for RA [23], and were treated with tocilizumab (8 mg/kg, every 4 weeks) preoperatively and postoperatively from 1999 to 2010, including phase III cases.

The timing of surgery for patients treated with tocilizumab was performed in accordance with the Japan College of Rheumatology (JCR) 2009 guidelines for use of tocilizumab. According to these guidelines, it is advisable that surgery be postponed until at least 14 days after the last tocilizumab infusion [24]. However, tocilizumab treatment was actually withheld for some weeks prior to surgery, and then restarted after reunion of the skin incision and confirmation of a lack of SSIs after surgery. The duration of withholding tocilizumab therapy was decided in each institute, and intermittent therapy from the last infusion until the restart of tocilizumab was also analysed in this study.

The diagnosis of SSIs was accomplished using the criteria for SSI definition in the Guideline for Prevention of Surgical Site Infection, 1999 [25]. The guide presents recommendations from the Centers for Disease Control and Prevention (CDC) for SSI prevention, comprising diagnosis of superficial or deep incisional SSIs by the operators, and intravenous or oral administration of antibiotics in all such cases.

It has also been suggested that flare-ups develop when administration of tocilizumab is interrupted during the perioperative period. Therefore, in this study, reappearance of arthralgia and swollen joints after surgery was defined as flare-up using subjective patient assessments by the attending doctors. It was considered that serological markers were not suitable for measuring disease activity during the perioperative period.

In this study, cases in which sutures could not be removed within 2 weeks after surgery or in which resuturing was needed were included in delayed wound healing.

To evaluate the clinical features before and after surgery, the CRP level, body temperature, white blood cell (WBC) count, neutrophil count and lymphocyte count were determined at the final infusion of tocilizumab (T-1), on the day before the operation (T-2), within a few days after the operation (T-3), between day 4 after surgery and immediately before the restart (T-4) and at the restart of tocilizumab (T-5).

Among the 161 patients, 67 had normal CRP levels at the final infusion of tocilizumab (T-1) and immediately before surgery (T-2) without complications such as postoperative infections and delayed wound healing. To further evaluate the clinical features during the perioperative period in cases in which tocilizumab was effective before surgery, changes in the postoperative CRP level and body temperature in the 67 cases were determined at each time point.

The TOPP study was a retrospective observational study using anonymized information, and conformed to the standard tocilizumab treatment proposed by the JCR. Written consent was obtained from the patients according to the Declaration of Helsinki. However, although the data had linking capability to the patients, all patients were anonymous and not identified, and the data never created disadvantages for the patients.

Statistical analysis

The Pearson chi-square test (for categorical variables) and Mann–Whitney *U* test (for continuous variables) were used to analyse flare-up development and delayed wound healing during the perioperative period. Multivariate logistic regression analysis was performed to test the association of delayed wound healing and flare-up with the putative risk factors [prior use of biologic agents, use of prednisolone, foot operation, spinal operation (for delayed wound healing analysis), and prior use of TNF inhibitors, days from final infusion of tocilizumab to operation, number of infusions before operation (for flare-up analysis)]. Values of $P < 0.05$ were considered statistically significant. All analyses were performed using the statistical package JMP version 9 (SAS Institute Japan, Tokyo, Japan).

Results

Postoperative events

A total of 161 case cards were collected. The major characteristics of the patients in the 161 cases were mean age of 56.9 years, mean duration of illness of 12.8 years, and 89 cases of joint replacement surgeries (Table 1). The duration of interruption of tocilizumab before surgery is specified in the guidelines, but there is no clear evidence for the duration. Therefore, the frequency of infusion of tocilizumab before surgery, duration from final infusion to surgery and duration from surgery to reinfusion were evaluated. The total number of tocilizumab infusions at the time of operation were 22.8 ± 26.6, 13 (median), and 1, 29 (min, max). The period from the final infusion of tocilizumab to the operation (days) was 23.5 ± 12.7, 21 (median), and 1, 71 (min, max). The period from the operation until reinfusion of tocilizumab (days) was 24.8 ± 12.7, 22.5 (median), and 0, 76 (min, max).

First, the cases with SSIs were evaluated. Only three cases with postoperative SSIs were identified among the total 161 surgeries, comprising two superficial incisional SSIs requiring use of antibiotics and one organ/space SSI requiring reoperation for removal of the hip prosthesis. In detail, one of two superficial SSIs was performed with the wrist fusion procedure infected by methicillin-sensitive

Table 1 Baseline characteristics of all patients

	Number	%	Mean	SD	Median	Min	Max
Total cases	161	–	–	–	–	–	–
Female	134	83.2	–	–	–	–	–
Age (years)	–	–	56.9	12.3	58	25	91
Weight (kg)	–	–	52.8	10.5	50.3	35	87.4
Disease duration (years)	–	–	12.8	8.5	11	1	44
Use of methotrexate	43	26.7					
Dosage of methotrexate (mg/week)	–		7.2	2.1	8	2	12.5
Use of prednisolone	120	74.5	–	–	–	–	–
Dosage of prednisolone (mg/day)	–		5.7	2.9	5	0.5	15
Prior use of biologic agents	88	54.7					
Infliximab	58						
Etanercept	57						
Adalimumab	5						
Others (ocrelizumab)	1						
Complications	74	46.0					
Diabetes mellitus	20						
Renal dysfunction	6						
Hypertension	22						
Respiratory disorder	12						
Cardiovascular disease	4						
Hepatic dysfunction	1						
Others	35						
Previous history	55	34.2	–	–	–	–	–
Infections	26						
Protracted wound healing	2						
Others	31						
Adverse events during tocilizumab therapy	11	6.8	–	–	–	–	–
Operations							
Total knee arthroplasty	40	24.8					
Total hip arthroplasty	35	21.7					
Total elbow arthroplasty	10	6.2					
Total ankle arthroplasty	3	1.9					
Total shoulder arthroplasty	1	0.6					
Spinal operations	4	2.5					
Others	68	42.2					

Staphylococcus aureus, and another was performed with total knee arthroplasty (TKA) infected by methicillin-resistant *Staphylococcus aureus* (MRSA). Moreover, one deep SSI occurred in a case performed with total hip arthroplasty (THA) infected by MRSA. In this deep infectious case, the total number of tocilizumab infusions at the time of operation were five, the period from the final infusion of tocilizumab to the operation was 27 days, and the period from the operation until reinfusion of tocilizumab was 16 days. A test comparing the groups with and without infection was not performed, because the number of patients with SSIs was too small.

Next, the cases with delayed wound healing were evaluated. Delayed wound healing was observed in 20 of the 161 cases (12.4 %) (Table 2). The number of cases with delayed wound healing was significantly greater after spinal surgery, and tended to be significantly greater after foot surgery and use of a steroid. No other factors led to a significant difference between the groups. Moreover, risk factors for delayed wound healing were analysed using a multiple logistic regression analysis, which identified use of prednisolone [$P = 0.046$, odds ratio (OR) = 5.49], foot operation ($P = 0.048$, OR = 3.07) and spinal operation ($P = 0.0002$, OR = 158.75) (Table 3).

Table 2 Baseline characteristics of patients with and without delayed wound healing

	Delayed wound healing (+), $n = 20$	Delayed wound healing (−), $n = 141$	P
Female, n (%)	16 (80)	118 (83.7)	0.680
Prior use of biologic agents, n (%)	8 (40)	80 (56.7)	0.126
Infliximab	6	52	–
Etanercept	4	53	–
Adalimumab	0	5	–
Age (years), mean (SD)	57 (12.3)	56.9 (12.4)	0.555
Median (min–max)	57 (36–91)	59 (25–79)	–
Weight (kg), mean (SD)	53.1 (10.9)	52.8 (10.5)	0.992
Median (min–max)	50 (40–87.4)	50.7 (35–87.4)	–
Disease duration (years), mean (SD)	12.8 (9.4)	12.8 (8.4)	0.802
Median (min–max)	9.5 (4–37)	11 (1–44)	–
Use of methotrexate, n (%)	6 (30)	37 (26.2)	0.736
Dosage of methotrexate (mg/week), mean (SD)	7 (2.1)	7.3 (2.2)	0.784
Median (min–max)	7 (4–10)	8 (2–12.5)	–
Use of prednisolone, n (%)	17 (85)	103 (73.0)	0.251
Dosage of prednisolone (mg/day), mean (SD)	7 (3.5)	5.5 (2.8)	0.099
Median (min–max)	6 (2–12.0)	5 (0.5–15)	–
Number of infusions before operation, mean (SD)	21 (22.9)	23 (27.2)	0.996
Median (min–max)	14 (2–98)	13 (1–129)	–
Days from final infusion of tocilizumab to operation, mean (SD)	25 (17.7)	23.3 (11.8)	0.802
Median (min–max)	21 (7–71)	21 (1–63)	–
Operations			
Total knee arthroplasty, n (%)	4 (20)	36 (25.5)	0.7838
Total hip arthroplasty, n (%)	5 (25)	30 (21.3)	0.7696
Foot, n (%)	8 (40)	32 (22.7)	0.089
Spinal operation, n (%)	3 (15)	1 (0.7)	0.0061

Table 3 Results of multiple logistic regression analysis examining factors related to delayed wound healing

Variables	Odds ratio	95 % confidence interval	P value
Prior use of biologic agents	0.490	0.154–1.461	0.2009
Use of prednisolone	5.494	1.026–101.926	0.0459
Foot operation	3.066	1.009–9.327	0.0483
Spinal operation	158.750	10.521–6993.007	0.0002

A rest period during the perioperative period may cause flare-ups of RA disease activity. Flare-ups were observed in 36 of the 161 cases (22.4 %) (Table 4), and there were significant differences between the groups with and without flare-ups in terms of use of prior TNF inhibitors in the pretreatment period and duration from final infusion to surgery. Multivariate logistic regression analysis of the risk factors that could have influenced flare-ups also revealed that prior use of TNF inhibitors agents ($P = 0.042$, OR = 2.88) and days from final infusion of tocilizumab to operation ($P = 0.0015$, OR = 1.03) were flare-up risk factors (Table 5).

A comparison of CRP levels at the restart of tocilizumab revealed a significant difference between the groups with and without flare-ups (3.5 ± 4.7 versus 0.9 ± 1.7 mg/dl; $P = 0.0010$) (Fig. 1), which was considered to verify that the diagnosis of flare-ups based on pain complaints by patients and physicians' determination was correct.

The perioperative clinical features of WBC count, neutrophil count and lymphocyte count were evaluated. The time-dependent changes of WBC count, neutrophil count and lymphocyte count before and after surgery are presented in Table 6.

Normal CRP levels group without events before surgery

The changes in CRP levels were evaluated in 67 cases in which the patients had normal range of CRP at T-1 and T-2 and did not have infection or delayed wound healing. To take into account the effects of rest periods in clinical practice, the cases were divided into the following three groups relative to the final infusion: group with surgery within 14 days (W-1), group with surgery between 15 and 28 days (W-2) and group with surgery after 29 days (W-3). The CRP levels at the time of final infusion and the day before surgery were within the normal range in all cases. The CRP levels were also within the normal range at T-3 and T-4 after surgery in many of the cases in the W-1 and W-2 groups. Moreover, almost none of the cases showed an increase in CRP, either transient or persistent, compared with that in the usual perioperative period, except in a few cases, mainly in the W-2 group, with a slight increase in CRP at T-5 (Fig. 2a). However, in some of cases in the W-3 group, in which the rest period was 29 days or more, CRP levels were increased at T-3 as with usual surgical pattern without tocilizumab treatment, and the CRP levels at T-4 and T-5 were higher than those immediately after surgery in more cases.

In all groups, body temperature rose to just around 37 °C at T-3, different from the usual pattern, but a shorter duration from final infusion to surgery as in W-1 group led to a more modest rise in temperature. Only a few cases

Table 4 Baseline characteristics of patients with and without flare-ups during discontinuation of tocilizumab

	Flare-up (+), $n = 36$	Flare-up (−), $n = 125$	P
Female, n (%)	29 (80.6)	105 (84.0)	0.626
Prior use of biologic agents, n (%)	27 (75)	69 (55.2)	0.041
Infliximab	17	41	
Etanercept	14	43	
Adalimumab	0	5	
Age (years), mean (SD)	55.1 (13.4)	57.5 (12.0)	0.473
Median (min–max)	57 (25–73)	59 (26–91)	
Weight (kg), mean (SD)	54.5 (13.1)	52.3 (9.7)	0.998
Median (min–max)	50 (39.9–87.4)	51 (35–84)	
Female, n (%)	12.5 (7.3)	12.9 (8.9)	0.805
Median (min–max)	10 (3–36)	11 (1–44)	
Use of methotrexate, n (%)	10 (27.8)	33 (26.4)	0.890
Dosage of methotrexate (mg/week), mean (SD)	7.6 (2.1)	7.1 (2.2)	0.509
Median (min–max)	8 (4–10)	8 (2–12.5)	
Use of prednisolone, n (%)	28 (77.8)	92 (73.6)	0.612
Dosage of prednisolone (mg/day), mean (SD)	6.1 (3.1)	5.6 (2.9)	0.499
Median (min–max)	5 (1–12)	5 (0.5–15)	
Number of infusions before operation, mean (SD)	19.9 (19.4)	23.6 (28.4)	0.277
Median (min–max)	16.5 (4–98)	12 (1–129)	
Days from final infusion of tocilizumab to operation, mean (SD)	30 (15.3)	21.6 (11.1)	0.002
Median (min–max)	26.5 (8–71)	19.5 (1–63)	

Table 5 Results of multiple logistic regression analysis examining factors related to flare-ups

Variables	Odds ratio	95 % confidence interval	P value
Prior use of TNF inhibitors	2.875	1.037–9.076	0.0420
Days from final infusion of tocilizumab to operation	1.033	1.013–1.055	0.0015
Number of infusions before operation	1.009	0.988–1.029	0.3940

showed a rise in temperature that was comparable to those in common cases after surgery (Fig. 2b).

Discussion

Since current clinical practice is to treat almost all RA patients with non-biological and/or biological DMARDs, the perioperative condition of RA patients must be carefully observed. In particular, biological DMARDs are associated with the possibility of increased susceptibility to infection, and it is therefore important to clarify the critical information as to whether a large proportion of RA patients who undergo orthopaedic surgery are at increased risk of SSIs.

In this study, we aim to clarify and analyse perioperative clinical features and complications after orthopaedic surgery in RA patients treated with tocilizumab. As described earlier, use of tocilizumab has succeeded in achieving more effective suppression of RA disease activity compared with conventional DMARDs. However, based on previous data, it has remained unknown whether tocilizumab also suppresses both surgery- and infection-related acute-phase responses and thereby leads to possible difficulties in early diagnosis of postoperative SSIs owing to a lack of observed clinical signs and symptoms in patients treated with tocilizumab. Therefore, it is very important to understand the details of the transition of data on blood tests and body temperature after joint surgery in tocilizumab-treated RA patients. Since it is important to detect postoperative SSIs as soon as possible, body temperature, CRP level, and WBC as well as local findings are considered to be indicators of infection. In the present study, we examined postoperative complications and clinical changes in body temperature and blood CRP levels in RA patients treated with tocilizumab.

Postoperative infections in patients treated with tocilizumab were observed in three of 161 cases (1.9 %). Of these three cases, only one case (0.6 %) had deep infection that required removal of the prosthetic joint, whereas the

remaining two cases (1.2 %) were controlled by antibiotics. The percentage did not seem to be extraordinarily high in view of the infection rates associated with RA surgeries reported to date and infection rates associated with TNF inhibitors [26, 27]. Kawakami et al. [26] reported that 12.5 % of patients treated with TNF inhibitors had SSI. In another study, a retrospective study of total hip arthroplasty (THA) and total knee arthroplasty (TKA) in RA patients treated with non-biologic and biologic DMARDs was previously performed during a 5-year period, and 5.7 % developed a superficial incisional SSI requiring use of antibiotics and 0.7 % developed an organ/space SSI necessitating surgical treatment to remove the artificial joint prosthesis [27]. The relationships of the number of days from the final tocilizumab infusion to surgery with the frequency of administration or preoperative CRP level could not be determined, because infections only occurred in three cases. Regarding postoperative laboratory values other than CRP and body temperature, WBCs increased after surgery in patients with infection (data not shown), and it may therefore be useful to monitor for an increase in WBCs even if the WBC count is within the normal range. Otherwise, Nagamine et al. [28] reported that WBC count along with neutrophil and lymphocyte counts significantly decreased immediately after tocilizumab administration. Therefore, measurement of complete blood cell count perioperatively may be helpful to identify patients with infection. Further studies are necessary to establish the perioperative risk for infection in RA patients treated with tocilizumab.

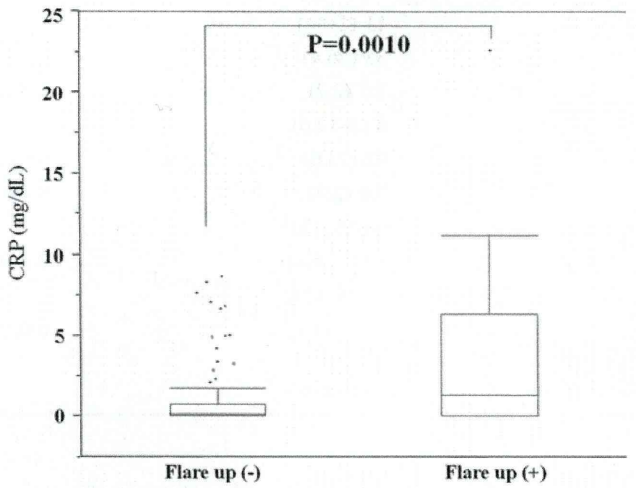

Fig. 1 CRP levels at restart of tocilizumab with and without flare-ups of RA symptoms

Many basic studies have reported that IL-6 plays a role in wound healing, and many investigators think that IL-6 modulates immune responses and is essential for timely wound healing [29–31]. In fact, skin wound healing is significantly delayed in IL-6 knockout mice [29, 31]. However, the effect of an anti-IL-6 receptor antibody on wound healing remains unknown in humans. In other words, the possibility of delayed wound healing after surgery during treatment with tocilizumab has been indicated. In the present study, delayed wound healing with tocilizumab treatment occurred in 20 of the 161 cases (12.4 %). Although this percentage seems slightly high,

Table 6 Perioperative changes in WBC count, neutrophil count and lymphocyte count

	Mean	SD	Median	Min	Max
White blood cell (/μl)					
T-1	6772.6	2680.3	6200	2400	14629
T-2	6301.8	2450.6	5600	2200	14700
T-3	7457.5	2497.7	7000	2860	14700
T-4	6272.8	2750.1	5705	1090	16300
T-5	6796.5	2737.6	6400	2100	16440
Neutrophil (%)					
T-1	67.57	13.11	68.0	32.4	95.0
T-2	65.78	14.41	68.0	10.1	94.5
T-3	72.24	11.98	74.1	35.8	92.7
T-4	63.03	12.85	61.2	28.2	86.5
T-5	65.82	14.45	66.8	29.7	95.0
Lymphocyte (%)					
T-1	23.15	10.74	21.5	3	53.9
T-2	23.67	10.82	22.3	2	54.8
T-3	19.67	9.85	18.0	4	50.5
T-4	25.37	10.47	24.4	5.4	54.3
T-5	24.09	12.08	21.8	2.5	60.0

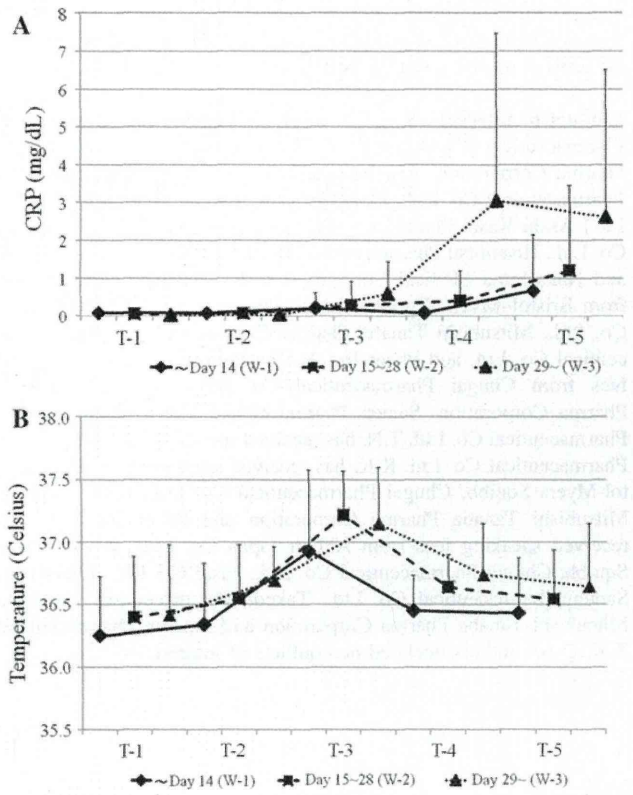

Fig. 2 Perioperative changes in CRP levels and body temperature. **a** Changes in CRP levels after joint surgery in patients with RA treated with perioperative interruption of tocilizumab. **b** Changes in febrile tendency after surgery in patients with RA treated with perioperative interruption of tocilizumab. *T-1* final infusion of tocilizumab, *T-2* day before operation, *T-3* within a few days after operation, *T-4* between day 4 after surgery and immediately before restart, *T-5* restart of tocilizumab. *Filled diamonds* group with surgery within 14 days (W-1); *filled squares* group with surgery between 15 and 28 days (W-2); *filled triangles* group with surgery after 29 days (W-3)

there were no associations between delayed wound healing and duration from final tocilizumab infusion to surgery, frequency of infusion before surgery or CRP level before surgery. The incidence of delayed wound healing was significantly higher in cases with surgical interventions such as foot and spinal surgeries, and it is unknown whether tocilizumab is directly related to the delay. On the other hand, it has been reported that surgical interventions such as foot surgery may be associated with high infection rates [13], indicating that the skin and subcutaneous tissues need to be sutured carefully in such surgical interventions.

Prolonged discontinuation of tocilizumab may lead to flare-ups of RA symptoms, primarily caused by decreases in the blood level of tocilizumab. In fact, we observed a significant difference between the groups with and without flare-ups only in terms of the duration from final infusion to surgery. In addition, CRP tended to increase in cases with flare-ups of RA symptoms. These findings suggest that flare-ups of RA symptoms should be noted after surgery, and if no problems have developed, it is desirable that tocilizumab be restarted immediately. Similarly, Kawakami et al. [26] reported that etanercept was more dependent on the rest period than infliximab.

Finally, the clinical features were evaluated. Previously published data showed that the cytokine IL-6 is upregulated by surgical trauma and involved in the febrile response [32]. The acute-phase response might be diminished by blocking the synthesis of IL-6 and has been reduced in genetically modified animals that do not produce this mediator [33, 34].

Hirao et al. [20] reported that the most recent infusion of tocilizumab was performed at 16.1 days (range 3–27 days) before surgery. Although the RA patients were treated with 4 weeks of perioperative interruption of tocilizumab, similar clinical results were reported by Hiroshima et al. [21]. These clinical manifestations should be considered in patients treated with tocilizumab when evaluating complications such as SSIs, even if withholding tocilizumab for 4 weeks prior to major surgical procedures. Therefore, the present study evaluated changes from baseline in CRP levels and body temperature in 67 cases in which tocilizumab was effective before surgery and no complications occurred. To investigate how these values were affected by the duration from final tocilizumab infusion to surgery in practice, the cases were divided into the following three groups relative to the final infusion: group with surgery within 14 days (W-1), group with surgery between 15 and 28 days (W-2) and group with surgery after 29 days (W-3). The CRP levels hardly changed after surgery but were slightly increased at the time of reinfusion in some cases in the W-1 and W-2 groups. On the other hand, in the W-3 group that underwent surgery at 29 days or more after the final infusion, the CRP levels immediately after surgery were within the normal range in most cases, but began to increase at 4 days or more after surgery, suggesting that the drug may continue to be effective in preventing the postoperative CRP level from increasing for up to 28 days after the final infusion, even if the surgical procedure is very invasive.

Similarly, it was shown that fever temporarily rose immediately after surgery in all groups, did not exceed 37 °C in the W-1 group, and rose to about 37 °C in most cases in the W-2 and W-3 groups.

In addition, IL-6 positively regulates the numbers of leucocytes and neutrophils, and inhibition of the IL-6 receptor leads to a decrease in leucocytes [9, 10]. In the present study, the WBC, neutrophil and lymphocyte counts were determined, and there were no significant changes in these values during the perioperative period (data not shown).

The above findings suggest that there are characteristic clinical features in the perioperative period in patients

treated with tocilizumab. In particular, the CRP level, body temperature and WBC count hardly changed after surgery in the patients who underwent surgery within 28 days of the final infusion. Careful observation of patients based on local findings and complaints may be necessary, instead of using these values as indicators of complications such as infections.

In this multicentre study, only patients treated with tocilizumab were enrolled, which resulted in a lack of controls in the study. Owing to this limitation, it was difficult to make comparisons with other drugs. However, according to other reports on TNF inhibitors etc. and clinical experience to date, the adverse reactions to tocilizumab and its clinical features during the perioperative period are very characteristic. Therefore, all the doctors who participated in this study believe that the conclusions drawn from the study are correct.

Another limitation was that the regimens of tocilizumab, such as rest periods during the perioperative period, varied from hospital to hospital because of the nature of a multicentre, retrospective study. However, use of antibiotics during the perioperative period is standardized in accordance with the recommendations by the Japanese Orthopaedic Association, and the unintentional variability in the duration from final infusion to surgery permitted a comparison of different conditions during the perioperative period.

In summary, it has been demonstrated that the infection rates in patients treated with tocilizumab are by no means high, suggesting that the rest period before surgery does not need to be very long and can be as described in the relevant guidelines. Of course, it is desirable that the rest period be sufficiently long for patients with complications and elderly patients. To reduce the risk of delayed wound healing, wounds should be sutured very carefully, especially during spine and foot surgeries, and in patients treated with high doses of steroids. If the duration from final infusion to restart is too long, flare-ups of symptoms may occur. For patients in good condition after surgery, it seems appropriate to restart tocilizumab as soon as their wounds heal. It should also be noted that, unlike with other conventional DMARDs or TNF blockers, the incidences of postoperative CRP increases and fever do not rise significantly in most cases even after major surgery, especially when the duration from final infusion to surgery is short. It is important to effectively use tocilizumab and successfully perform surgery, by understanding that some patients may have normal CRP and may not have fever even immediately after major surgery.

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Conflict of interest S.M. has received speaking fees from Chugai Pharmaceutical Co. Ltd., Eisai Co. Ltd. and Mitsubishi Tanabe Pharma Corporation. J.H. has received speaking fees from Chugai Pharmaceutical Co. Ltd., Eisai Co. Ltd., Takeda Pharmaceutical Co. Ltd., Asahi Kasei Pharma Co. Ltd., Taisho Toyama Pharmaceutical Co. Ltd., Hisamitsu Pharmaceutical Co. Ltd., Eli Lilly Japan Co. Ltd. and Nakashima Medical Co. Ltd. A.K. has received speaking fees from Bristol–Myers Squibb, Chugai Pharmaceutical Co. Ltd., Eisai Co. Ltd., Mitsubishi Tanabe Pharma Corporation, Takeda Pharmaceutical Co. Ltd. and Pfizer Inc. H. Matsuno has received speaking fees from Chugai Pharmaceutical Co. Ltd., Mitsubishi Tanabe Pharma Corporation, Santen Pharmaceutical Co. Ltd. and Takeda Pharmaceutical Co. Ltd. T.N. has received speaking fees from Chugai Pharmaceutical Co. Ltd. K.K. has received speaking fees from Bristol-Myers Squibb, Chugai Pharmaceutical Co. Ltd., Eisai Co. Ltd., Mitsubishi Tanabe Pharma Corporation and Pfizer Inc. T.S. has received speaking fees from Abbott Japan Co. Ltd., Bristol-Myers Squibb, Chugai Pharmaceutical Co. Ltd., Eisai Co. Ltd., Pfizer Inc., Santen Pharmaceutical Co. Ltd., Takeda Pharmaceutical Co. Ltd., Mitsubishi Tanabe Pharma Corporation and Janssen Pharmaceutical K.K. Other authors declared no conflicts of interest.

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PADI4 and HLA-DRB1 Are Genetic Risks for Radiographic Progression in RA Patients, Independent of ACPA Status: Results from the IORRA Cohort Study

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Abstract

Introduction: Rheumatoid arthritis (RA) is a systemic, chronic inflammatory disease influenced by both genetic and environmental factors, leading to joint destruction and functional impairment. Recently, a large-scaled GWAS meta-analysis using more than 37,000 Japanese samples were conducted and 13 RA susceptibility loci were identified. However, it is not clear whether these loci have significant impact on joint destruction or not. This is the first study focused on the 13 loci to investigate independent genetic risk factors for radiographic progression in the first five years from onset of RA.

Methods: Sharp/van der Heijde score of hands at 5-year disease duration, which represents joint damage, were measured retrospectively and used as an outcome variable in 865 Japanese RA patients. Genetic factors regarded as putative risk factors were RA-susceptible polymorphisms identified by the Japanese GWAS meta-analysis, including HLA-DRB1 (shared epitope, SE), rs2240340 (*PADI4*), rs2230926 (*TNFAIP3*), rs3093024 (*CCR6*), rs11900673 (*B3GNT2*), rs2867461 (*ANXA3*), rs657075 (*CSF2*), rs12529514 (*CD83*), rs2233434 (*NFKBIE*), rs10821944 (*ARID5B*), rs3781913 (*PDE2A-ARAP1*), rs2841277 (*PLD4*) and rs2847297 (*PTPN2*). These putative genetic risk factors were assessed by a stepwise multiple regression analysis adjusted for possible non-genetic risk factors: autoantibody positivity (anti-citrullinated peptide antibody [ACPA] and rheumatoid factor), history of smoking, gender and age at disease onset.

Results: The number of SE alleles ($P = 0.002$) and risk alleles of peptidyl arginine deiminase type IV gene (*PADI4*, $P = 0.04$) had significant impact on progressive joint destruction, as well as following non-genetic factors: ACPA positive ($P = 0.0006$), female sex ($P = 0.006$) and younger age of onset ($P = 0.02$).

Conclusions: In the present study, we found that *PADI4* risk allele and HLA-DRB1 shared epitope are independent genetic risks for radiographic progression in Japanese rheumatoid arthritis patients. The results of this study give important knowledge of the risks on progressive joint damage in RA patients.

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Introduction

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by the chronic synovitis and the localized destruction of cartilage and bone resulting in deteriorated physical function and reduced quality of life. It has been recognized that early therapeutic intervention can prevent progress of joint damage and provide long-term benefits to the patients of RA. The therapeutic recommendations for the management of RA indicate patients may use non-biologic and/or biologic disease-modifying anti-rheumatic drugs (DMARDs) in consideration of the presence of poor prognostic factors.[1–3].

To date, prognostic markers of joint damage have been studied extensively and reported; anti-cyclic citrullinated peptides antibody (ACPA) positive,[4–7] rheumatoid factor (RF) positive, [6,7] the history of smoking, [8,9] the high level of disease activity measured using composite measures,[10–12] gender [4,13] and the age of disease onset.[13–15].

Since RA is a complex disease influenced by both genetic and environmental factors, susceptibility genes to the disease have been widely investigated and identified, especially in the era of genome-wide association studies (GWAS) and GWAS meta-analyses.[16–18] Recently, a large-scaled GWAS meta-analysis was conducted using samples from more than 9,000 Japanese RA patients and 38,000 controls. As a result, nine novel RA susceptibility loci were identified; *B3GNT2*, *ANXA3*, *CSF2*, *CD83*, *NFKBIE*, *ARID5B*, *PDE2A-ARAP1*, *PLD4* and *PTPN2*. [16] The study also showed that some previously reported RA susceptibility genes satisfied the genome-wide significance threshold ($P<5.0 \times 10^{-8}$); HLA-DRB1, *PADI4*, *TNFAIP3* and *CCR6*. [16] Of these 13 RA-susceptible loci, HLA-DRB1 shared epitope (SE) have been reported to have impact on disease severity.[19–21] However, the question remains whether if the other RA-susceptible genes have significant impact on joint destruction.

The purpose of this study is to explore genetic risk factors associated with radiographic progression in RA patients.

Methods

Patients and Evaluation of Radiographic Joint Damage

Tokyo Women's Medical University Genome Ethics Committee approved the present study and each individual signed an informed consent form after receiving a verbal explanation of the study. All the patients satisfied the American College of Rheumatology 1987 revised criteria for RA. [22] DNA samples from RA patients were obtained from the IORRA (Institute of Rheumatology Rheumatoid Arthritis cohort study) DNA collection. [16] IORRA is a project of observational RA cohort with an enrollment of over 5,000 Japanese RA patients, and DNA samples were collected from 2,068 patients. [23,24] All these DNA samples were included in the Japanese GWAS meta-analysis. [16].

Radiographic data at 5-year disease duration were collected retrospectively from the medical records of the patients. Of the patients who donated DNA samples, Sharp/van der Heijde score (SHS) of the hands representing radiographic joint damage (a higher score indicating more damage) was available in 865 patients who have not received biologic agents. [25] Proper anteroposterior radiographs of the hands were scored by a single experienced reader as described elsewhere. [26] Since it has been well known that the rate of radiologic progression develops rapidly in early disease course of RA, joint damage scores of the same disease duration, 5 years, were used. Interobserver and intraobserver agreements (0.85 and 0.95, respectively) indicated good reliability.

The reasons of the exclusion for the patients who treated with biologic agents were as follows: the apparent reported dissociation between clinical and radiologic outcomes in patients with RA who are treated with biologic agents, which could be a confounding factor for the study; [27] the year of RA onset for most patients in this study was before 2000 (70.2%), while the first biologic agent was not launched in the Japanese market until 2003, and the number of the patients who have ever used biologic agents in the first 5-year of disease duration was not sufficient for the sub-analysis targeted on biologic agents.

Assessment Measures, Non-genetic Factors

From the IORRA database and medical records of the patients, demographic, clinical, biological and therapeutic data during the first 5-year after onset of RA were collected, including ACPA status (ACPA titers were measured with second [MESACUP CCP test, Medical and biological laboratories] or third generation [QUANTA Lite CCP3 IgG ELISA, Inova Diagnostics] kit), [28] RF status (determined by a latex agglutination turbidimetric immunoassay method), history of smoking, gender and the age at onset. The age at onset was defined as the age at the onset of first symptoms, according to the patient's self-report, and it did not mean the age that satisfied the 1987 ACR criteria.

ACPA, RF, history of smoking and gender were categorized into two dichotomous variables: ACPA (positive [> = 4.5 IU/ml] = 1, negative = 0), RF (positive [> = 15.0 IU/ml] = 1, negative = 0; maximum value in the first 5 years was used), history of smoking (ever smoked = 1, never = 0) and gender (female = 1, male = 0). Data of age at onset was used as continuous variables.

Assessment Measures, Genetic Factors

HLA-DRB1 SE and twelve single nucleotide polymorphisms that have been reported as RA susceptibility polymorphisms using a large-scaled GWAS meta-analysis of Japanese were chosen for the study. [16] There were rs2240340 (*PADI4*, peptidyl arginine deiminase type IV), rs2230926 (*TNFAIP3*, tumor necrosis factor, alpha-induced protein 3), rs3093024 (*CCR6*, C-C chemokine receptor type 6), rs11900673 (*B3GNT2*, UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 2), rs2867461 (*ANXA3*, annexin A3), rs657075 (*CSF2*, colony stimulating factor 2), rs12529514 (*CD83*, CD83 molecule), rs2233434 (*NFKBIE*, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon), rs10821944 (*ARID5B*, AT rich interactive domain 5B [MRF1-like]), rs3781913 (*PDE2A-ARAP1*, *PDE2A*; phosphodiesterase 2A, cGMP-stimulated, *ARAP1*; ArfGAP with RhoGAP domain, ankyrin repeat and PH domain 1), rs2841277 (*PLD4*, phospholipase D family, member 4) and rs2847297 (*PTPN2*, protein tyrosine phosphatase, non-receptor type 2). The risk alleles were defined as the allele that increases the risk of RA based on a prior report. [16].

Genotyping

Duplicate samples and negative controls were included to ensure accuracy of genotyping. High-resolution polymerase chain reaction (PCR) based DNA typing of HLA-DRB1 locus was performed using the sequence-based typing method with the AlleleSEQR DRB1 typing kit (Abbott Japan), according to the manufacturer's instructions. Assignment of HLA-DRB1 alleles was performed using Assign software. HLA-DRB1 SE were defined as alleles encoding amino acid sequences of QKRAA/QRRAA/RRRAA in positions 70–74 of HLA-DRB1. Genotyping of non-HLA RA susceptibility single-nucleotide polymorphisms (SNPs) were performed using the TaqMan fluorogenic 5′ nuclease assay according to the manufacturer's instructions (Applied Biosystems,