RESEARCH ARTICLE

Potential application of measuring serum infliximab levels in rheumatoid arthritis management: A retrospective study based on KURAMA cohort data

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Abstract

Infliximab (IFX) therapy has considerably improved the treatment of rheumatoid arthritis (RA). However, some patients still do not respond adequately to IFX therapy, or the efficacy of the treatment diminishes over time. Although previous studies have reported a relationship between serum IFX levels and therapeutic efficacy, the potential applications of IFX therapeutic drug monitoring (TDM) in clinical practice remain unclear. The purpose of this study was to investigate the potential applications of IFX TDM by analyzing a Japanese cohort database. Data were collected retrospectively from the Kyoto University Rheumatoid Arthritis Management Alliance cohort between January 1, 2011, and December 31, 2018. Serum IFX levels were measured using a liquid chromatography-tandem mass spectrometer. Out of the 311 RA patients that used IFX, 41 were eligible for the analysis. Serum IFX levels were significantly higher in responders than in non-responders. An optimal cut-off value was determined to be 0.32 μg/mL based on a receiver operating characteristic curve.

At the IFX measurement point, a better therapeutic response was observed in the high IFX group (n = 32) than in the low IFX group (n = 9). Conversely, at the maximum effect point, when DAS28-ESR was the lowest between IFX introduction and measurement points, there were no differences in responder proportions between the low and high IFX groups. IFX primary ineffectiveness could be avoided with appropriate dose escalation without blood concentration measurement in clinical practice. In conclusion, IFX TDM could facilitate the identification of secondary non-responders and in turn, proper IFX use.

Citation: Nakae K, Masui S, Yonezawa A, Hashimoto M, Watanabe R, Murata K, et al. (2021) Potential application of measuring serum infliximab levels in rheumatoid arthritis management: A retrospective study based on KURAMA cohort data. PLoS ONE 16(10): e0258601. https://doi.org/10.1371/journal.pone.0258601

Editor: Masataka Kuwana, Nippon Medical School, JAPAN

Received: May 27, 2021
Accepted: September 30, 2021
Published: October 13, 2021

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Data Availability Statement: There are ethical and legal restrictions on sharing a de-identified data set, because the data contain potentially sensitive information of the patients. Data are available from Ethics Committee (ethcom@kuhp.kyoto-u.ac.jp) for researchers who meet the criteria for access to confidential data.

Funding: This study was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports,
Science and Technology of Japan (Grants-in-Aid for Scientific Research (B) [19H03389]), by Japan Agency for Medical Research and Development (AMED) under Grant Number 20mk0101152h0502 and the Nakatomi Foundation to AY. KURAMA cohort study is supported by a grant from Daiichi Sankyo. Daiichi Sankyo had no role in the design of the study, the collection or analysis of the data, the writing of the manuscript or decision to submit the manuscript for publication.

Competing interests: M.H., R.W., K.Murata, M.T., and H.I. are associated with a department financially supported by two local governments in Japan (Nagahama City, Shiga and Toyooka City, Hyogo) and five pharmaceutical companies (Mitsubishi Tanabe Pharma Corp., Chugai Pharmaceutical Co., Ltd., Ayumi Pharmaceutical Corp., Asahi Kasei Pharma Corp., and UCB Japan Co., Ltd.). A.Y. and K.Matsubara has received a research grant from Shimadzu Corporation. M.H. received a research grant and/or speaker fee from Bristol-Myers Squibb Co., Eisai Co., Ltd., Eli Lilly Japan K.K., and Mitsubishi Tanabe Pharma Corp. R.W. has received speaker’s fee from Mitsubishi Tanabe Pharma Corp., Pfizer Inc., Santoli K.K., AbbVie GK, Asahi-Kasei Corp., Eisai Co., Ltd., Eli Lilly Japan K.K., Bristol-Myers Squibb Co., and Janssen Pharmaceutical K.K. K.Murata received a speaking fee from Eisai Co., Ltd., Mochida Pharmaceutical Co., Ltd., and Astellas Pharma Inc. M.T. has received research grants and/or speaker fees from AbbVie GK, Asahi Kasei Pharma Corp., Astellas Pharma Inc., Ayumi Pharmaceutical Corp., Chugai Pharmaceutical Co., Ltd., Eisai Co., Ltd., Eli Lilly Japan K.K., Janssen Pharmaceutical K.K., Mitsubishi Tanabe Pharma Corp., Novartis Pharma K.K., Pfizer Inc., Taisho Phama Co., Ltd., and UCB Japan Co., Ltd. H.I. has received a research grant and/or speaker fees from Bristol-Myers Squibb Co., Eisai Co., Ltd., Taisho Pharmaceutical Co., Ltd., Mochida Pharmaceutical Co., Ltd., and Asahi Kasei Corp. The other authors declare no conflicts of interest.

Introduction

Infliximab (IFX) is a chimeric monoclonal antibody composed of human constant and murine variable regions that specifically bind to tumor necrosis factor alpha (TNF-α). IFX therapy has substantially improved the treatment of rheumatoid arthritis (RA). The result of Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT) study has revealed that IFX therapy provided clinical benefits and halted joint damage progression [1, 2]. However, in some patients, the efficacy of IFX therapy is not adequate, or is gradually lost with the lapse of the treatment [3–6]. It has also been reported that secondary non-response occurs in approximately a half of RA patients during the first year of its treatment [7]. In addition, another study has shown that IFX discontinuation rate due to inefficacy was 32.1% at 36 months [8]. One of the current challenges in IFX therapy is to avoid secondary non-response in long-term treatment.

The pharmacokinetic mechanisms of therapeutic antibodies have largely been clarified. The development of anti-drug antibodies (ADAs) is associated with low serum drug levels and non-response [9–11]. Previous studies have shown that approximately 10–60% of RA patients receiving IFX developed ADAs against IFX within the first 6 months [12–15]. In addition to ADAs, baseline TNF-α level is another factor that reduces serum IFX levels [16]. Furthermore, FcRn (neonatal Fc receptor) function influences the pharmacokinetics of therapeutic antibodies [17, 18]. High inter- and intra-individual variabilities in monoclonal antibody pharmacokinetics have been reported [19]. Consequently, therapeutic strategies that take into account IFX pharmacokinetics variability should be developed.

Therapeutic drug monitoring (TDM) has facilitated the optimal and appropriate use of immunosuppressive drugs and antiepileptic drugs, etc. Based on the serum concentrations of drugs, dosages can be adjusted to appropriate therapeutic concentrations and ranges. Some studies have demonstrated that clinical responses to IFX therapy are associated with serum IFX levels. A prospective, randomized, double-blind study (the RISING study) has reported a significant correlation between serum IFX levels and disease activity score in 28 joints (DAS28)-remission [20]. A non-interventional retrospective study has also reported that high serum IFX levels are related to good responses at 52 weeks from baseline [15]. Although a relationship between serum IFX levels and its therapeutic benefits has been described in several studies [15, 20–22], it remains unclear how IFX TDM could be applied in clinical practice.

Here, we conducted a retrospective cohort study by enrolling consecutive RA patients treated with IFX in a cohort, and investigated the practicality of IFX TDM in clinical practice. Furthermore, we measured ADA levels to evaluate its correlation with serum IFX levels.

Materials and methods

Patients

The study subjects were enrolled from the Kyoto University Rheumatoid Arthritis Management Alliance (KURAMA) cohort, which was established in 2011 by the Center for Rheumatic Diseases at Kyoto University Hospital. The cohort aims to provide strict RA control and to use patient clinical and laboratory data in clinical investigations, as described previously [23]. All patients fulfilled the revised 1987 American College of Rheumatology (ACR) or the 2010 ACR/European League Against Rheumatism (EULAR) classification criteria for RA. Written informed consent to enroll in this retrospective cohort study was obtained from all the patients. The present study adhered to the principles of the Declaration of Helsinki, and was approved by the Medical Ethics Committee of Kyoto University Graduate School and Faculty of Medicine (R0357).
KURAMA cohort data between January 1, 2011 and December 31, 2018 were used in the present study. IFX was administered at 0, 2, and 6 weeks in the induction phase and thereafter every 8 weeks in the maintenance phase. In this study, 112 days (16 weeks) after the initiation was defined as the initiation of the maintenance phase. Out of the 311 RA patients with IFX therapy, 210 were excluded, because their serum IFX levels were not obtained during maintenance therapy (at least 112 days after IFX introduction). In addition, 55 patients were excluded due to lack of the 28-joint disease activity score incorporating erythrocyte sedimentation rate (DAS28-ESR) data at initiation and measurement point of IFX. When DAS28-ESR was not recorded on the IFX initiation and measurement days, DAS28-ESR at the visit before IFX initiation and the visit before or after IFX measurement were allowed to be used. Five patients who had already completed clinical remission (DAS28-ESR < 2.6) before IFX introduction were also excluded, and 41 patients were eligible for further analysis (Fig 1).

### Data collection and evaluation of disease activity

Clinical characteristics included age, body weight, sex, RA disease duration, IFX treatment duration, weekly methotrexate (MTX) dose, oral glucocorticoid use, conventional synthetic disease modifying anti-rheumatic-drug (csDMARD) use, tender joint count, swollen joint count, C-reactive protein (CRP) level, and rheumatoid factor (RF). Actarit, aurothiomalate, auranofin, bucillamine, igratimod, leflunomide, mizoribine, salazosulfapyridin, cyclosporine, and tacrolimus were considered as csDMARDs. RA disease activity was evaluated based on clinical disease activity index (CDAI), simplified disease activity index (SDAI), physical...
Measurement of serum IFX levels

Blood samples for measuring trough serum IFX levels were collected immediately before a new infusion. Serum IFX levels were measured using an LCMS-8060 quadrupole mass spectrometer (SHIMADZU, Kyoto, Japan), as previously reported, with some modifications [24–26]. Briefly, to obtain the peptides from the fragment antigen-binding (Fab) region of immunoglobulin G, serum samples were pretreated using the nSMOL™ Antibody BA Kit (SHIMADZU, Kyoto, Japan) according to the provided protocol. The lower limit of quantitation was 0.293 μg/mL.

Detection of ADAs in serum

ADA analysis was performed by the electrochemiluminescence (ECL) method [27, 28]. A microplate coated with streptavidin (MSD GOLD 96-well Streptavidin QUICKPLEX Plate, Meso Scale Diagnostics [MSD], Rockville, MD, USA) was blocked with 150 μL blocking solution (3% MSD Blocker A) overnight at 4 °C. A master mixture of 20 μg/mL biotinylated IFX and 20 μg/mL ruthenium-labeled IFX was prepared in assay diluent (1% MSD Blocker A) at a ratio of 1:1. Subsequently, 25 μL of a diluted sample and 50 μL of the master mixture were added to each well in a 96-well plate, and incubated for 2 h under gentle agitation. After three washes with 200 μL of wash buffer (phosphate-buffered saline with 0.05% Tween 20), 50 μL of premix solution was transferred to each corresponding streptavidin-coated plate well, and the plates were incubated for 1 h under agitation. The plates were then washed three times, and 150 μL of read buffer (MSD Read Buffer T [4×] diluted two-fold in ultrapure water) was added to each well. The ECL signal from the solution was measured using a MESO QuickPlex SQ120 (MSD).

Statistical analysis

Statistical analyses were performed using GraphPad Prism v7.0 (GraphPad Software, Inc., La Jolla, CA, USA). Non-normally distributed data were summarized with medians and analyzed using nonparametric tests (Mann-Whitney U test or Wilcoxon signed-rank test). Categorical data summarized with percentages were analyzed using Fisher’s exact test with continuity correction, where necessary. Results were considered statistically significant at p-value ≤0.05. The Kaplan-Meier method was performed to evaluate time to first response and time to loss of response.

To define an optimal cut-off value for predicting clinical response, a receiver operating characteristic (ROC) curve was plotted using JMP® Pro14 (SAS Institute, Inc., Cary, NC, USA).

Results

Clinical efficacy and serum levels of IFX in RA patients

Fig 2A shows a change in DAS28-ESR after introduction of IFX. Large inter- and intra-individual differences in daily disease activities were observed. Kaplan-Meier curves indicated that more than 80% of total patients responded within 12 weeks after IFX introduction in clinical
practice (Fig 2B), and around 40% of responders exhibited loss of response within 48 weeks after the first response (Fig 2C).

There were 34 responders and 7 non-responders at the measurement point (Fig 3A). Serum IFX levels were significantly higher in responders than in non-responders (Fig 3B). The area under the curve (AUC) of the ROC curve was 0.87, and the cut-off value that distinguished EULAR responders from non-responders was 0.319 μg/mL (sensitivity: 94.1%, specificity: 85.7%, Fig 3C). Based on this, the cutoff value was set to 0.32 μg/mL for subsequent analyses.

Background demographics and clinical characteristics of patients

Out of the 311 RA patients who received IFX therapy in the KURAMA cohort, 41 were eligible for the analysis. We assessed the risk of bias by comparing the baseline demographics and clinical characteristics of the patients between the total population and study cohort (S1 Table). There was a difference in year of IFX initiation, age, and swollen joint count (SJC). Patients were divided into two groups based on serum IFX levels, that is, patients with serum IFX level <0.32 μg/mL (Low-IFX group, n = 9) and patients with serum IFX level ≥0.32 μg/mL (High-IFX group, n = 32). The baseline demographics and clinical characteristics of the patients in the two groups are summarized in Table 1. At the measurement point, the mean duration of IFX treatment was around 1 year. Age, tender joint count, CDAS, SDAI, HAQ-DI and
DAS28-ESR were significantly lower in the Low-IFX group than in the High-IFX group. Only patients in the High-IFX group used oral glucocorticoids, but there was no significant association between the use of glucocorticoids and disease severity. There were no significant differences in body weight, sex, disease duration, duration of IFX treatment, SJC, CRP level, RF-positive patients and concomitant MTX and csDMARDs use.

Disease activity markers in Low-IFX group and High-IFX group

In the present study, the “maximum effect point” was defined as the date when DAS28-ESR was the lowest during the IFX therapy between after its introduction point and at the measurement point. At the maximum effect point, only two patients (4.9%) were non-responders; there were no differences in proportions of responders between the Low-IFX and High-IFX groups ($p = 0.40$, Table 2). However, at the measurement point, five patients additionally
Table 1. Baseline demographics and clinical characteristics of the patients.

| Characteristics                        | IFX ≤ 0.32 μg/mL (n = 9) | IFX > 0.32 μg/mL (n = 32) | p-value |
|----------------------------------------|--------------------------|--------------------------|---------|
| Age, mean (SD), (years)                | 47.4 (19.4)              | 61.6 (12.1)              | 0.03    |
| Body weight, mean (SD), (kg)           | 57.8 (12.8)              | 55.8 (8.9)               | 0.85    |
| Women, no. (%)                         | 7 (77.8)                 | 25 (78.1)                | 1.00    |
| Disease duration, mean (SD), (years)   | 3.28 (2.01)              | 4.14 (3.71)              | 0.95    |
| Duration of IFX treatment, median (Min-Max), (days) | 332 (147–539)            | 429 (112–882)            | 0.34    |
| Weekly MTX dose, mean (SD), (mg/week)  | 9.3 (3.0)                | 8.7 (3.5)                | 0.63    |
| Oral glucocorticoid use, no. (%)       | 0 (0.0)                  | 13 (40.6)                | 0.04    |
| csDMARDs use, no. (%)                  | 2 (22.2)                 | 8 (25.0)                 | 1.00    |
| Tender joint count, mean (SD)          | 1.4 (1.2)                | 5.4 (5.8)                | 0.01    |
| Swollen joint count, mean (SD)         | 1.9 (1.4)                | 5.4 (5.3)                | 0.07    |
| CRP level, mean (SD), (mg/dL)          | 0.56 (0.61)              | 2.48 (3.42)              | 0.18    |
| RF positive, no. (%)                   | 8 (88.9)                 | 22 (68.8)                | 0.24    |
| CDAI, mean (SD)                        | 9.9 (3.9)                | 20.4 (13.7)              | <0.01   |
| SDAI, mean (SD)                        | 10.5 (4.1)               | 22.9 (15.9)              | <0.01   |
| HAQ-DI, mean (SD)                      | 0.47 (0.35)              | 1.22 (0.99)              | 0.04    |
| DAS28-ESR, mean (SD)                   | 3.69 (0.65)              | 4.93 (1.43)              | 0.02    |

The patients were divided into two groups; Low-IFX (IFX ≤ 0.32 μg/mL) and High-IFX (IFX > 0.32 μg/mL). Demographics and clinical characteristics at baseline are represented as means ± standard deviation (SD) for continuous data and numbers (percentages) for categorical data. Analysis of variance and Fisher’s exact test were used to compare the clinical characteristics among the different groups for continuous variables and categorical variables, respectively. csDMARDs include actarit, aurothiomalate, auranofin, bucillamine, igeratumod, leflunomide, mizoribine, salazosulfapyridin, cyclosporine, and tacrolimus. Abbreviations: ACPA, anticyclic citrullinated peptide antibody; CDAI, clinical disease activity index; csDMARDs, conventional synthetic disease modifying anti-rheumatic drugs; CRP, C-reactive protein; DAS28-ESR, the 28 joint disease activity score incorporating erythrocyte sedimentation rate; HAQ, physical disability by health assessment questionnaire-disability index; IFX, infliximab; MTX, methotrexate; SDAI, simplified disease activity index; RF, rheumatoid factor.

https://doi.org/10.1371/journal.pone.0258601.t001

Table 2. Number (percentages) of responders and non-responders at maximum effect and measurement point in each group.

|                                    | Responders | Non-responders | p-value |
|------------------------------------|------------|----------------|---------|
| **<maximum effect point>**         |            |                |         |
| IFX <0.32 μg/mL, n (%)             | 8 (88.9)   | 1 (11.1)       | 0.40    |
| IFX >0.32 μg/mL, n (%)             | 31 (96.9)  | 1 (3.1)        |         |
| Total (%)                          | 39 (95.1)  | 2 (4.9)        |         |
| **<measurement point>**            |            |                |         |
| IFX <0.32 μg/mL, n (%)             | 3 (33.3)   | 6 (66.7)       | <0.01   |
| IFX >0.32 μg/mL, n (%)             | 31 (96.9)  | 1 (3.1)        |         |
| Total (%)                          | 34 (82.9)  | 7 (17.1)       |         |
| **<measurement point>**            |            |                |         |
| ADA-positive, n (%)                | 2 (50.0)   | 2 (50.0)       | 0.14    |
| ADA-negative, n (%)                | 30 (85.7)  | 5 (14.3)       |         |
| Total (%)                          | 32 (82.1)  | 7 (17.9)       |         |

Responders had “good and moderate responses,” and non-responders had “no responses” based on the EULAR response criteria. ADAs in two patients could not be examined due to sample shortage. Values were considered statistically significant at a p value less than 0.05, based on two-sided Fisher’s exact test. Abbreviations: ADA, anti-drug antibody; EULAR, European League Against Rheumatism; IFX, infliximab.

https://doi.org/10.1371/journal.pone.0258601.t002

https://doi.org/10.1371/journal.pone.0258601.t001
turned into non-responder status, accordingly, there were significant differences in responder proportions between the Low-IFX group and the High-IFX group based on Fisher’s exact test ($p < 0.01$). One non-responder in the High-IFX group had finally attained efficacy after the measurement point. Disease activity marker trends between the introduction and measurement points in the two groups are illustrated in Fig 4. CDAI and SDAI in the Low-IFX group improved significantly. In addition, CDAI, SDAI, CRP, and HAQ-DI scores in the High-IFX group exhibited notable improvements.

Fig 4. Changes in (A) CDAI, (B) SDAI, (C) CRP, and (D) HAQ-DI from the baseline to the IFX measurement point. The left figures show the data of patients with IFX level $<0.32 \mu g/mL$ (closed circles), and the right figures show the data of patients with IFX level $\geq 0.32 \mu g/mL$ (open circles). Each line corresponds to each patient. The data were analyzed by Wilcoxon signed-rank test. Abbreviations: CDAI, clinical disease activity index; CRP, C-reactive protein; DAS28-ESR, the 28 joint disease activity score incorporating erythrocyte sedimentation rate; HAQ-DI, physical disability by health assessment questionnaire(disability index); IFX, infliximab; SDAI, simplified disease activity index.

https://doi.org/10.1371/journal.pone.0258601.g004
Correlation between serum IFX levels and ADA positivity

In 39 of the 41 investigated patients, serum samples were sufficient amount for the ADA determination. ADA was detected in four patients (10.3%) at the measurement point. All ADA-positive patients belonged to the steroid-free group. The IFX levels in the ADA-positive group were significantly lower than that in the ADA-negative group \( (p < 0.01, \text{Fig } 5) \). Although two patients in the ADA-positive group (50.0%) were responders, 30 patients in the ADA-negative group (85.7%) were responders. There were no significant differences in proportions of responders between the ADA-positive group and ADA-negative group (Table 2).

**Discussion**

In a previous intervention study (the RISING study), RA patients were randomly assigned to three treatment groups (3, 6, and 10 mg/kg IFX infusions) at week 10 after receiving 3 mg/kg IFX at weeks 0, 2, and 6 [20]. The rates of responders at week 54 for 3, 6 and 10 mg/kg were 10%, 56% and 100%, respectively. Better response was obtained in patients with higher dose of IFX. In addition, when the serum IFX concentration was \( \geq \)1.0 \( \mu \text{g/mL} \), a clinical response was observed in 98.8% of patients. Although the exact therapeutic window of IFX is yet to be clearly defined, a higher trough level has been associated with improved clinical outcomes in several observational studies and post-hoc analyses of clinical trials for RA [21, 22] and other chronic inflammatory diseases [29–32]. Notably, our real-world cohort data indicated the effectiveness of IFX treatment in 39 of the 41 target patients (95.1%) at the point of maximum effect. The
results obtained from this study strongly suggested that physicians increased IFX doses to appropriate levels in each patient even without measuring blood levels, and that primary ineffectiveness could be avoided in clinical practice.

Conversely, some patients showed secondary loss of response to IFX with the lapse of the continuous use. Notably, at the measurement point, the efficacy was significantly lower in the Low-IFX group than in the High-IFX group, strongly suggesting that the effect of this therapy was potentially decreased by lower blood IFX level. It is challenging to reliably determine secondary ineffectiveness under long-term use. Information on serum IFX levels might be helpful for physicians in the assessment of patients. Overall, we propose the development of a treatment algorithm based on IFX TDM, wherein IFX therapeutic efficacy would be extensively re-evaluated when blood IFX concentrations are low under continuous use.

The determination of a cut-off value for predicting clinical response is a key challenge. In the RISING study, a trough serum IFX level of 1.0 μg/mL was the threshold level for eliciting clinical responses [20]. Wolbink et al. [33] reported similar results, where patients with low trough serum IFX levels (less than 1.2 μg/mL) showed relatively low improvements in DAS28 score. From the result of ROC analysis in this study, an optimal cut-off value of ≥0.32 μg/mL was determined. Our study also revealed almost similar results when the cut-off value was determined to be at serum IFX level ≥1.0 μg/mL (S2 Table), which is largely consistent with RISING study [20]. Enzyme-linked immunosorbent assay method has been extensively used to quantify serum therapeutic antibodies. However, by use of this technique, nonspecific signals could be detected [34, 35]. In the present study, we employed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with nano-surface and molecular-orientation limited proteolysis to monitor IFX-specific peptides, based on Food and Drug Administration (FDA) criteria [36]. The analytical methods used should be taken into account to set cut-off values in clinical practice. Further studies are required to determine the optimal cut-off values across several analytical methods.

Previous studies have shown that ADA is one of the factors influencing IFX pharmacokinetics [9–11]. ADA formation increases IFX clearance, which can, in turn, reduce serum IFX levels. In the present study, 4 out of 39 patients (10.3%) were ADA-positive. Compared to the ADA-negative patients, the ADA-positive patients had significantly lower serum IFX levels. All ADA-positive patients belonged to the steroid-free group, which may have caused the significant difference in glucocorticoid use between the High- and Low-IFX groups. The proportion of patients satisfying the EULAR response criteria tended to be lower in the ADA-positive group. Although ADA could influence IFX pharmacokinetics, the key factor influencing IFX efficacy is serum IFX level. Although there are factors other than ADA to influence blood IFX levels, monitoring IFX levels is the potentially optimal tool for evaluating its clinical efficacy. Conversely, it has been reported that dose escalation of IFX in patients with IBD could be less successful for improving treatment efficacy in ADA-positive patients compared to that in ADA-negative patients [37, 38]. Measurement of ADA as well as serum IFX concentrations, could facilitate determination of the next appropriate therapeutic strategy between dose escalation or switching therapies in patients exhibiting secondary loss of response.

The present study had some limitations. First, the sample size was small. We had to exclude numerous patients with no information on serum IFX level or DAS28-ESR data around the IFX administration date as clinical data prior to 2011 was not included in the KURAMA cohort. Baseline demographics and clinical characteristics of the patients between the total population and study cohort were almost similar; however, selection bias cannot be completely precluded. Second, we did not measure serum IFX levels at the maximum effect point and were unable to investigate the association between reduction in DAS28-ESR and serum IFX levels at the maximum effect point. Third, background characteristics in several patients were
different between the High-IFX and Low-IFX groups. The present study was an observational study, and we could not employ randomization to control or eliminate confounding factors. However, more than 90% of patients in both the High- and Low-IFX groups exhibited a primary response. The skewed patient characteristic distributions could have had relatively less impact on our results associated with secondary non-response.

Conclusions

The present study demonstrated that serum IFX levels were correlated with IFX therapeutic efficacy under continuous use, based on real-world cohort data. In clinical practice, the IFX primary ineffectiveness could be avoided via appropriate dose escalation without measuring the blood concentrations. However, IFX TDM could facilitate the identification of secondary non-response and, in turn, proper IFX use.

Supporting information

S1 Table. Baseline demographics and clinical characteristics of the patients in total and study cohort. Demographics and clinical characteristics at baseline are represented as means ± standard deviation (SD) for continuous data and numbers (percentages) for categorical data. Analysis of variance and Chi-square test were used to compare the clinical characteristics among the different groups for continuous variables and categorical variables, respectively. csDMARDs include actarit, aurothiomalate, auranofin, bucillamine, iguratimod, leflunomide, mizoribine, salazosulfapyridin, cyclosporine, and tacrolimus. Abbreviations: CDAI, clinical disease activity index; csDMARDs, conventional synthetic disease modifying anti-rheumatic drugs; CRP, C-reactive protein; DAS28-ESR, the 28 joint disease activity score incorporating erythrocyte sedimentation rate; HAQ-DI, physical disability by health assessment questionnaire-disability index; IFX, infliximab; MTX, methotrexate; SDAI, simplified disease activity index; RF, rheumatoid factor. (DOCX)

S2 Table. Number of responders and non-responders at maximum effect and measurement points in High/Low-IFX group. The cut-off value was determined to be at serum IFX level ≥1.0 μg/mL. Responders had “good and moderate responses” and non-responders had “no responses” based on the EULAR response criteria. Values were considered statistically significant at a p value less than 0.05, based on two-sided Fisher’s exact test. Abbreviations: EULAR, European League Against Rheumatism; IFX, infliximab. (DOCX)

Acknowledgments

The authors are grateful to Dr. Akiko Ishii-Watabe, Dr. Hiroko Shibata, and Ms. Kazuko Nishimura, National Institute of Health Sciences, for advice on ADA analysis, and thank all the medical staff of the Rheumatic Disease Center, Kyoto University Hospital.

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