Original Research

The Impact of Early Optimization of Infliximab Blood Concentrations >1 μg/mL on Therapeutic Effectiveness in Rheumatoid Arthritis

Yuji Nozaki[1,*], Takuya Kotani[2], Tohru Takeuchi[2], Toshihiko Hidaka[3], Hirofumi Miyake[4], Kazuhiro Hatta[4], Yoichi Kurosawa[5,6], Masanori Sudo[5,6], Satoshi Ito[6], Koji Kinoshita[1], Itaru Matsumura[1]

[1]Department of Hematology and Rheumatology, Kindai University Faculty of Medicine, Osaka-sayama, 589-8511 Osaka, Japan
[2]Department of Internal Medicine IV, Division of Rheumatology, Osaka Medical and Pharmaceutical University, Takatsuki, 569-8686 Osaka, Japan
[3]Institute of Rheumatology, Miyazaki Zenjinkai Hospital, 880-0834 Miyazaki, Japan
[4]Department of General Internal Medicine, Tenri Hospital, 632-8552 Nara, Japan
[5]Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, 951-8520 Niigata, Japan
[6]Department of Rheumatology, Niigata Rheumatic Center, 957-0054 Niigata, Japan
*Correspondence: yuji0516@med.kindai.ac.jp (Yuji Nozaki)
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Abstract

Background: Infliximab is a human-murine chimeric monoclonal IgG antibody against tumor necrosis factor that is used in combination with methotrexate for the treatment of moderate to severe rheumatoid arthritis (RA). The trough concentration of serum infliximab required to control disease activity in RA is ≥ 1 μg/mL, and we investigated whether this trough concentration can predict the effectiveness of RA treatment. **Methods**: We retrospectively analyzed the cases of 76 patients with RA. The REMICHECK Q® (REMIQ) is a kit that can check for serum infliximab concentrations. Infliximab concentrations >1 μg/mL at 14 weeks after an initial infliximab induction is considered REMIQ-positive, otherwise considered REMIQ-negative. Here, we determined the retention rates and investigated the clinical and serologic features of REMIQ-positive and REMIQ-negative patients. **Results**: At 14 weeks, significantly more of the REMIQ-positive patients (n = 46) were responders compared to the non-responders (n = 30). The retention rate at 54 weeks was also significantly higher in the REMIQ-positive group versus the negative group. After 14 weeks, more patients in the REMIQ-negative group were considered inadequate responders, and their infliximab doses were escalated. At baseline, the REMIQ-positive group had significantly lower C-reactive protein (CRP) levels compared to the negative group. Cox regression analysis with multiple variables showed that the positivity of REMIQ (hazard ratio [HR] 2.10 and 95% confidence interval [CI]: 1.55–5.71) at baseline was associated with the achievement of low disease activity. The positivities of rheumatoid factor and anti-CCP antibody at baseline were associated with the achievement of remission with infliximab treatment (HR 0.44, 95% CI: 0.09–0.82 and HR 0.35, 95% CI: 0.04–0.48, respectively). **Conclusions**: The results of this study suggest that the control of RA disease activity may be facilitated by using the REMIQ kit at 14 weeks to check whether it is necessary to increase a patient's infliximab dose to ensure a therapeutic blood concentration that will help the patient achieve low disease activity.

Keywords: rheumatoid arthritis; infliximab; blood concentration; therapeutic predictor

1. Introduction

Infliximab is a human-murine monoclonal IgG antibody against tumor necrosis factor (TNF) that is used in combination with methotrexate (MTX) for the treatment of moderate to severe rheumatoid arthritis (RA) in patients who have had an inadequate response to one or more conventional synthetic disease modifying anti-rheumatic drugs [1]. In a major multinational clinical trial, i.e., the Anti-TNF Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT), repeated doses of infliximab at 3 mg or 10 mg/kg reduced disease activity in RA better than MTX alone, reducing joint damage, and improving the patients' physical function [2,3]. In routine practice, it is well known that some RA patients require dose escalation from the initial dose of infliximab in order to control disease activity.

A dose-escalation investigation (the RISING study) that investigated the effect of infliximab + MTX on radiographic and clinical responses in RA patients classified based on their serum infliximab trough concentrations also showed a significant association between the serum infliximab trough concentration and the European League Against Rheumatism (EULAR) response and 28-joint DAS28 (Disease Activity Score) remission [4].

Regarding infliximab concentrations, the median serum trough concentration at 54 weeks of treatment among EULAR non-responders was reported to be <0.1 μg/mL, whereas that for EULAR responders (with a good or moderate response) was reported to be >1.1 μg/mL. This is consistent with values reported in the ATTRACT and START trials, suggesting that a serum trough concentration of 1.0

μg/mL is the threshold for a clinical response in RA [5,6].

The REMICHECK Q® (REMIQ) kit is used to determine whether an individual's infliximab serum concentration is ≥ 1.0 μg/mL, and the REMIQ kit has been covered by Japan health insurance program since October 1, 2017. The REMIQ kit had 0.96 sensitivity and 1.00 specificity for an infliximab serum concentration of ≥ 1 μg/mL when measured by an enzyme-linked immunosorbent assay [REMIQ package insert, LSI Medience Corp, Tokyo; 2016]. However, although the use of the REMIQ kit is now available in Japan, the optimal timing of its use and its potential as a predictor of therapeutic efficacy in RA are not yet known.

We conducted the present study to determine whether an infliximab blood concentration above or below 1 μg/mL could be a predictor of the therapeutic efficacy of infliximab treatment in RA. The REMIQ kit was used at 14 weeks after the initial induction of infliximab treatment, as this time point was the first 8-week interval of the patients' infliximab regimen.

2. Materials and Methods

2.1 Patients

This multicenter study was conducted at Kindai University Hospital, Osaka Medical and Pharmaceutical University, Zenjinkai Miyazaki Hospital, Tenri Hospital, and Niigata Rheumatic Center, all of which are in Japan's Niigata prefecture. We collected the clinical and laboratory data of the 76 RA patients for whom the REMIQ kit was applied at 14 weeks after their initial infliximab induction during the period 2015–2021.

To be eligible for the study, the patient's diagnosis of RA had to be based on the American College of Rheumatology (ACR) criteria [7] before 2009 and the 2010 ACR and EULAR classification criteria [8] after 2010. The patients were also screened for latent and active tuberculosis. Patients with other connective tissue disease with joint symptoms or had a dose escalation within 14 weeks of infliximab induction were excluded. The infliximab dose escalation protocol is to increase the dose from 3 mg/kg to 6 mg/kg and 10 mg/kg, with dosing intervals of 8 weeks after week 14.

All of the patients were treated with infliximab + MTX at a maximum dose of 4–12 mg/week. The dose assignments were determined by the patients' treating physicians. A patient's dose of prednisolone (PSL) could be decreased when treatment efficacy was observed. The follow-up period was 54 weeks, starting at the patient's initial dose of infliximab 3 mg/kg.

2.2 Demographic Characteristics and the Assessment of Clinical and Laboratory Data

Table 1 summarizes the patients' background. The group clinical data recorded at baseline and at weeks 14, 24, and 54 included the following: the 28 swollen joint count and tender joint counts (SJC28 and TJC28); a patient visual analogue scale for pain (Pt-VAS) in which 0 = best and 100

= worst; the Steinbrocker stage and class, and the Health Assessment Questionnaire Disability Index (HAQDI). We obtained the following laboratory data: the rheumatoid factor (RF) and anti-citrullinated protein/peptide antibody (ACPA) titers and positivity, the C-reactive protein (CRP) value, the erythrocyte sedimentation rate (ESR), and the metalloproteinase-3 (MMP-3) value. The DAS28 using the ESR (DAS28-ESR) and Clinical Disease Activity Index (CDAI) were obtained from the patient's clinical examination and calculated with/without laboratory data. With respect to the DAS28-ESR disease activity categories, we divided the patients into those at remission (DAS28-ESR <2.6) and those in non-remission (DAS28-ESR low: ≥ 2.6 to <3.2, moderate: $3.2–5.1$, high: >5.1). In the the REMIQ kit, an infliximab blood concentration of ≥ 1.0 μg/mL was defined as positive, and a concentration <1.0 μg/mL was defined as negative.

2.3 Clinical Assessment

The endpoint for clinical response was the DAS28-ESR in the REMIQ-positive (n = 46) and REMIQ-negative (n = 30) groups at baseline and 14, 24, and 54 weeks after the start of infliximab treatment. The EULAR response [9] was also evaluated at 14, 24, and 54 weeks. The treatment efficacy parameters included the proportion of patients with a complete set of data of the DAS28-ESR, CDAI, and TJC28 and SJC28 (both of which were determined by the patient's treating physician), the Pt-VAS, and the laboratory parameters of CRP (mg/dL), ESR (mm/hr), RF (U/mL), and MMP-3 (ng/mL) assessed at every visit during the follow-up period. The patient's physical function at baseline was evaluated based on his or her HAQDI score [9].

2.4 Safety

The treating physicians recorded adverse events (AEs) if any occurred and made treatment adjustments at his/her discretion. AEs were defined as any adverse reaction associated with the infliximab treatment.

2.5 Statistical Analyses

Summary statistics of the mean $\pm$ standard deviation (SD) or the median and interquartile range (IQR) are presented for continuous variables as appropriate. Categorical variables are presented as percentages. Comparisons between independent means were analyzed using the Mann-Whitney test and paired t-test. The retention rates of the REMIQ-positive and -negative groups were assessed by the Kaplan-Meier method, and the difference in retention curves was examined by log-rank test. Multivariate logistic regression was applied in a Cox regression analysis of the achievement of low disease activity and remission in order to calculate the hazard ratios (HRs) and 95% confidence intervals (95% CIs) including the REMIQ test result at 14 weeks, the CRP and ESR values as inflammatory biomark-

ers, and RF and ACPA as immunological tests at baseline [10].

A receiver-operator characteristic (ROC) curve was constructed using CRP and ESR at baseline and 14 weeks in both the positive- and negative-REMIQ patients. Sensitivity, specificity, and predictive values were calculated using ROC curves. The last observation carried forward (LOCF) method was applied to assess the patients who discontinued the infliximab therapy [11]. p-values < 0.05 were considered significant. Statistical analysis software (GraphPad Prism, GraphPad Software, San Diego, CA, USA) and JMP Statistical Software (SAS Institute, Cary, NC, USA) were used for the statistical analyses.

3. Results

3.1 Baseline Characteristics and Patient Disposition

Table 1 summarizes the baseline characteristics, clinical assessments, laboratory results, and doses and/or the percentage of MTX and PSL for all patients (n = 76) and the REMIQ- positive (n = 46) and -negative groups (n = 30) at 14 weeks after their initial infliximab induction. The disease duration in the REMIQ-negative group (54.0 months) tended to be longer than that in the REMIQ-positive group (12.0 months). At baseline, the REMIQ-positive group had significantly lower CRP levels than the REMIQ-negative group (**$p < 0.01$). The MTX doses also tended to be higher in the REMIQ-positive group (10.0 mg/week) compared to the -negative group (8.0 mg/week). The Steinbrocker class proportions were significantly different between the two groups (*$p < 0.05$).

3.2 Retention Rate

The RA patients' retention rates at 54 weeks are illustrated in Fig. 1. The overall retention rate at 54 weeks based on withdrawal from infliximab treatment for any reason (e.g., lack of efficacy, remission, at the patient's request due to economic problems or moving away from the area) or sustained remission is illustrated in Fig. 1A. The infliximab retention rates in the REMIQ- positive and -negative groups were 100.0% and 100.0% at 14 weeks, 100.0% and 90.0% at 24 weeks, 93.5% and 73.3% at 36 weeks, and 93.5% and 70.0% at 54 weeks, respectively. Thus, the overall retention rate at 54 weeks in the two groups was significantly higher for the REMIQ-positive group. Fig. 1B depicts the retention rate for infliximab 3 mg/kg at 54 weeks. The patients who received infliximab 3 mg/kg but whose dose was escalated by the treating physician's decision due to an inadequate response were considered withdrawals. The REMIQ-positive and -negative groups' retention rates for infliximab 3 mg/kg were 91.3% and 80.0% at 14 weeks, 87.0% and 26.7% at 24 weeks, 84.7% and 26.7% at 36 weeks, and 82.6% and 6.7% at 54 weeks ($p < 0.0005$), respectively. The details of the infliximab dose escalation procedure in the REMIQ-positive and -negative groups are provided in **Supplementary Figs. 1,2**.

Table 1. The 76 RA patients' baseline clinical and laboratory data and treatment information.

	All patients	REMIQ (+)	REMIQ (−)
	n = 76	n = 46	n = 30
Age, years	61.7 ± 10.4	61.5 ± 12.5	61.9 ± 7.8
Female, %	74.5	82.8	65.4
Disease duration, months	36.0 [8.0–73.0]	12.0 [6.5–63.0]	54.0 [17.0–90.0]
bDMARDs-naïve, %	84.6	90.0	83.3
RF, positive %	81.8	82.8	80.1
RF titer, IU/mL [IQR]	52.0 [20.0–112.0]	51.0 [20.2–112.2]	53.9 [17.3–118.8]
ACPA, %	81.5	79.3	84.0
ACPA titer, Al/mL [IQR]	86.1 [12.7–287.3]	86.4 [8.6–324.3]	81.9 [16.7–297.9]
CRP, mg/dL [IQR]	1.8 [0.3–4.7]	1.3 [0.2–3.9]	2.7** [0.8–5.7]
ESR, mm/hr [IQR]	46.4 ± 29.4	45.4 ± 29.8	45.8 ± 28.5
MMP-3, ng/mL [IQR]	281.9 [105.8–552.9]	250.6 [69.6–455.4]	297.5 [145.8–682.7]
Tender joints, range 0–28 [IQR]	4.0 [2.0–8.0]	4.0 [2.0–8.0]	3.0 [2.0–8.0]
Swollen joints, range 0–28 [IQR]	5.0 [3.0–10.0]	5.0 [3.0–8.0]	7.0 [3.0–10.0]
Pt VAS, 0–100 mm	52.9 ± 29.3	50.0 [30.0–72.5]	59.0 [27.0–87.5]
DAS28-ESR	5.1 ± 1.4	5.0 ± 1.1	5.2 ± 1.5
CDAI score	23.1 ± 15.0	20.8 ± 11.2	25.8 ± 17.6
Steinbrocker stage I/II/III/IV	43.6/27.2/23.6/0.0	44.8/24.1/24.1/6.9	46.2/30.8/19.2/3.9
Steinbrocker class I/II/III/IV	43.6/36.4/20.0/0.0	34.5/51.7/13.8/0.0	53.9/15.4/30.8/0.0*
HAQDI, range 0–3	0.9 [0.3–1.3]	0.7 [0.2–1.3]	1.1 [0.3–1.3]
MTX mg/week [IQR]	8.0 [8.0–10.0]	10.0 [8.0–10.0]	8.0 [6.0–10.0]
PSL, %, mg/day [IQR]	45.1, 0.0 [0.0–5.0]	41.3, 0.0 [0.0–5.0]	50.0, 2.5 [0.0–9.5]

Values are median [25th–75th centiles] or mean (SD), unless otherwise indicated. ACPA, anticitrullinated peptide antibody; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DAS, disease activity score; ESR, erythrocyte sedimentation rate; HAQDI, Health Assessment Questionnaire Disability Index; IQR, interquartile range; MMP-3, matrix metalloproteinase-3; MTX, methotrexate; PSL, prednisolone; Pt-VAS, patient visual analogue scale; RA, rheumatoid arthritis; bDMARDs, biologic disease-modifying antirheumatic drugs; RF, rheumatoid factor. *$p < 0.05$, **$p < 0.01$.
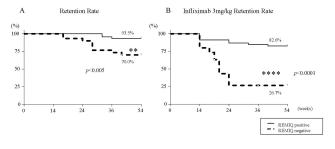

Fig. 1. Retention rates of the 76 patients with rheumatoid arthritis (RA). Kaplan-Meier curves for the REMIQ-positive (n = 46) and REMIQ-negative (n = 30) groups treated with infliximab 3, 6, and 12 mg/kg regarding the lenghts of time to continue or withdraw for any reason, e.g., due to lack of efficacy, remission, or the patient's request (A) and the length of time to continue at infliximab 3 mg/kg (B) from week 0 to week 54. **$p < 0.01$ and ****$p<0.0001$, REMIQ-positive group vs. negative.

3.3 Clinical Efficacy

We analyzed the changes from the two groups' baseline values in the clinical assessment. Fig. 2 illustrates the disease activity and clinical response at 14, 24, and 54 weeks: the DAS28-ESR score, the CDAI, the TJC28 and SJC28 values, and the Pt-VAS ratings. The DAS28-ESR score, CDAI, TJC28, and SJC28 of both the REMIQ-positive and -negative patients were significantly reduced compared to the baseline values. At 14 weeks, the DAS28-ESR, TJC, and Pt-VAS in the REMIQ-positive group were significantly reduced compared to those in the REMIQ-negative group. The REMIQ-positive patients also showed a decrease in Pt-VAS ratings at all observation points compared to the REMIQ-negative group.

The ESR and MMP-3 values at 14 and 54 weeks in the REMIQ-positive group were significantly reduced compared to those in the -negative group (Fig. 3). At 14, 24, and 54 weeks, the CRP, ESR, and MMP-3 of both the REMIQ-positive and -negative groups were significantly reduced compared to the baseline values. The RF titers at each observation period were reduced from the baseline values in both groups but not significantly different between the groups.

When we evaluated the disease activity based on the DAS28 criteria at 14, 24, and 54 weeks (Fig. 4A), we observed that the proportions of patients achieving low disease activity including remission at 14 weeks was significantly increased for the DAS28 in the REMIQ-positive patients
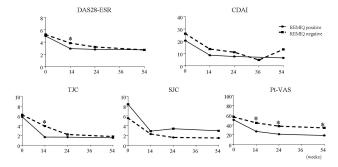
Fig. 2. Serial changes in the DAS28-ESR, CDAI, TJC28 (0–28 joints), SJC28 (0–28 joints), and Pt-VAS (cm) in the REMIQ-positive and -negative groups at baseline, 14, 24, 36, and 54 weeks. The LOCF method was applied to assess the group who discontinued the treatment. Comparisons between the two groups' mean values were analyzed using the Mann-Whitney test: *$p <$ 0.05, REMIQ-positive vs. -negative. CDAI, Clinical Disease Activity Index; DAS28-ESR, Disease Activity Score assessing 28 joints with erythrocyte sedimentation rate; Pt-VAS, patient Visual Analogue Scale; SJC, swollen joint count; TJC, tender joint count.
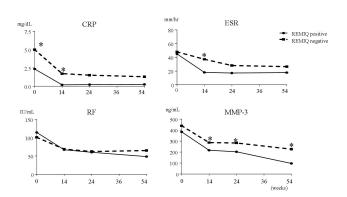

Fig. 3. Serial changes in the C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and rheumatoid factor (RF) values in the REMIQ-positive and -negative groups at baseline, 14, 24, 36, and 54 weeks. The LOCF method was applied to assess the group who discontinued treatment. Comparisons between the two groups' mean values were analyzed using the Mann-Whitney test: *$p <$ 0.05, REMIQ-positive vs. -negative.

compared to the -negative patients (63.0% vs. 28.6%, respectively). At 24 and 54 weeks, the percentage of patients achieving low disease activity tended to increase with the escalating infliximab dose in the REMIQ-negative group at 24 and 54 weeks. The rate of responders (good response) at 14 weeks was 58.7% in the REMIQ-positive group but only 32.1% in the REMIQ-negative group (Fig. 4B). However, the percentage of responders (good response) at week 54 was 70.0% in the REMIQ-positive group and 52.9% in the REMIQ-negative group, with a smaller difference between the two groups when infliximab was escalated in the REMIQ-negative group based on physicians' judgments of disease activity. The percentages of non-responders at 12,

24, and 54 weeks were not significantly different between the REMIQ-positive and -negative groups.
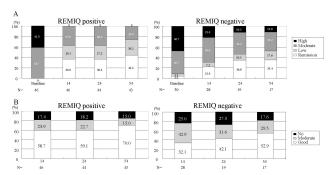

Fig. 4. Disease activity in DAS28-ESR and the EULAR (European League Against Rheumatism) response. (A) Time course of the disease activity in DAS28-ESR through 54 weeks following the REMIQ-positive and -negative groups. (B) By comparing the groups' DAS28-ESR scores at 14, 24, and 54 weeks, it is possible to define an improved response. The disease activity and DAS28-ESR criteria were categorized as follows. The DAS28-ESR criteria: High: >5.1, Moderate: 3.2–5.1, Low: 2.6–3.2. Remission: <2.6. The EULAR responses were categorized as follows: No response: DAS28-ESR improvement ≤0.6 and DAS28-ESR improvement >0.6 and ≤1.2 in present DAS28-ESR >5.1. Moderate response: DAS28-ESR improvement >0.6 and ≤1.2 in present DAS28-ESR ≤3.2, and >3.2 and ≤5.1; DAS28-ESR improvement >1.2 in present DAS28-ESR >5.1, and >3.2 and ≤5.1. Good response: DAS28-ESR improvement >1.2 in present DAS28-ESR ≤3.2. DAS28-ESR: Disease Activity Score assessing 28 joints with erythrocyte sedimentation rate.

When the percentage of patients achieving low disease activity was examined in the REMIQ-positive and -negative groups, the REMIQ-positive group showed a significant increase compared to the -negative group after 14 weeks (Fig. 5). Conversely, there was no significant between-group difference in the length of time to the achievement of remission.
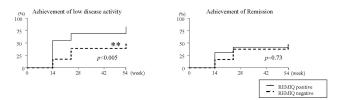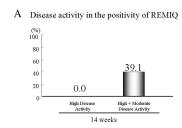

Fig. 5. Achievement of low disease activity and the patients' infliximab blood concentrations. Kaplan-Meier curves for the REMIQ positive- (n = 46) and -negative (n = 30) groups treated with infliximab 3, 6, and 12 mg/kg regarding the length of time to achieve low disease activity and remission in the 76 RA patients. **$p <$ 0.01, REMIQ-positive vs. -negative group.

We also obtained the Cox proportional HRs for the number of days to the achievement of low disease activity and the number of days to remission. The HR to achieve low disease activity in the REMIQ-positive group was significantly higher than that in the REMIQ-negative group (HR 2.10, 95% CI: 1.55–5.71). Conversely, the number of days to remission was not significantly different between the REMIQ-positive and -negative groups; nor was the HR (HR 1.13, 95% CI: 0.53–2.55).

3.4 Predictors for Positive REMIQ Results

The REMIQ kit was used to determine the infliximab blood concentrations and RA disease activity at 14 weeks (Fig. 6A). The percentage of patients with positive REMIQ at 14 weeks was 0.0% in the high disease activity group and 39.1% in the high/moderate disease activity group. We examined the ROC curves for CRP and ESR at 14 weeks and observed that at CRP <0.45 mg/dL and ESR <20.0 mm/hr, the ROC curves showed 31.0% and 26.9% specificity and 93.1% and 67.9% sensitivity (area under the curve [AUC] 0.78 and 0.70) (Fig. 6B). The Cox regression analysis with multiple variables showed that REMIQ positivity at baseline (HR 2.10, 95% CI: 1.55–5.71) and ACPA negativity at baseline (HR 0.53, 95% CI: 0.11–0.87) were each associated with the length of time to achieve low disease activity (Fig. 5, Table 2). In addition, RF positivity and ACPA positivity at baseline were each associated with the length of time to achieve remission (HR 0.44, 95% CI: 0.09–0.82) and (HR 0.35, 95% CI: 0.04–0.48), respectively. Conversely, the patients' CRP and ESR values at baseline were not significantly associated with the lengths of time to achieve low disease activity or remission.
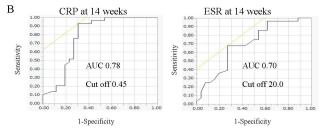
Table 2. Cox regression analysis for the achievement of low disease activity and remission in infliximab treatment.

Factor	Low disease activity		Remission	
	HR	95% CI	HR	95% CI
CRP <2.0	1.3	0.60–3.00	0.97	0.44–2.11
ESR <50	1.4	0.80–3.09	1.10	0.53–2.57
RF positivity	0.79	0.28–1.70	0.44	0.09–0.82
ACPA positivity	0.53	0.11–0.87	0.35	0.04–0.48

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; ACPA, anticitrullinated peptide antibody.

3.5 Safety Assessments

Table 3 lists the adverse events over the 54 weeks in the REMIQ positive- and -negative groups. At 54 weeks, an AE had occurred in 13.3% of the REMIQ-negative patients and in 6.5% of REMIQ-positive patients. None of the patients in either group experienced an AE that was serious enough to result in treatment discontinuation or hospitalization. Infusion reactions occurred in three of the REMIQ-negative patients and one REMIQ-positive patient. No significant between-group differences were detected in the occurrence of other AEs such as rash, gastrointestinal disorders, and liver disorders.

Table 3. Adverse events observed over the 54-week follow-up in the REMIQ positive- and negative groups.

	REMIQ (+) n = 46	REMIQ (−) n = 30
sAE	0	0
Discontinuation due to AE	0	0
Infusion reaction	1	3
Rash	1	0
Gastrointestinal disorder	1	0
Liver disorder	0	1
Total	3 (6.5%)	4 (13.3%)

AE, adverse event; sAE, serious adverse event.

4. Discussion

Examinations of RA registries have estimated that (*i*) 20%–30% of RA patients do not respond to treatment with a first TNF inhibitor, and (*ii*) >20% of those who initially respond lose efficacy within 2 years of starting treatment [12]. In their study of a cohort of 105 RA patients, Wolbink *et al*. [13] observed that the serum infliximab trough concentrations at 14 weeks were correlated with the clinical response to treatment but negatively correlated with the patients' baseline CRP levels. Similarly, in a series of 106 infliximab-treated RA patients, Bendtzen *et al*. [14] showed that serum infliximab trough concentrations after the first two infusions were clearly influenced by the patients' baseline CRP levels. Importantly, high disease activity with elevated baseline CRP levels and DAS values were

Fig. 6. Predictors for the achievement to the low disease activity. (A) The patients in the REMIQ-positive group were divided based on their disease activity at 14 weeks of infliximab treatment. (B) The ROC curves for the examination of the CRP and ESR cut-off values for predicting REMIQ positivity were assessed.

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associated with low infliximab trough concentrations after the first two infusions, and these low trough concentrations were observed to indicate an increased risk of anti-drug antibodies (ADAbs) to infliximab after 6 months [15,16]. Although it is not possible to measure ADAbs to infliximab directly, we recognized that measuring infliximab blood concentrations at an early stage is important for the purpose of estimating the presence of ADAbs. The REMIQ test kit determines whether the serum concentration of infliximab is 1.0 μg/mL or higher.

In the RISING study, a serum infliximab trough value of 1.0 μg/mL was considered the threshold for the development of clinical efficacy [6]. It was also recently reported that the optimal therapeutic range concentration of infliximab in the blood has a cut-off value of ≥ 0.32 μg/mL [17]. The present study's Cox regression analysis with multiple variables showed that the patients' baseline CRP and ESR values were not significantly associated with the achievement of low disease activity or remission. Conversely, REMIQ positivity was associated with the length of time to achieve low disease activity (HR 2.10, 95% CI: 1.55–5.71). Interestingly, not all of the present REMIQ-positive patients showed high disease activity at 14 weeks; however, all of these patients received escalating doses of infliximab within 24 weeks, and none of them stopped treatment early due to ineffectiveness, and the mean duration of the treatment for these patients was 38 weeks (data not shown). These results suggest that adequate dose escalation could suppress the production of ADAbs and prolong the rates of the retention of ADAbs by improving treatment efficacy. No increase in the incidence of serious AEs such as infections was associated with higher infliximab doses.

This retrospective study has several limitations. It was not randomized or blinded, which may have allowed bias in the selection of patients and treatments and in the judgment of treatment efficacy. Physicians at five medical centers independently identified RA patients who had an inadequate response to infliximab treatment, and the physicians used their judgment to decide whether to increase the infliximab dose. It should also be noted that the maximum oral dosage of MTX in Japan differs from that in Western countries. Since the maximum clinical dose of MTX in Japan is 16 mg/week, the present study was performed with MTX 4–12 mg/day. Reports on the maximum MTX dosages outside of Japan recommend increasing the dosage up to 20–30 mg/week if the effect is inadequate, while taking safety into consideration [18]. The results for RA patients outside of Japan who are treated with up to 20–30 mg/week of MTX are not known.

In summary, positivity on the REMIQ test at 14 weeks was shown to be an important predictor of the infliximab treatment response among patients with RA. A positive REMIQ results after the first two loading doses, i.e., an infliximab blood concentration of ≥ 1 μg/mL, was associated with a good clinical course of treatment with suppressions

of current and future RA disease activity. Conversely, if the REMIQ result is negative at 14 weeks, there is concern that the target cytokine may be not TNF but rather another inflammatory cytokine such as interleukin-6 or -17, or that discontinuation due to secondary ineffectiveness may occur by the appearance of ADAbs with continued infliximab treatment at the same dose in patients with blood concentrations <1 μg/mL. The infliximab dose escalation protocol in the blood concentrations, as suggested by the present results, is shown in **Supplementary Fig. 3**. Our findings indicate that the early assessment of blood concentrations of infliximab, a TNF inhibitor, is predictive of future therapeutic efficacy, and the REMIQ kit has demonstrated its usefulness as a testing method.

5. Conclusions

These results suggest that the control of RA disease activity may be facilitated by using the REMIQ kit at 14 weeks to check whether it is necessary to increase a patient's infliximab dose to ensure a therapeutic blood concentration that will help the patient achieve low disease activity.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. The data analyzed and displayed in the present manuscript are available from the corresponding author upon reasonable request.

Author Contributions

Conceptualization—YN; Data collection—YN; Formal analysis—YN; Investigation—TK, TT, TH, HM, KH, YK, MS, SI, KK and IM; Project administration—YN. All authors contributed to editorial changes in the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

The patients were enrolled and treated at the five institutions mentioned above. The study was conducted in accord with the principles of the Helsinki Declaration of 1983 and was approved by the Research Ethics Committee of Kindai University of Medicine (approval no. R03–048). For this retrospective cohort study, the patient's formal consent was not required. However, at Kindai University Faculty of Medicine, we obtain written consent from all patients to use their medical records, blood tests, and imaging findings for any clinical research. We also provided the details of this study on our website and at the participating hospitals, making the study information accessible to all patients, and we gave them the option to refuse to have their data used. The other facilities either obtained consent directly from the patients in writing or published an opt-out on their websites, giving patients notice and the right to refuse the disclosure of their information.

Acknowledgment

Conflict of Interest

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2804068.

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