Table 1 Patient demographics and baseline disease characteristics

Patient characteristics	Total ($n = 123$)	Short duration ($n = 41$) (≤4.8 years)	Medium duration ($n = 40$) (4.8–12.0 years)	Long duration ($n = 42$) (≥12.0 years)	*P* value
Age (years)	57 ± 13	52 ± 14	56 ± 14	61 ± 10	0.009*
Women (%)	78	71	75	88	0.14
Disease duration (years)	10.0 ± 8.5	2.2 ± 1.4	8.3 ± 2.1	19.4 ± 7.5	<0.0001*
No. of previous anti-TNF agents	48 (39)	18 (44)	12 (30)	18 (43)	0.61
0 (%)	50 (41)	17 (41)	19 (48)	14 (33)	
1 (%)	21 (17)	6 (15)	7 (17)	8 (19)	
2 (%)	4 (3)	0 (0)	2 (5)	2 (5)	
3 (%)					
RF positive (%)	97	95	94	100	0.47
MTX use at baseline (%)	39	41	45	31	0.4
Weekly MTX dose at baseline (mg)	7.1 ± 2.1	7.6 ± 2.5	7.0 ± 1.3	6.7 ± 2.3	0.58
Corticosteroid use at baseline (%)	63	56	85	69	0.018**
Prednisolone dose (mg/day)	4.7 ± 2.2	5.2 ± 1.9	4.9 ± 2.4	4.0 ± 1.9	0.06
Tender joint count (28 joints assessed)	9.4 ± 7.8	8.2 ± 6.4	9.3 ± 8.0	9.1 ± 8.0	0.94
Swollen joint count (28 joints assessed)	7.4 ± 6.3	8.2 ± 6.2	6.5 ± 5.0	6.6 ± 6.1	0.22
General health (VAS, mm)	53 ± 25	53 ± 26	57 ± 24	57 ± 26	0.66
ESR (mm/h)	68.5 ± 35.1	66.8 ± 33.5	71.8 ± 30.9	63.9 ± 37.3	0.42
CRP (mg/dl)	3.1 ± 3.1	3.5 ± 3.4	3.8 ± 2.3	3.0 ± 2.8	0.11
DAS28-ESR	5.8 ± 1.4	5.8 ± 1.4	5.8 ± 1.2	5.7 ± 1.4	0.83

CRP C-reactive protein, *DAS* disease activity score, *ESR* erythrocyte sedimentation rate, *MTX* methotrexate, *RF* rheumatoid factor

* Kruskal–Wallis test, ** chi-square test

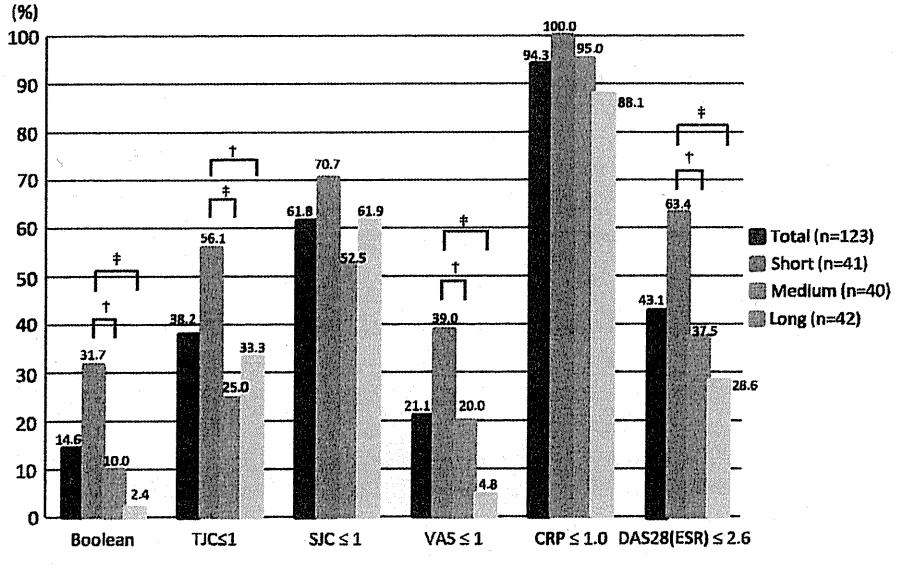

Fig. 1 Boolean core measure set of patients categorized by disease duration at 52 weeks. *CRP* C-reactive protein, *DAS* disease activity score, *SJC* swollen joint count, *TJC* tender joint count. *$P < 0.05$; **$P < 0.01$ (Fisher's exact test, JMP Ver. 8.0)

85.4%, medium 25%, long 7.1%: $P < 0.001$) and daily dysfunction (Steinblocker class short 92.7%, medium 60%, and long 35.7%; $P < 0.001$) by disease duration.

Tocilizumab treatment effectiveness is summarized in Fig. 1. Remission rate according to the Boolean criteria was significantly higher for patients with short (31.4%) than medium (10%) and long (2.4%) duration. Similarly, DAS28-ESR-defined remission was significantly higher in the short- (63.4%) than in the medium- (37.5%) and long- (28.6%) duration groups.

The proportion of patients with SJC ≤1 did not differ among patient groups; however, the proportion with TJC ≤1 and VAS-GH ≤1 was significantly lower, with longer disease duration. Serum CRP levels were normalized by tocilizumab treatment in almost all patients in the three groups. One hundred fifteen patients (94%) achieved CRP ≤1 mg/dl. Achievement of remission criteria in objective values (SJC ≤1, TJC ≤1, and CRP ≤1 mg/dl) was related to achievement of subjective values (VAS-GH ≤1 cm) (Fig. 2). The proportion of patients who achieved remission criteria in objective values (SJC ≤1, TJC ≤1, and CRP ≤1 mg/dl) was significant higher in the short disease duration group compared with patients in the medium and long disease duration groups (Fig. 2a). Interestingly, the proportion of patients who achieved all remission criteria (SJC ≤1, TJC ≤1, CRP ≤1 mg/dl, and VAS-GH ≤1 cm) among patients who achieved remission criteria in objective values (SJC ≤1, TJC ≤1, and CRP ≤1 mg/dl) was significantly higher in the short (65%) than in the long (9%) disease duration group (Fig. 2b). Inversely, 26% of patients with CRP ≤1 mg/dl did not achieve any other criteria (SJC ≤1, TJC ≤1, VAS-GH ≤1 cm).

Univariate regression analysis revealed that achieving Boolean-defined remission with tocilizumab therapy was associated with short disease duration [<4.8 years; odds ratio (OR) 7.2, 95% confidence interval (95% CI) 2.3–21.9], lower disease activity (DAS28-ESR <5.23; OR 3.9, 95% CI 1.4–11.1), and no concomitant use of corticosteroids (OR 3.4, 95% CI 1.2–9.6). Multivariate logistic analysis confirmed that short disease duration (OR 2.5, 95% CI 1.4–4.7) and lower disease activity (OR 2.5, 95% CI 1.2–5.1) were independent factors for achieving Boolean-defined remission with tocilizumab therapy (Table 2).

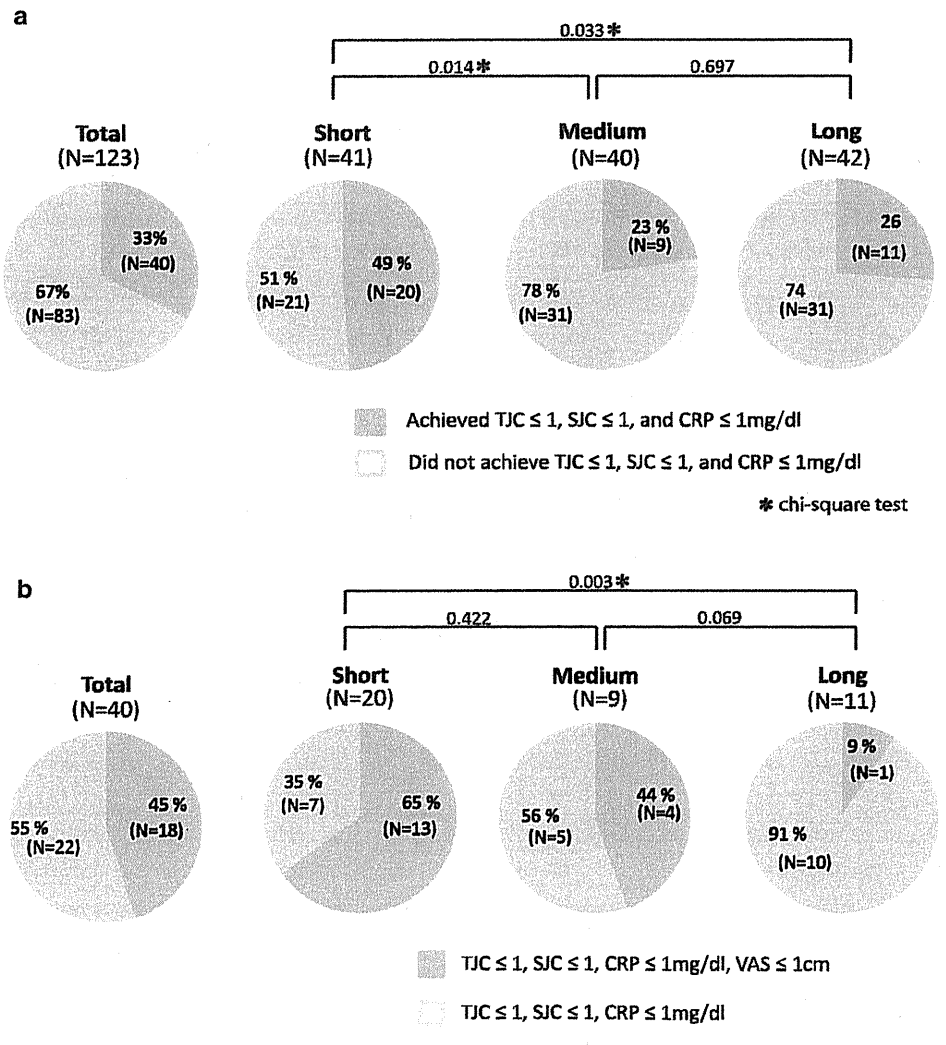

Fig. 2 Relationship between achieving remission criteria in objective and subjective values. **a** Percentage of patients who met tender joint count (TJC) ≤1, swollen joint count (SJC) ≤1, and C-reactive protein (CRP) ≤1 mg/dl in each disease duration group. *Asterisk* indicates chi-square test. **b** Proportion of patients with visual analog scale–general health (VAS-GH) ≤1 cm among patients who met TJC ≤1, SJC ≤1, and CRP ≤1 mg/dl. *Asterisk* chi-square test

Table 2 Univariate and multivariate analysis of factors associated with remission (Boolean approach) at 52 weeks

Variables	Univariate analysis Odds ratio (95% CI)	Multivariate analysis Odds ratio (95% CI)
Disease duration (first tertile, <4.8 years)	**7.15 (2.34–21.88)**	**2.53 (1.35–4.72)**
DAS28-ESR (lowest tertile, <5.23)	**3.93 (1.39–11.09)**	**2.51 (1.23–5.12)**
No corticosteroid use	**3.44 (1.23–9.58)**	1.63 (0.87–3.03)
Age (youngest tertile, 24–52 years)	1.75 (0.63–4.83)	1.42 (0.76–2.72)
No previous use of anti-TNF agent	1.69 (0.62–4.62)	1.15 (0.61–2.16)
CRP (lowest tertile, <1.28 mg/dl)	1.33 (0.47–3.73)	1.46 (0.70–3.06)
MTX use	1.30 (0.47–3.57)	1.02 (0.54–1.95)
Gender (female)	0.98 (0.29–3.27)	1.28 (0.60–2.72)

Significance for the bold values: In univariate analysis, p value for disease duration, 0.002, for DAS-ESR, 0.046, for no corticisteroid use, 0.004. In multivariate analysis, p value for disease duration, 0.002, for DAS-ESR, 0.009

CI Confidence interval, *CRP* C-reactive protein, *DAS* disease activity score, *MTX* methotrexate, *TNF* tumor necrosis factor

Discussion

We demonstrated that disease duration <5 years is one of the most important factors for achieving RA remission with tocilizumab in actual clinical practice. Tocilizumab is thought to directly suppress CRP expression [13]. We found that serum CRP levels were completely normalized in almost all patients, indicating that tocilizumab was pharmacologically active in these patients. In addition, the SJC was reduced to less than one joint in patients with short disease duration and those with longer disease duration, suggesting that tocilizumab exerts effects regardless of disease duration.

Our results demonstrate that the patient global assessment rated on a VAS was the most critical component of remission according to the Boolean-based definition; however, we do not know which factors influenced this assessment. As mentioned in the ACR/EULAR statement of the newly proposed definition of remission [7], it is not clear whether patient global assessment is sufficient to capture their experience of outcomes. In several clinical trials, patient-reported pain was suggested to be another important factor influencing remission [7]. Irreversible structural damage, which may be related to longer disease duration, can cause mechanical pain despite suppression of inflammation. Smolen et al. [14] reported that irreversible structural damage should be related to physical function (joint damage-related physical disability), i.e., physical dysfunction remaining after suppression of inflammation. This dysfunction could impact VAS-GH. Although we did not precisely determine joint structural damage, Steinbrocker joint damage and daily dysfunction were clearly related to disease duration.

Furthermore, we previously reported relationships among psychosocial factors, disease status, and quality of life in patients with RA [15] and demonstrated that the combined effects of inflammation and depression in the presence of severe pain were linearly increased by serum CRP levels and depression severity, independently [16]. The two types of persistent pain are nociceptive/inflammatory and neuropathic [17]. Neuropathic pain is produced by a lesion or dysfunction of the peripheral or central nervous system [18]. Nociceptive/inflammatory pain is responsive to anti-inflammatory therapy, whereas neuropathic pain is complex and difficult to treat. Our findings indicated that both types of pain coexist in RA patients. Long-standing pain in patients with longer disease duration could cause this neuropathic pain.

The main limitation of this study is that we evaluated only patients who underwent tocilizumab therapy. An important issue is the difference in remission achievement between tocilizumab and antitumor necrosis factor alpha (anti-TNF-α) agents. However, comparing the two is difficult in clinical practice. One reason is that levels of acute-phase reactants such as CRP are improved with tocilizumab treatment compared with treatment with anti-TNF-α agents [19]. Based on the definition of remission using a Boolean approach, CRP ≤1 should be achieved in most cases involving tocilizumab treatment. Another reason is that, in addition to disease duration, critical factors may affect efficacy and remission in treatment with anti-TNF-α agents, such as MTX dose and previous failure of other anti-TNF-α agents. MTX dose is critical for the efficacy of anti-TNF-α agents; however, MTX dose was limited to 8 mg/week in Japan until 2011. Accordingly, we do not yet have sufficient patient data on doses of MTX >8 mg/week in our register. The previous failure of anti-TNF-α agents may also be involved in efficacy. Although these points can also be argued for tocilizumab, MTX use and previous use of anti-TNF-α agents were not associated with achievement of remission in this study. For these reasons, we limited our analysis to patients treated with tocilizumab.

It would be interesting to determine whether differences exist in the mechanisms of pain reduction between nociceptive/inflammatory pain and neuropathic pain when targeting TNF-α and IL-6. Further studies are needed to evaluate the ability of patient-reported outcomes, including mental and physical function, to fully capture patient satisfaction with treatment.

In conclusion, we demonstrated that remission, as newly defined, may be a realistic goal for patients with short

disease duration in actual clinical practice. Thus, patients who have a good chance of achieving remission may benefit from aggressive therapy including biologics.

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Conflict of interest N. Ishiguro, T. Kojima, and A. Kaneko received lecture fees (less than $5,000) from Chugai Pharma Corporation. The other authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Study protocol of a multicenter registry of patients with rheumatoid arthritis starting biologic therapy in Japan: Tsurumai Biologics Communication Registry (TBCR) Study

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Abstract Biologic agents have proven to be effective against rheumatoid arthritis (RA) in clinical trials and post-marketing surveillance (PMS) studies. However, limited follow-up periods and strict criteria for recruitment might lead to an underestimation of adverse events. To document the long-term course of patients with RA treated with biologics in clinical settings, we established the Tsurumai Biologics Communication Registry (TBCR). First, we retrospectively collected data of patients registered for any biologic PMS study or clinical trial at participating

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institutes. Thus far, thirteen institutes have joined the registry and 860 patients have been identified. Comparing baseline characteristics by age and initiation year of biologics, young patients had significantly less joint damage and dysfunction and a higher dose of concomitant methotrexate (MTX) compared to older patients. Older age and functional class were significantly related to the incidence of adverse events that resulted in discontinuation of the 1st biologic treatment. The TBCR is in its initial stages, and information on all patients newly starting biologic therapy at participating institutes is being collected prospectively. Differences in baseline characteristics by age and initiation year of biologics need to be carefully evaluated in order to report on drug-related survival and long-term prognosis, using follow-up data in the near future.

Keywords Rheumatoid arthritis · Biologics · Methotrexate · Registry · Age

Introduction

Biologic agents have proven to be effective against rheumatoid arthritis (RA) in a number of clinical trials and post-marketing surveillance (PMS) studies. In Japan, PMS for biologics including infliximab, etanercept, adalimumab, and tocilizumab has provided variable information for clinical practice [1, 2]. However, limited follow-up periods and strict criteria for recruitment might lead to an underestimation of adverse events. Long-term clinical data are necessary to evaluate the effectiveness of biologics in the clinical setting. To this end, we developed the Tsurumai Biologics Communication Registry (TBCR), a prospective, observational, multicenter registry of patients with RA starting biologic therapy; the registry became active in October 2008. The aim of this registry is to document the course and outcome of RA patients newly starting biologic therapy in Japan.

Treatments using biologics are expensive. Considering the significant economic burden on society, the efficacy, safety, and cost-effectiveness of biologics need to be determined. Age is one of the most important factors that influence health outcomes and socioeconomic status. Information from clinical trials and other registries is also becoming increasingly available. Thus, assessing changes in patient characteristics will prove to be invaluable for a better understanding of the clinical outcomes of treatment with biologics. In this study, we describe the study protocol of the TBCR and evaluate the baseline characteristics of patients who initiated treatment with biologics by October 2008 and registered with the TBCR. We evaluated the patients' baseline characteristics stratified by age and initiation year of biologics, as well as determining the incidence of adverse events within 1 year from the initiation of biologics.

Patients, materials, and methods

Registration of patients treated with biologic agents

Thirteen institutes throughout Japan have joined this project. All institutes have already conducted PMS for at least one of infliximab, etanercept, adalimumab, or tocilizumab, continued the follow-up of registered patients after the completion of PMS, and continue to register patients with RA newly starting biologic therapy after PMS. Therefore, we collected retrospective data of patients registered for any biologic PMS or clinical trial at each of the participating institutes. We identified a total of 1,037 patients with RA who began biologic therapy at the participating institutes, and we transferred all their information from each institute's established database to the new TBCR database. We also initiated recruitment of all patients who had newly started biologic therapy since October 2008.

Baseline data

Baseline data were collected in two ways. Data for patients registered through the established database system at each institute were obtained from the institutional databases. Data for patients recruited after TBCR implementation were collected from hospital charts at each institute. Documented variables at baseline were as follows: demographic variables, including sex, age, years of disease duration; Steinbrocker stage of joint damage and class of dysfunction in daily life [3]; disease activity assessed by the 28-joint disease activity score using C-reactive protein (DAS28-CRP), including swollen and tender joint counts; visual analog scale (VAS) scores for patient's global assessment of health status; serum CRP levels; and concomitant treatment with methotrexate (MTX). In addition to these variables, we have been collecting physicians' global assessment scores and information on concomitant treatment with prednisolone (PSL), Health Assessment of Questionnaire disability index (HAQ-DI) scores, and serum matrix metalloproteinase-3 (MMP-3) levels of newly registered patients since October 2008 for baseline data.

Follow-up

Registered patients are followed until they discontinue biologic therapy; the reasons for discontinuation are recorded: serious adverse events, non-serious adverse events, insufficient effectiveness, patient's convenience, etc. If a patient changes biologics, collected data include disease activity (DAS28-CRP), physician's global assessment, MMP-3, and information regarding current biologic use and concomitant treatment. Follow-up is continued by rheumatologists during regular visits at each institute. Data

of registered patients are collected at 3, 6, and 12 months after initiation of biologic therapy and recorded annually at participating institutes.

Database management

Data are maintained by the TBCR Center (hereafter, the Center) located in the Department of Orthopedic Surgery and Rheumatology at the Nagoya University School of Medicine. In addition, all data regarding registered patients collected at participating institutes are transferred to the Center every July. The Center checks all data and combines them for statistical analysis, which is conducted at the Department of Public Health, Nagoya City University, Graduate School of Medical Science. Access to combined data and its release are controlled by the Center; however, all members of the management committee can access the data. The registry was initially funded by a grant from the Ministry of Health, Labour and Welfare of Japan, "Study for mortality-based optimal management of patients with rheumatoid arthritis in the biologic era" (chaired by Professor H. Yamanaka, Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan).

The registry and study design were approved by the Ethics Committee of Nagoya University, School of Medicine. Patient anonymity was maintained during data collection, and the security of personal information is strictly controlled.

Statistical analysis

To examine baseline characteristics by age and initiation period of biologics, patients who were retrospectively registered were divided into three groups based on age tertiles: the young- ($\leq$53 years, $n = 292$), middle- (>53 to $\leq$64 years, $n = 309$), and old-aged (>64 years, $n = 259$) groups. To examine baseline characteristics by initiation period of biologics, we divided registered patients into four groups based on the year of initiation ($\leq$2005, 2006, 2007, and 2008). Differences among the groups were analyzed with general linear models for continuous variables and the χ^2test for categorical variables. As for the incidence of adverse events, we collected information on adverse events that resulted in the discontinuation of the 1st biologic treatment within 1 year and classified them according to the International Statistical Classification of Diseases and Related Health Problems 10th Revision Version for 2007 (ICD-10). Baseline characteristics for the incidence of adverse events were analyzed using the Cox proportional-hazards regression model. All data were analyzed using SPSS version 19.0 (IBM, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Results

A total of 964 patients were identified retrospectively through the established database system at thirteen institutes. After excluding 98 subjects with missing values and 6 patients younger than 17 years of age, the mean age and disease duration $\pm$ SD of the 860 subjects were 56.4 $\pm$ 13.8 and 11.5 $\pm$ 9.8 years, respectively.

Baseline characteristics are summarized by age group in Table 1. Significant differences by age were observed in sex, disease duration, stage of joint damage and class of dysfunction, patient's global assessment score for health, CRP, concomitant use of MTX, and the MTX dose at first administration of biologics. Patients in the young age group were more likely to be female, have shorter disease duration, less severe joint damage, and less dysfunction in daily life, and lower CRP levels. In particular, the proportion of patients with disease duration of <2 years was significantly higher in the young age group. There was no difference in DAS28-CRP by age. The use of MTX as a concomitant drug was more frequent and the dose higher in the young- and middle-aged groups than in the old-aged group.

The most popular of the biologics were etanercept (62.8%) and infliximab (32.0%). The proportion of etanercept as the first prescribed biologic treatment was higher in the young and old age groups than in the middle age group.

Differences in baseline characteristics by initiation year of treatment with biologics are shown in Table 2. Significant differences by initiation year were observed in the stage of joint damage, DAS28-CRP, CRP, concomitant use of PSL, and the PSL dose at first biologic administration. The proportion of etanercept as the 1st prescribed biologic treatment increased until 2007. In 2008, when other new biologics (adalimumab and tocilizumab) became available, the proportion of etanercept decreased while that of infliximab did not change significantly.

Adverse events within 1 year from the initiation of biologics which resulted in discontinuation of the 1st biologic treatment are summarized in Table 3. Notably, 75% of the adverse events that occurred within 1 year from the initiation of biologics occurred within 6 months of their initiation. Diseases of the respiratory system and infection (43.0%) were the main adverse events. Also included in the adverse events were one case (0.11%) of tuberculosis, two cases of *Pneumocystis jiroveci* pneumonia (0.23%), and three cases (0.35%) of nontuberculous mycobacterial infection. From 6 months to 1 year, 63% of the adverse events were categorized as infectious and parasitic diseases and diseases of the respiratory system.

We also examined the factors that influenced the incidence of adverse events (Table 4). Older age (per year) was

Table 1 Differences in baseline patient characteristics by age at initiation of biologics

Variables		Total all ages (n = 860)	Young ≤53 (n = 292)	Middle >53 to ≤64 (n = 309)	Old >64 (n = 259)	P value
Age (years)		56.4 (13.8)	40.8 (9.4)	59.1 (3.2)	70.9 (4.7)	
Women (%)		82.8	88.7	82.7	76.0	0.001
Disease duration (years)		11.5 (9.8)	9.0 (7.6)	13.0 (10.7)	12.5 (10.5)	<0.001
Disease duration ≤2 years (%)		16.8	22.9	12.0	15.7	0.004
Stage (%)	I	8.2	12.8	5.8	6.1	0.002
	II	14.1	14.6	13.9	13.8	
	III	37.0	41.2	35.6	34.0	
	IV	40.7	31.4	44.7	46.2	
Class (%)	I	15.6	25.9	14.6	5.3	<0.001
	II	46.8	53.3	46.4	40.1	
	III	36.0	20.4	36.9	52.2	
	IV	1.6	0.4	2.0	2.4	
DAS28-CRP		4.98 (1.14)	4.86 (1.18)	5.04 (1.14)	5.04 (1.09)	ns
Patient's global score (mm)		60.5 (21.8)	57.0 (23.6)	61.5 (20.4)	63.5 (21.0)	0.045
CRP (mg/dl)		3.8 (3.2)	3.3 (2.9)	3.9 (3.4)	4.2 (3.3)	0.02
MTX use (%)		74.9	81.9	80.9	59.3	<0.001
MTX dosage (mg/week)		7.2 (1.9)	7.6 (2.0)	7.1 (1.7)	6.7 (1.8)	<0.001
PSL use (%)		82.5	78.8	83.0	86.1	ns
PSL dosage (mg/day)		5.1 (2.3)	5.0 (2.0)	5.1 (2.7)	5.2 (1.9)	ns
Biologics						
Infliximab (%)		32.0	26.0	38.5	30.9	0.025
Etanercept (%)		62.8	68.5	57.0	63.3	
Others (adalimumab, tocilizumab, abatacept) (%)		5.2	5.5	4.5	5.8	

Except where indicated otherwise, values are means (SD). Stage and class were defined using Steinbrocker's classification

DAS28-CRP, Disease activity score in 28 joints (DAS28) based on C-reactive protein (CRP) levels with 4 variables; *MTX*, methotrexate; *PSL*, prednisolone

P values for continuous variables were determined with the general linear model; P values for categorical variables were determined with the χ^2test

significantly related to a higher incidence of discontinuation because of adverse events [hazard ratio (HR) = 1.041, 95% confidence interval (CI) 1.006–1.076] while lower level of daily dysfunction (Class I and II) were clearly related to a lower incidence of discontinuation because of adverse events [HR = 0.406, 95% CI 0.193–0.856].

Discussion

We confirmed significant age differences in the baseline characteristics of patients registered with the TBCR retrospectively. Compared to old-aged patients, young-aged patients tended to have started biologic therapy at an early stage of RA and more aggressively with high-dose MTX. We found significant differences in patient characteristics by the initiation year of biologics. The profile of adverse events within 1 year from initiation of biologics and related baseline characteristics were also examined.

Biologics confer good disease control to many patients with RA, but are associated with rare but severe adverse events such as serious infections, lymphoma, or chronic heart failure [4]. Adverse events clearly increase with age. In fact, the British Society for Rheumatology Biologics Register (BSRBR) reported that incidences of severe infection markedly increased with age, although no significant difference was observed in the relative risk of infection in patients undergoing anti-tumor necrosis factor (TNF)-α therapy compared to patients treated with non-biologic disease-modifying anti-rheumatic drugs (DMARDs) among older populations [5]. In the present study, older age had a significant impact on the incidence of adverse events that resulted in the discontinuation of the 1st biologic treatment.

On the other hand, the BSRBR reported that age and disease duration did not predict response to anti-TNF-α therapy, while a better response to this therapy was associated with the concurrent use of nonsteroidal anti-inflammatory drugs (NSAIDs) and MTX in 2,879 patients

Table 2 Differences in baseline patient characteristics by year at initiation of biologics

Variables		≤2005 (n = 193)	2006 (n = 221)	2007 (n = 250)	2008 (n = 196)	P value
Age (years)		57.0 (12.1)	57.4 (13.5)	56.2 (14.1)	55.2 (15.1)	ns
Women (%)		82.0	82.1	82.4	84.7	ns
Disease duration (years)		11.2 (9.0)	12.1 (10.1)	12.2 (10.7)	10.3 (9.1)	ns
Disease duration ≤2 years (%)		22.4	20.8	29.6	27.2	ns
Stage (%)	I	4.4	4.2	10.4	14.0	0.001
	II	9.9	12.6	15.4	18.4	
	III	42.9	39.1	33.3	33.5	
	IV	42.9	44.2	40.8	34.1	
Class (%)	I	11.5	13.5	16.30	21.2	ns
	II	48.4	47.0	48.3	43.0	
	III	38.5	37.2	34.6	34.1	
	IV	1.6	2.3	.8	1.7	
DAS28-CRP		5.4 (1.2)	4.9 (1.2)	4.8 (.9)	4.8 (1.1)	<0.001
Patient's global score (mm)		64.8 (20.0)	61.4 (23.2)	59.8 (21.1)	56.5 (21.9)	ns
CRP (mg/dl)		4.9 (3.3)	3.8 (3.5)	3.4 (2.8)	3.1 (2.9)	<0.001
MTX use (%)		74.4	73.6	74.4	77.5	ns
MTX dosage (mg/week)		7.4 (1.8)	7.2 (1.7)	6.9 (1.9)	7.3 (2.0)	ns
PSL use (%)		91.5	89.3	83.5	67.8	<0.001
PSL dosage (mg/day)		6.2 (3.4)	5.1 (1.7)	4.7 (1.4)	4.4 (1.6)	<0.001
Biologics						
Infliximab (%)		49.2	30.8	25.2	25.0	<0.001
Etanercept (%)		47.7	68.3	74.0	57.1	
Others (adalimumab, tocilizumab, abatacept) (%)		3.1	0.9	0.8	17.9	

Except where indicated otherwise, values are means (SD). Stage and class were defined using Steinbrocker's classification

DAS28-CRP, Disease activity score in 28 joints (DAS28) based on C-reactive protein (CRP) levels with 4 variables; *MTX*, methotrexate; *PSL*, prednisolone

P values for continuous variables were determined with the general linear model; P values for categorical variables were determined with the χ^2 test

Table 3 Adverse events within 1 year from initiation of biologics

ICD-10 categories	Events within 6 months n (%)	Events within 6 months to 1 year n (%)	Total events within 1 year n (%)
Certain infectious and parasitic diseases	7 (12.3)	4 (21.1)	11 (14.5)
Diseases of the respiratory system	14 (24.6)	8 (42.1)	22 (28.9)
Diseases of the skin and subcutaneous tissue	9 (15.8)	1 (5.3)	10 (13.2)
Diseases of the musculoskeletal system and connective tissue	6 (10.5)	1 (5.3)	7 (9.2)
Injury, poisoning, and certain other consequences of external causes	8 (14.0)	0 (0.0)	8 (10.5)
Diseases of the circulatory system	1 (1.8)	1 (5.3)	2 (2.6)
Neoplasms	3 (5.3)	1 (5.3)	4 (5.3)
Diseases of the genitourinary system	1 (1.8)	2 (10.5)	3 (3.9)
Others	8 (14.0)	1 (5.3)	9 (11.8)
All categories	57 (100.0)	19 (100.0)	76 (100.0)

ICD-10: International Statistical Classification of Diseases and Related Health Problems 10th Revision Version for 2007. Infusion reaction is included in the category "Injury, poisoning and certain other consequences of external causes"

with RA [6]. Consistent with this finding, the South Swedish Arthritis Treatment Group Register reported that MTX use was associated with a good response to anti-TNF therapy [7]. In our cohorts, doctors may have been hesitant to aggressively treat older patients, given the fear of adverse side effects, while younger patients could have

Table 4 Factors associated with incidence of adverse events within 1 year from initiation of biologics

Factors	HR	95% CI	*P* value
Age/year	1.041	1.006–1.076	0.019
Disease duration/year	0.990	0.954–1.026	0.571
Stage I and II	1.292	0.535–3.123	0.569
Class I and II	0.406	0.193–0.856	0.018
PSL no use	0.257	0.061–1.083	0.064
MTX no use	0.735	0.310–1.745	0.485

HR was determined using the Cox proportional-hazards regression model. Stage and class were defined using Steinbrocker's classification

CI confidence interval, *MTX* methotrexate, *PSL* prednisolone

been treated more aggressively. However, the results of the British and Swedish studies suggest that these older patients potentially could have been brought to remission without initiating aggressive treatments by using biologics and concomitant MTX at an early stage. Based on accumulated evidence, the European League Against Rheumatism (EULAR) published recommendations in 2010 for the management of RA with synthetic and biological DMARDs, stating that the aim of treatment should be achieving remission or low disease activity as soon as possible in every patient [8, 9]. Further careful observation will be needed to determine the safety and efficacy of aggressive therapy in older patients.

Biologics have been available in Japan since 2003, which is later than their introduction in North America and Europe. Moreover, in Japan, the MTX dose for RA treatment has been much lower than the doses used in North America and Europe, because the approved upper limit was 8 mg/week. The mean dose of MTX in the present study was 7.2 mg/week, which is comparable to that in the PMS studies of infliximab (mean 7.3 mg/week) [1] and etanercept (mean 6.58 mg/week) [2]. In response to frequent and persistent requests by rheumatologists and RA patients, the Japanese government raised the upper dose limit of MTX to 16 mg/week in January 2011. As such, ongoing Japanese cohort data with RA patients will provide valuable information on various treatment courses and disease states.

Wolfe et al. [10] reported that the administration of PSL in RA treatment had a significant impact on the incidence of serious pneumonia (i.e., pneumonia that required hospitalization). We did not find a significant relationship between the concomitant use of PSL with biologics and the incidence of adverse events, although there was trend (*P* value = 0.064). In this analysis, the number of cases for determining the incidence of adverse events was small and the observation period was limited. Further studies with longer follow-up periods are needed.

To date, three observational cohort studies have been established in Japan: Institute of Rheumatology Rheumatoid Arthritis (IORRA) (since 2000) [11], NinJa (National Database of Rheumatic Diseases by iR-net in Japan; http://www.ninja-ra.jp) (since 2002) [12], and REAL (the Registry of Japanese Rheumatoid Arthritis Patients on Biologics for Long-term Safety; http://www.real-study.jp) (since 2005) [13]. Compared with these cohorts, the TBCR is unique in that it includes a variety of participating institutes, spanning a single university hospital, a national medical center, urban county hospitals, and clinics throughout Japan. Thus, the TBCR more accurately reflects the clinical setting with regard to patients with RA in Japan. However, members of the TBCR study group are orthopedic surgeons, which represents a unique situation for RA treatment. Thus, there is the possibility of selection bias for treatments, although the baseline patient characteristics and incidence of adverse events in our registry are comparable to those of the PMS study of infliximab and etanercept in Japan.

The TBCR is in its initial stages, and information on all patients newly starting biologic therapy at the participating institutes is being collected prospectively. Drug-related survival and long-term prognosis based on follow-up data will be reported in the near future.

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CASE REPORT

Anti-tumor necrosis factor therapy in rheumatoid arthritis patients with a history of deep prosthetic joint infection: a report of four cases

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Abstract Four rheumatoid arthritis patients (three women and one man) who had a history of prosthetic joint infection were treated with anti-tumor necrosis factor (TNF) agents after treatment of the infection. The anti-TNF therapy was subsequently discontinued in three patients. The reason for discontinuation was not the reactivation of infection, but disseminated tuberculosis, *Pneumocystis jiroveci* pneumonia, and interstitial pneumonia, respectively. These cases suggest that a history of prosthetic joint infection may be a contraindication for treatment with anti-TNF agents.

Keywords Rheumatoid arthritis · Anti-tumor necrosis factor therapy · Prosthetic joint infection · Disseminated tuberculosis · Pneumocystis pneumonia

Introduction

The development of anti-tumor necrosis factor (TNF) agents has dramatically changed the treatment strategy for patients with rheumatoid arthritis (RA) [1, 2]. The administration of anti-TNF agents during the early stages of RA is recommended if disease activity remains high despite treatment with methotrexate (MTX) [3]. However, some patients are unable to continue with anti-TNF therapy due to adverse events. Severe infection is a particular concern, and the risk factors for developing a severe infection during anti-TNF therapy are not clear.

Joint destruction has progressed significantly in patients in the later stages of RA. Joint replacement surgery can improve the function of the patient's affected joints and, consequently, their activities of daily living (ADL). One potential severe complication of joint replacement surgery is deep infection. Deep prosthetic joint infections often require lengthy treatment with antibiotics, and follow-up operations, such as debridement, implant removal, and revision surgery [4]. In addition, it is difficult to determine when prosthetic joint infections have been successfully treated because inflammatory signs and symptoms often disappear, even when bacteria are still present. Because most RA patients undergoing joint replacement surgery have high disease activity, treatment of their RA is needed during and after treatment of a deep infection. Due to the small number of such cases, it is rare that anti-TNF agents are used. It is unclear whether anti-TNF therapy is appropriate for such patients and what the outcome of this therapy is in such cases. Here we report the clinical courses of four RA patients (three women and one man). All patients had the same RA classification (Steinbrocker stage IV, class 3), a history of deep prosthetic joint infection, and were treated with anti-TNF agents following treatment of the infection.

Case reports

Case 1

The patient was a 55-year-old woman diagnosed with RA in 1979 when she was 23 years old. Despite treatment

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with conventional disease-modifying anti-rheumatic drugs (DMARDs), her right knee required replacement, and a total knee arthroplasty (TKA) was performed in December 2002. Although her clinical course after surgery was satisfactory, right gonalgia with localized heat was noted in January 2003. Blood tests revealed a C-reactive protein (CRP) level of 8.8 mg/dl. Infection of the TKA was suspected and emergency joint debridement without implant removal was performed. Although culture from the joint fluid was negative, signs and symptoms were consistent with infection, and antibiotic treatment was initiated, first with cefazolin (CEZ), followed by cefotiam (CTM), cefmetazon (CMZ), and minocycline (MINO). Blood hemoglobin (Hb), serum total protein (TP), and serum albumin (Alb) just before the infection occurred were 12.7, 7.3, and 4.4 g/dl, respectively. The infection was well controlled and the implant was not removed. RA disease activity remained high and the disease activity score in 28 joints using CRP (DAS28-CRP) was 6.11. Treatment with etanercept (ETA) was initiated in April 2007 after consent was obtained in regard to the risks and benefits of anti-TNF therapy. Her tuberculin skin test was negative and no abnormality was found on her chest X-ray. However, ETA was not effective and she was switched to infliximab (INF) with tacrolimus (TAC) 2 mg/day in January 2008. Although INF with TAC was effective over a 5-month period, she reported abdominal discomfort in June 2008 after the 5th infusion of INF. She lost 5 kg in 2 months just before the occurrence of abdominal discomfort. Abdominal computed tomography (CT) imaging showed apparent ascites (Fig. 1a, b), and chest X-ray showed fluid in the right pleural cavity (Fig. 1c). The treatment with INF and TAC was discontinued. A QuantiFERON-TB2G test (QFT; Cellestis, Carnegie, Victoria, Australia) was positive and culture examination of sputum and stomach fluid was positive for *Mycobacterium tuberculosis*. A pleural biopsy was performed and showed granulomas with Langhans giant cells. She was diagnosed with disseminated tuberculosis (TB), and anti-tuberculosis chemotherapy was initiated with isoniazid (INH), ethambutol (EB), rifampicin (REP), and pyrazinamide (PZA). Signs and symptoms of TB were well controlled with the combination drug therapy.

Case 2

The patient was a 34-year-old woman diagnosed with juvenile idiopathic arthritis (JIA) in 1986 when she was 12 years old. Total hip arthroplasty (THA) was performed for her right hip joint in April 2001 (Fig. 2a) and for her left hip joint in July 2001. Late infection of the right THA occurred in July 2002, and this was treated with antibiotics and surgical debridement without implant removal. Blood Hb, serum TP, and serum Alb just before the infection occurred were 8.3, 6.6, and 3.5 g/dl, respectively. *Staphylococcus aureus* was detected by culture examination.

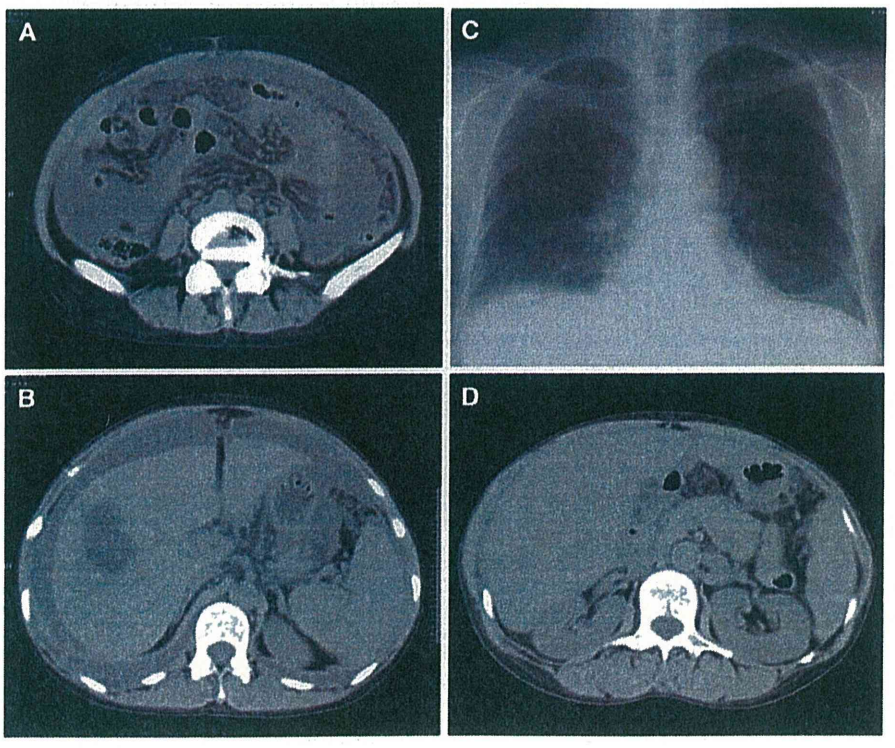

Fig. 1 Radiological images of the patient in Case 1. **a**, **b** Abdominal computed tomography (CT) before treatment of disseminated tuberculosis (TB). **c** Chest X-ray before treatment of TB. **d** Abdominal CT after treatment of TB

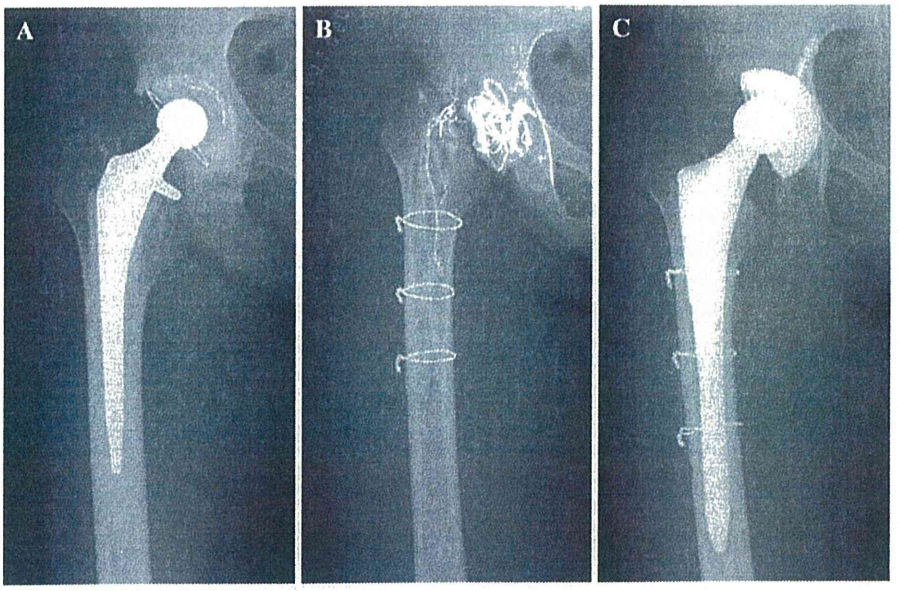

Fig. 2 Radiological images of the right hip joint of the patient in Case 2. **a** Primary total hip arthroplasty (THA). **b** Treatment of infection with cement beads following implant removal. **c** Revision THA

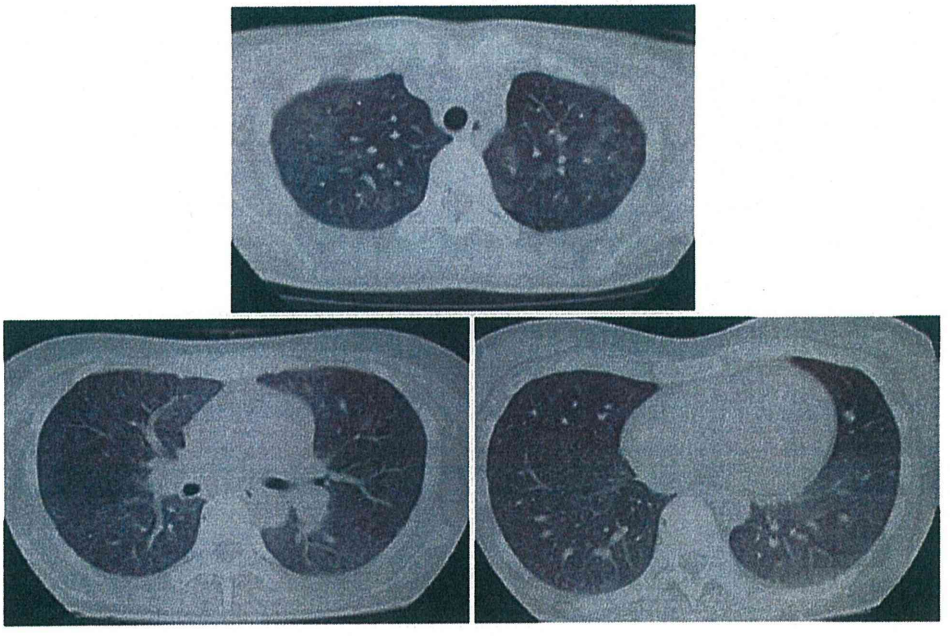

Fig. 3 Chest computed tomography (CT) at the onset of *Pneumocystis jiroveci* pneumonia in Case 2. A ground-glass pattern is seen bilaterally in the lungs

Although the infection seemed to be well controlled, reactivation of infection occurred in March 2004 and was treated again by surgical debridement without implant removal. The implants loosened in July 2005, resulting in implant removal and treatment with cement beads (Fig. 2b). Revision surgery of the right hip joint was performed in February 2006 (Fig. 2c). RA disease activity remained high, and DAS28-CRP was 5.71. ETA with MTX was initiated in May 2006 after consent was obtained in regard to the risks and benefits of anti-TNF therapy. Her chest X-ray was normal before ETA administration. Although eight injections of ETA were given, Herpes zoster infection and *Pneumocystis jiroveci* pneumonia (PCP) were evident after a month (Fig. 3) and ETA with MTX was discontinued. PCP was diagnosed by a positive polymerase chain reaction test of bronchial alveolar lavage fluid. The Herpes zoster infection and PCP were successfully treated with aciclovir, trimethoprim-sulfamethoxazole, and subsequently, with pentamidine. No signs of infection around the right THA have been seen to date. She is currently being treated with MTX (8 mg/week), TAC (0.5 mg/day), and prednisolone (PSL) (2.5 mg/day).

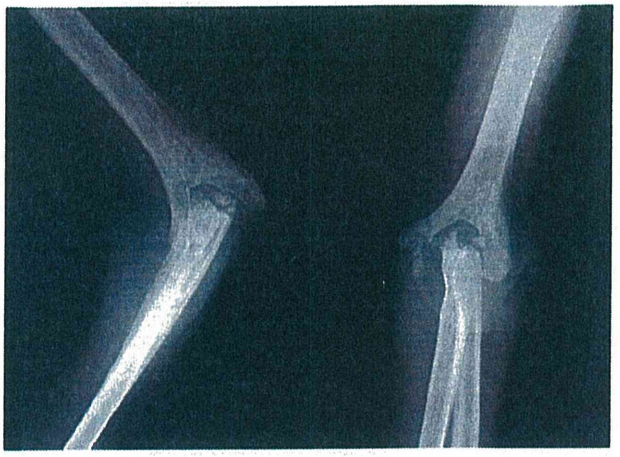

Fig. 4 Radiological images of the left elbow after implant removal in Case 3. No revision surgery was performed, and orthosis was used to stabilize the left elbow

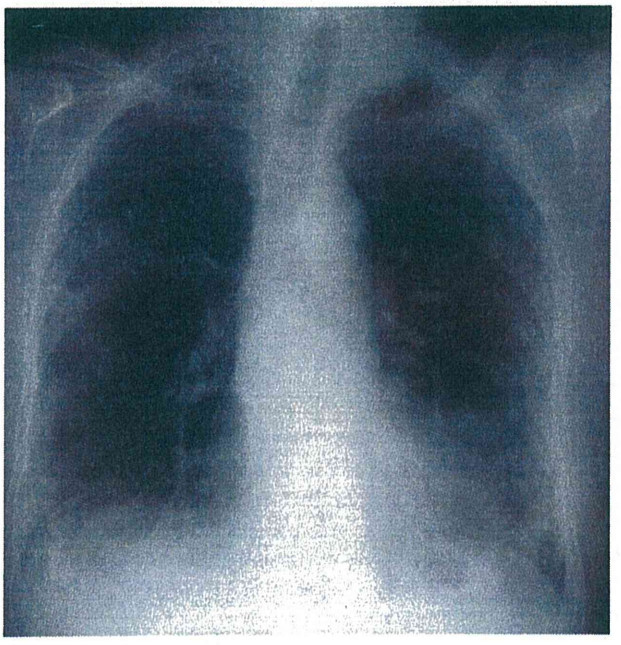

Fig. 5 Chest X-ray of the patient in Case 3. Infliximab was discontinued due to interstitial pneumonia

Case 3

The patient was a 67-year-old man diagnosed with RA in 1989 when he was 48 years old. A total elbow arthroplasty (TEA) was performed on his left elbow in February 2002. Infection of the TEA occurred in November 2002. Blood Hb and serum TP just before the infection occurred were 11.2 and 7.6 g/dl, respectively. Emergency surgical debridement without implant removal was performed and culture examination was positive for *S. aureus*. The infection was well controlled with antibiotic treatment (CEZ, CTM, and CMZ), but reactivation occurred in January 2003, and the implants were removed. Revision surgery was not performed (Fig. 4) and orthosis was used to stabilize the left elbow. RA disease activity remained high, and DAS28-CRP was 5.65. INF with MTX was initiated in August 2004 after consent was obtained in regard to the risks and benefits of anti-TNF therapy. Bronchiectasis was noted prior to the initiation of INF administration. He was referred to another hospital because of a change of address. After that, deterioration, with interstitial pneumonia (IP), occurred in 2006, and INF and MTX were discontinued (Fig. 5). The total duration of treatment with INF was 24 months.

Case 4

The patient was a 69-year-old woman diagnosed with RA when she was 30 years old. Disease activity remained high despite treatment with MTX (6 mg/week), bucillamine (BUC; 100 mg/day), and PSL (5 mg/day). DAS28-CRP was 5.70. INF was added to MTX, BUC, and PSL in November 2005. She had right gonalgia, which worsened, and joint space narrowing was apparent on X-ray images. TKA was performed on her right knee in January 2007 (Fig. 6). The duration of INF treatment was 1 year and 2 months, with the last dose administered 21 days before the surgery. She had a high fever just after the surgery, and discharge from the wound continued, although bacteria were not detected by culture examination. Finally, surgical debridement without implant removal was performed, and *Capnocytophaga* was detected from a synovial specimen taken during surgery. Blood Hb, serum TP, and serum Alb just before the infection occurred were 11.0, 6.4, and 3.2 g/dl, respectively. Ampicillin/sulbactam (ABPC/SBT) 6 g/day was administered for 4 weeks and this effectively treated the infection. INF was discontinued after the infection of the TKA. She was treated with oral levofloxacin (LVFX) for 6 months, and reactivation did not occur after the LVFX treatment ended. RA disease activity remained high, and DAS28-CRP was 5.47. Administration of ETA with MTX was initiated in August 2008 after consent was obtained in regard to the risks and benefits of anti-TNF therapy. DAS28-CRP had decreased to 3.73 1 month after the initiation of ETA and MTX. No signs of infection around her right TKA have been seen to date, and the anti-TNF therapy was re-initiated 1 year and 8 months after it was discontinued.

Discussion

In this case series, we have described the clinical courses of four patients with RA who had a history of prosthetic joint infection and were treated with anti-TNF therapy. Anti-TNF therapy was discontinued in three of the four patients

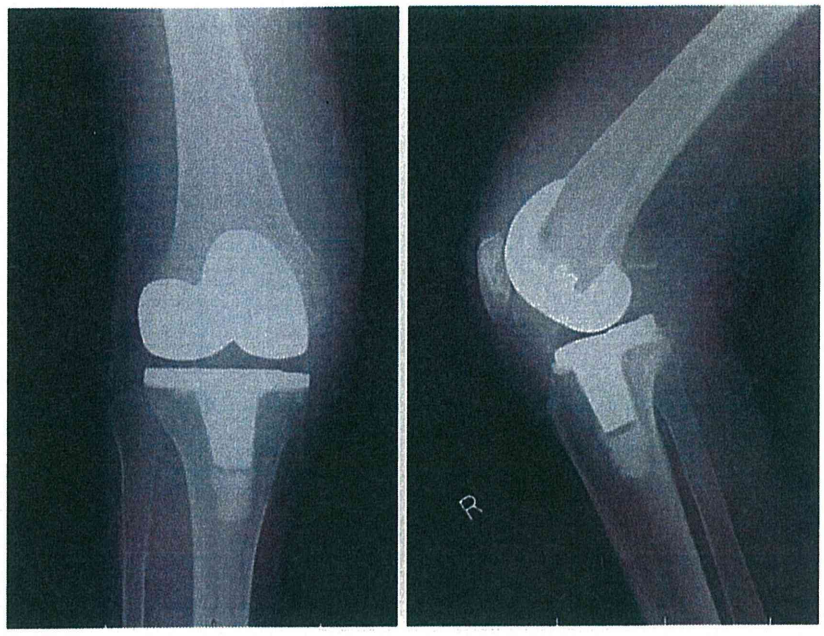

Fig. 6 Total knee arthroplasty of the patient in Case 4. High fever and discharge from the right (*R*) knee continued immediately after surgery. *Capnocytophaga* was detected from a specimen of synovial tissue

for reasons other than reactivation of the infection, showing that the prosthetic joint infections were treated successfully. We are very interested in the fact that these patients could not continue anti-TNF therapy for other reasons. The causes for discontinuation were disseminated TB in Case 1, PCP in Case 2, and IP in Case 3. These findings suggest that patients with a history of prosthetic joint infection may be at higher risk of adverse events during treatment with anti-TNF agents. Although immunological factors in such patients may be one of the reasons for the failure of anti-TNF therapy, the precise mechanisms of this failure are currently unknown. Prospective studies including more patients with a history of prosthetic joint infection are needed.

Prosthetic joint infection is one of the most miserable complications of joint replacement surgery. Its prevalence in patients in the United States was reported to be 2% [5]. The risk of prosthetic joint infection is higher for patients with RA than for patients with osteoarthritis [6, 7]. In the present report, bacteria were not detected in Case 1, and *Capnocytophaga*, an opportunistic pathogen, was detected in Case 4. Although prostheses were not removed in Cases 1 and 4, successful treatment of the infection required both surgical debridement and the administration of antibiotics.

In clinical studies, the influence of anti-TNF therapy on the manifestations of surgical site infection (SSI) is controversial. Bibbo and Goldberg [8] reported that the use of anti-TNF agents might be safely undertaken in the perioperative period without increasing risks to healing or risks of infectious complications in RA patients undergoing elective foot and ankle surgery. den Broeder et al. [9] investigated postoperative SSIs in 1,219 operations carried out in 768 patients, and found that the crude infection risks were 4.0, 5.8, and 8.7% in patients who did not use anti-TNF agents, patients who did but then stopped, and patients who continued anti-TNF preoperatively, respectively. However, there were no significant differences in infection rates among the three groups. Also, we previously reported that anti-TNF therapy did not increase the rates of postoperative infection of orthopedic surgery sites in patients with RA [10]. By contrast, Giles et al. reported that anti-TNF agents increased the rate of infection in elective orthopedic operations [11]. Kawakami and Momohara also reported that anti-TNF therapy was a risk factor for SSI following major orthopedic surgery [12]. Reactivation of the infection may be induced if anti-TNF therapy is initiated. There is currently no definitive treatment strategy for patients with very active RA who have a history of prosthetic joint infection.

Anti-TNF agents have revolutionized the treatment of severe RA [1, 2], but they are not indicated for all patients with RA. For example, they are contraindicated in patients with signs of infection. However, it is difficult to decide whether anti-TNF therapy should be initiated in RA patients with a history of prosthetic joint infection, because it is difficult to determine whether the infection has been successfully treated. The usefulness of inflammatory markers, such as CRP or the erythrocyte sedimentation rate (ESR), is limited in patients with RA. We decided that anti-TNF agents could be administered after a long observation period without reactivation of infection. Neutrophil CD64 expression is a promising candidate for use as a marker of bacterial infection when using anti-TNF agents to treat RA patients with a history of prosthetic joint infection [13].

In conclusion, we have reported the clinical courses of four RA patients with a history of deep prosthetic joint infection who were treated with anti-TNF agents following treatment of the infection. Anti-TNF therapy could not be continued in three of the four patients, not because the infection had reactivated, but because of disseminated tuberculosis, *Pneumocystis jiroveci* pneumonia, and deterioration of interstitial pneumonia (IP), respectively. This case series suggests that a history of prosthetic joint infection is a contraindication for treatment with anti-TNF agents, possibly due to abnormalities in the immune systems of such patients.

Conflict of interest Y. Hirano has received speaking fees from Abbot Japan Co. Ltd.; Eisai Co. Ltd.; Mitsubishi Tanabe Pharma Corporation; and Pfizer Co. Ltd. T. Kojima has received speaking fees from Mitsubishi Tanabe Pharma Corporation; Takeda Pharmaceutical Company Limited; Pfizer Co. Ltd.; and Wyeth K.K. N. Ishiguro has received speaking fees from Abbot Japan Co. Ltd.; Eisai Co. Ltd.; Mitsubishi Tanabe Pharma Corporation.; Takeda Pharmaceutical Company Limited; and Pfizer Co. Ltd. The other authors have declared no conflict of interest.

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Keratan sulfate and related murine glycosylation can suppress murine cartilage damage *in vitro* and *in vivo*

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ABSTRACT

Keratan sulfate (KS) proteoglycan side chains are abundant in the human cartilage matrix, but these chains have been said to be absent in murine skeletal tissues. We previously showed that KS suppresses cartilage damage and ameliorates inflammation in mice arthritis model. Because mice deficient of *N*-acetylglucosamine 6-*O*-sulfotransferase-1 (GlcNAc6ST-1) (KS biosynthesis enzyme) are now available, we decided to do further examinations.

We examined, in culture, the difference between GlcNAc6ST-1$^{-/-}$ and wild-type (WT) mice for interleukin (IL)-1α-induced glycosaminoglycan (GAG) release from the articular cartilage. Arthritis was induced by intravenous administration of an anti-type II collagen antibody cocktail and subsequent intraperitoneal injection of lipopolysaccharide. We examined the differences in arthritis severities in the two genotypes. After intraperitoneal KS administration in phosphate-buffered saline (PBS) or PBS alone, we evaluated the potential of KS in ameliorating arthritis and protecting against cartilage damage in deficient mice.

GAG release induced by IL-1α in the explants, and severity of arthritis were greater in GlcNAc6ST-1$^{-/-}$ mice than their WT littermates. Intraperitoneal KS administration effectively suppressed arthritis induction in GlcNAc6ST-1$^{-/-}$ mice. Thus, GlcNAc6ST-1$^{-/-}$ mice cartilage is more fragile than WT mice cartilage, and exogenous KS can suppress arthritis induction in GlcNAc6ST-1$^{-/-}$ mice. Vestigial KS chain or altered glycosylation in articular cartilage in GlcNAc6ST-1$^{-/-}$ mice may be protective against arthritis and associated cartilage damage as well as cartilage damage in culture. KS may offer therapeutic opportunities for chondroprotection and suppression of joint damage in inflammatory arthritis and may become a therapeutic agent for treating rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) causes joint destruction, especially to the articular cartilages, and negatively affects the quality of life [1]. Articular cartilage is composed of chondrocytes embedded in an extensive extracellular matrix (ECM), which provides the biomechanical properties essential for joint motion, enabling the dissipation of load of compressive forces. Type II collagen and proteoglycan aggrecan are responsible components of ECM for the presence of these biomechanical properties. Aggrecan is composed of two types of glycosaminoglycan (GAG) side chains in human hyaline cartilages, namely chondroitin sulfate (CS) and keratan sulfate (KS). The GAG chains contain carboxyl and sulfate residues and are thus negatively charged. The negative charge enables water retention in the ECM and provides resistance to compressive forces. Because most contribution for the ability to bind water comes from CS, the role of the shorter, less charged KS chains remains unclear.

KS is composed of repeating disaccharide units of galactose and *N*-acetylglucosamine (GlcNAc), and the C6 position of the GlcNAc is always sulfated. The reaction sequence for KS biosynthesis consists of *N*-acetylglucosaminylation, 6-sulfation of a GlcNAc residue exposed at the nonreducing end, and galactosylation [2,3]. The GlcNAc sulfation at the C6 position is crucial for the elongation of the KS chain without which KS synthesis fails to occur. In humans, deficiency in *N*-acetylglucosamine 6-*O*-sulfotransferase-5 (GlcNAc6ST-5) leads to the loss of corneal KS synthesis [4], and in GlcNAc6ST-1-deficient mice, the lack causes the loss of 5D4-reactive KS expression in the central nervous system [5]. This suggests that KS deficiency occurs in these sites as a result of the absence of this transferase. Whether this also happens in the murine articular cartilages of enzyme-deficient mice is not known.

Abbreviations: KS, keratan sulfate; CS, chondroitin sulfate; GAG, glycosaminoglycan; GlcNAc6ST, *N*-acetylglucosamine 6-*O*-sulfotransferase.

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Unlike the clusters of CS stubs created from the depletion of CS side chains which provoke a strong B-cell response, the presence of KS side chains in adult proteoglycans inhibits the recognition of arthritogenic T-cell epitopes, thus preventing the development of a T-cell response, and protecting the animal from arthritis [6]. But the KS side chains found in humans and other mammals are not found in rodent tissues [7], and are only discussed in the costal cartilages and lumbar disks of rats and mice [8].

We previously showed that KS suppresses cartilage damage and ameliorates inflammation in mice arthritis model [9]. Because mice deficient in the enzyme GlcNAc6ST-1 are now available for study, we decided to examine the effects of any KS deficiency on articular cartilage damage *in vitro* and *in vivo*.

2. Materials and methods

2.1. Mice

DBA/1J mice, widely used in various arthritis models, were purchased from Japan SLC (Shizuoka, Japan). GlcNAc6ST-1$^{-/-}$ DBA/1J mice were obtained using a previously described method [10]. Briefly, heterozygous mutant C57BL/6 mice (GlcNAc6ST-1$^{-/+}$) were backcrossed with DBA/1J mice for 7–8 generations. The offspring were then inbred to obtain WT and GlcNAc6ST-1$^{-/-}$ mice, which were then used for subsequent experiments. We analyzed the genotyping, as previously described [10]. All mice were maintained with sterilized food, water, and bedding at the Animal Facility of Nagoya University School of Medicine (Nagoya, Japan). All experiments were approved by the Animal Ethics Committee of Nagoya University.

2.2. Culture of ex vivo murine cartilage explants

Femoral hip articular cartilage explants were isolated from WT and transferase-deficient mice as previously described [11]. Femoral head cartilage explants were harvested from 3–4-week-old mice and preincubated in 1 ml Dulbecco's modified Eagle's medium (DMEM), containing 10% fetal bovine serum per well in 12-well test plates (TPP, Trasadingen, Switzerland), at 37 °C in 5% CO_2 in air for 48 h. Explants were then washed and cultured for an additional 72 h in serum-free DMEM with 10 ng/ml mouse interleukin-1α (IL-1α) (R&D Systems, Minneapolis, MN) under the same conditions. Media and residual cartilages were then collected.

2.3. Measurement of GAG content and release from explants of WT mice compared with GlcNAc6ST-1$^{-/-}$ mice in the presence of IL-1α

To examine the differences between GlcNAc6ST-1$^{-/-}$ and WT mice in the IL-1α–induced degradation of aggrecan, cartilage explants were examined 72 h after incubation (n = 13 mice/group) in 12-well test plates (TPP), with 1 cartilage explant/well in the presence of IL-1α at 37 °C in 5% CO_2 in air. After the supernatants were harvested, the explants were completely digested with proteinase K (Sigma, St. Louis, MO), pulsed in 1 ml of DMEM, and centrifuged [11]. The proteoglycan content in the media and the digested cartilage supernatant containing sulfated GAG were measured using a colorimetric assay kit utilizing dimethylmethylene blue (Seikagaku, Tokyo, Japan). Results were expressed as the percentage of GAG release relative to the total GAG in the media and digested cartilage. All analyses were performed in duplicate.

2.4. Mouse arthritis model and arthritis score

In the arthritis challenges, we used 6–7-week-old DBA/1J mice. On day 0, each mouse received in the tail vein an intravenous injection of 2 mg of arthritogenic monoclonal antibody cocktail (Iwai Chemicals, Tokyo, Japan) containing anti-type II collagen antibodies [12]. On day 3, 100 μl of 500 μg/ml lipopolysaccharide (packed with the arthritogenic monoclonal antibody cocktail) in phosphate-buffered saline (PBS) was administered intraperitoneally. First, we examined the two littermate genotypes during this time course, then GlcNAc6ST-1$^{-/-}$ mice received a daily intraperitoneal injection of either 40 μg of KS (from bovine cornea; Seikagaku) in 40 μl PBS or 40 μl PBS only (as a control). The severity of arthritis was graded clinically on a scale of 0–3 as follows: grade 0, normal; grade 1, swelling of 1 digit; grade 2, swelling of 2 or more digits; and grade 3, swelling of the entire paw [9,13]. Values obtained for the four limbs were added. Grading was performed blinded to treatment; body weight was monitored daily. The maximum loss of body weight was calculated for each mouse as a percentage of change from baseline.

2.5. Histologic analysis

Mice were anesthetized and then sacrificed by cervical dislocation on day 7 (n = 6/group). All 4 paws were removed, fixed for 7 days in 10% formalin solution (Wako Pure Chemicals, Tokyo, Japan), and decalcified with 0.5 mol/l ethylenediaminetetraacetic acid. After dehydration, the tissues were embedded in paraffin and cut into 3 μm sagittal sections. Serial sections were mounted on slides, dried overnight, and stored in an airtight container. Sections were stained with hematoxylin and eosin and labeled before examination. We evaluated the erosion of the cartilage and bone as well as infiltration of inflammatory cells for each mouse. All sections from each mouse were graded separately by an observer blinded to treatment on a scale of 0–4, with the highest total score being 16/mouse: grade 0, normal; grade 1, synovial hypertrophy; grade 2, pannus, erosion of cartilage; grade 3, erosion of bone; and grade 4, complete ankylosis [14].

2.6. Statistical analysis

All data are presented as mean ± SEM. Between-group differences were determined using the Student's t-test, and multiple treatment groups were compared within individual experiments by analysis of variance. P values <0.05 were considered statistically significant.

3. Results

3.1. Increased release of GAG induced by IL-1α from articular cartilage explants of GlcNAc6ST-1$^{-/-}$ mice compared with that from their WT littermates

The percentage of GAG release from the cartilage explants of GlcNAc6ST-1$^{-/-}$ mice exposed to IL-1α was higher than that of their WT littermates statistically (n = 13/group) (46.5 ± 3.01 vs. 32.9 ± 1.01) (Fig. 1).

3.2. Antibody-induced arthritis was exacerbated in GlcNAc6ST-1$^{-/-}$ mice compared with their WT littermates

Arthritis scores increased in a time-dependent manner in both the mice genotypes after administration of the antibody cocktail (n = 10/group) (Fig. 2A). The scores peaked around day 8–10 and remained high. Body weight of all mice of both genotypes decreased, showed its lowest value around day 6–7 and then recovered subsequently (n = 10/group) (Fig. 2B). Maximum body weight loss (percentage compared to baseline on day 0) was determined for each mouse, and the mean ± SEM (n = 10/group) weight

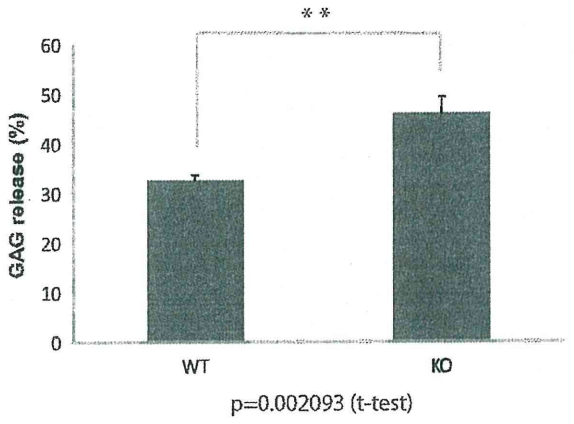

Fig. 1. Total sulfated GAG release from cultured femoral head cartilage. The percentage of GAG release from the cartilage explants of GlcNAc6ST-1$^{-/-}$ exposed to IL-1α was higher than that of their WT littermates (n = 13/group).

loss was significantly greater in GlcNAc6ST-1$^{-/-}$ mice than in their WT littermates (34.31 ± 2.29 vs. 25.22 ± 2.59) (Fig. 2C). Histologic analysis revealed that cartilage degradation was more severe in GlcNAc6ST-1$^{-/-}$ mice compared with their littermate WT mice (9.89 ± 0.47 vs. 5.10 ± 0.70) (Fig. 2D). Neutrophils were the predominant infiltrating inflammatory cells observed (Fig. 2E) [13] with comparable neutrophil infiltration seen between the two genotypes. We observed extensive neutrophil infiltration without lymphocyte infiltration on the slides from both genotypes on day 7 and day 14 after antibody challenge [15].

3.3. Suppression of antibody-induced arthritis by KS administration in GlcNAc6ST-1$^{-/-}$ mice

Finally, we assessed the potential therapeutic effect of KS on arthritis in GlcNAc6ST-1$^{-/-}$ mice. Arthritis scores increased in a time-dependent manner in mice with and without KS administration after antibody administration (Fig. 3A). The scores peaked around day 7–10 and remained high. The scores were significantly higher in the control mice not administered KS injections. Body weight decreased in all mice and subsequently recovered, with the lowest value obtained around day 5–7 (n = 10/group) (Fig. 3B). The maximum body weight loss was determined for each mouse, and the mean weight loss ± SEM (n = 10/group) was greater for mice not administered KS than for mice administered KS

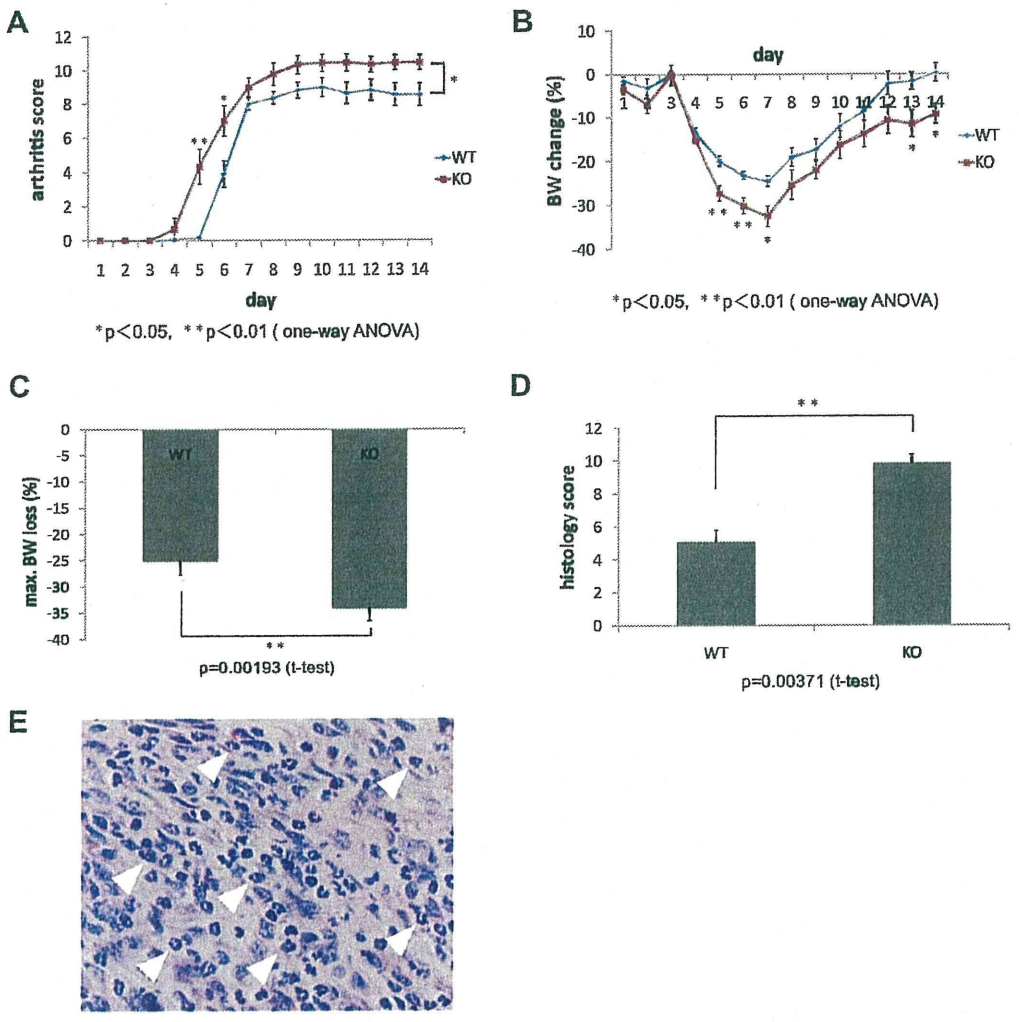

Fig. 2. Greater severity of arthritis seen in GlcNAc6ST-1$^{-/-}$ mice than in their WT littermates. (A) Time course of the arthritis development in GlcNAc6ST-1$^{-/-}$ mice and their WT littermate mice (n = 10/group). (B) Time course for the change in body weight (percentage compared to baseline on day 0; n = 10/group). (C) Maximum body weight loss (n = 10/group). (D) Histology scores on day 7 (n = 6/group). (E) Representative slide from day 14, showing a massive infiltration of neutrophils into the synovial tissues. The arrowheads indicate neutrophils.