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Research Article

Are Trough Levels of Infliximab Superior to Antibodies to Infliximab for Assessing Loss of Response in Crohn's Disease? A Prospective Cohort Study

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- Crohn's disease
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Abstract

Background/Aims: Decreased trough levels of IFX (TLI) and antibodies to infliximab (ATI) are associated with loss of response (LOR) in Crohn's disease. Two prospective studies were conducted to determine whether TLI or ATI correlates better with LOR (Study 1), and whether TLI could become a predictor of mucosal healing (MH) (Study 2).

Methods: Study 1 was conducted in 108 patients, including those with LOR and remission to compare ATI and TLI (two assay ways were compared) in discriminating the two conditions based on receiver operating characteristic (ROC) curve analyses. Study 2 involved 35 patients who were evaluating dendoscopically.

Results: Study 1: There were no differences between the two assays in ROC curve analyses; the TLI cutoff value for LOR was 2.6 μg/ml (sensitivity 70.9%, specificity 79.2%), and the ATI cutoff value was 4.9 μg/ml (sensitivity 65.5%, specificity 67.9%). The AUROC (area under the ROC curve) of TLI was greater than that of ATI. AUROC was useful for discriminating between the two conditions. Study 2: The TLI was significantly higher in the colonic MH group than in the non-MH group.

Conclusion: TLI is better than ATI for clinically diagnosing LOR, and a correlation was observed between TLI and colonic MH.

ABBREVIATIONS

CD: Crohn's Disease; IFX: Infliximab; TLI: Trough Levels of IFX; ATI: Antibodies to Infliximab; MH: Mucosal Healing, ROC: Receiver Operating Characteristic; AUCROC: The Area Under the Receiver Operating Characteristic Curve; TNF: Tumor Necrosis Factor; CDAI: CD Activity Index; CS: Colonoscopy; DBE: Double-Balloon Enteroscopy; IR: Infusion Reactions; ADA: Adalimumab

INTRODUCTION

Infliximab (IFX) is a chimeric antibody preparation against tumor necrosis factor (TNF)-alpha, and, although it demonstrates a strong therapeutic effect in Crohn's disease (CD), loss of response (LOR) occurs in about 30-50% of patients during IFX maintenance therapy after remission induction [1,2]. It is said that LOR occurs in about 10% of patients undergoing IFX maintenance therapy every year [2-4]. It has also been reported that, because LOR occurs in more than 70% of patients overall, the IFX dose must be doubled or the patient switched to another anti-TNF-alpha antibody to re-induce and maintain remission [1]. The presence of antibodies to IFX (ATI), which correlate strongly

to infusion reactions, is believed to be a factor inducing LOR [5]. However, Maser et al. reported that the clinical effects of IFX treatment correlate more strongly to the trough level of IFX (TLI) than to the presence of ATI. Specifically, they concluded that TLI is linked to higher remission and endoscopic mucosal healing rates [6]. In any case, TLI must be monitored to determine the clinical effect.

Despite the fact that many studies mention the necessity of monitoring, and although many methods for measuring IFX blood concentrations have been reported, measurement standards and appropriate values for TLI and ATI values have yet to be established. In particular, evaluation of the ATI assay has been incomplete in the clinical setting. Moreover, recently, the goal of CD treatment has been shifting away from achieving clinical remission through IFX treatment and toward mucosal healing (MH), though the TLI required to achieve this goal has yet to be established.

Accordingly, in the present study, a novel assay was compared with a conventional assay in a prospective analysis to determine whether TLI and ATI are associated with LOR and MH.

MATERIALS AND METHODS

Patients and study design

The current study was a single-site, prospective study that was conducted in 215 CD patients who received IFX maintenance therapy [IFX infusions (5 mg/kg or 10 mg/kg) every 6 to 8 weeks] at Fukuoka University Chikushi Hospital, Department of Gastroenterology, between November 2012 and November 2014. The protocol was approved by the Institutional Review Board for Clinical Research of Fukuoka University Chikushi Hospital (November 2012, R12-036).

The subjects were patients 18 years and older in whom initial treatment induced remission, were undergoing maintenance therapy, and had been receiving IFX treatment for a minimum of 14 weeks and no longer than 5 years. The IFX dose (IFX 5 mg/kg or 10 mg/kg) and concomitant immunomodulatory use were not criteria for exclusion.

In addition, the TLI and ATI measurements used in this study and the assessment of endoscopic mucosal activity were performed blind, without knowledge of the results of either.

A total of 108 patients were enrolled in Study 1, in which the objective was to investigate the relationships of TLI and ATI with the clinical demographics. In study 2, 35 patients were enrolled to investigate the relationships of TLI and ATI with endoscopic mucosal healing (MH). The inclusion criteria for each of these studies are shown in Figure (1). Study 1 included 108 patients who met the following criteria: i) efficacy of initial infusion of IFX was response, were undergoing maintenance therapy, had received at least 4 treatments, and had been receiving treatments for ≤ 5 years; ii) provided informed consent to blood sampling to measure IFX blood concentrations; iii) their course could be followed up sufficiently; and iv) their CD activity index (CDAI) could be measured. Eleven of the 108 patients (10.2%) were receiving a dose of IFX of 10 mg/kg.

The 35 consecutive patients in Study 2 met the following

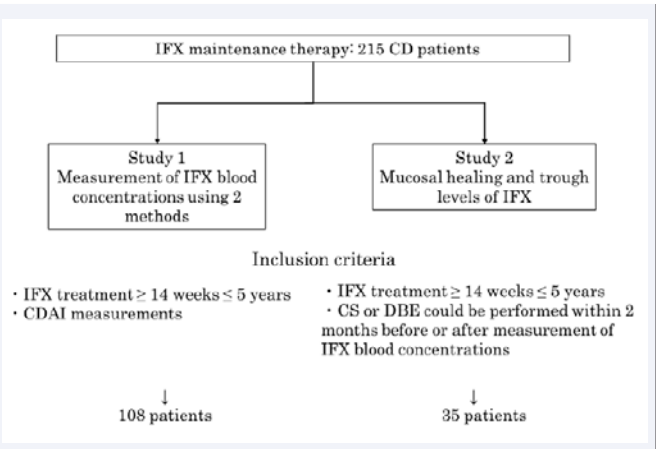

Figure 1 Subject selection and inclusion criteria.
Abbreviations: CD: Crohn's Disease; IFX: Infliximab; CDAI: Crohn's Disease Activity Index; TCS: Colonoscopy; DBE: Double-Balloon Endoscopy.

criteria: i) efficacy of initial infusion of IFX was response, and they had been undergoing maintenance therapy for at least 14 weeks; ii) were able to undergo colonoscopy (CS) or double-balloon enteroscopy (DBE) within 2 months before or after the date of IFX blood concentration measurement; iii) provided informed consent to blood sampling to measure IFX blood concentrations and to endoscopy; and iv) did not have an artificial anus. Nine patients (25.7%) were receiving an IFX dose of 10 mg/kg.

Each study was done according to the study design shown in Figure (2). In Study 1 the first assay (assay A vs. assay B) was done with patients divided clinically into a remission group and an LOR group after patient enrollment. Then, after about one year, we conducted a follow-up evaluation of patients among the remission group whose course had been closely followed.

In Study 2, assay A and endoscopy findings were compared after patient enrollment.

Measurements of IFX concentrations

Serum taken immediately before IFX infusion was used for TLI measurements. TLI measurements were conducted at Tanabe R&D Service Co., Ltd (assay A) and Shiga University of Medical Science (assay B). Measurements were performed blind, without disclosing patient background or clinical results.

Serum TLI measurements with assay A were conducted with an enzyme-linked immunosorbent assay using a monoclonal antibody against IFX obtained from Jansen Biotech Inc. (Horsham, PA, USA). The detection limit was 0.1 μg/ml [7].

Serum TLI measurements with assay B were conducted using an ELISA system using an avidin ELISA plate® (blocking-less type; Sumitomo Bakelite Co., Ltd, Tokyo, Japan) [8].

ATI measurement

With assay A, measurements were performed using an ELISA method based on a double-antigen format. If IFX is present in the blood it will compete with the labeled-IFX, making accurate measurement of ATI impossible. As a result, to obtain a positive

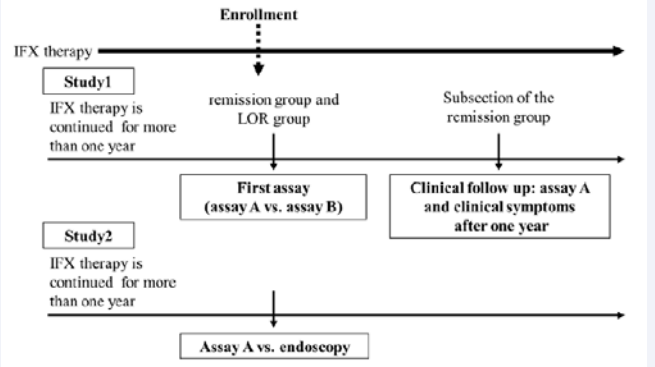

Figure 2 Overview of the study protocol in study1 and study2. The subjects were patients in whom IFX administration had been continued for more than one year. In Study 1, the first assay was done with the patients enrolled in the study divided into a remission group and an LOR group. Clinical follow-up was done for patients among the remission group whose detailed course was followed after one year. In Study 2, assay A and endoscopy were compared.

or negative result for ATI, the determination can only be made under conditions in which IFX is not present in the blood.

On the other hand, with assay B, ATI measurements were conducted using an original method developed by Shiga University of Medical Science called modified Direct-ELISA [9].

Measurement of clinical laboratory data

Biochemical markers such as C-reactive protein (CRP) were measured by the Laboratory Test Department of Fukuoka University Chikushi Hospital. Blood samples taken immediately before IFX infusion were also used for these measurements.

Assessment of clinical activity

The clinical activity index for IFX was assessed according to the CDAI [10]. A CDAI ≤ 150 indicates a clinically inactive state, while ≥ 150 indicates the active phase. In this study, the CDAI was measured within 8 weeks of the time IFX trough levels were measured, following infusion of IFX.

In Study 1, because the objective was to evaluate the clinical usefulness for diagnosing LOR, patients were classified as LOR or remission strictly based on the CRP level and CDAI score at the time IFX blood concentrations were measured. Remission was defined as CDAI < 150 points and CRP< 0.3 mg/dl. LOR was defined as CDAI ≥ 150 points and/or CRP ≥ 0.3 mg/dl.

Endoscopic Examination

The DBE models used were the Fujinon EN-580T, EN-450P5, and EN-450T5 (Fujinon Inc., Saitama, Japan); the CS models used were the Olympus PCF-240AI, PCF-PQ260I, PCF-Q260AI, and PCF-290I (Olympus, Tokyo, Japan). A transanal approach was used in all patients. DBE was performed in 18 patients, and CS was performed in 17 patients. The mean distance of small intestinal observation after passing through Bauhin's valve was 62 (7-150) cm. Lesions were assessed at the site where activity was the strongest that could be confirmed endoscopically.

Measurement of endoscopic activity

The Fukuoka index was used to evaluate endoscopic mucosal activity. There are essentially 3 components to this index: stenosis, polyposis, and ulcer [11]. In this study, ulcer scores were used to assess ileal and colorectal mucosa. Without using the polyposis score, Beppu et al. reported no link between the stenosis score and MH assessment [12]. For ileal and colorectal lesions, the sites where activity was the highest were assessed for the activity index. No lesion (0 point) or ulcer scarring (1 point) was defined as "mucosal healing (MH)," and an ulcer score of 2 to

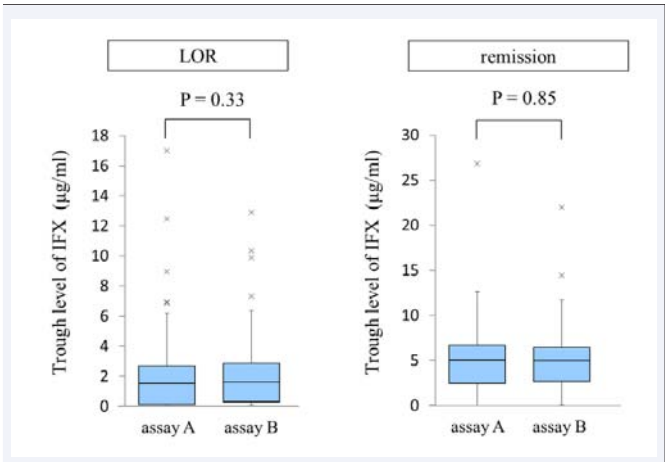

Figure 3a Comparison of trough levels of IFX between assay A and assay B.
Trough levels of infliximab (TLI) were measured in the LOR and remission groups using assays A and B. Serum drawn immediately before infliximab (IFX) infusion was used for TLI measurements. Mean TLI values with assays A and B are 2.4 ± 3.2 μg/ml vs. 2.3 ± 2.7μg/ml (P = 0.33) in the LOR group, and 5.2 ± 4.2 μg/ml vs. 5.2 ± 3.8μg/ml (P = 0.85) in the remission group, respectively.

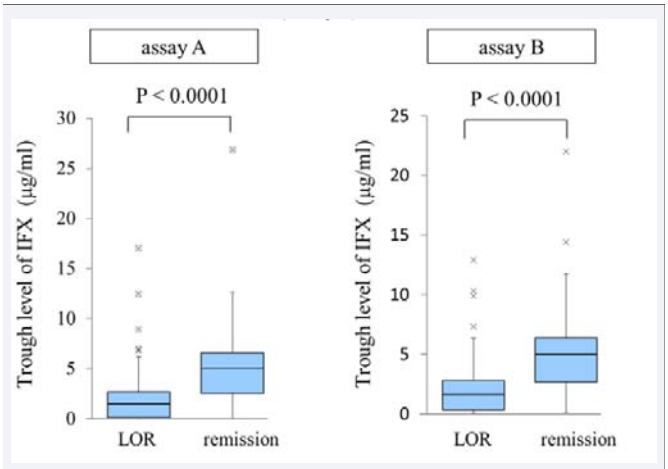

Figure 3b Comparison of trough levels of IFX between the LOR and remission groups
Assay A and assay B were used to measure trough levels of infliximab (TLI) in both the LOR and remission groups. Serum drawn immediately before infliximab (IFX) infusion was used for TLI measurements. Mean TLI values in the LOR and remission groups are 2.4 ± 3.2 μg/ml vs. 5.3 ± 4.2μg/ml (P < 0.0001) with assay A and 2.3 ± 2.7 μg/ml vs. 5.2 ± 3.8μg/ml (P < 0.0001) with assay B, respectively.

4 points was defined as "non-mucosal healing (nMH)." For small intestinal lesions, the sites evaluated were the small intestinal mucosa in patients with ileitis CD and ileocolitis CD. For colonic lesions, the colonic mucosa in colitis CD and the colonic mucosa in ileocolitis CD were the sites evaluated.

Statistical analyses

Fisher's exact test or the Mann-Whitney U-test was used in two-group comparisons, and to analyze the diagnostic ability of TLI and ATI, cutoff values were established for each using the minimum distance criteria from the area under the receiver operating characteristic curve (AUROC). Significance was defined as a p value ≤ 0.05. Statistical analysis was performed using SPSS Statistics 21.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Study 1

Characteristics: Based on CDAI and CRP, the 108 CD patients were categorized and placed in either the LOR group or the remission group. The characteristics of the patients in the two groups are shown in Table (1). There were no clear significant differences in the male to female ratio, age at initial IFX infusion, surgical history, or anal lesions. However, disease duration was slightly longer in the LOR group than in the remission group (9.5 vs. 6.8 years, P = 0.051). Ileocolitis tended to be the most common type of disease in both groups. The duration of IFX treatment was approximately 3 years in both groups (3.1 vs. 3.6 years, P = 0.0658). There were no significant differences in the concomitant medications used at the time IFX blood concentrations were measured. IFX 10 mg/kg infusions were used significantly more frequently in the LOR group than in the remission group (18.2% vs. 1.9%, P = 0.0082).

Comparison of trough levels of IFX between assay A and assay B: TLI was compared in the LOR and remission groups using both assay A and assay B Figure (3 a,b). The overall results showed no differences between the groups in TLI (LOR: 2.4 ± 3.2 vs. 2.3 ± 2.7 μg/ml, P = 0.33; remission: 5.3 ± 4.2 vs. 5.2 ± 3.8 μg/ml, P = 0.85). When analyzed by assay, values were significantly lower in the LOR group than in the remission group with each assay (assay A: 2.4 ± 3.2 vs. 5.3 ± 4.2 μg/ml, P < 0.0001; assay B: 2.3 ± 2.7 vs. 5.2 ± 3.8 μg/ml, P < 0.0001).

Comparison of results of ATI by assay A and assay B: The results of ATI measured in assay A are shown in Figure (4). The numbers of ATI positive and ATI negative patients were small in both the LOR group and the remission group. Many patients in both the LOR group and the remission group were inconclusive ATI.

Next, 108 patients whose ATI values were measured using assay B are shown in Fig. 5. A comparison of ATI levels in the LOR and remission groups showed that ATI was significantly higher in the LOR group (18.4 ± 30.1 vs. 6.5 ± 9.2 μg/ml, P = 0.0014).

Comparison of TLI and ATI measurements: TLI and ATI values as determined assay B were compared using AUROC to determine their associations with LOR Figure (6a,b). The LOR cutoff value for TLI was 2.6 μg/ml (sensitivity 70.9%, specificity 79.2%, PPV 77.6%, NPV 71.2%), while that for ATI, was 4.9 μg/ml (sensitivity 65.5%, specificity 67.9%, PPV 67.9%, NPV 65.5%).

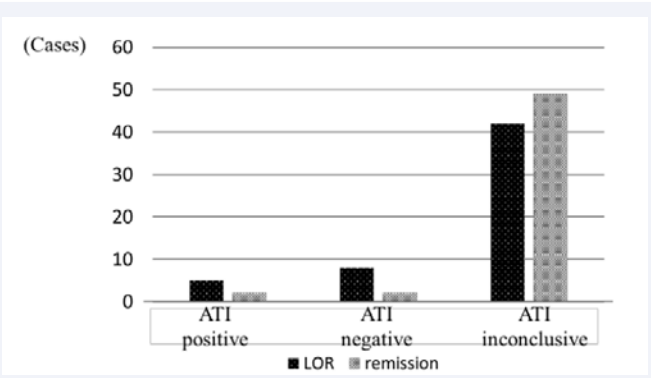

Figure 4 Comparison of ATI levels by assay A in the LOR and remission groups (Study 1).
This is a graph of ATI assessed using assay A. In the LOR group there were 5 ATI positive patients, 8 ATI negative patients, and 42 inconclusive ATI patients. In the remission group there were 2 ATI positive patients, 2 ATI negative patients, and 49 inconclusive ATI patients. In assay A, ATI measurements were inconclusive in both the LOR group and the remission group.

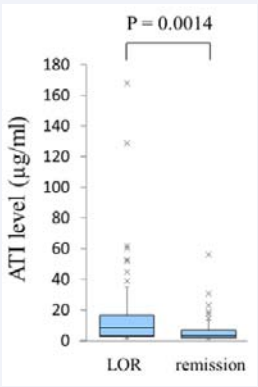

Figure 5 Comparison of ATI levels by assay B in the LOR and remission groups.
Assay B was used to measure antibodies to infliximab (ATI) levels in both the LOR and remission groups. The mean ATI level is 18.4 ± 30.1 μg/ml in the LOR group and 6.5 ± 9.2 μg/ml in the remission group (P = 0.0014). IFX trough levels < 0.1 μg/ml were recorded as 0 μg/ml.

First, using the ATI results from the previous assay A and the ATI cutoff value results from assay B, we compared the sensitivity, specificity, positive predictive value, and negative predictive value for ATI from assays A and B Table (2). The results showed that the sensitivity, specificity, and negative predictive value were lower in assay A than in assay B.

Next, a comparison of the AUROC for TLI and ATI revealed that the AUROC of TLI was larger than that of ATI (77.8% vs. 67.9%). These results showed that TLI has a high capacity for discrimination.

The percentage of patients positive for ATI in the LOR and remission groups was also investigated (Figure 7). With assay A,

Table 1: Characteristics of CD patients with infliximab maintenance treatment (Study 1).

	LOR (n=55)	Continued remission (n=53)	P value
Female/male	41 / 14	Oct-43	n.s
Duration of symptoms (year) [mean (range)]	9.5 (0-31)	6.8 (0-31)	0.0512
Type of disease			
Ileitis/Ileocolitis/Colitis	16 / 33 / 6	20 / 31 / 2	
Age (years) at initial infusion [mean (range)]	32.8 (16-64)	29.9 (13-55)	0.0829
Duration of IFX treatment (years) [mean (range)]	3.1 (1-5)	3.6 (1-5)	0.0658
Prior CD surgery (%)	35 (63.6)	29 (54.7)	n.s
Anal fistula (%)	25 (45.5)	17 (32.1)	n.s
Total CDAI score ≥150 (%)	29 (54.7)	0 (0.0)	<0.0001
CRP ≥ 0.3 mg/dl (%)	45 (81.8)	0 (0.0)	<0.0001
Concomitant medications at IFX initial infusion (%)			
5-Aminosalicylates	44 (80.0)	38 (71.7)	n.s
Prednisolone	5 (9.1)	5 (9.4)	n.s
Current immunosuppressant	15 (27.3)	11 (20.8)	n.s
Elemental diet	13 (23.6)	13 (24.5)	n.s
Number of IFX 10mg/kg (%)	10 (18.2)	1 (1.9)	0.0082

Abbreviations: CD: Crohn's Disease; IFX: Infliximab; CDAI: Crohn's Disease Activity Index

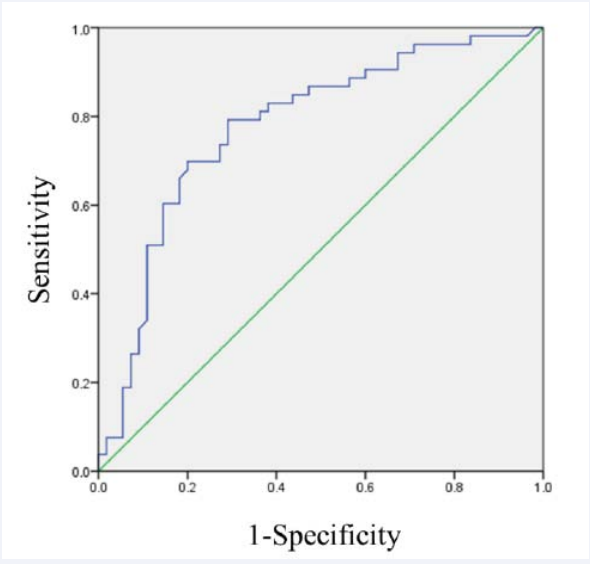

Figure 6a ROC curve and cutoff value of the IFX trough level by assay B (Study 1)
Receiver operating characteristic (ROC) curve-cutoff value of the infliximab trough level in Crohn's disease was calculated as was association between the infliximab trough level and loss of response, with corresponding sensitivity and specificity for Crohn's disease. Cutoff value, 2.6 μg/ml; AUROC, 77.8%; sensitivity, 70.9%; specificity, 79.2%; PPV, 77.6%; NPV, 71.2%. AUROC, Area under the ROC curve; PPV, positive predictive value; NPV, negative predictive value.

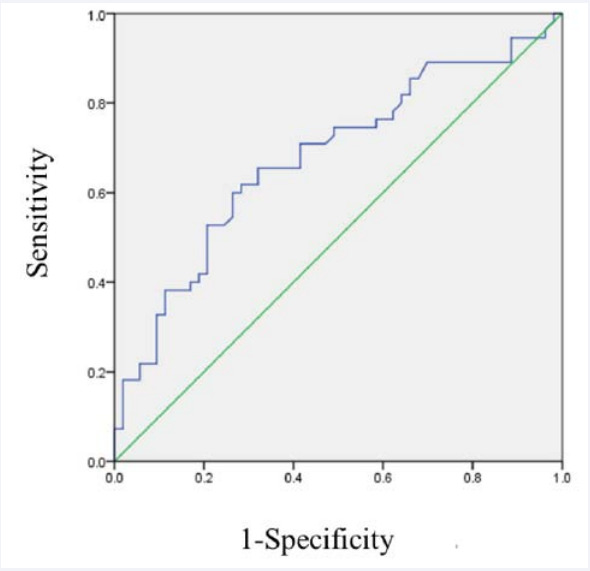

Figure 6b Cutoff value of ATI by assay B
Receiver operating characteristic (ROC) curve-cutoff value of antibodies to infliximab (ATI) in Crohn's disease was calculated as was association between ATI and loss of response, with corresponding sensitivity and specificity for Crohn's disease. Cutoff value, 4.9 μg/ml; AUROC, 67.9%; sensitivity, 65.5%; specificity, 67.9%; PPV, 67.9%; NPV, 65.5%. AUROC, area under the ROC curve; PPV, positive predictive value; NPV, negative predictive value.

it was not possible to accurately compare ATI-positive and ATI-negative cases, because a relatively large number of patients were inconclusive for ATI. Looking at the results of assay B, the rate of ATI was seen to be significantly higher in the LOR group than in the remission group (65.5% vs. 32.1%, P = 0.0006). However, these data also showed that ATI was positive in a high percentage (32.1%) of the remission group.

Follow-up of the ATI-positive group in remission: In the remission group, patients with an ATI ≥ 4.9 μg/ml with assay B (n = 17) were categorized as ATI-positive, and those with ATI ≤ 4.9 μg/ml (n = 34) were categorized as ATI-negative, and the incidence of infusion reactions (IR), incidence of LOR, and percent decrease in TLI were investigated in patients whose course could be followed in detail after 1 year (Figure 8). An infusion reaction (IR) was defined as unable to continue IFX. LOR was defined as in study 1 and was evaluated by measuring CDAI and CRP after 1 year. A TLI decrease was defined as a ≥50% decrease in TLI from the initial measurements. The results of these investigations showed that IR tended to occur more readily in ATI-positive patients (17.6% vs. 2.9%, P = 0.0967), but otherwise no differences were observed in the incidence of LOR (6.7 vs. 2.9%, P = 0.523) or in the decrease in TLI (7.7% vs. 0%, P = 1.0000). In addition, 3 of the 17 patients who were positive for ATI and 1 of the 34 patients negative for ATI discontinued IFX or switched to ADA due to an IR. However, during the course of 1 year, ATI-positive patients did not trend in a major way toward LOR.

Study 2

Based on the results of study 1, because TLI showed a better ability to discriminate than ATI, and there were no differences

Table 2: Se, Sp, PPV, NPV of ATI by assay A and Assay B (Study 1).

	Sensitivity	Specificity	PPV	NPV
ATI of assay A	0.38	0.5	0.71	0.2
ATI of assay B	0.68	0.65	0.65	0.68
Assay A				
	LOR group	Remission group		Total
ATI poistive	5	2		7
ATI negative	8	2		10
Total	13	4		17
ATI inconclusive	42	49		91
Assay B				
	LOR group	Remission group		Total
ATI poistive	36	17		53
ATI negative	19	36		55
Total	55	53		108

Abbreviations: Se: Sensitivity; Sp: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value

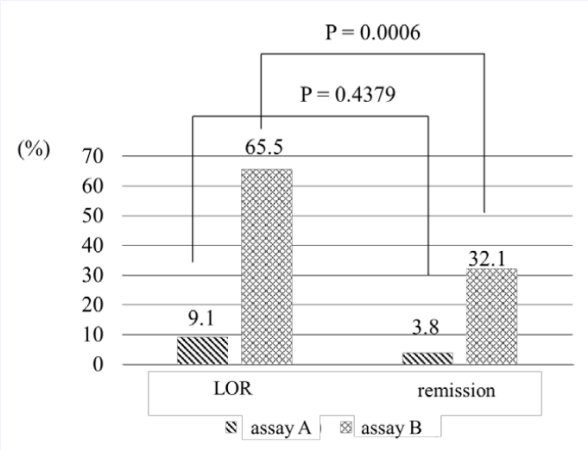

Figure 7 ATI-positive rates in the LOR group and in the remission group

Assay B was used to measure the percentage of patients with antibodies to infliximab (ATI) in both the LOR and remission groups. ATI-positive rates with assays A and B, respectively, are 9.1% vs. 65.5% in the LOR group, and 3.8% vs. 32.1% in the remission group. Comparison of ATI-positive rates in the LOR and remission groups shows p = 0.4379 with assay A, compared to p = 0.0006 with assay B.

between the assay methods in terms of the results, the relationship between TLI and mucosal assessment was investigated using assay A.

Characteristics of the patients in study 2: Table (3) shows the characteristics of the 35 CD patients of study 2. The cohort trended toward patients with a relatively young age at diagnosis (22 years), by sex toward men, and by disease type toward ileocolitis. IFX treatment duration was approximately 3 years. Time between measurement of TLI and the endoscopy

Table 3:

Age at diagnosis (years)[mean (range)]	22.0±7.0 (11-48)
Female/male	4 /31
Type of disease [n]	
Ileitis/Ileocolitis/Colitis	14/19/2
Duration of IFX treatment (years) [mean (range)]	2.8 ± 1.8 (0-5)
Number of IFX 10 mg/kg [n (%)]	9 (25.7)
Time from IFX concentration measurement to endoscopy (months) [mean (range)]	0.3 ± 0.5 (0-2)
The median length of ileum inserted (cm) [mean (range)] (n=21)	62.0 ± 50.0 (7-150)
CS*/DBE	17/18
CDAI [mean (range)]	121.9 ± 74.9 (25-299)
CRP [mean mg/dl (range)]	0.9 ± 1.5 (0.01-7.8)
Concomitant therapy (%)	
5-Aminosalicylate	25 (71.4)
Prednisolone	1 (2.9)
Enteral nutrition (>900 kcal/day)	8 (22.9)
Immunomodulators	15 (42.9)
Previous major abdominal surgery [n (%)]	26 (74.3)
Anal fistula [n(%)]	16 (45.7)

Abbreviations: IFX: Infliximab; CDAI: Crohn's Disease Activity Index; CS: Colonoscopy; DBE: Double-Balloon Endoscopy.

procedure was 0.3 months. In contrast to CDAI, which was in a state of remission at the time of IFX measurement, CRP levels were high (CDAI 122 and CRP 0.9 mg/dl). With regard to concomitant therapy, 8 patients (22.9%) were receiving ≥ 900 kcal/day enteral nutrition, and 15 patients (42.9%) were taking an immunomodulator.

Comparison of IFX trough levels between the MH group and the nMH group: The 31 patients who had small intestinal lesions were classified and assigned to the MH group (10 patients) or the nMH group (21 patients) (Figure 9a). Comparison of patients with small intestinal lesions revealed no significant difference between the MH and nMH groups in terms of TLI (2.5 vs. 1.8 μg/ml, P = 0.38). No relationship between the MH group and nMH group was seen with regard to patients taking IFX 10 mg/kg (30.0% vs. 23.3%, P = 1.000) or patients positive for ATI (10.0% vs. 14.3%, P = 1.000).

Next, the 21 patients with large intestinal lesions were classified and assigned to either the MH group (13 patients) or the nMH group (8 patients) (Figure 9b). TLI levels were significantly higher in the MH group than in the nMH group (2.7 vs. 0.5 μg/ml, P = 0.032). However, no relationship between the MH group and the nMH group was seen with regard to patients taking IFX 10 mg/kg (15.4 vs. 37.5%, P = 0.3254) or patients positive for ATIs (0 vs. 25.0%, P = 0.3333).

Based on the above, it was concluded that colorectal mucosal healing (MH) is obtained with TLI ≥ 2.7 μg/ml.

Comparison of the characteristics of the MH and nMH groups of the small intestine and colon: We investigated

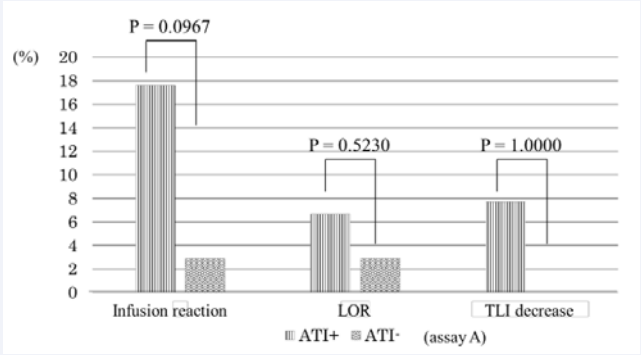

Figure 8 Follow-up of patients in the remission group for one year after initial TLI measurements.
Patients in the remission group were separated into groups that were ATI-positive (ATI > 4.9 μg/ml) and ATI-negative (ATI ≤ 4.9 μg/ml). The follow-up observation period was 12.1 ± 2.0 months (mean ± SD). Infusion reactions occurred in 3 of 17 (17.6%) ATI-positive patients and 1 of 34 (2.9%) ATI-negative patients. LOR after 1 year: With LOR defined as CDAI ≥ 150 and CRP ≥ 0.3 mg/dl, CDAI and CPR were measured after approximately 1 year, and the percentages of patients who developed LOR were measured. After 1 year, LOR had occurred in 1 of 15 (6.7%) ATI-positive patients and 1 of 34 (2.9%) ATI-negative patients. TLI decrease: TLI had decreased ≥ 50% in 1 of 13 (7.7%) ATI-positive patients and in 0 of 7 (0%) ATI-negative patients. ATI, antibodies to infliximab; IR, infusion reaction; LOR, loss of response.

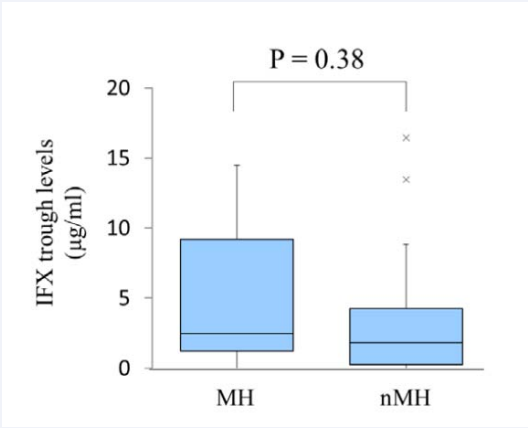

Figure 9a Comparison of IFX trough levels between the MH group and the nMH group with lesions of the small intestine. (Study 2)
Mucosal healing (MH) has occurred in 10 patients, and there are 21 patients in the nMH group. TLI (median values) in the MH and nMH groups are 2.5 vs. 1.8 μg/ml, respectively. TLI in the MH and nMH groups shows no significant difference (P = 0.38). There are 3 patients (30.0%) in the MH group and 5 patients (23.3%) in the nMH group receiving infliximab (IFX) 10 mg/kg. Number of patients positive for antibodies to infliximab (ATI) with assay A: 1 patient (10.0%) in the MH group and 3 patients (14.3%) in the nMH group.

whether there were any significant differences between the MH group and the nMH group in small intestine and colon lesions (Table 4). There were no background factors that showed a significant difference between the MH group and the nMH group for small intestinal lesions. For the colon, however, a tendency was seen for the CRP value to be lower in the MH group than in the nMH group (0.5±0.6 vs. 0.9±1.0 mg/dl; P=0.0597).

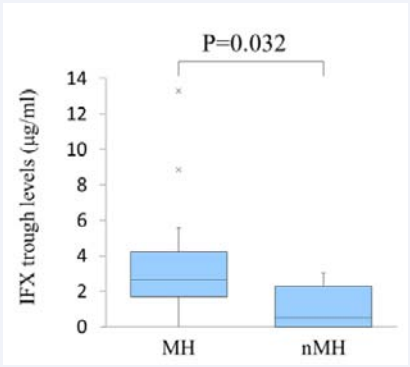

Figure 9b Study 2 Comparison of IFX trough levels between the MH group and the nMH group with lesions of the large intestine
There are 13 patients with mucosal healing (MH) and 8 patients in the nMH group. TLI (median values) in the MH and nMH groups are 2.7 vs. 0.5 μg/ml, respectively. Comparison of TLI between the two groups shows asignificant difference (P = 0.032). There are 2 patients (15.4%) in the MH group and 3 patients (37.5%) in the nMH group receiving infliximab (IFX) 10 mg/kg. Number of patients positive for antibodies to infliximab (ATI) with Assay A: 0 patients (0.0%) in the MH group and 2 patients (25.0%) in the nMH group.

Table 4: Comparision of the characteristics of the MH and nMH groups of small intestine and colon in study 2

Small intestine	MH	nMH	P value
patients	10	21	
Age at diagnosis (years) [mean(range)]	18.6(15-32)	29.9(11-48)	0.0898
Female/ Male	1//9	2//19	n.s
Duration of IFX treatment (years) [mean(range)]	2.5(0-5)	2.8(0-5)	0.5882
Number of IFX 10mg/kg	3	5	n.s
The median length of ileum inserted (cm) [mean(range)]	68 ± 57.6 (7-150)	45.1 ± 48.4(9-150)	0.2811
Total CDAI Score [mean (range)]	103.5 ± 57.4(42-285)	134 ± 77.9(25-299)	0.2907
CRP [Mean(range)]	1 ± 2.4(0.1-7.8)	0.8 ± 1.5(0.0-2.4)	0.3102
Number of current immunosuppressant	5	6	0.4232
Colon	MH	nMH	P value
Patients	13	8	
Age at diagnosis (years) [mean(range)]	22.1 (11-34)	21.8 (16-26)	0.942
Female/ Male	1//12	1//7	n.s
Duration of IFX treatment (years) [mean(range)]	2.3(0-5)	2.4 (0-5)	0.6556
Number of IFX 10mg/kg	2	3	0.3254
Total CDAI Score [mean (range)]	128.6 ± 85.9 (31-299)	126.5 ± 75.4 (25-207)	0.828
CRP [Mean(range)]	0.5 ± 0.6 (0.0-2.3)	0.9 ± 1.0(0.1-3.3)	0.0597
Number of current immunosuppressant	5	4	0.6731

Abbreviations: CD: Crohn's Disease; IFX: Infliximab; CDAI: Crohn's Disease Activity Index

DISCUSSION

This prospective study examined whether TLI or ATI was useful for the evaluation of LOR. Furthermore, our study is the first report to clearly demonstrate that MH of the colon is related to TLI.

In the present study, TLI and ATI were measured after approximately three years of IFX maintenance treatment. In an early study, many CD cases (23-46%) who became LOR within one year received IFX [2]. However, the period of IFX administration was 2.7 years in the report by Yamada in 2010[13]. In the report by Warman and others in 2014, the period of IFX administration was about 3.4 years [14].Based on these earlier reports, it was thought that three years of IFX administration was appropriate for the present study.

In the present study, CDAI and CRP were combined, clinical remission and LOR were defined strictly, and the results of TLI and ATI were collated. Whether TLI or ATI was useful for discrimination of LOR was determined using AUROC analysis.

There are various methods for the measurement of TLI and ATI. However, reports that use two or more measuring methods in a clinical trial are few. The present study compared the results of two typical measurement methods (Tanabe R & D, assay A; Shiga University of Medical Science, assay B) using the same serum. This was done because there is a significant problem in ATI measurement with assay A. It is known that there are inconclusive cases in which ATI cannot be measured, although it can be measured in 54-70% of cases; there are cases, however, that do not satisfy the requirements of assay A for measuring ATI [4,5,7].Therefore, development of a method to measure ATI that does not depend on the serum IFX density was urgently needed. Imaeda may have solved this problem by raising the sensitivity of the ATI value using a new measurement method (Direct ELISA; DA ELISA and IC-based ELISA).The present study examined the clinical value of ATI measurement using this method and the DA ELISA method.

The results showed that assay A had clearly lower sensitivity, specificity, and negative predictive value for ATI than assay B. In the LOR group, the ATI positive rate was higher in assay B than in assay A. We therefore judged that clinical activity could not be satisfactorily evaluated with the ATI results from assay A.

In addition, the ATI level in assay B was significantly higher in the LOR group, while TLI levels were significantly lower in the LOR group than in the remission group. These results show that the presence of ATI is related to a drop in TLI when the ATI level does not depend on serum IFX, as is the case with assay B. However, there have been few reports that compared TLI and ATI evaluations of clinical LOR with high discrimination ability. AUROC analysis is useful for evaluating discrimination ability. Nanda performed a meta-analysis to compare ATI and TLI in a current study and a past report using an AUROC approach. Only DA ELISA was used for most ATI measurements and 5 high-quality reports of ATI sensitivity as high as that in the present study have been published [15]. There have been only 3 reports, including the present study that compared TLI with ATI by AUROC analysis (Table 5) [16,17]. Both sensitivities and specificities of TLI and ATI were at least 80% in the report of Steenholdt, and AUROC was high, at about 90%; the conclusion was that both ATI and TLI should be used in the evaluation of clinical LOR. On the other hand, Vande Casteele and others measured the cut-off levels of ATI and TLI in 483 cases that were combined from four studies. TLI appeared to have a strong relationship with remission, and ATI had a strong relationship with the active phase (CRP>5 mg/L) [17]. It was again concluded that both TLI and ATI should be measured, although an AUROC analysis to compare the usefulness of TLI and ATI for the clinical outcome was not performed. However, the AUROC of TLI was high in each case. This is certain to depend on the measurement technique of ATI and has the possibility of greatly contributing to the clarification of LOR. However, the present results suggest that measurement of TLI alone is sufficient for the evaluation of clinical LOR.ATI appears to have a supplementary role; it may be appropriate to measure ATI when the TLI value is low and clinical LOR is suspected or when an IR may have occurred. A low ATI did not affect the long-term prognosis when the investigation of ATI positivity included many cases of ATI positivity in the remission group in the present study and in the remission group at one year.

In Study 2, the relationship between endoscopic MH and TLI was examined based on the results of Study 1.The TLI necessary for small intestinal MH and colonic MH was examined in this study based on the supposition that it was different. In a recent report MH was defined using a different endoscopic score than that used in the present study. However, most reports define MH as disappearance of the ulcer lesion. The definition of MH in the present study used the ulcer score of the Fukuoka index. The reason why this definition was used in the present study was that, using the Fukuoka index, Beppu enumerated the points for calculating the scores at which the small intestinal lesions and the colon change to a morbid state were separately appreciable. Additionally, it was reported that small intestinal MH and colonic MH were related to clinical remission [12]. Moreover, the average IFX administration period in the present study was 2.8 years. When complete MH of 1-2 years was a predictive factor for remission maintenance for more than four years, Beppu and others reported that it occurred with IFX treatment. In addition, it has been reported that steroid-free remission was obtained

Table 5: Comparison of TLI and AT I studies using ROC curve analysis.

Author	year	n	LOR vs remission	TLI	AUC	Se/Sp (%)	ATI level	AUC	Se/Sp (%)
Steenholdt et al., [11]	2011	85	26 vs 59	0.5	0.93	86/85	10U/ml	0.89	81/90
Vanda Casteele et al., [17]	2014	483	N/A	2.79	0.681	52.5/77.6	3.15U/ml	0.632	38/87.4
Present Study	2015	108	55 vs 53	2.6	0.778	70.9/79.2	4.9μg/ml	0.679	65.5/67.9

Abbreviations: LOR: Loss of Response; TLI: Trough Level of Infliximab; AUC: Area Under the Curve; Se: Sensitivity; Sp: Specificity; ROC: Receiver Operating Characteristic Curve; ATI: Antibody to Infliximab.

in four years when complete mucous membrane recovery was reached at an early stage [18]. Imaeda and others reported the association of MH and TLI on examination at a median of approximately three years [19]. From this result, it was thought that endoscopy should be performed at approximately three years in the present study; furthermore, it is valuable to have performed endoscopic evaluation in cases with long-term IFX use. The results of Study 2 appeared to show that colonic MH and TLI were causally related, while there was no significant relationship between small intestinal MH and TLI.

The reasons why no significant differences were seen in TLI and MH in small intestinal lesions are thought to be the following i) Endoscopic observation is easy in large intestinal lesions and detailed lesions can be identified. As a result, findings that agree with clinical symptoms can be obtained. With small intestinal lesions, however, it is not easy to observe the entire small intestine and the lesion areas are small. It is possible that clinical symptoms and small intestinal lesions do not agree because of the tendency to identify very small lesions. ii) The effectiveness of IFX for small intestinal lesions may be lower than that in the large intestine. Imaeda et al. reported that TLI of ≥ 4.0 μg/ml was needed in MH [13]. Additionally, Ungar et al. reported that 80-90% of patients achieve MH with a TLI of 6-10 μg/ml and that the MH achievement rate becomes higher as the TLI value increases [20]. Although those authors did not classify and score small intestinal lesions and large intestinal lesions, considering those reports our findings suggest that higher TLI is needed in order to achieve small intestinal MH. The above reasons may therefore explain why no significant differences were seen between small intestinal MH and TLI. At the same time, no significant relationship was seen between the MH group and the nMH group in either the small intestine or the large intestine with ATI, although this was a comparison using assay A. In the large intestine there was also a tendency to achieve MH when the CRP value was low, but no relationship was seen between other background factors and achieving MH. Imaeda et al reported that ATI and MH had only a weak relationship [19]; in the present study, MH and ATI also had a weak relationship.

Whether combined therapy with an immunomodulator is related to TLI was evaluated.TLI was not higher in LOR and remission groups even with combined use of an immunomodulator.

In conclusion, the present study showed that TLI was more useful for diagnosis and the evaluation of LOR in CD during IFX maintenance therapy than ATI; ATI appears to have a supporting role in LOR evaluation. In addition, remission could be evaluated only by TLI.As for colonic MH, a relationship with TLI was observed; for remission, TLI needed to be greater than 2.6 μg/ml.

This study has several limitations. Although Study 1 was a prospective study there was no follow up from the first IFX administration. Furthermore, with limitation to the cross-sectional period only patients receiving long-term IFX were enrolled. Recent reports have shown that ATI can exist as stable ATI or transient ATI, and that transient ATI sometimes appears coincidentally during the time a patient is receiving IFX and does not affect LOR. Stable ATI, however, is reported to affect LOR. Therefore, multiple ATI measurements are recommended since it cannot be determined whether ATI is stable or transient with a single measurement. There are also reports that in determining LOR a more accurate prediction is possible with a combination of CRP, TLI, and stable ATI [21,22,23]. Since ATI was measured only once in this study, it could not be determined whether it was stable or transient ATI. Moreover, ATI-positive patients in the remission group were taken to be patients who would not experience LOR in at least one year and in whom TLI would not significantly decrease; however, the possibility cannot be ruled out that many cases of transient ATI were also included. Nevertheless, from reports that transient ATI does not affect LOR and that multiple ATI measurements are recommended, we recommend measurement of TLI for clinical purposes. TLI measurement means that LOR can be determined with a single measurement, rather than having to perform multiple measurements to determine whether ATI is stable or transient. The limitations of Study 2 are thought to be that the number of patients who could participate in the study was small and that the entire small intestine could not be observed.

CONCLUSION

In conclusion, the present study showed that TLI was more useful for diagnosis and the evaluation of LOR in CD during IFX maintenance therapy than ATI; ATI appears to have a supporting role in LOR evaluation. In addition, remission could be evaluated only by TLI.As for colonic MH, a relationship with TLI was observed; for remission, TLI needed to be greater than 2.6 μg/ml.

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REFERENCES

1. Gisbert JP, Panés J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. Am J Gastroenterol. 2009; 104: 760-767.

2. Ben-Horin S, Chowers Y. Review article: loss of response to anti-TNF treatments in Crohn's disease. Aliment Pharmacol Ther. 2011; 33: 987-995.

3. Schnitzler F, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, et al. Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. Gut. 2009; 58: 492-500.

4. Hanauer SB, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Maintenance infliximab for Crohn's disease: ACCENT1 randomised trial. Lancet. 2002; 359: 1541-1549.

5. Danese S, Fiorino G, Reinisch W. Review article: Causative factors and the clinical management of patients with Crohn's disease who lose response to anti-TNF-α therapy. Aliment Pharmacol Ther. 2011; 34: 1-10.

6. Maser EA, Villela R, Silverberg MS, Greenberg GR. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. Clin Gastroenterol Hepatol. 2006; 4: 1248-1254.

7. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, et al. Therapeutic efficacy of multiple intravenous infusions of anti-

tumor necrosis factor a monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. Arthritis Rheum. 1998; 41:1552-1563.

8. Imaeda H, Takahashi K, Fujimoto T, Bamba S, Tsujikawa T, Sasaki M, et al. Clinical utility of newly developed immunoassays for serum concentrations of adalimumab and anti-adalimumab antibodies in patients with Crohn's disease. J Gastroenterol. 2014; 49: 100-109.

9. Imaeda H, Andoh A, Fujiyama Y. Development of a new immunoassay for the accurate determination of anti-infliximab antibodies in inflammatory bowel disease. J Gastroenterol. 2012; 47: 136-143.

10. Best WR, Becktel JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology. 1976; 70: 439-444.

11. Sou S, Matsui T, Yao T, Yorioka M, Tsuda S, Kikuchi Y, et al: Clinical and endoscopic healing after infliximab treatment in patients with Crohn's disease. Dig Endosc. 2006; 18: 29-33.

12. Beppu T, Ono Y, Matsui T, Hirai F, Yano Y, Takatsu N, et al. Mucosal healing of ileal lesions is associated with long-term clinical remission after infliximab maintenance treatment in patients with Crohn's disease. Dig Endos. 2015; 27: 73-81.

13. Yamada A, Sono K, Hosoe N, Takada N, Suzuki Y. Monitoring functional serum antitumor necrosis factor antibody level in Crohn's disease patients who maintained and those who lost response to anti-TNF. Inflamm Bowel Dis. 2010; 16: 1898-1904.

14. 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: 242-248.

15. Nanda KS, Cheifetz AS, Moss AC. Impact of antibodies to infliximab on clinical outcomes and serum infliximab levels in patients with inflammatory bowel disease (IBD): a meta-analysis. Am J Gastroenterol. 2013; 108: 40-47.

16. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OØ, Ainsworth MA. Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. Scand J Gastroenterol. 2011; 46: 310-318.

17. Vande Casteele N, Khanna R, Levesque BG, Stitt L, Zou GY, Singh S, et al. The relationship between infliximab concentrations, antibodies to infliximab and disease activity in Crohn's disease. Gut. 2015; 64: 1539-1545.

18. Baert F, Moortgat L, Van Assche G, Caenepeel P, Vergauwe P, De Vos M, et al. Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease. Gastroenterology. 2010; 138: 463-468.

19. Imaeda H, Bamba S, Takahashi K, Fujimoto T, Ban H, Tsujikawa T, et al. Relationship between serum infliximab trough levels and endoscopic activities in patients with Crohn's disease under scheduled maintenance treatment. J Gastroenterol. 2014; 49: 674-682.

20. Ungar B, Levy I, Yavne Y, Yavzori M, Picard O, Fudim E, et al. Optimizing Anti-TNF-α Therapy: Serum Levels of Infliximab and Adalimumab are Associated with Mucosal Healing in Patients with Inflammatory Bowel Diseases. Clin Gastroenterol Hepatol. 2016; 14: 550-557.

21. Roblin X, Marotte H, Leclerc M, Del Tedesco E, Phelip JM, Peyrin-Biroulet L, et al. Combination of C-reactive protein, infliximab trough levels, and stable but not transient antibodies to infliximab are associated with loss of response to infliximab in inflammatory bowel disease. J Crohns Colitis. 2015; 9: 525-531.

22. Vande Casteele N, Gils A, Singh S, Ohrmund L, Hauenstein S, Rutgeerts P, et al. Antibody response to infliximab and its impact on pharmacokinetics can be transient. Am J Gastroenterol. 2013; 108: 962-971.

23. Ungar B, Chowers Y, Yavzori M, Picard O, Fudim E, Har-Noy O, et al. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. Gut. 2014; 63: 1258-1264.

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