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Development of a C1q-immobilized (Cim) assay to measure total antibodies to infliximab and its clinical relevance in patients with inflammatory bowel disease

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ABSTRACT

Objective: Determination of antibodies to infliximab (ATI) is desirable for the management of patients with inflammatory bowel disease (IBD) who receive infliximab. Conventional ligand-binding ATI-assays detect only free-form of ATI, potentially increasing the proportion of patients with undetectable ATI, but with adequate trough infliximab (TRI) level who experience loss of response (LOR) to infliximab. We investigated this assertion using a novel ATI-Cim assay.

Methods: An ATI-Cim assay was developed by utilizing a C1q-immobilized plate, detecting free-form and ATI-infliximab complexes. Plasma ATI in 137 consecutive IBD patients, 56 with sustained clinical response (SCR), 76 with LOR and 5 with infusion reactions was measured.

Results: ATI levels reached a plateau following addition of up to 25 μg/mL infliximab to different concentrations of free-form ATI. ATI concentration did not significantly change during infliximab infusion ($P = 0.4316$). ATI concentration > 0.153 μg/mL was associated with LOR (odds ratio 3.0: 95%, confidence interval 1.5 to 6.1, $P = 0.0029$). The number of patients with undetectable ATI was higher in SCR than in LOR, 53.6% vs 22.4% ($P = 0.0004$). Patients with SCR and LOR were divided into 4 subgroups by combined cut-off ATI and TRI values. (A) ATI > 0.153 μg/mL and TRI ≤ 2 μg/mL; (B) ATI > 0.153 μg/mL and TRI > 2 μg/mL; (C) ATI ≤ 0.153 μg/mL and TRI ≤ 2 μg/mL; (D) ATI ≤ 0.153 μg/mL and TRI > 2 μg/mL. The frequency of LOR showed a decreasing trend from subgroup A to D, 80.8%, 64.1%, 55.2% and 36.8%, respectively ($P = 0.0003$).

Conclusions: The measured ATI level appeared to define the patients' response to infliximab. Combining ATI and trough infliximab levels should help to understand the mechanism of LOR and make therapeutic algorithms.

1. Introduction

The anti-tumour necrosis factor (TNF)-α antibody, infliximab is widely used to induce and maintain remission in patients with inflammatory bowel diseases (IBD) [1–3]. However, between 20% and 50% of patients experience loss of response (LOR) to infliximab within one month to 2 years following initiation of infliximab therapy [4,5]. The generation of antibodies to infliximab (ATI) due to the immunogenicity of this anti-TNF-α is believed to be a major factor for the diminished clinical response and an increased risk of adverse side effects [6].

Hitherto, attempts to restore an adequate clinical response to infliximab in patients with LOR have included dose-escalation, shortening of the infusion interval [7,8], and switching to another anti-TNF-α

Abbreviations: ATI, antibodies to infliximab; CD, Crohn's disease; CDAI, Crohn's disease activity index; CRP, C-reactive protein; EDTA, ethylenediaminetetraacetic; IBD, inflammatory bowel disease; IFX, infliximab; LOR, loss of response; SCR, sustained clinical response; TNF, tumour necrosis factor; UC, ulcerative colitis

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[9–13]. An ATI measuring assay that adequately reflects the immunogenicity of infliximab has been desirable as such method can help optimize treatment of patients with LOR [6,14]. However, the principles of various anti-drug antibody (ADA) measuring assays are basically ligand-binding [15–19]. For example, one major limitation of these assays is the gradual decline in the assay yield caused by interference by the drug present in a patient's test sample [20]. Further, current assays including those developed to measure ATI may detect free-form of ADA, and not pre-existing ADA-drug complex. Therefore conventional ATI-assays are potentially causing an increase in the proportion of patients with undetectable ATI, but with a clinically relevant level of trough infliximab who have developed LOR to infliximab. The new ATI-Cim assay we have developed is designed to measure total ATI in patients under infliximab therapy. We have assessed the performance and clinical relevance of this ATI measuring assay in patients with IBD who were under maintenance infliximab therapy.

2. Methods

2.1. Patients and study design

This was a case-control study of infliximab-treated IBD patients at 6 independent institutes treated between June 2016 and September 2017. The eligible patients had initially received 3 infusions of 5 mg/kg bodyweight infliximab at weeks 0, 2, and 6. Those who achieved remission received maintenance infliximab at an 8-week interval. The study population comprised patients without primary non-responder to infliximab. Blood samples were obtained before infliximab infusion, and EDTA-plasma was harvested after centrifugation and stored at −80 °C until assay. Fig. 1 shows a flow diagram of the study design and summary of the outcomes.

2.2. Definition of response to infliximab

Currently, there is no broadly accepted consensus on the definition of LOR to infliximab. Patients were classified according to Roblin, et al. [21]. LOR was defined as the presence of moderate to severe IBD with Crohn's disease activity index (CDAI) ≥220 in patients with Crohn's disease (CD) and partial Mayo score ≥5 in patients with ulcerative colitis (UC), or CRP ≥ 0.3 mg/dL. Intolerance to infliximab was assumed when acute or delayed infusion reactions (IR) were observed. Otherwise, patients were showing a sustained clinical response (SCR) to infliximab.

2.3. The ATI-Cim assay

An ATI-Cim assay with C1q-immobilized plate was developed as follows. Briefly, a 50 µL of EDTA-plasma was diluted by adding to 150 µL of diluent buffer which was phosphate buffered saline (PBS) containing 0.5% boiled casein (Sigma-aldrich, USA) and 0.5% Tween20. The test was then pre-incubated with 50 µL of infliximab (125 µg/mL) or 50 µL of diluent as control, at 4 °C overnight. After incubation, the tests were diluted 5-fold and then, a 100 µL of the test was added to the ELISA plate (Maxisorp F8X12; BioLegend Inc., CA, USA), which had been coated with C1q (1 µg/well; C1740, Sigma-Aldrich, USA), and was incubated for 2 h at 25 °C. Following washing in PBS containing 0.5% Tween20, a 10 ng/100 µL of horseradish peroxidase (HRP) labelled anti-infliximab monoclonal antibody was added to the plate and incubated for a further 1 hr at 25 °C. The plates were developed by using tetramethylbenzidine substrate (TMB-Blue, S1601, Dako, Denmark) and the reaction was stopped by adding 2 M H_2SO_4. The test optical density (OD) was measured at 450/650 nm in a Multiscan Well Leader (SK603, Seikagaku Corporation Tokyo, Japan). Results were read on a titration curve constructed with rabbit anti-human IgG(H + L) antibody (ab7155, Abcam plc., UK) as an ATI standard. The lower limit of detection was 0.078 µg/mL. The ATI values below this

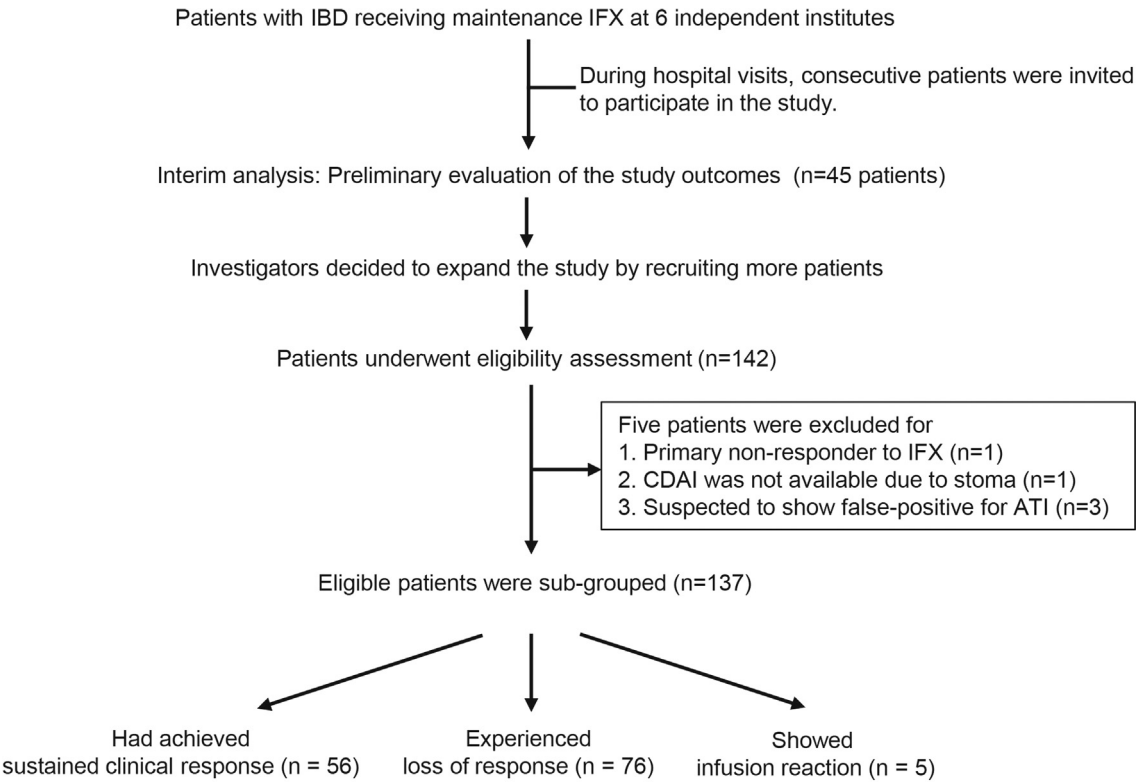

Fig. 1. A flow diagram summarizing the overall experimental design and the outcomes in this study. Abbreviations: ATI, antibodies to infliximab; CDAI, Crohn's disease activity index; IBD, inflammatory bowel disease; IFX, infliximab.

limit of quantification were taken as 0.039 μg/mL in data analysis. The anti-infliximab monoclonal antibody we used was prepared according to a published method [22].

2.4. The ATI-bridging ELISA

We compared the performance of the ATI-Cim assay with a conventional ATI-bridging ELISA during an interim period in this study. A commercially available kit (SHIKARI Q-ATI) was purchased from Matriks Biotek Laboratories (Ankara, Turkey), and all practical steps were according to the manufacturer's instructions. The results were based on a positive cut-off value which was estimated by multiplying the mean OD of the negative control at 450 nm by 3.

2.5. Determination of trough infliximab level

Measurement of infliximab level in patients was based on a previously described method [23]. Briefly, a 100 μL of 1:200 diluted EDTA-plasma was added to an ELISA plate (Maxisorp F8X12; BioLegend Inc., CA, USA), which had been pre-coated with anti-infliximab monoclonal antibody (100 ng/well) and incubated for 1hr at 25 °C, with gentle agitation on a plate mixer. After washing in PBS containing 0.5% Tween20, a 10 ng/100 μL of HRP labelled anti-infliximab monoclonal antibody was added, and incubated for 1 hr at 25 °C. The plates were developed by using tetramethylbenzidine substrate (TMB-Blue, S1601, Dako, Denmark) and the reaction was stopped by adding 2 M H_2SO_4. The test OD at 450/650 nm was determined in a Multiscan Well Leader (SK603, Seikagaku Corporation, Tokyo, Japan). The results were read on a titration curve constructed with infliximab. The lower limit of measurement was 0.6 μg/ml test. The infliximab levels below the limit of assay were taken as 0.3 μg/mL during data analysis.

2.6. Statistical analysis

When appropriate, numerical data are presented as median and interquartile range (IQR). Different groups were compared by the Wilcoxon-Mann-Whitney test for continuous variables and by the Fisher's exact test for categorical variables unless stated otherwise. Paired data sets were compared by Wilcoxon-Signed-Rank test. Correlation between data sets was assessed by applying the Spearman's rank correlation. The measured ATI level was reviewed to see its relevance for predicting an LOR by applying a receiver operating characteristic (ROC) model. Trends between LOR rate in the stratified subgroups with cut-off value of ATI and trough infliximab were verified by the Cochran-Armitage trend test. All P values are two-tailed with the significance level set at $P < 0.05$ by using a statistical software package (JMP, SAS Institute, Cary, NC).

2.7. Ethical considerations

Prior to initiating this study, our investigation protocol was reviewed and approved by the ethics committees at each of the 6 participating medical institutes. Patients were informed of the study purpose, the nature of the procedures involved, and were asked to provide informed consent. Further, the study was conducted with strict adherence to the Helsinki Declaration.

3. Results

3.1. The principle and analytical validation of the ATI-Cim assay

The principle of the ATI-Cim assay is illustrated in Fig. 2, in comparison with a conventional ligand-binding assay. The test specimens from patients who have been exposed to infliximab may contain ATI in free-form and infliximab together with ATI-infliximab immune complexes. In ligand-binding assay, test specimens were incubated with labelled infliximab to form ATI-labelled infliximab immune complex. However, we believe that in a conventional ligand-binding assay, any pre-existing ATI-infliximab immune complexe may not be detected, but only the free-form of ATI be detected (Fig. 2A). In contrast, for the Cim assay (Fig. 2B), the test specimens were pre-incubated with infliximab to convert the free-form of ATI into ATI-infliximab immune complexes. The C1q-immobilized plate then captures the ATI-infliximab immune complexes, and are detected with labelled anti-infliximab monoclonal antibody. Fig. 3A shows the optimum dose of infliximab required to convert all of the free-form ATI into ATI-infliximab immune complex by using infliximab-immunized rabbit serum as ATI. All ATI samples reached a plateau with addition of infliximab above 25 μg/mL. Data presented in Fig. 3B confirmed the selectivity of the ATI-Cim assay, detecting only ATI-infliximab immune complex, and not free-form of ATI, also not free-form of antibodies to adalimumab (ATA), or ATA-adalimumab immune complexes. The dose response curve showed a linear increase in signal up to 500 ng/mL of ATI-standard. There was no significant signal with addition of infliximab, adalimumab or heat aggregated human IgG (HAG-IgG) in the assay system (Fig. 3C). Intra-assay and inter-assay precision had a coefficient of variation less than 12% in quality control tests.

3.2. The outcomes of an interim analysis

We carried out an interim investigation to compare the efficiency of the present ATI-Cim assay relative to a conventional ATI-bridging ELISA by using EDTA-plasma obtained from 45 consecutive patients following the initiation of the study (Fig. 4). To check for drug-tolerance and specificity in both ATI-assay methods, a 25 μg/mL of infliximab or diluent as control was added to each of the 45 patients' EDTA-plasma, and incubated at 4 °C overnight. Then measurements were done with both assays. In the conventional bridging ELISA, ATI values were significantly ($P < 0.0001$) decreased when a 25 μg/mL of infliximab was added to the test plasma instead of diluent (Fig. 4A, lower panel). Only 3 of 45 patients were ATI-positive based on plasma to which infliximab was not added ('a', 'b', 'c' in Fig. 4A, lower panel). In contrast, there was a significant ($P = 0.0006$) increase in ATI level in tests to which a 25 μg/mL of infliximab had been added as compared with the ATI level without addition of infliximab in the ATI-Cim assay (Fig. 4A, upper panel). However, even with the addition of infliximab, ATI in 48.9% of the patients (22 of 45) was undetectable in the ATI-Cim assay. The percentage changes following addition of infliximab in comparison to control specimens in the bridging ELISA and Cim assay in patients with ATI-positive were −73.8 ± 4.9% and 108.2 ± 46.6%, respectively. Addition of infliximab to patient's plasma to verify its specificity for ATI in both assays, but this reduced the ATI-value in the bridging ELISA, while increasing it in the Cim assay. Therefore, the data from plasma to which infliximab was not added in the bridging ELISA, and those to which infliximab was added for ATI-Cim assay were used to compare the ATI levels in the 22 patients with SCR and 23 with LOR. Unexpectedly, the ATI levels in patients with LOR measured by the bridging ELISA tended to be lower than in the SCR subgroup. In the ATI-Cim assay, the ATI level in the LOR subgroup was significantly ($P = 0.0116$) higher than in the SCR subgroup (Fig. 4B, upper panel). Additionally, the measured ATI value correlated with C-reactive protein (CRP) levels in the Cim assay but not in the bridging ELISA (Fig. 4C). Based on these observations, addition of 25 μg/mL infliximab to plasma was considered to be optimum in the ATI-Cim assay protocol.

3.3. Plasma infliximab and ATI level during infliximab infusion

Twelve paired plasma samples from 5 patients were prepared during infliximab infusion for determining the changes in infliximab and ATI levels (Fig. 5A). The median [IQR] value of infliximab were significantly increased relative to pre-infusion, 1.9 μg/mL [0.4–2.7 μg/mL]

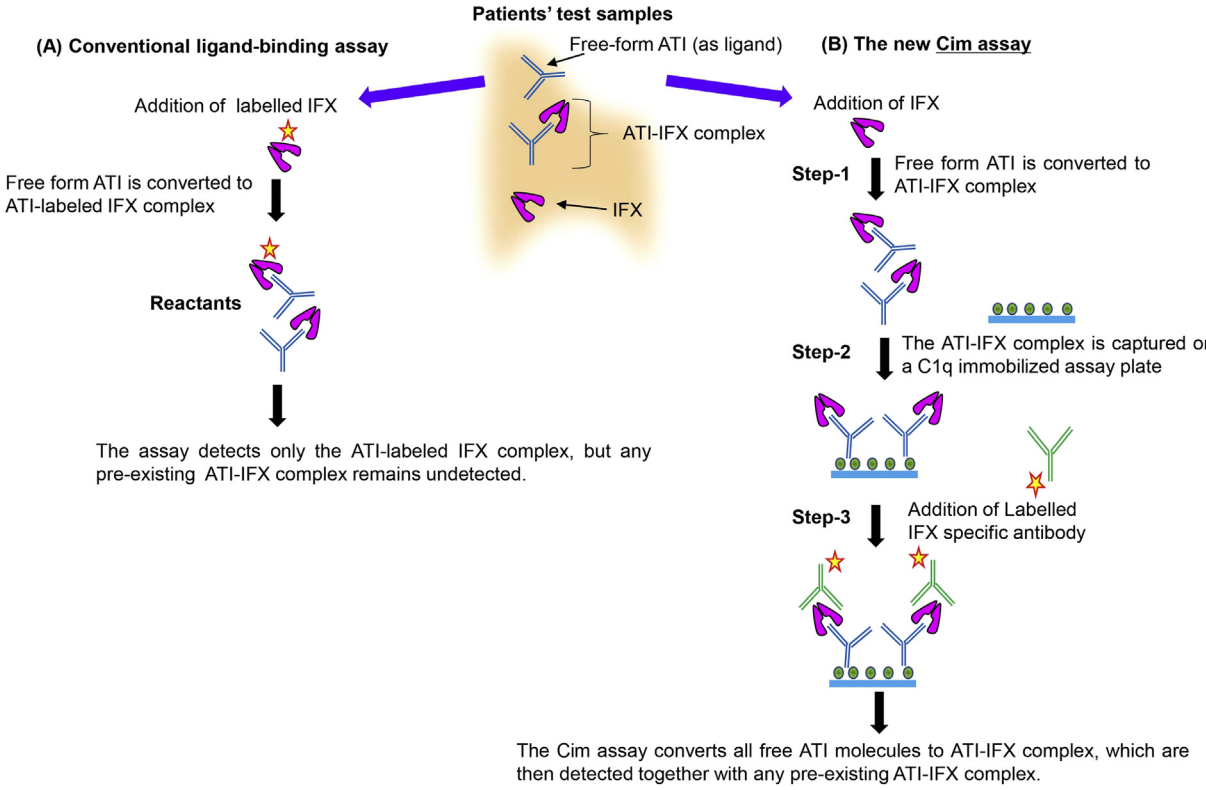

Fig. 2. Schematic presentation of the principle of conventional ligand-binding assay (A) and the present ATI-Cim assay (B).

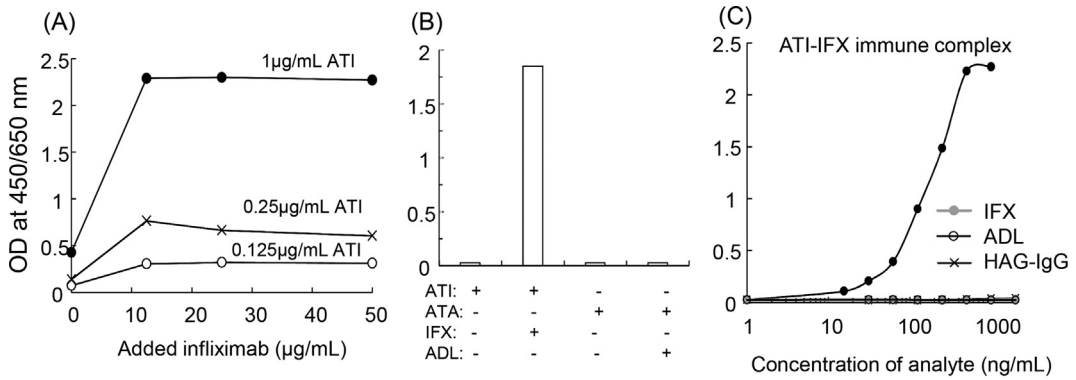

Fig. 3. (A) the effects of adding infliximab to free-form of ATI to prepare ATI-infliximab immune complex. A 200 μL aliquot of ATI (156, 312.5 and 1250 ng/mL) were incubated with 50 μL of infliximab (0, 62.5, 125 and 250 μg/mL) at 4 °C overnight, and then used in the ATI-Cim assay. (B) detection of ATI-infliximab immune complex by the ATI-Cim assay. A 50 μL of ATI (25 μg/mL) alone or with a 50 μL of infliximab (125 μg/mL) was incubated at 4 °C overnight in a total volume of 250 μL. Also, tests containing a 50 μL of ATA (12.5 μg/mL) alone or together with a 50 μL of adalimumab (125 μg/mL) in a total volume of 250 μL were incubated at 4 °C overnight. The pre-incubated specimens were used in the ATI-Cim assay (see materials and methods). The monoclonal antibodies to infliximab and adalimumab were prepared according to a published method.[22] (C) the dose response curves to show the specificity of the ATI-Cim assay for ATI. Constructions were made with 1000 ng/mL to 15.6 ng/mL of ATI-infliximab immune complex, and with 2000 ng/mL to 31.2 ng/mL of infliximab, adalimumab or HAG-IgG. Abbreviations: ADL, adalimumab; ATA, antibodies to adalimumab; ATI, antibodies to infliximab; HAG-IgG, heat aggregated IgG; HRP, horseradish peroxidase; OD, optical density.

at pre-infusion and 47.3 μg/mL [38.3–53.0 μg/mL] at post-infusion (P = 0.0005). In contrast, there was no significant change in ATI level between the pre- and post-infusion, 0.189 μg/mL [0.093–0.245 μg/mL] at pre infusion and 0.160 μg/mL [0.096–0.238 μg/mL] at post infusion (P = 0.4316).

3.4. Patients with sustained response or loss of response to infliximab

The 137 eligible patients were sub-divided based on the definition of SCR, LOR or IR (Fig. 1). The clinical features of patients with SCR and LOR are summarized in Table 1. The duration of infliximab therapy was longer in patients with SCR than in those with LOR (P = 0.0082) in UC. Patients with LOR had significantly higher ATI levels than patients with SCR, 0.197 μg/mL [0.080–0.503 μg/mL] vs 0.039 μg/mL [0.039–0.259 μg/mL], respectively (P = 0.0004, Fig. 5B, lower panel). Regarding trough infliximab, patients with LOR showed significantly decreased trough infliximab levels than patients with SCR, 2.0 μg/mL [1.4–2.7 μg/mL] vs 2.5 μg/mL [1.5–3.4 μg /mL], respectively (P = 0.0292, Fig. 5B, upper panel). Based on the receiver operating characteristic (ROC) curve analysis, an ATI cut-off value > 0.153 μg/mL (AUC = 0.6781, with 66.1% specificity and 60.5% sensitivity) was associated with LOR (odds ratio 2.986; 95% confidence interval 1.454 to 6.131, P = 0.0029). The 132 patients with SCR and LOR were divided into 4 subgroups by combined cut-off ATI value and trough

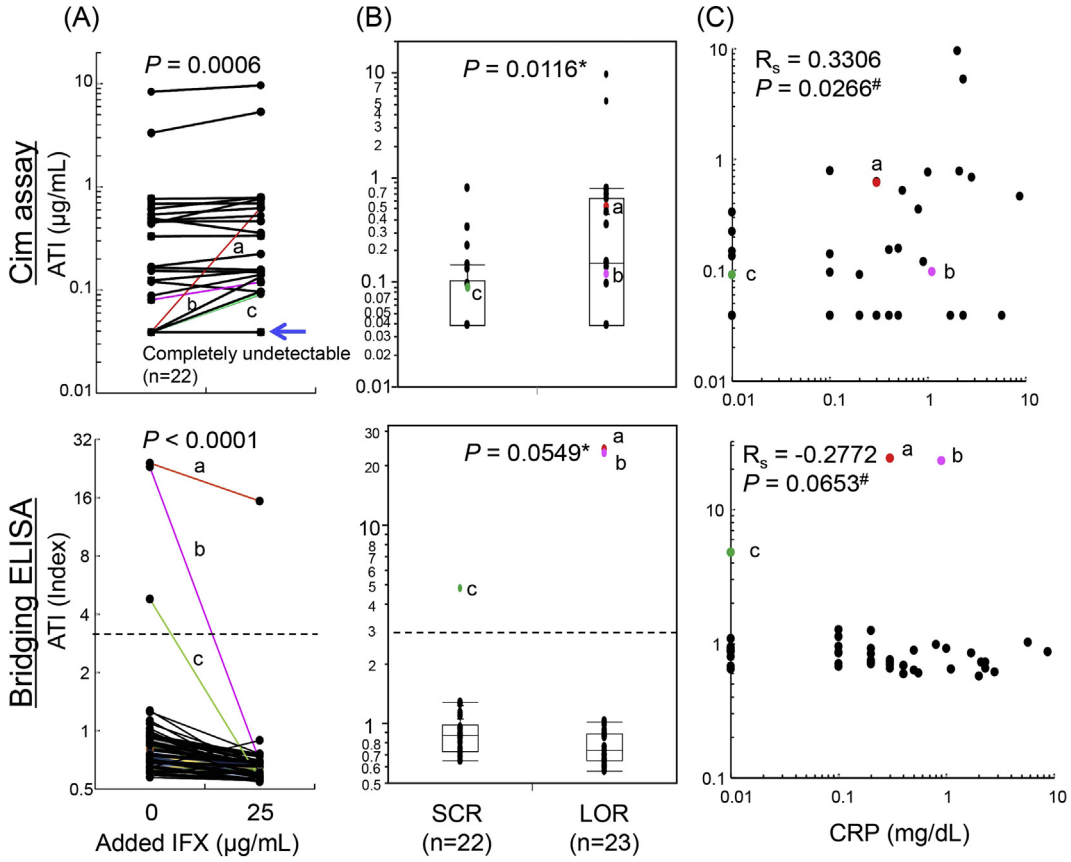

Fig. 4. Comparison of the conventional ATI-bridging-ELISA and the ATI-Cim assay in 45 patients receiving maintenance infliximab. Drug-tolerance and specificity in the bridging-ELISA (A, lower panel) and the present ATI-Cim assay (A, upper panel) are presented. ATI levels in patients marked 'a', 'b' and 'c' showed a dramatic increase from undetectable level to 0.628 μg/mL, from 0.08 μg/mL to 0.119 μg/mL and from undetectable to 0.091 μg/mL respectively following addition of 25 μg/mL infliximab to the patients' plasma in the ATI-Cim assay. ATI levels in 22 patients who achieved a sustained clinical response (SCR) and 23 who experienced loss of response (LOR) to infliximab were measured with the conventional method (B, lower panel) or with the ATI-Cim assay (B, upper panel). Correlation between the measured ATI value and C-reactive protein (CRP) levels in both assays (C). In (A), comparisons were made by applying the Wilcoxon-Signed-Rank test. *In (B) by the Wilcoxon-Mann-Whitney test. # (C) by the Spearman's rank correlation test. The dotted lines in (A) and (B) indicate the positive cut-off point for the ATI-bridging-ELISA. The results with the ATI-bridging-ELISA are expressed as ATI (index), and were calculated by the test OD450nm/average OD450nm for negative control tests. Abbreviations: ATI, antibody to infliximab.

infliximab (Fig. 6A). The frequency of LOR in each subgroup showed a decreasing trend from subgroup A to D of Fig. 6A, 80.8%, 64.1%, 55.2% and 36.8%, respectively ($P = 0.0003$). The LOR in subgroups A, C and D was assumed to be immunogenic, pharmacokinetic or pharmacodynamic factor, respectively. A pharmacodynamic factor together with immunogenic reaction may define subgroup B.

3.5. Patients with undetectable ATI

Among the 132 patients, 47 (35.6%) were found to have undetectable ATI. The rate of finding patients with undetectable ATI was significantly higher in the SCR subgroup than in those with LOR, 53.6% vs 22.4% ($P = 0.0004$). Likewise, the frequency of CRP being below 0.3 mg/dL was significantly higher in patients with undetectable ATI than in those with detectable ATI, 66.0% vs 44.7% ($P = 0.0285$, Fig. 6B, upper panel). Further, patients with undetectable ATI had significantly ($P = 0.0232$) longer duration of infliximab therapy than patients with detectable ATI (Fig. 6B, lower panel).

3.6. Hypersensitivity reaction to infliximab

Among the 137 patients, 5 (3.6%) showed infusion reaction to infliximab, 2 UC, 1 CD and another 2 CD patients showed delayed hypersensitivity reaction to infliximab, one with psoriatic lesions and the other developed polyarthralgia together with myalgia. ATI level in one patient with UC was 0.091 μg/mL, but in the other 4 patients, ATI levels were elevated with a mean of 1.797 μg/mL, range 0.236–4.229 μg/mL. However, CRP level in these 5 patients ranged from 0.01 mg/dL to 0.15 mg/dL, while their CDAI and partial Mayo score were 118 to 189 (in CD), and 4 to 0 (in UC).

4. Discussion

The ATI-Cim assay requires reagents and instruments, which are the same as conventional enzyme-immunoassays. The assay is suitable for therapeutic drug monitoring (TDM) in clinical test samples using an auto-analyzer. Further, to our knowledge, this is the first observation of ATI levels not being changed during infliximab-infusion, even though the concentration of infliximab in the patients' blood was increasing. However, ATI levels were significantly elevated in patients with LOR as compared with patients who had achieved SCR. Additionally, we found that up to 35.6% of patients had undetectable ATI; the frequency of finding a patient with undetectable ATI was higher in patients with SCR than in those with LOR. Likewise, patients with undetectable ATI had a longer duration of infliximab therapy and a higher proportion of them had normal CRP levels as compared with patients who had detectable ATI. This observations may suggest that patients with undetectable ATI are likely to response to infliximab and stay longer in the therapy.

Based on the combined cut-off values of ATI and trough infliximab, 4 subgroups could be identified with different response profiles to

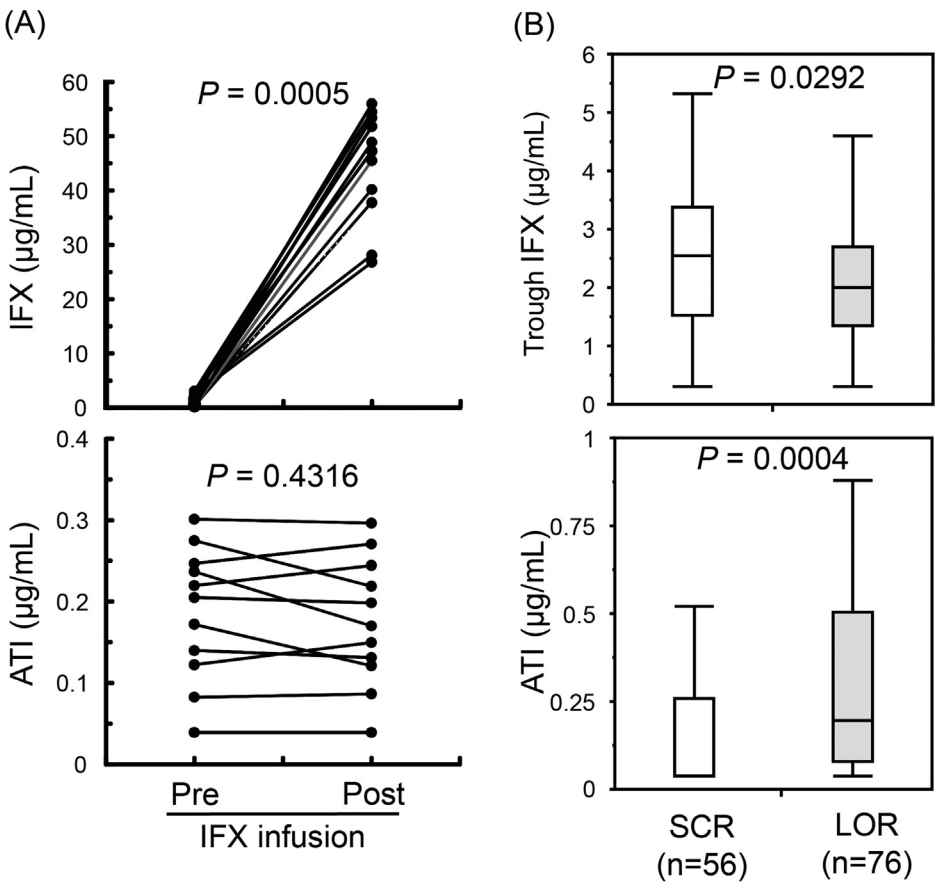

Fig. 5. (A) Changes in infliximab (IFX) and antibody to infliximab (ATI) levels during infusion of infliximab in 5 patients as a test. The infliximab and ATI levels were measured up to 4 times during the infliximab infusion (n = 12 tests). Pre = blood samples were taken immediately prior to infliximab infusion; Post = blood samples were taken at the end of infliximab infusion. Comparisons were made by the Wilcoxon-Signed-Rank test. (B) ATI and trough infliximab levels in patients who achieved a sustained clinical response (SCR) and those who experienced loss of response (LOR) to infliximab, compared by the Wilcoxon-Mann-Whitney test.

infliximab. We thought that a classification based on the concept of TDM may help to better understand the mechanism of LOR onset, and make therapeutic algorithms to optimize treatments in IBD settings. LOR in subgroups B and D of Fig. 6A who were found to have therapeutic infliximab levels might be suspected to have been caused by a pharmacodynamic factor or by a non-TNF driven mechanism, a decreased expression of membrane-bound TNF-α in the effector T-cells [24], or by inflammation driven by another cytokine, like interleukin-6

Table 1
Baseline characteristics of the 132 eligible patients.

Demography	UC (n = 48)		*P* Value	CD (n = 84)		*P* Value
	SCR (n = 23)	LOR (n = 25)		SCR (n = 33)	LOR (n = 51)	
Male gender – no. (%)	12 (52.2)	17 (68.0)	0.3767	21 (63.6)	37 (72.6)	0.4706
Age, year	40.0 [31.0–47.0]	37.0 [23.5–51.0]	0.4762	30.0 [26.0–45.5]	34.0 [29.0–43.0]	0.9197
Duration of disease, year	8.4 [5.5–12.6]	8.7 [5.2–11.9]	0.6059	8.5 [5.4–11.2]	9.0 [5.3–12.1]	0.6514
Duration of infliximab therapy, year	4.1 [3.6–5.9]	3.0 [1.1–4.8]	0.0082	7.4 [4.1–9.0]	5.8 [3.5–7.8]	0.2318
CRP (mg/dL)	0.05 [0.01–0.10]	0.30 [0.10–0.96]	0.0001	0.03 [0.01–0.10]	0.85 [0.47–2.03]	< 0.0001
Partial Mayo score	1 [0–2]	5 [2–5]	< 0.0001	.	.	N/A
CDAI	.	.	N/A	71 [38–133]	173 [133–227]	< 0.0001
Disease location –no. (%)						
Proctitis	0 (0)	1 (4.0)	1.0000	.	.	N/A
Left-sided	7 (30.4)	4 (16.0)	0.3105	.	.	N/A
Pan-colitis	16 (69.6)	20 (80.0)	0.5112	.	.	N/A
Ileum	.	.	N/A	9 (27.3)	8 (15.7)	0.2671
Ileocolonic	.	.	N/A	14 (42.4)	32 (62.8)	0.0772
Colon	.	.	N/A	9 (27.3)	11 (21.6)	0.6052
Concomitant medication – no. (%)						
5-Aminosalicylates	19 (82.6)	23 (92.0)	0.4073	31 (93.9)	41 (80.4)	0.1140
Corticosteroids	1 (4.4)	4 (16.0)	0.3499	0 (0)	5 (9.8)	0.1514
Thiopurines	4 (17.4)	10 (40.0)	0.1167	5 (15.2)	15 (29.4)	0.1906
Smoking status – no. (%)						
Currents smoker	0 (0)	3 (12.5)	0.2348	5 (15.2)	9 (18.8)	0.7708
Former smoker	9 (40.9)	4 (16.7)	0.1028	6 (18.2)	9 (18.8)	1.0000
Nonsmoker	13 (59.1)	17 (70.8)	0.5379	22 (66.7)	30 (62.5)	0.8147

CDAI, Crohn's disease activity index; CRP, C-reactive protein; LOR, loss of response; SCR, sustained clinical response. Certain values are presented as the median [interquartile range] compared by Wilcoxon Mann Whitney test. Categorical values were compared by the Fisher's exact test.

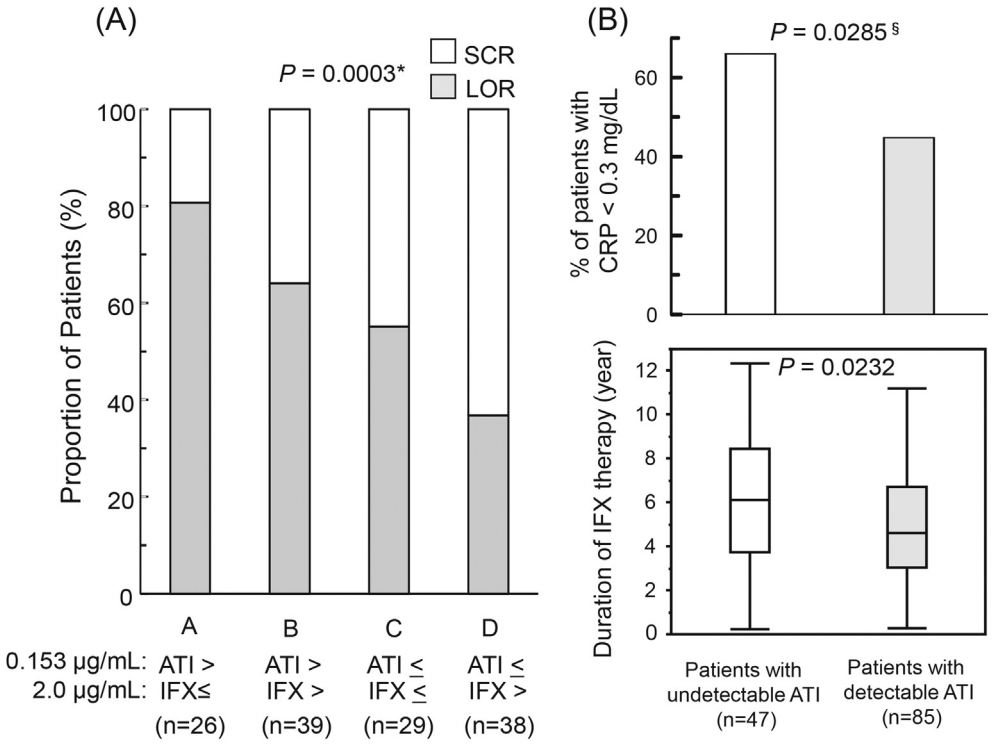

Fig. 6. (A) Subgrouping of the 132 eligible patients based on the cut-off values of ATI and trough infliximab levels. (B) the proportion of patients with C-reactive protein (CRP) below 0.3 mg/dL and the duration of infliximab therapy in the subgroup with undetectable ATI vs the subgroup with detectable ATI. Comparisons were done by applying the Wilcoxon-Mann-Whitney test, * by Cochran-Armitage trend test or § by the Fisher's exact test.

at local mucosal sites [25]. Consequently, stopping the anti-TNF-α and switching to another class of biologic or optimizing the treatment with immunosuppressive medication like a glucocorticoid are conceivable options.

Further, among the patients in subgroup B, up to 29.5% (39/132) showed an atypical levels of ATI and trough infliximab, which were well above the cut-off values in this investigation. In contrast, Steenhold, et al. [26] in their fluid-phase radioimmunoassay of ATI and infliximab reported that only 4% of their patients showed similar ATI and infliximab levels as in subgroup B of this study. As an explanation for this discrepancy, one may speculate that the ATI-Cim assay measures total ATI. Another possibility might be false-positive readings due to the formation of immune complexes between infliximab and TNF-α as a soluble target. We verified that infliximab-TNF-α immune complex was not detected as ATI by adding up to 1000 pg/mL TNF-α to 5 μg/mL of infliximab. Yet, a third possibility could be that the patients' blood contained traces of free-form ATI due to a low binding affinity of ATI for infliximab in vivo [27]. In fact, we observed a significant elevation of ATI levels following addition of infliximab to the patients' test samples (seen Fig. 4A, upper panel).

At this point, we should mention certain limitations of this study, which potentially might have impacted our findings. Firstly, the method we applied to capture ATI-infliximab immune complex by immobilized C1q detects only IgG and IgM of the immunoglobulin isotypes of ATI due to the binding feature of the C1q moiety. Secondly, we could not find a relationship between the time of ATI-detection and the onset of LOR due to retrospective nature of this observational study. Finally, the definition of LOR was made by a modified method of Robin, et al. [21] by combining with the CRP concentration due to lack of a widely accepted consensus at present.

In conclusion, to our knowledge, this is the first assay, which can be applied to detect and measure total ATI in IBD patients during infliximab therapy, without being affected by the concentration of free infliximab in the patients' test samples. Further, undetectable ATI level was associated with a sustained clinical response, together with longer duration of infliximab therapy and normal CRP levels, not seen in a similar number of patients with measurable ATI level. Accordingly, the ATI level measured by the ATI-Cim assay appeared to define patients' clinical response to infliximab therapy. Therefore, monitoring ATI may help to understand how patients develop LOR and to plan better therapeutic strategies for LOR. Further prospective studies should strengthen our findings.

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Potential competing interests

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Specific author contributions

Study concept and design: NY, YY, NA, and YS. Data recordings, administration of interventions, and interpretation of the results: NY, YY, MS, NA, FH, KS and YS. Analysis and interpretation of the data: NA, NK, and YY. Drafting of the initial manuscript version: NY, YY, NK, and NA. All authors critically reviewed and approved the final version of the manuscript prior to submission.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2019.02.014.

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