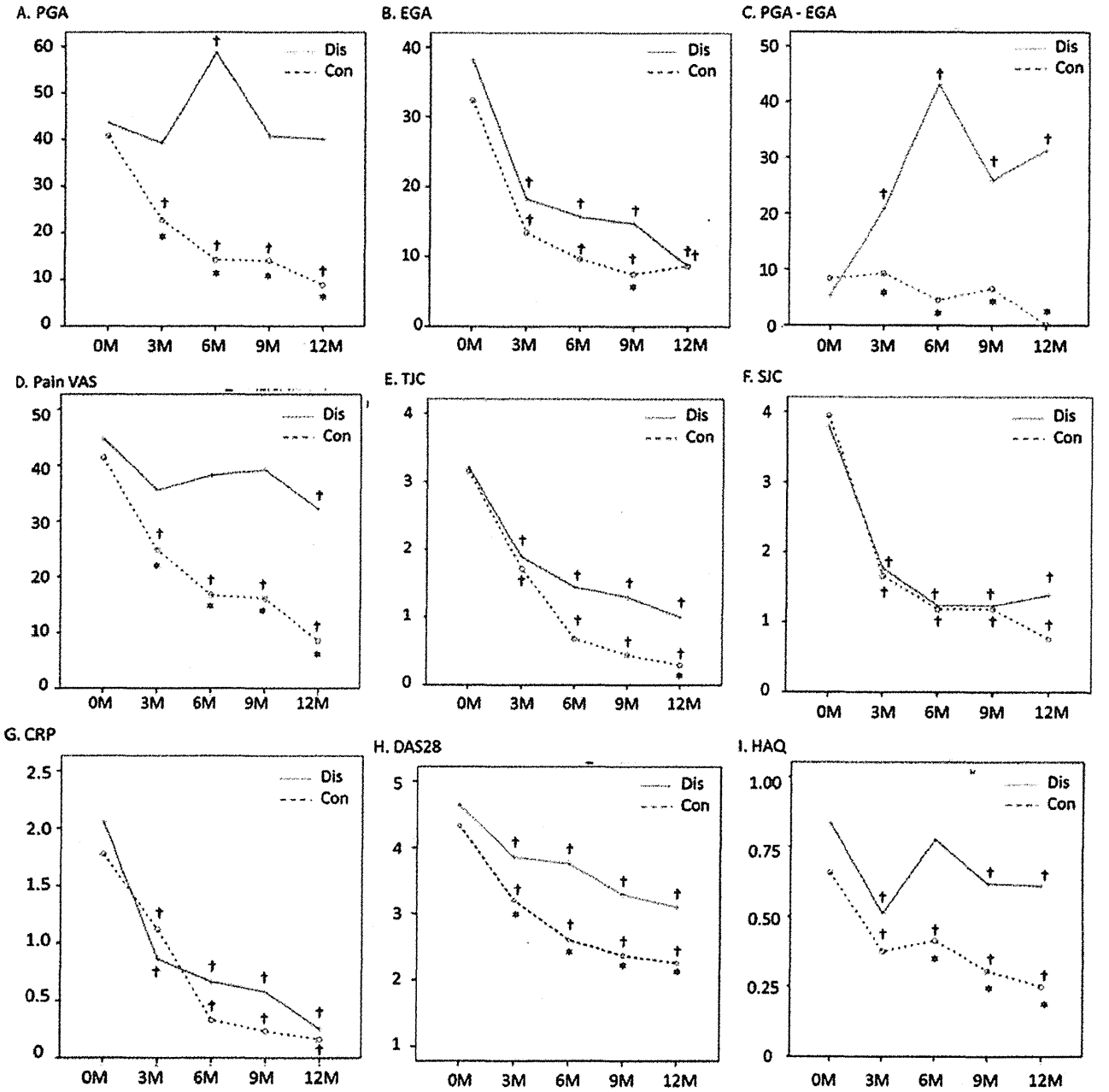

Figure 2. Changes in variables pertinent to disease activity over 12 months. A. Patient's global assessment (PGA). B. Evaluator global assessment (EGA). C. PGA-EGA. D. Pain visual analog scale (VAS). E. Tender joint count (TJC). F. Swollen joint count (SJC). G. C-reactive protein (CRP). H. 28-joint Disease Activity Score (DAS28). I. Health Assessment Questionnaire (HAQ). A discordance between the PGA and the EGA at 12 months was defined as 10 mm. The concordance group is indicated by solid lines, the higher PGA group by dotted lines. * $p < 0.05$ compared to the corresponding time point; † $p < 0.05$ compared to basal values.

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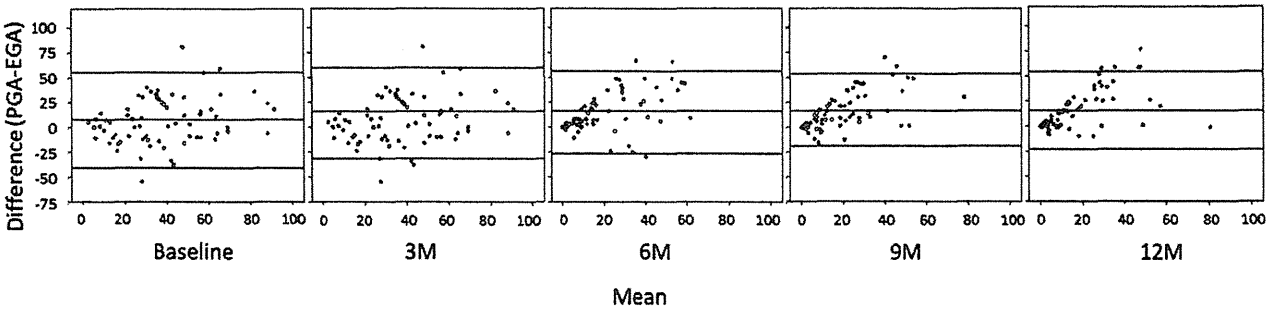
APPENDIX 1. The changes in PGA and EGA over 12 months were analyzed using Bland-Altman plots. The difference between PGA and EGA was assigned as the vertical value, and the mean of the PGA and EGA as the horizontal value, and t. Of 3 horizontal lines, the center one presented the mean value of the difference between the two, the upper was the mean plus 2 SD, and the lower the mean minus 2 SD. PGA: patient's global assessment; EGA: evaluator global assessment.



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ORIGINAL ARTICLE

Drug free REmission/low disease activity after cessation of tocilizumab (Actemra) Monotherapy (DREAM) study

Norihiro Nishimoto · Koichi Amano · Yasuhiko Hirabayashi · Takahiko Horiuchi · Tomonori Ishii · Mitsuhiro Iwahashi · Masahiro Iwamoto · Hitoshi Kohsaka · Masakazu Kondo · Tsukasa Matsubara · Toshihide Mimura · Hisaaki Miyahara · Shuji Ohta · Yukihiko Saeki · Kazuyoshi Saito · Hajime Sano · Kiyoshi Takasugi · Tsutomu Takeuchi · Shigeto Tohma · Tomomi Tsuru · Yukitaka Ueki · Jiro Yamana · Jun Hashimoto · Takaji Matsutani · Miho Murakami · Nobuhiro Takagi

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Abstract
Objectives To investigate the duration of remission and low disease activity (LDA) after cessation of tocilizumab (TCZ) treatment in rheumatoid arthritis patients who showed remission or LDA as assessed by DAS28 in response to preceding TCZ monotherapy, and to explore the factors contributing to prolonged efficacy duration.
Methods Disease activity was monitored for 56 weeks. The rate of continued efficacy was estimated by Kaplan–Meier curves.
Results A total of 187 patients were eligible. At baseline of this study, median disease duration was 7.8 years, preceding TCZ treatment period was 4.0 years and DAS28 was 1.5. The rate of continued LDA at 52 weeks was 13.4 % according to the Kaplan–Meier estimate. 19 patients (10 %) were completely drug-free and 17 patients (9.1 %) fulfilled DAS28 remission at 52 weeks. Multivariate Cox regression analysis identified low serum IL-6 and normalisation of MMP-3 levels at cessation of TCZ as independent predictive markers for longer duration of LDA. In patients with low serum IL-6 (<12.9 pg/mL) and

For the MRA Study Group for RA.

N. Nishimoto (✉)
Osaka Rheumatology Clinic, Tatsuno-Sinsaibashi-Building 5th Floor, 4-4-10 Minamisenba Chuo-ku, Osaka 542-0081, Japan
e-mail: norichan@wakayama-med.ac.jp;
nishimot@tokyo-med.ac.jp

N. Nishimoto · M. Murakami
Department of Molecular Regulation for Intractable Disease, Institute of Medical Science, Tokyo Medical University, Tokyo, Japan

N. Nishimoto · T. Matsutani · M. Murakami
Laboratory of Immune Regulation, Wakayama Medical University, Wakayama, Japan

K. Amano
Department of Rheumatology/Clinical Immunology, Saitama Medical Centre, Saitama Medical University, Saitama, Japan

Y. Hirabayashi
Department of Rheumatology, Hikarigaoka Spellman Hospital, Sendai, Japan

T. Horiuchi
Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan

T. Ishii
Department of Hematology and Rheumatology, Tohoku University Graduate School of Medicine, Miyagi, Japan

M. Iwahashi · J. Yamana
Higashihiroshima Memorial Hospital, Higashihiroshima, Japan

M. Iwamoto
Division of Rheumatology and Clinical Immunology, Jichi Medical University, Tochigi, Japan

H. Kohsaka
Department of Medicine and Rheumatology, Tokyo Medical and Dental University, Tokyo, Japan

M. Kondo
Kondo Clinic of Rheumatology and Orthopaedic Surgery, Fukuoka, Japan

T. Matsubara
Matsubara Mayflower Hospital, Hyogo, Japan

T. Mimura
Department of Rheumatology and Applied Immunology, Saitama Medical University, Saitama, Japan

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normal MMP-3 levels, the rate of continued LDA reached 38.0 % at 52 weeks.
Conclusions TCZ monotherapy may induce biologics-free remission or LDA without concomitant use of synthetic DMARDs. Serum levels of IL-6 and MMP-3 are useful markers for identifying patients who could discontinue TCZ without acute disease flare.

Keywords Tocilizumab · Rheumatoid arthritis · Duration of efficacy · Drug free · Interleukin 6 · Matrix metalloproteinase 3

Introduction

Newly licensed medications, especially biological agents, have enabled the attainment of unprecedented outcomes for patients with rheumatoid arthritis (RA) [1–4], and structured patient management aiming to achieve remission is an achievable goal in many patients in clinical trials and in actual clinical practice [5–8]. However, because biologics are more expensive than conventional synthetic DMARDs, continuous therapy with biologics strains medical finances; the next step in research on the treatment of RA should be to evaluate the possibility of sustaining remission without the use of biologics.

Tocilizumab (TCZ) is a humanised anti-human IL-6 receptor (IL-6R) monoclonal antibody that inhibits IL-6 binding to IL-6R [9]. TCZ as monotherapy and in combination with methotrexate (MTX) has been demonstrated to frequently induce remission according to the 28-joint disease activity score (DAS28)-erythrocyte sedimentation rate (ESR) and also to prevent joint damage [10–20].

In a previous study, we showed that the degree of abnormality of serum IL-6 levels in RA patients was positively correlated with RA disease activity, and that serum IL-6 levels were decreased in patients who sustained DAS28 remission by TCZ monotherapy [21, 22]. This evidence suggests that TCZ may be able to be discontinued without acute flare of disease activity in patients whose serum IL-6 has normalised. Based on this assumption, we planned an open-labelled, single-arm, multicentre clinical trial to investigate *D*rug-free *RE*mission/low disease activity (LDA) after cessation of tocilizumab (*A*ctemra as a product name) *M*onotherapy (DREAM study) in RA patients.

Method

Patients

Eligible patients were those who had participated in previous long-term clinical studies of TCZ monotherapy conducted in Japan, and the inclusion criteria and study design for each of these studies have already been reported [23]. Briefly, eligible patients were ≥20 years of age and fulfilled the 1987 American Rheumatism Association criteria for RA[24] with a disease history of 6 months or longer (with the exception of the SAMURAI study [14], in which the eligible disease duration was restricted to between 6 months and 5 years). All subjects failed to respond satisfactorily to treatment with at least one DMARD, including MTX or immunosuppressants. At enrolment in the initial trials, the patients had active RA, defined as the presence of six or more swollen joints and six or more tender joints. Patients receiving corticosteroids

H. Miyahara
National Hospital Organization Kyushu Medical Center, Fukuoka, Japan

S. Ohta
Department of Rheumatology, Taga General Hospital, Ibaraki, Japan

Y. Saeki · J. Hashimoto
National Hospital Organization Osaka-Minami Medical Center, Osaka, Japan

K. Saito
The First Department of Internal Medicine, University of Occupational and Environmental Health Japan, Kitakyushu, Japan

H. Sano
Division of Rheumatology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan

K. Takasugi
Dohgo Spa Hospital, Ehime, Japan

T. Takeuchi
Division of Rheumatology and Clinical Immunology, Department of Internal Medicine, Faculty of Medicine, Keio University, Tokyo, Japan

S. Tohma
Sagamihara National Hospital, National Hospital Organization, Kanagawa, Japan

T. Tsuru
PS Clinic, Fukuoka, Japan

Y. Ueki
Sasebo Chuo Hospital, Nagasaki, Japan

N. Takagi
Chugai Pharmaceutical Co. Ltd., Tokyo, Japan

(≤10 mg/day as prednisolone equivalent) and/or non-steroidal anti-inflammatory drugs (NSAIDs) were eligible if the dose had not increased during the 1-month washout period. Sexually active premenopausal women were required to have a negative urine pregnancy test at entry and periodically thereafter and to use effective contraception during the study period.

All patients were registered in this study within 4 weeks of the last observation in each preceding long-term extension study of TCZ monotherapy. Patients were enrolled if their DAS28-ESR was <2.6 at two or three of three consecutive assessment points, including the last observation point in the preceding study. Patients with DAS28-ESR ≤3.2 at two or three of three consecutive assessment points were additionally enrolled to know if the disease activity at TCZ discontinuation might influence the duration of DAS28 remission or LDA. Patients were excluded if they had received DMARDs, immunosuppressants, oral corticosteroids in excess of the dose at the initial infusion of TCZ, intravenous or intramuscular injections of corticosteroids, or plasmapheresis before being enrolled in this study. The baseline for each enrolled patient was defined as the time of the last TCZ infusion in the preceding clinical trial.

Study protocol

The study protocol was approved by the Ministry of Health, Labour and Welfare of Japan, and by the local ethical committees. All patients gave their written informed consent. This study is registered with http://clinicaltrials.gov/ (NCT00661284).

The primary endpoint of this study was the rate of DAS28 remission (DAS28-ESR <2.6) or LDA (DAS28-ESR ≤3.2) at 52 weeks after cessation of TCZ monotherapy, which was estimated from Kaplan–Meier curves prepared with the duration of continued efficacy for each patient defined as the time from the last infusion of TCZ in the preceding clinical study until loss of efficacy.

Nineteen hospitals in Japan participated in this study. Disease activities were monitored every 4 weeks for 56 weeks after cessation of TCZ for RA disease activity. During the study period, concomitant uses of NSAIDs and oral corticosteroid were allowed if the doses were not increased. Intra-articular injections of corticosteroids and hyaluronate preparations were avoided as far as possible, but surgical treatments were not limited. Additional RA treatments, including DMARDs, increases in oral corticosteroid dose, intravenous or intramuscular injections of corticosteroids, or plasmapheresis, were not allowed throughout the discontinuation period. Criteria for loss of efficacy was defined as DAS28-ESR >3.2 at two consecutive observations, initiation of additional RA treatments including increase in oral corticosteroid dose, the patient's request for retreatment, or the treating physician judging that retreatment was necessary. If patients met the criteria for loss of efficacy, observations in the study period were terminated.

Statistical analysis

Patients who had maintained a DAS28-ESR ≤3.2 at the last observation point in this study were handled as censored at that time. DAS28 remission and the 2011 American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) remission criteria (Boolean approach) were also considered [25].

The rate of continued efficacy at each time point was also estimated from Kaplan–Meier curves. The factors contributing to the duration of efficacy were estimated from univariate and multivariate Cox regression analyses using the following patient background data for this study: age, gender, disease duration, American College of Rheumatology functional class, RA stage determined by Steinbrocker's criteria, corticosteroid dose, rheumatoid factor (RF), DAS28-ESR, modified health assessment questionnaire (MHAQ) score, serum IL-6 concentration, and serum matrix metalloproteinase (MMP)-3 concentration. The receiver operating characteristic (ROC) curve was used to determine the most sensitive and specific cut-off value for the serum IL-6 level. Ineligible patients were excluded from efficacy evaluations.

Results

Characteristics of patients

We enrolled 189 patients and 187 of them were eligible. The two patients who did not meet DAS28-ESR LDA at the last observation of preceding long-term extension studies were excluded from this study from 189 patients. At the baseline of this study, the median disease duration was 7.8 years and the median preceding TCZ treatment period was 4.0 years (min–max = 1.9–8.6 years). Of the patients, 126 (67.4 %) received TCZ with 8 mg/kg every 4 weeks before enrolling in this study, 45 (24.1 %) extended the treatment interval (39.7 ± 10.9 days, mean ± SD) and 3 reduced the TCZ dosage (2 for 4 mg/kg; 1 for 6 mg/kg every 4 week), mostly due to sufficient efficacy, while 13 patients (7.0 %) shortened the interval (10 for patient's convenience; 3 for insufficient efficacy).

Oral corticosteroids were being taken by 64 patients (34.2 %), with a mean dose of 2.8 mg/day for those patients; 143 patients (76.5 %) had no swollen joints; 137 patients (73.3 %) had no tender joints; 115 patients (61.5 %) had no swollen and no tender joints. The medina serum IL-6 concentration and serum MMP-3 concentration were also decreased at enrolment in this study compared

Table 1 Demographic and clinical characteristics of patients at baseline

Total of 187 patients	Before first TCZ infusion (baseline of previous studies)	At cessation of TCZ treatment (baseline of this study)
Age, years (range)	52 (21–75)	57 (26–78)
Gender, female (%)	164 (87.7)	164 (87.7)
Disease duration, years	3.1 (0.4–20.9)	7.8 (3.7–24)
Functional class[a], 1:2:3:4	16:154:17:0	91:94:2:0
RA stage[a], I:II:III:IV	9:90:48:40	13:76:40:58
Number of prior use of DMARDs	2 (1–9)	
Corticosteroid dose, mg/d	5 (0–15.0)	0 (0–7.0)
No of patients who used MTX previously (%)	169 (90.4)	
RF positive, RF ≥20 IU/mL (%)	160 (85.6)	No data
TCZ treatment period (years)		4.0 (1.9–8.6)
DAS28-ESR	6.2 (2.2–8.8)	1.5 (0–3.2)
Tender joint count (28-joint count)	9 (0–28)	0 (0–7)
Swollen joint count (28-joint count)	9 (0–26)	0 (0–6)
CRP, mg/dL	3.29 (0.3–20.1)	0.02 (0–5.2)
ESR, mm/h	57 (11–165)	5 (1–28)
MHAQ score	0.6 (0–2.0)	0 (0–1.4)
IL-6, pg/mL	32 (1.6–611)	19 (3.3–431)
MMP-3, ng/mL	346 (38–800)	55 (23–697)

Values are median (range) except where indicated otherwise

RA rheumatoid arthritis, *RF* rheumatoid factor, *DAS28* 28-joint disease activity score, *ESR* erythrocyte sedimentation rate, *MHAQ* modified health assessment questionnaire, *IL-6* interleukin 6, *MMP-3* matrix metalloproteinase 3

[a] RA functional status determined by the American College of Rheumatology criteria. RA stage determined by Steinbrocker's criteria

with the patient background before starting the TCZ treatment (Table 1). At enrolment in this study, 169 (90.4 %) met DAS28 remission, and 107 (57.2 %) met Boolean remission. The DAS28 remission and LDA were kept more than 24 weeks in 133 patients (71.1 %) and 159 patients (85.0 %), respectively, before enrolment into this study.

Continuation rate of DAS28 remission and LDA efficacy after cessation of TCZ treatment

The rate of continued efficacy LDA without concomitant use of synthetic DMARDs was 35.1 % [95 % confidence interval (CI) 28.2–42.0 %] at 24 weeks and 13.4 % (95 % CI 8.4–18.3 %) at 52 weeks according to the Kaplan–Meier estimate (Fig. 1). DAS28 remission and 2011 ACR/EULAR remission criteria (Boolean approach) were maintained in 17 patients (9.1 %) and 14 patients (7.5 %), respectively, at 52 weeks. Furthermore, efficacy continued throughout the study period without concomitant use of corticosteroids or NSAIDs during the study period in 19 patients (10.2 %). The mean DAS28-ESR of these 19 patients was 2.2 at week 52.

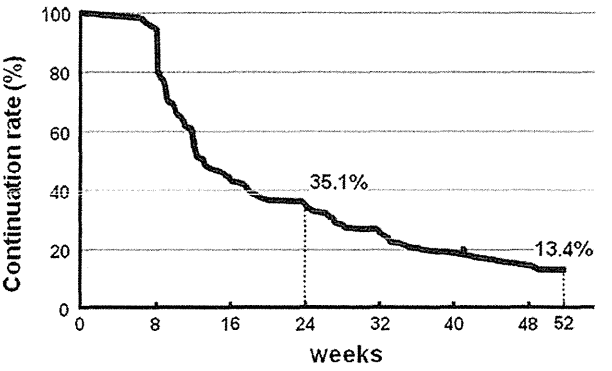

Fig. 1 Rate of continued LDA after cessation of tocilizumab treatment estimated by Kaplan–Meier method over 52 weeks

When we estimated the LDA continuation rates by the Kaplan–Meier method in the patients with DAS28 remission and new stringent Boolean-based remission at the cessation of TCZ, LDA continuation rates (95 % CI) at 52 weeks were 14.2 % (8.9–19.5 %) and 16.1 % (9.1–23.1 %), respectively. Further analysis for the factors contributing to the prolongation of efficacy duration is described later.

In total, 161 patients were withdrawn from this study. The major reason for the loss of efficacy was DAS28-ESR >3.2 at two consecutive visits in 44.7 % of patients (72/161 patients) and investigator's judgment in 39.8 % of patients (64/161 patients). The major reason of investigator's judgement was DAS28-ESR >3.2 at one visit in 84.4 % of patients (54/64 patients). However, there were no patients in whom disease activity flared up at the end of the observational period. Only 6.8 % (11/161 patients) were patients' request.

In terms of disease activity at cessation of TCZ monotherapy, the patients who completed the 52 weeks of this study period were comparable to the patients who withdrew from the study and restarted anti-rheumatic therapy before 52 weeks (Table 2).The serum IL-6 levels at baseline in the patients who restarted anti-rheumatic therapy before 52 weeks were higher than those in the patients who completed the 52-week study period [19.3 (range 0.7–431.0) pg/mL vs 10.9 (range 0.9–32.6) pg/mL]. In addition, the percentage of patients whose MMP-3 levels

Table 2 Comparison of disease activity between patients who completed the 52-week TCZ-free period and those who restarted anti-rheumatic treatment

	Before first TCZ infusion (baseline of previous studies)	*n*	At cessation of TCZ treatment (baseline of this study)	*n*	Last observation point of this study	*n*	Difference[a] (95 % CI)
DAS28-ESR, median (range)							
Completed	6.3 (2.2–7.5)	24	1.0 (0.0–2.7)	24	2.4 (0.5–3.3)	23	1.05 (0.75–1.35)
Restarted treatment	6.2 (3.2–8.8)	160	1.5 (0.1–3.2)	161	4.3 (0.8–7.8)	161	2.85 (2.67–3.03)
Tender joint count, median (range), 28-joint count							
Completed	9 (0–19)	24	0 (0–2)	24	0 (0–3)	23	0.2 (−0.1 to 0.4)
Restarted treatment	9 (1–28)	160	0 (0–7)	161	3 (0–27)	161	3.9 (3.2–4.5)
Swollen joint count, median (range), 28-joint count							
Completed	8 (0–26)	24	0 (0–2)	24	0 (0–4)	23	0.3 (−0.1 to 0.7)
Restarted treatment	9 (1–25)	160	0 (0–6)	161	2 (0–16)	161	2.8 (2.3–3.3)
CRP, median (range), mg/dL							
Completed	4.9 (0.5–9.3)	24	0.0 (0.0–0.7)	24	0.1 (0.0–2.3)	23	0.23 (0.02–0.45)
Restarted treatment	3.1 (0.3–20.1)	161	0.0 (0.0–5.2)	161	0.8 (0.0–13.5)	161	1.52 (1.18–1.86)
ESR, median (range), mm/h							
Completed	62 (16–123)	24	4 (1–28)	24	14 (2–53)	23	13.7 (8.0–19.3)
Restarted treatment	57 (11–165)	161	5 (1–26)	161	36 (2–115)	161	34.3 (30.7–37.8)
MHAQ scores, median (range)							
Completed	0.3 (0.0–2.0)	24	0.0 (0.0–0.4)	24	0.0 (0.0–0.5)	23	0.02 (−0.01 to 0.05)
Restarted treatment	0.8 (0.0–2.0)	161	0.0 (0.0–1.4)	161	0.3 (0.0–2.1)	161	0.29 (0.23–0.35)
MMP-3, median (range), ng/mL							
Completed	262.0 (38–800)	17	47.7 (32–225)	24	57.1 (13–109)	23	5.7 (0.6–10.8)
Restarted treatment	365.0 (38–800)	88	58.7 (23–697)	157	98.9 (36–800)	142	86.9 (64.4–109.5)
Percentage of patients whose MMP-3 levels were within normal range (%)							
Completed	5.9		91.7		73.9		–
Restarted treatment	3.4		56.7		24.6		–

[a] Difference: mean of the difference between the value at cessation of TCZ treatment (baseline of this study) and at the last observation point of this study

DAS28 28-joint disease activity score, *ESR* erythrocyte sedimentation rate, *MHAQ* modified health assessment questionnaire, *MMP-3* matrix metalloproteinase 3, *Completed* patients who completed the 52-week observational period without anti-rheumatic treatment, *Restarted treatment* patients who restarted anti-rheumatic treatment

were within normal range was lower in the group of patients who restarted anti-rheumatic therapy (56.7 %) than in the group who completed the 52-week study period (91.7 %).

Even though the median DAS28 was slightly increased from 1.0 at baseline of this study to 2.4 at the last observation point of this study (Table 2), tender joint count and swollen joint count at week 52 did not meaningfully worsen from the baseline of this study in the patients who completed the 52-week study period. MMP-3 concentration at week 52 was also almost stable during the study period in these patients.

In the patients who restarted anti-rheumatic therapy, disease activity and MMP-3 levels had worsened compared to the baseline of this study. Nevertheless, the values of those parameters were no worse than they had been before the initiation of TCZ treatment in previous studies (Table 2).

Factors contributing to the prolongation of duration of DAS28 remission and LDA

Univariate Cox regression analysis showed the following variables to be associated with the rate of continued efficacy: negative RF at baseline of the previous study and low serum IL-6 level (<35 pg/mL), under upper limit of the normal MMP-3 level, no concomitant corticosteroid use, DAS28-ESR <median, and an MHAQ score of zero at TCZ discontinuation.In contrast, disease duration, gender, functional class, and RA stage were not associated with continued efficacy (Fig. 2a). Multivariate Cox regression analysis showed that low serum IL-6 (<35 pg/mL) and normalisation of MMP-3 levels at TCZ cessation were independently associated with continued efficacy (Fig. 2b).

Based on this result, we examined the effects that IL-6 and MMP-3 levels at cessation of TCZ treatment had on the rate of continued efficacy. We found that the rate of continued efficacy in the patients with low serum IL-6 (<35 pg/mL) was 39.3 % (95 % CI 31.1–47.4) at 24 weeks and 15.9 % (95 % CI 9.7–22.0) at 52 weeks (Fig. 3a). In contrast, 69.7 % of the patients with serum IL-6 levels ≥35 pg/mL met the criteria for loss of efficacy within 12 weeks, and in none was efficacy maintained until 52 weeks. Analysis of the ROC curve identified the most sensitive and specific cut-off value for the serum IL-6 level to be 12.9 pg/mL. The rate of continued efficacy in patients whose serum IL-6 levels were less than 12.9 pg/mL was 63.2 % (95 % CI 48.8–77.5) at 24 weeks and 30.2 % (95 % CI 16.4–44.0) at 52 weeks (Fig. 3b).

The rate of continued efficacy in those with normalised MMP-3 levels was 50.9 % (95 % CI 41.6–60.2) at 24 weeks and 20.3 % (95 % CI 12.8–27.8) at 52 weeks (Fig. 3c), compared with 11.8 % at 24 weeks and 3.0 % at 52 weeks in patients with abnormal MMP-3 levels.

In patients with both serum IL-6 <12.9 pg/mL and normalised MMP-3 level, the rate of continued efficacy reached 70.6 % (95 % CI 55.3–85.9) at 24 weeks and 38.0 % (95 % CI 21.6–54.4) at 52 weeks (Fig. 3d).

Discussion

This study indicated that, in about 13 % of patients who achieve LDA (70.8 % of them were DAS28 remission) during long-term TCZ monotherapy, efficacy can be sustained for 1 year after cessation of TCZ treatment without concomitant use of synthetic DMARDs or immune suppressants; and in 79 % of them (19 patients), efficacy was maintained without concomitant use of corticosteroids or NSAIDs. To the best of our knowledge, this is the first report to show evidence that anti-IL-6 therapy can induce drug-free remission/LDA for 1 year in RA patients. The treatment recommendations of the EULAR state that, in patients who achieve remission with biological products, it may be possible to taper off the biological product after tapering off the corticosteroid. However, at present,

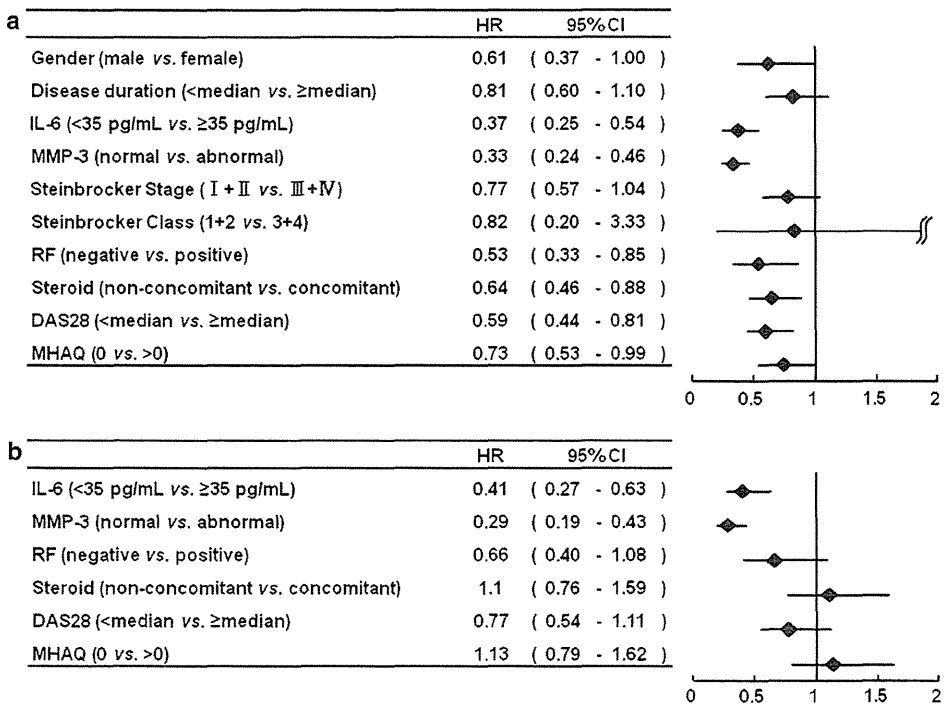

Fig. 2 Factors associated with continued LDA. **a** Univariate Cox regression analysis, **b** multivariate Cox regression analysis. *HR* hazard ratio, *CI* confidence interval, *RF* rheumatoid factor, *DAS28* 28-joint disease activity score, *MHAQ* modified health assessment questionnaire, *IL-6* interleukin 6, *MMP-3* matrix metalloproteinase 3

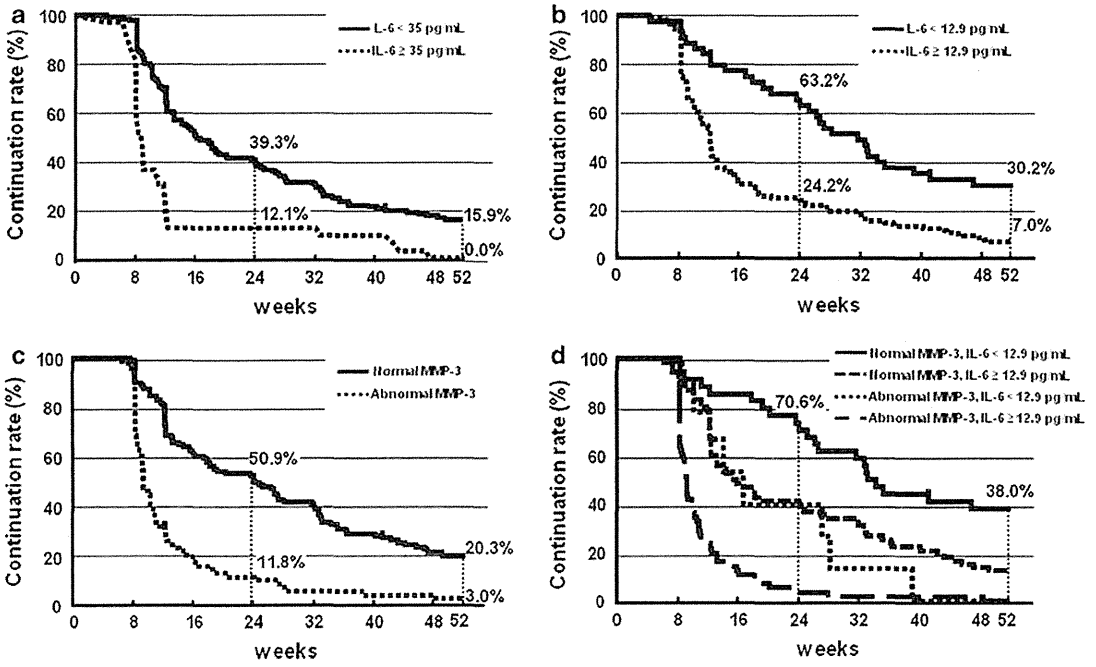

Fig. 3 Effects of serum IL-6 and MMP-3 levels on rate of continued LDA after cessation of tocilizumab treatment estimated by Kaplan–Meier method over 52 weeks. Contributing factors: **a** serum IL-6 level (cut-off level: 35 pg/mL), **b** serum IL-6 level (cut-off level: 12.9 pg/mL), **c** MMP-3 level (normal vs abnormal), **d** combinations of MMP-3 (normal vs abnormal) and serum IL-6 levels (cut-off level: 12.9 pg/mL). Space between *curves* represents the contribution of each factor to rate of continued efficacy

evidence in support of this conjecture is insufficient [26]. We believe that this report supports the possibility of discontinuing biological products as per the ACR/EULAR recommendations.

In a previous study (the BeST study), van der Kooij et al. [27] indicated that 18 % of patients could discontinue infliximab and synthetic DMARDs. Even though the characteristics of the patients in our study differed from those in BeSt study, the success rate of discontinuing TCZ without synthetic DMARDs in our study is comparable to that of the BeSt study. Moreover, with the use of synthetic DMARDs including MTX, a high rate of continued efficacy was shown after discontinuation of infliximab in the BeSt study [27]. A similar result was shown in the Japanese RRR study [28]. Therefore, it can be expected that introducing the use of synthetic DMARDs would similarly result in an increased rate of continued DAS28 remission or LDA after cessation of TCZ.

Because multivariate Cox regression analysis identified low serum IL-6 and normalised MMP-3 levels at the start of cessation of TCZ to be factors associated with continued efficacy, it can be considered that these factors may predict continued efficacy of a preceding TCZ treatment. With long-term TCZ treatment, reduced serum IL-6 levels are observed in some patients although TCZ does not directly inhibit IL-6 production but blocks IL-6R. We previously reported that, during blockade of IL-6R by TCZ, the serum IL-6 level represents the true IL-6 production *in vivo* and correlates well with true disease activity in RA patients [21, 22]. Therefore, TCZ treatment may improve not only inflammation-related symptoms but also the underlying cause of RA in patients whose serum IL-6 levels decrease. This implies that TCZ could be discontinued without acute disease flare in patients with normalised serum IL-6 levels. IL-6, as such a biomarker, is available only for anti-IL-6R antibody therapy but for anti-IL-6 neutralizing antibody therapies.

MMP-3 is deeply involved in cartilage destruction in RA and is also correlated with disease activity [29]. Since normalisation of the MMP-3 level is thought to reflect inhibition of excessive cartilage and bone destruction in the joints, normalisation of the MMP-3 level may indicate an improvement in the underlying cause of RA as well as synovial inflammation. In this study, we did not examine the progression of joint damage by imaging after cessation of TCZ. However, since the MMP-3 level during the TCZ-free period did not increase in the majority of the patients showing continued efficacy, it can be inferred that there was no sudden progression of joint destruction during the cessation of TCZ treatment. Further study will be necessary to evaluate this question.

In conclusion, these results showed that TCZ monotherapy can induce biologics-free remission/LDA without concomitant use of conventional DMARDs. Serum levels

of IL-6 and MMP-3 are useful markers for identifying patients who could possibly discontinue TCZ without acute disease flare. This evidence has also encouraged us to taper and adjust the interval of TCZ treatment in patients who show good response and normalisation of serum IL-6 and MMP-3 levels.

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Conflict of interest N. Nishimoto has served as a consultant to and received honoraria from Chugai Pharmaceutical Co., Ltd. N.N. also works as a scientific advisor to F. Hoffmann–La Roche, which is developing TCZ in collaboration with Chugai Pharmaceutical Co., Ltd. N.N. also has received research grants from Chugai Pharmaceutical Co. Ltd., Bristol–Myers Japan, and Pfizer Japan Inc. K. Amano has received research grants from Chugai Pharmaceutical Co. Ltd., Astellas Pharm Inc., and Mitsubishi Tanabe Pharma. Y. Hirabayashi has received speakers' bureau honoraria from Chugai Pharmaceutical Co. Ltd. M. Iwamoto has received a Royalty from Chugai Pharmaceutical Co. Ltd. H. Kohsaka has received research grants, consultant fees, and/or speakers' bureau honoraria from, Bristol-Myers Japan, Pfizer Japan Inc., and Takeda Pharmaceutical Co. Ltd. T. Mimura received research grants from Abbott Japan, Chugai Pharmaceutical Co. Ltd., Mitsubishi Tanabe Pharma, and Takeda Pharmaceutical Co. Ltd. T. Takeuchi has received research grants, consultant fees, and/or speakers' bureau honoraria from Abbott Japan, Bristol-Myers Squibb, Chugai Pharmaceutical Co. Ltd., Eisai Co. Ltd., Janssen Pharmaceutical KK, Mitsubishi Tanabe Pharma, Novartis, Pfizer Japan Inc., and Takeda Pharmaceutical Co. Ltd. S. Tohma has received a research grant from Pfizer Japan Inc. and has received subsidies or donations from Health and Labour Sciences Research Grants for Research on Allergic Disease and Immunology, and Chugai Pharmaceutical Co. Ltd. N. Takagi is a full-time employee of Chugai Pharmaceutical Co., Ltd. All other authors have declared no conflicts of interest.

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ORIGINAL ARTICLE

Effects of Smoking and Shared Epitope on the Production of Anti–Citrullinated Peptide Antibody in a Japanese Adult Population

CHIKASHI TERAO,[1] KOICHIRO OHMURA,[1] KATSUNORI IKARI,[2] TAKAHISA KAWAGUCHI,[1] MEIKO TAKAHASHI,[1] KAZUYA SETOH,[1] TAKEO NAKAYAMA,[1] SHINJI KOSUGI,[1] AKIHIRO SEKINE,[1] YASUHARU TABARA,[1] ATSUO TANIGUCHI,[2] SHIGEKI MOMOHARA,[2] HISASHI YAMANAKA,[2] RYO YAMADA,[1] FUMIHIKO MATSUDA,[1] AND TSUNEYO MIMORI,[1] ON BEHALF OF THE NAGAHAMA STUDY GROUP

Objective. Anti–citrullinated peptide antibody (ACPA) and rheumatoid factor (RF) are markers to rheumatoid arthritis (RA). Smoking and shared epitope (SE) in HLA–DRB1 are associated with the production of these autoantibodies in RA. Detailed distribution and characterization of ACPA and RF in the general population have remained unclear. We aimed to evaluate positivity of ACPA and RF in a general Japanese population and to detect correlates, including genetic components.

Methods. ACPA and RF were quantified in 9,804 Japanese volunteers ages 30–75 years. Logistic regression analyses were performed to evaluate the effects of candidates of correlates on the autoantibody positivity. A genome-wide association study (GWAS) was performed using 394,239 single nucleotide polymorphisms for 3,170 participants, and HLA–DRB1 alleles were imputed based on the GWAS data.

Results. A total of 1.7% and 6.4% of subjects were positive for ACPA and RF, respectively, and the 2 markers showed a significant correlation ($P = 2.0 \times 10^{-23}$). Old age was associated with ACPA positivity ($P = 0.00062$). Sex, smoking, SE, and other candidates of correlates did not have significant effects. Interaction between smoking and SE positivity was not apparent, but smoking showed a significant association with high levels of ACPA ($P = 0.0019$).

Conclusion. ACPA and RF could be detected in 1.7% and 6.4% of the Japanese adult population without RA, respectively. ACPA and RF were suggested to share mechanisms even in healthy populations. Old age was associated with increasing ACPA positivity. While positivity of ACPA and RF was not associated with SE and smoking, an association between high ACPA and smoking was observed.

INTRODUCTION

Rheumatoid factor (RF), an IgM autoantibody against the Fc fraction of IgG, is a serum marker of rheumatoid arthritis (RA) (1,2). In spite of its specificity to RA, RF appears in other diseases, especially connective tissue diseases, hepatic disorders, and even in healthy populations (3–9). Recently, anti–citrullinated protein antibody (ACPA) was found to show high specificity to RA and was able to distinguish RA from other connective tissue diseases with higher accuracy compared with RF (1,10). Although some studies reported functional pathogenicity of ACPA (11), pathogenicity and production mechanisms of ACPA and RF are largely unknown. Vigorous studies that address associations with the positivity and levels of ACPA and RF in patients with RA identified a wide range of factors. Some are disease-specific factors, such as disease

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[1]Chikashi Terao, MD, PhD, Koichiro Ohmura, MD, PhD, Takahisa Kawaguchi, MSc, Meiko Takahashi, PhD, Kazuya Setoh, MSc, Takeo Nakayama, MD, PhD, Shinji Kosugi, MD, PhD, Akihiro Sekine, PhD, Yasuharu Tabara, PhD, Ryo Yamada, MD, PhD, Fumihiko Matsuda, PhD, Tsuneyo Mimori, MD, PhD: Kyoto University, Kyoto, Japan; [2]Katsunori Ikari, MD, PhD, Atsuo Taniguchi, MD, PhD, Shigeki Momohara, MD, PhD, Hisashi Yamanaka, MD, PhD: Tokyo Women's Medical University, Tokyo, Japan.

Address correspondence to Chikashi Terao, MD, PhD, Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan. E-mail: a0001101@kuhp.kyoto-u.ac.jp.

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Significance & Innovations

- Positivity of anti–citrullinated peptide antibody (ACPA) in the general population is associated with aging and high C-reactive protein level.
- Smoking and shared epitope do not have comparable effect in the general population on the production of ACPA and rheumatoid factor (RF) as with patients with rheumatoid arthritis.
- Smoking may be associated with a high level of ACPA, even in healthy subjects.
- Correlates should be taken into account for RF and ACPA positivity in the general population. Novel findings of RF and ACPA production in general populations would provide clues to uncover the pathophysiology of the production of these autoantibodies.

activity and extraarticular symptoms (12–14) and others are disease–non-specific factors such as age, smoking, and common variants of HLA alleles (8,15–17). Smoking was shown to have an effect on the susceptibility to seropositive RA, especially in men (18). HLA–DRB1 is the strongest susceptibility locus to RA and is associated with ACPA or RF positivity in patients with RA (19). In particular, shared epitope (SE), an allelic group with a common amino acid pattern from the 70th to the 74th amino acid of the HLA–DRB1 protein (20), is strongly associated with RA susceptibility and production of ACPA and RF in patients with RA (15,17).

However, the distribution of these antibodies and whether the correlates are associated with positivity of ACPA or RF in the general population is largely unknown. There are no reports where ACPA levels were quantified and correlates of ACPA were analyzed in a large-scale study of healthy individuals. Although there are reports suggesting that the positivity of RF in healthy individuals is influenced by age and smoking in a European population (8,21–25), the positivity of RF and its correlates in healthy individuals is not known in Asian populations. If the likelihood of having RA based on positivity of ACPA or RF is different between subgroups with and without correlates, determining the distribution and correlates of ACPA and RF in a healthy population would lead to efficient screening to identify subjects at risk of RA. Moreover, determining the distribution and correlates would give clues for novel insights of mechanisms of production for ACPA and RF.

Here, we quantified circulating levels of ACPA and RF in 9,804 healthy Japanese subjects, identified prevalence, and estimated correlates, including genetic factors, of these 2 autoantibodies.

PATIENTS AND METHODS

Study population. This study was conducted as a part of the Nagahama Prospective Genome Cohort for Comprehensive Human Bioscience (The Nagahama Study) (26), a community-based prospective multiomics cohort study conducted by Kyoto University. A total of 9,804 volunteers in Nagahama City, Shiga Prefecture, Japan were recruited in this study from 2008 to 2010. All participants were asked to complete a detailed questionnaire about their present symptoms, present illness, past history of illness, family history, and smoking status. Written informed consent was obtained from all of the participants. This study was approved by Kyoto University Graduate School and Faculty of Medicine Ethics Committee.

Exclusion of samples. We excluded volunteers from the association studies if they had or have had autoimmune diseases. Individuals who were judged from their answers to the questionnaire to possibly have autoimmune diseases were also excluded from the analyses. As a result, a total of 9,575 subjects were recruited for the analysis.

RA patients. A total of 2,067 patients with RA in Tokyo Women's Medical University, whose age at onset, sex, and data of ACPA and RF were available, were registered in this study. A total of 1,237 patients with RA in Kyoto University were used for correlation analysis of genetic components.

Quantifying of circulating autoantibody. Serum samples were obtained from all the participants. ACPA was quantified as second-generation anti–cyclic citrullinated peptide (anti-CCP) antibody by MesaCup CCP enzyme-linked immunosorbent assay kit (Medical and Biological Laboratories) (27,28). IgM-RF was quantified by latex turbidimetric immunoassay, Iatro-RF II (Mitsubishi Kagaku Medience) (29). Both autoantibodies were quantified by SRL for healthy individuals and in Tokyo Women's Medical University for patients with RA. The cutoff levels of the autoantibodies were according to manufacturer's instructions (ACPA <4.5 units/ml, RF ≤ 20 IU/ml).

Candidates of correlates for ACPA and RF. Age, sex, smoking status, Brinkman index (BI; number of cigarettes a day $\times$ smoking years) as a quantitative measure of smoking, alcohol consumption, body mass index (BMI), and serum level of C-reactive protein (CRP) were selected as candidates of correlates for ACPA and RF. They were selected based on the previous reports of significant association between RA and smoking and a study from the US analyzing correlates of anti–nuclear antibody in the general population (30). We classified all the included participants into 5 groups according to their age at 10-year intervals. Logistic linear regression analysis or chi-square test was performed to analyze the influence of candidates of correlates on the positivity of autoantibodies. The effects of smoking in conditions with alcohol consumption were also analyzed.

Genome-wide association study (GWAS). GWAS was performed for 3,710 samples of participants who joined the Nagahama Study during 2008 to 2009. A series of BeadChip DNA array was used for the genotyping and

several samples were repeatedly genotyped using different arrays. All samples were scanned by at least 1 of 3 arrays, namely, Illumina HumanHap610Quad, Omni2.5-4, and Omni2.5-8 (see Supplementary Table 1, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). A total of 394,239 markers that were common across the 3 arrays were used for the current study. Genotyping quality was controlled by excluding single-nucleotide polymorphisms (SNPs) with a call rate below 95%, with minor allele frequency below 5%, and deviating from Hardy-Weinberg equilibrium ($P < 1.0 \times 10^{-7}$). Excluded from the analysis were 162 samples with a call rate <95%, 295 individuals estimated to have kinship within this population (PI-hat more than 0.35), and 7 ancestry outliers identified by principal component analysis, with HapMap Phase 2, release 28, data set as reference. A total of 83 individuals were excluded, because of having or being suspected of having connective tissue diseases from their answers to the questionnaire. As a result, 3,170 samples were analyzed for GWAS. Logistic regression analyses were performed by using positivity of ACPA and RF as dependent variables, each SNP as an independent variable, and age and sex as covariates.

HLA imputation. Alleles for HLA–DRB1 were imputed based on genotypes in the GWAS by using HLA–IMP2 (31). We imputed HLA–DRB1 alleles for 589 patients with RA for a test set as reported previously (28). Imputed HLA–DRB1 alleles were compared with genotyped HLA–DRB1 alleles and an algorithm for determining HLA–DRB1 alleles was established (See Supplementary Appendix A, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). Next, HLA–DRB1 alleles were determined based on the same algorithm for 932 healthy individuals as described previously (32), and compared with the genotyped HLA–DRB1 alleles. The algorithms for HLA–DRB1 based on imputation provided more than 93.5% of sensitivity and 99.8% of specificity for SE. HLA–DRB1 alleles were inferred for the 3,170 individuals in the current GWAS using the same algorithm.

Correlation analysis. Effect sizes of SNPs in the logistic regression analysis for the autoantibody positivity in the healthy population were compared with those in the association study for RA susceptibility, recruiting 1,237 cases and 2,087 controls in Kyoto University and previously described elsewhere (19,32). The 259,249 SNPs that were common across the current study and the previous study were pruned by linkage disequilibrium of $r^2 > 0.3$. As a result, there were 82,445 SNPs remaining for further analysis. Correlation analysis was performed by using Pearson's correlation coefficients with 8 intervals, according to the P values in each study.

Power analysis. Power analysis was performed by an online power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/).

Statistical analysis. Logistic regression analyses in genetic studies were performed by Plink, version 1.07 (33). Other statistical analyses were performed using the R statistic system (http://www.R-project.org) or SPSS (version 18). We regarded P values less than 0.005 as significant to assess correlations in a conservative manner. A stringent cutoff value of $P < 5 \times 10^{-8}$ was adopted for the GWAS.

RESULTS

Characteristics of ACPA and RF. In the current study, 1.7% and 6.4% of the study population (n = 9,575) showed positive ACPA and RF, respectively (Tables 1 and 2). The distribution of titers is shown in Table 1. We also found 0.44% of subjects being positive for both ACPA and RF, and a significant association between ACPA and RF positivity ($P = 2.0 \times 10^{-23}$ in chi-square test [odds ratio (OR) 5.19 (95% confidence interval [95% CI] 3.62–7.44)]) (see Supplementary Table 2, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). The individuals who were positive for both ACPA and RF showed a significant correlation of the titers of these autoantibodies ($\rho = 0.60$) (see Supplementary Figure 1, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). When we analyzed effects of candidates of correlates on positivity of these autoantibodies, we did not observe a significant difference in positivity for RF and ACPA between men and women. We found that ACPA positivity increased with respect to older age ($P = 0.00045$ in logistic linear regression analysis), especially for those in their 70s ($P = 0.00062$) (Table 2). While people in their 50s showed an increase of RF positivity ($P = 5.4 \times 10^{-5}$) (Table 2), no linear effect of age on RF positivity was observed ($P = 0.093$ in logistic linear regression analysis). The associations between age and ACPA or increase of RF for those in their 50s were observed mainly in women (see Supplementary Table 3, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). Next, BMI, smoking, alcohol consumption, and serum level of CRP were analyzed for associations with ACPA and RF positivity. While we did not find

Table 1. Distribution of titers in ACPA and RF in the general population*

	No.	Ratio, %
ACPA (units/ml)		
<4.5	9,408	98.3
4.5–13.5	100	1.0
>13.5–45	33	0.3
>45	34	0.4
RF (IU/ml)		
≤20	8,961	93.6
20–60	486	5.0
>60–200	87	0.9
>200	41	0.4

* ACPA = anti–citrullinated peptide antibody; RF = rheumatoid factor.