Potential Application of Measuring Serum Infliximab Levels in Rheumatoid Arthritis Management: A Retrospective Study based on KURAMA Cohort Data

Kazuto Nakae

Kyoto University

Sho Masui

Kyoto University

Atsushi Yonezawa (✉ ayone@kuhp.kyoto-u.ac.jp)

Kyoto University

Motomu Hashimoto

Kyoto University

Ryu Watanabe

Kyoto University

Koichi Murata

Kyoto University

Kosaku Murakami

Kyoto University

Masao Tanaka

Kyoto University

Hiromu Ito

Kyoto University

Kotoko Yokoyama

Shimadzu Corporation (Japan)

Noriko Iwamoto

Shimadzu Corporation (Japan)

Takashi Shimada

Shimadzu Corporation (Japan)

Miyuki Nakamura

Kyoto University

Masaya Denda

Kyoto University

Kotaro Itohara

Kyoto University

Shunsaku Nakagawa

Kyoto University

Yasuaki Ikemi

Kyoto University

Satoshi Imai

Kyoto University

Takayuki Nakagawa

Kyoto University

Makoto Hayakari

Kyoto University

Kazuo Matsubara

Kyoto University

Research Article

Keywords: Rheumatoid arthritis, Infliximab, Therapeutic Drug Monitoring, Cohort study

Posted Date: April 8th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-396661/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

1   **Potential application of measuring serum infliximab levels in rheumatoid arthritis**

2   **management: A retrospective study based on KURAMA cohort data**

3

4   Kazuto Nakae^{1,2#}, Sho Masui^{1,2#}, Atsushi Yonezawa^{1,2*}, Motomu Hashimoto³, Ryu

5   Watanabe³, Koichi Murata³, Kosaku Murakami⁴, Masao Tanaka³, Hiromu Ito³, Kotoko

6   Yokoyama⁵, Noriko Iwamoto^{5,6}, Takashi Shimada^{5,6}, Miyuki Nakamura^{1,2}, Masaya

7   Denda^{1,2}, Kotaro Itoharu¹, Shunsaku Nakagawa¹, Yasuaki Ikemi¹, Satoshi Imai¹,

8   Takayuki Nakagawa¹, Makoto Hayakari¹, Kazuo Matsubara¹

9

10   **Author Affiliations:**

11   1.   Department of Clinical Pharmacology and Therapeutics, Kyoto University

12   Hospital, Japan

13   2.   Graduate School of Pharmaceutical Sciences, Kyoto University, Japan

14   3.   Department of Advanced Medicine for Rheumatic Diseases, Graduate School of

15   Medicine, Kyoto University, Japan

16   4.   Department of Rheumatology and Clinical Immunology, Graduate School of

17   Medicine, Kyoto University, Japan.

18   5.   Shimadzu Corporation, Kyoto, Japan

19   6.   Shimadzu Scientific Instruments, Bothell, Washington, USA

20 # equal contributions

21

22 *Corresponding author: Atsushi Yonezawa, Ph.D.

23 Department of Clinical Pharmacology and Therapeutics, Kyoto

24 University Hospital, 54 Shogoin Kawahara-cho, Sakyo-Ku, Kyoto

25 606-8507, Japan

26 TEL number: +81-75-751-3582

27 FAX number: +81-75-751-4207

28 E-mail: ayone@kuhp.kyoto-u.ac.jp

29

Abstract

Background

Infliximab (IFX) therapy has considerably improved the treatment of rheumatoid arthritis (RA). On the other hand, in some patients, the efficacy of IFX therapy is not adequate, or gradually diminishes with the lapse of the treatment. Although previous studies have reported a positive relationship between serum IFX levels and therapeutic efficacy, the potential application of IFX therapeutic drug monitoring (TDM) in clinical practice remains unclear. The purpose of this study was to investigate the potential applications of IFX TDM by analyzing a Japanese cohort database.

Methods

Data were collected retrospectively from the Kyoto University Rheumatoid Arthritis Management Alliance, KURAMA, cohort between January 1, 2011, and December 31, 2018. Serum IFX levels were measured using liquid chromatography-tandem mass spectrometry.

Results

Out of the 311 RA patients who received IFX therapy, 41 were eligible for analysis. Serum IFX levels were significantly higher in responders than in non-responders. An optimal cut-off value was determined to be 0.4 $\mu\text{g/mL}$ based on a receiver operating characteristic curve. At the IFX measurement point, a better therapeutic response was

observed in the High-IFX group (n = 31) than in the Low-IFX group (n = 10). Conversely, at the maximum effect point, when DAS28-ESR (the 28 joint disease activity score incorporating erythrocyte sedimentation rate) was the lowest between IFX introduction and measurement points, there were no differences in responder proportions between the Low- and High-IFX groups.

Conclusions

In clinical practice, IFX primary ineffectiveness could be avoided with appropriate dose escalation without blood concentration measurement. However, IFX TDM could facilitate the identification of secondary non-responders, and in turn, proper IFX use.

Keywords: Rheumatoid arthritis; Infliximab; Therapeutic Drug Monitoring; Cohort study

Background

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]. The current challenge in IFX therapy is the optimization of 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.

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. Informed consent to enroll in this retrospective cohort study was obtained from all the patients. All data were de-identified and analyzed anonymously. 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. 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 56 days before IFX introduction and 56 days before or after IFX measurement.

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 (Figure 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, iguratimod, 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 disability by health assessment questionnaire-disability index (HAQ-DI), and DAS28-ESR. Baseline was defined as the last data within 3 months before IFX introduction. Patients achieving good or moderate responses to IFX therapy according to the EULAR response criteria were defined as “responders,” and patients with no response were defined as “non-responders.”

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 $p\text{-value} \leq 0.05$. 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

Figure 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 (Figure 2b), and around 40 % of responders exhibited loss of response within 48 weeks after the first response (Figure 2c).

There were 34 responders and 7 non-responders at the measurement point (Figure 3a). Serum IFX levels were significantly higher in responders than in non-responders (Figure 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 $\mu\text{g/mL}$ (sensitivity: 94.1 %, specificity: 85.7 %, Figure 3c). Considering clinical usefulness, 0.4 $\mu\text{g/mL}$ was selected as the cut-off value in subsequent analyses.

Background demographics and clinical characteristics of patients

Patients were divided into two groups based on serum IFX levels, that is, patients with serum IFX level $< 0.4 \mu\text{g/mL}$ (Low-IFX group, $n = 10$) and patients with serum IFX level $\geq 0.4 \mu\text{g/mL}$ (High-IFX group, $n = 31$). 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, CDAI, and SDAI were significantly lower in the Low-IFX group than in the High-IFX group, and only patients in the High-IFX group used oral glucocorticoids. There were no

significant differences in body weight, sex, disease duration, duration of treatment, tender joint count, swollen joint count, CRP level, number of antibodies to citrullinated peptide antigens-positive or RF-positive patients, HAQ-DI, DAS28-ESR, and concomitant MTX and csDMARD 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 ($p = 0.43$, Table 2). However, at the measurement point, five patients additionally 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 Figure 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.

214

215 **Correlation between serum IFX levels and ADA positivity**

216 In 39 of the 41 investigated patients, serum samples were sufficient amount for the
217 ADA determination. ADA was detected in four patients (10.3 %) at the measurement
218 point. The IFX levels in the ADA-positive group were significantly lower than that in
219 the ADA-negative group ($p < 0.01$, Figure 5). Although two patients in the ADA-
220 positive group (50.0 %) were responders, 30 patients in the ADA-negative group
221 (85.7 %) were responders. There were no significant differences in proportions of
222 responders between the ADA-positive group and ADA-negative group (Table 2).

223

224 **Discussion**

225 In a previous intervention study (the RISING study), RA patients were randomly
226 assigned to three treatment groups (3, 6, and 10 mg/kg IFX infusions) at week 10 after
227 receiving 3 mg/kg IFX at weeks 0, 2, and 6 [20]. The rates of responders at week 54 for
228 3, 6 and 10 mg/kg were 10%, 56% and 100%, respectively. Better response was obtained
229 in patients with higher dose of IFX. In addition, when the serum IFX concentration was
230 $\geq 1.0 \mu\text{g/mL}$, a clinical response was observed in 98.8 % of patients. Although the exact
231 therapeutic window of IFX is yet to be clearly defined, a higher trough level has been
232 associated with improved clinical outcomes in several observational studies and post-hoc

analyses of clinical trials across different diseases [21, 22, 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 successfully 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. In clinical practice, checking DAS28-ESR is somewhat difficult for physicians due to requiring considerable time for examination, especially on busy patients. Large inter- and intra-individual differences in disease activities were observed. Consequently, it is challenging to reliably determine secondary ineffectiveness under long-term use. 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 ≥ 0.4 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 (Additional file 1 Table S1), 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. 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, dose escalation of IFX 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. 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 patient 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

In the present study, we 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.

List of abbreviations

ACR: American College of Rheumatology; ADA: Anti-drug antibody; CDAI: Clinical disease activity index; CRP: C-reactive protein; csDMARD: Conventional synthetic disease modifying anti-rheumatic drug; DAS28-ESR: The 28 joint disease activity score incorporating erythrocyte sedimentation rate; ECL: Electrochemiluminescence; EULAR: European League Against Rheumatism; FcRn; Neonatal Fc receptor; FDA: Food and Drug Administration; HAQ-DI: Health assessment questionnaire-disability index; IFX: Infliximab; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MTX: Methotrexate ; RA: Rheumatoid arthritis; RF: Rheumatoid factor; ROC: Receiver

operating characteristic; SD: Standard deviation; SDAI: Simplified disease activity index;

TDM: Therapeutic drug monitoring; TNF- α : Tumor necrosis factor alpha

Declarations

Ethics approval and consent to participate: 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). Informed consent to enroll in the retrospective cohort study was obtained from all the patients. All data were de-identified and analyzed anonymously.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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., Sanofi 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 Pharma 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.

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.

Authors' contributions: A.Y., M.H., and K.Matsubara. conceived and designed the research. K.N., S.M., A.Y., M.H., and K.Matsubara. mainly contributed to writing the manuscript. M.H., R.W., K.Murata., K.Mmurakami., M.T., and H.I. recruited the patients and obtained samples. K.Y., N.I., and T.S. measured the plasma concentrations of IFX. K.N., S.M., A.Y., M.N., M.D, K.I., S.N., Y.I., S.I., T.N., and M.H. carried out and analyzed all experiments. All the authors participated in the discussion of the results, and all had reviewed the manuscript.

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.

References

1. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, et al. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT study group. *Lancet*. 1999;354(9194):1932-9.
2. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, et al. Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study group. *N Engl J Med*. 2000;343(22):1594-602.
3. Hanauer S, Wagner CL, Bala M, Mayer L, Travers S, Diamond R, et al. Incidence and importance of antibody responses to infliximab after maintenance or episodic treatment in Crohn's disease. *Clin Gastroenterol Hepatol*. 2004;2(7):542-53.
4. Chaudhari U, Romano P, Mulcahy LD, Dooley LT, Baker DG, Gottlieb AB. Efficacy and safety of infliximab monotherapy for plaque-type psoriasis: a randomised trial. *Lancet*. 2001;357(9271):1842-7.
5. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med*. 2005;353(23):2462-76.
6. Yanai H, Hanauer SB. Assessing response and loss of response to biological

therapies in IBD. Am J Gastroenterol. 2011;106(4):685-98.

7. Buch MH, Bingham SJ, Bryer D, Emery P. Long-term infliximab treatment in rheumatoid arthritis: subsequent outcome of initial responders. Rheumatology (Oxford). 2007;46(7):1153-6.

8. Ebina K, Hashimoto M, Yamamoto W, Ohnishi A, Kabata D, Hirano T, et al. Drug retention and discontinuation reasons between seven biologics in patients with rheumatoid arthritis -The ANSWER cohort study. PLoS One. 2018;13(3):e0194130.

9. Baert F, Noman M, Vermeire S, Van Assche G, D' Haens G, Carbonez A, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. N Engl J Med. 2003;348(7):601-8.

10. Plasencia C, Jurado T, Villalba A, Peitedado D, Casla MT, Nuno L, et al. Effect of infliximab dose increase in rheumatoid arthritis at different trough concentrations: A cohort study in clinical practice conditions. Front Med. 2015;2:71.

11. Kalden JR, Schulze-Koops H. Immunogenicity and loss of response to TNF inhibitors: implications for rheumatoid arthritis treatment. Nat Rev Rheumatol. 2017;13(12):707-18.

12. Bendtzen K, Geborek P, Svenson M, Larsson L, Kapetanovic MC, Saxne T. Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor alpha inhibitor

infliximab. *Arthritis Rheum.* 2006;54(12):3782-9.

13. Pascual-Salcedo D, Plasencia C, Ramiro S, Nuno L, Bonilla G, Nagore D, et al.

Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis. *Rheumatology (Oxford)*. 2011;50(8):1445-52.

14. Strand V, Balsa A, Al-Saleh J, Barile-Fabris L, Horiuchi T, Takeuchi T, et al.

Immunogenicity of biologics in chronic inflammatory diseases: a systematic review. *BioDrugs*. 2017;31(4):299-316.

15. Siljehult F, Arlestig L, Eriksson C, Rantapaa-Dahlqvist S. Concentrations of

infliximab and anti-drug antibodies in relation to clinical response in patients with rheumatoid arthritis. *Scand J Rheumatol*. 2018;47(5):345-50.

16. Takeuchi T, Miyasaka N, Tatsuki Y, Yano T, Yoshinari T, Abe T, et al. Baseline

tumour necrosis factor alpha levels predict the necessity for dose escalation of infliximab therapy in patients with rheumatoid arthritis. *Ann Rheum Dis*. 2011;70(7):1208-15.

17. Tzaban S, Massol RH, Yen E, Hamman W, Frank SR, Lapierre LA, et al. The

recycling and transcytotic pathways for IgG transport by FcRn are distinct and display an inherent polarity. *J Cell Bio*. 2009;185(4):673-84.

18. Kuo TT, Aveson VG. Neonatal Fc receptor and IgG-based therapeutics. *MAbs*.

2011;3(5):422-30.

19. Gill KL, Machavaram KK, Rose RH, Chetty M. Potential sources of inter-subject variability in monoclonal antibody pharmacokinetics. *Clin Pharmacokinet.* 2016;55(7):789-805.
20. Takeuchi T, Miyasaka N, Inoue K, Abe T, Koike T. Impact of trough serum level on radiographic and clinical response to infliximab plus methotrexate in patients with rheumatoid arthritis: results from the RISING study. *Mod Rheumatol.* 2009;19(5):478-87.
21. Mulleman D, Meric JC, Paintaud G, Ducourau E, Magdelaine-Beuzelin C, Valat JP, et al. Infliximab concentration monitoring improves the control of disease activity in rheumatoid arthritis. *Arthritis Res Ther.* 2009;11(6):R178.
22. Price S. Rheumatoid arthritis: Monitoring serum concentration of infliximab might improve ra disease control. *Nat Rev Rheumatol.* 2010;6(2):66.
23. Terao C, Hashimoto M, Yamamoto K, Murakami K, Ohmura K, Nakashima R, et al. Three groups in the 28 joints for rheumatoid arthritis synovitis--analysis using more than 17,000 assessments in the KURAMA database. *PLoS One.* 2013;8(3):e59341.
24. Iwamoto N, Shimada T, Umino Y, Aoki C, Aoki Y, Sato TA, et al. Selective detection of complementarity-determining regions of monoclonal antibody by limiting protease access to the substrate: nano-surface and molecular-orientation limited proteolysis. *Analyst.* 2014;139(3):576-80.

- 441 25. Iwamoto N, Yokoyama K, Takanashi M, Yonezawa A, Matsubara K, Shimada T.
442 Verification between original and biosimilar therapeutic antibody infliximab using
443 nSMOL coupled LC-MS bioanalysis in human serum. *Curr Pharm Biotechnol*.
444 2018;19(6):495-505.
- 445 26. Iwamoto N, Takanashi M, Yokoyama K, Yonezawa A, Denda M, Hashimoto M, et
446 al. Multiplexed monitoring of therapeutic antibodies for inflammatory diseases using
447 Fab-selective proteolysis nSMOL coupled with LC-MS. *J Immunol Methods*.
448 2019;472:44-54.
- 449 27. Reinisch W, Jahnsen J, Schreiber S, Danese S, Panés J, Balsa A, et al. Evaluation of
450 the cross-reactivity of antidrug antibodies to CT-P13 and infliximab reference
451 product (Remicade): an analysis using immunoassays tagged with both agents.
452 *BioDrugs*. 2017;31(3):223-37.
- 453 28. Shibata H, Nishimura K, Miyama C, Tada M, Suzuki T, Saito Y, et al. Comparison
454 of different immunoassay methods to detect human anti-drug antibody using the
455 WHO erythropoietin antibody reference panel for analytes. *J Immunol Methods*.
456 2018;452:73-7.
- 457 29. Pallagi-Kunstar E, Farkas K, Szepes Z, Nagy F, Szucs M, Kui R, et al. Utility of
458 serum TNF- α , infliximab trough level, and antibody titers in inflammatory bowel
459 disease. *World J Gastroenterol*. 2014;20(17):5031-5.

30. Dannepond C, Maruani A, Machet L, Ternant D, Paintaud G, Samimi M. Serum
infliximab concentrations in psoriatic patients treated with infliximab: a systematic
review. *Acta Derm Venereol.* 2015;95(4):401-6.
31. Vande Casteele NV, Ferrante M, Van Assche G, Ballet V, Compernelle G, Van Steen
K, et al. Trough concentrations of infliximab guide dosing for patients with
inflammatory bowel disease. *Gastroenterology.* 2015;148(7):1320-9.e3.
32. Warman A, Straathof JW, Derijks LJ. Therapeutic drug monitoring of infliximab in
inflammatory bowel disease patients in a teaching hospital setting: results of a
prospective cohort study. *Eur J Gastroenterol Hepatol.* 2015;27(3):242-8.
33. Wolbink GJ, Voskuyl AE, Lems WF, De Groot E, Nurmohamed MT, Tak PP, et al.
Relationship between serum trough infliximab levels, pretreatment C reactive
protein levels, and clinical response to infliximab treatment in patients with
rheumatoid arthritis. *Ann Rheum Dis.* 2005;64(5):704-7.
34. Güven E, Duus K, Lydolph M, Jørgensen CS, Laursen I, Houen G. Non-specific
binding in solid phase immunoassays for autoantibodies correlates with
inflammation markers. *J Immunol Methods.* 2014;403(1-2):26-36.
35. Jourdil JF, Lebert D, Gautier-Veyret E, Lemaitre F, Bonaz B, Picard G, et al.
Infliximab quantitation in human plasma by liquid chromatography-tandem mass
spectrometry: Towards a standardization of the methods? *Anal Bioanal Chem.*

2017;409(5):1195-205.

36. U.S. Food and Drug Administration. Bioanalytical Method Validation Guidance for Industry. 2018. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry>. Accessed 5 Jan 2021.

37. Kothari MM, Nguyen DL, Parekh NK. Strategies for overcoming anti-tumor necrosis factor drug antibodies in inflammatory bowel disease: Case series and review of literature. World J Gastrointest Pharmacol Ther. 2017;8(3):155-61.

38. Afif W, Loftus Jr EV, Faubion WA, Kane SV, Bruining DH, Hanson KA, et al. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. Am J Gastroenterol. 2010;105(5):1133-9.

Figure legends

Fig. 1 Flowchart on patient inclusion and exclusion.

Abbreviations: IFX, infliximab; DAS28-ESR, the 28 joint disease activity score incorporating erythrocyte sedimentation rate; RA, rheumatoid arthritis.

Fig. 2 Clinical efficacy of IFX. (a) Change in DAS28-ESR over time. The x-axis represents time after introduction of IFX. The y-axis represents values of DAS28-ESR. (b) Kaplan-Meier curve showing time to first response. Responders represent patients with “good or moderate response” based on the EULAR response criteria. The baseline (Day 0) was defined as the day of IFX introduction. (c) Kaplan-Meier curve showing time to loss of response. A change from responder to non-responder was defined as “loss of response.” The baseline (Day 0) was defined as the time point when the first response was observed. Patients who never showed any response during observation periods were excluded (n = 40).

Abbreviations: DAS28-ESR, the 28 joint disease activity score incorporating erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; IFX, infliximab.

Fig. 3 DAS28-ESR values and serum IFX concentrations in responders and non-responders. (a) DAS28-ESR values at baseline and at IFX measurement point. Responders (open circle) represent patients with “good or moderate response” and non-responders (closed circle) represent those with “no response” based on the European League Against Rheumatism (EULAR) response criteria. (b) Serum IFX levels were measured in non-responders and responders. Each dot represents each patient’s serum IFX level and the bars indicate the median. Differences between the groups were assessed

by Mann-Whitney U test. (c) Receiver operating characteristic (ROC) curve for the determination of the optimal IFX cut-off value for predicting persistent responder (area under the curve (AUC) = 0.87).

Abbreviations: DAS28-ESR, the 28 joint disease activity score incorporating erythrocyte sedimentation rate; IFX, infliximab.

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 < 0.4 µg/mL (closed circles), and the right figures show the data of patients with IFX level ≥ 0.4 µg/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.

Fig. 5 Comparison of serum IFX levels between ADA positive group (ADA (+)) and negative group (ADA (-)). Each dot represents each patient's serum IFX level and the bars indicate the median. Differences between groups were assessed by Mann-Whitney

U test.

Abbreviations: ADA, anti-drug antibody; IFX, infliximab.

Supplementary information

Additional file 1: Table S1. Number of responders and non-responders at maximum effect and measurement point in High/Low-IFX groups.

The cut-off value was determined to be at serum IFX level $\geq 1.0 \mu\text{g/mL}$. 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.

548 **Table 1.** Baseline demographics and clinical characteristics of the patients.

Characteristics	IFX < 0.4 µg/mL (n = 10)	IFX ≥ 0.4 µg/mL (n = 31)	<i>p</i> -value
Age, mean (SD), (years)	47.7 (17.4)	61.9 (11.9)	< 0.01
Body weight, mean (SD), (kg)	59.9 (13.0)	55.1 (7.9)	0.46
Women, no. (%)	7 (70.0)	25 (80.6)	0.66
Disease duration, mean (SD), (years)	3.02 (1.96)	4.25 (3.65)	0.51
Duration of IFX treatment, median (Min-Max), (days)	336 (142-539)	388 (112-882)	0.14
Weekly MTX dose, mean (SD), (mg/week)	9.4 (2.7)	8.6 (2.5)	0.39
Oral glucocorticoid use, no. (%)	0 (0.0)	13 (41.9)	< 0.01
csDMARDs use, no. (%)	2 (20.0)	8 (25.8)	> 0.99
Tender joint count, mean (SD)	2.6 (3.6)	5.1 (5.6)	0.06
Swollen joint count, mean (SD)	2.9 (3.3)	5.2 (5.2)	0.22
CRP level, mean (SD), (mg/dL)	1.6 (3.3)	2.2 (3.0)	0.49
RF positive, no. (%)	9 (90.0)	21 (67.7)	0.24
CDAI, mean (SD)	12.7 (8.9)	19.9 (13.4)	0.03
SDAI, mean (SD)	14.3 (12.0)	22.1 (15.2)	0.03
HAQ-DI, mean (SD)	0.70 (0.75)	1.17 (0.95)	0.14
DAS28-ESR, mean (SD)	4.04 (1.19)	4.86 (1.37)	0.08

549

550 The patients were divided into two groups; Low-IFX (serum IFX level < 0.4 µg/mL) and
551 High-IFX (serum IFX level ≥ 0.4 µg/mL). Demographics and clinical characteristics at
552 baseline are represented as means ± standard deviation (SD) for continuous data and
553 numbers (percentages) for categorical data. Analysis of variance and Fisher's exact test
554 were used to compare the clinical characteristics among the different groups for
555 continuous variables and categorical variables, respectively. csDMARDs include actarit,
556 aurothiomalate, auranofin, bucillamine, iguratimod, leflunomide, mizoribine,

557 salazosulfapyridin, cyclosporine, and tacrolimus.

558 Abbreviations: CDAI, clinical disease activity index; csDMARDs, conventional
559 synthetic disease modifying anti-rheumatic drugs; CRP, C-reactive protein; DAS28-ESR,
560 the 28 joint disease activity score incorporating erythrocyte sedimentation rate; HAQ-DI,
561 physical disability by health assessment questionnaire-disability index; IFX, infliximab;
562 MTX, methotrexate; SDAI, simplified disease activity index; RF, rheumatoid factor.

563

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

	Responders	Non-responders	<i>p</i> -value
<maximum effect point>			
IFX < 0.4 µg/mL, n (%)	9 (90.0)	1 (10.0)	0.43
IFX ≥ 0.4 µg/mL, n (%)	30 (96.8)	1 (3.2)	
Total (%)	39 (95.1)	2 (4.9)	
<measurement point>			
IFX < 0.4 µg/mL, n (%)	4 (40.0)	6 (60.0)	< 0.01
IFX ≥ 0.4 µg/mL, n (%)	30 (96.8)	1 (3.2)	
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.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figures

Figure 1

Figure 1

Please see the Manuscript PDF file for the complete figure caption

Figure 2

Figure 2

Please see the Manuscript PDF file for the complete figure caption

Figure 3

Figure 3

Please see the Manuscript PDF file for the complete figure caption

Figure 4

Figure 4

Please see the Manuscript PDF file for the complete figure caption

Figure 5

Figure 5

Please see the Manuscript PDF file for the complete figure caption

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplement.docx](#)