OPEN

Comparison of Ustekinumab Trough Concentrations Measured by 2 ELISA Kits and Evaluation of Clinical Response in Crohn's Disease

Yiyoung Kwon, MD, Ben Kang, MD,† Eun Sil Kim, MD,* Yon Ho Choe, MD, PhD,* and Mi Jin Kim, MD, PhD**

Background: Ustekinumab is a recently introduced biological agent for the treatment of Crohn's disease. The clinical use of the trough concentration of ustekinumab is not as standardized as that of infliximab. The authors aimed to introduce a measurement method and the results of trough concentrations of ustekinumab in clinical applications.

Methods: Thirty-two blood samples from 10 young adult patients diagnosed with Crohn's disease were analyzed. During the maintenance treatment, injection intervals were shortened from 12 weeks to 8 weeks in 4 patients who exhibited a loss of response. Ustekinumab trough concentrations were measured using 2 commercial ELISA kits, kit A and kit B.

Results: The median trough concentrations measured with kits A and B were 0.26 and 0.38 mcg/mL, respectively. In the case of kit A, low trough concentrations were undetected on many occasions and measured as zero, whereas kit B displayed their relative values even at low concentrations. Poor clinical parameters, elevated erythrocyte sedimentation rate, C-reactive protein, and calprotectin levels were significantly correlated with lower trough concentrations ($P < 0.05$). The area under the receiver operating characteristics curve of kit B (0.921) was greater than that of kit A (0.744). The optimal cutoff values for prediction clinical responses were 0.17 and 0.41 mcg/mL for kit A and kit B, respectively.

Conclusions: The trough concentration of ustekinumab measured by the 2 ELISA kits correlated with laboratory results that indicated the activity of Crohn's disease. Furthermore, kit B detected even minute changes in trough concentrations.

Key Words: ustekinumab, therapeutic drug monitoring, commercial ELISA kit, Crohn's disease

(*Ther Drug Monit* 2022;44:535–542)

INTRODUCTION

With the introduction of biological agents, complications in Crohn's disease treatment have decreased, and the quality of life has increased with increased disease control. However, some patients develop a loss of response to classical biological agents such as anti–tumor necrosis factor (TNF) alpha.[1] Therefore, new drugs that target different elements of the inflammatory cascade are being developed. Ustekinumab is a recently introduced biological agent approved in Korea in 2018 for adult patients with moderate to severe Crohn's disease. Ustekinumab is a human IgG1k monoclonal antibody that inhibits the biological activities of interleukin 12 and interleukin 23 through their common p40 subunits.[2,3]

Various studies have been conducted to determine the appropriate drug dosage for induction treatment and the appropriate week intervals for maintenance treatments, but none have been determined using standard methods. After the UNITI-1, UNITI-2, and IM-UNITI studies,[4–6] a Crohn disease treatment policy using ustekinumab was established in Korea, which states that maintenance treatment should be administered every 12 weeks after induction treatment. For patients whose symptoms are not well controlled at 12-week intervals, dose intensification can be shortened at 8-week intervals.

Received for publication September 15, 2021; accepted January 27, 2022.

From the *Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; and †Department of Pediatrics, School of Medicine, Kyungpook National University Children's Hospital, Kyungpook National University, Daegu, Korea.

Y. Kwon and B. Kang contributed equally to this work.

The authors declare no conflict of interest.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.drug-monitoring.com).

Ethics approval and consent to participate: Patients' medical records were reviewed retrospectively with approval from the Clinical Research Ethics Committee and have undergone appropriate written consent procedures for blood collection and analysis.

Name of the ethics committee and reference: Samsung Medical Center. IRB File No.: SMC 2020-10-064.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

All authors read and approved the manuscript. Conception or design: M. J. Kim and Y. H. Choe. Acquisition, analysis, or interpretation of data: Y. Kwon, B. Kang, Y. H. Choe, and E. S. Kim. Drafting the work or revising: Y. Kwon, B. Kang, and M. J. Kim. Final approval of the manuscript: Y. Kwon, B. Kang, and M. J. Kim.

Correspondence: Mi Jin Kim, MD, PhD, Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea (e-mail: mijin1217.kim@samsung.com).

In the case of infliximab, another conventional biological agent that has been long used, measuring the trough concentration and determining the dose intensification therapy according to the target level, has become a treatment strategy.[7–10] In the case of ustekinumab, research on the clinical correlation between trough concentration and clinical outcomes has recently been conducted. We reviewed articles that monitored ustekinumab trough concentration using unstandardized and varying measurement methods, such as enzyme-linked immunosorbent assay (ELISA), liquid phase homogeneous mobility shift assay, and liquid chromatography–tandem mass spectrometry. Due to differences in measurement methods, the reported trough concentration ranges, units, and cutoff values varied.[11–17]

In this study, the trough concentrations were measured using 2 experimental kits for young adult patients undergoing ustekinumab maintenance therapy for 12 or 8 weeks. We aimed to introduce an appropriate measurement method and assess the relationship between trough concentrations and antibodies of ustekinumab for the first time in Korea.

MATERIALS AND METHODS

Patients

Young adult patients with Crohn's disease who started receiving ustekinumab treatment were included in the study. This study was conducted from January 2019 to July 2020, and patients who had been using ustekinumab since March 2019 were observed. Twelve patients on ustekinumab were screened, but only 10 patients on maintenance treatment for more than 24 weeks were enrolled in the study. All methods of this study were performed in accordance with the relevant guidelines and regulations and were approved by the Clinical Research Ethics Committee of Samsung Medical Center. Written informed consent for blood collection and analysis of clinical data were provided (IRB File No.: SMC 2020-10-064).

The clinical characteristics of the 10 patients were that they were diagnosed with Crohn's disease when they were children; therefore, the patients had a long disease period (median value of 9.5 years) and were all in their early 20s. All patients had a history of treatment with mesalazine and azathioprine, but some discontinued the drugs due to complications or poor compliance. Of the 10 patients, 4 were treated with mesalazine and 3 were treated with azathioprine at the time of this study. Some patients had a history of treatment with systemic steroids in the past, but patients who used systemic steroids at the onset of ustekinumab were excluded from the study because it might affect the evaluation of the effectiveness of ustekinumab. All patients had a history of treatment with a biological agent, infliximab, or adalimumab. The patients were switched to ustekinumab due to secondary loss of response to anti-TNF alpha agent or the formation of antibodies to anti-TNF alpha.

Study Design

When the patients were switched to ustekinumab, laboratory tests of albumin (normal range: 3.5–5.2 g/dL), erythrocyte sedimentation rate (ESR) (normal range: 0–22 mm/h), and C-reactive protein (CRP) (normal range: 0–0.5 mg/dL) were performed, and fecal examinations such as calprotectin (normal range: $<$50 mcg/g), colonoscopy with simple endoscopic score for Crohn's disease (SES-CD) assessment, and magnetic resonance enteroclysis (MRE) tests were conducted, and the Crohn's disease activity index (CDAI) was checked[18,19]

For induction treatment, a single, intravenous, induction-tiered dose of ustekinumab, approximately 6 mg/kg [260 mg (weight $\leq$ 55 kg), 390 mg (weight $>$ 55 kg and $\leq$85 kg), or 520 mg (weight $>$ 85 kg)] was injected. After 8 weeks, the patients received a second ustekinumab injection at a dose of 90 mg subcutaneously. The response to induction therapy and initiation of maintenance treatment were evaluated using the CDAI score at 16 weeks. Maintenance treatment was maintained when either CDAI was decreased by more than 70 points or by more than 25% of the total CDAI score according to the insurance permit criteria.

After induction therapy, the patients began maintenance therapy with a dose of 90 mg subcutaneously every 12 weeks. However, patients whose symptoms did not improve with the injections every 12 weeks were administered injections at 8-week intervals. The decision to change the injection interval to 8 weeks was made based on the laboratory test results (albumin, ESR, and CRP), calprotectin level, and CDAI score.[20,21] We defined these patients requiring dose intensification with injection at 8-week intervals to be in a loss of response state during the maintenance therapy, and they were defined by (1) ESR, CRP elevation, or not normalization, (2) lack of improvement in fecal calprotectin level, or (3) CDAI score $>$ 150 or increase more than 30 points over the baseline score.

Trough and antibody concentrations of 32 blood samples obtained from 10 patients immediately before the maintenance injection were measured twice using 2 experimental techniques (see **Figure 1**, **Supplemental Digital Content**, http://links.lww.com/TDM/A550). Trough and antibody concentrations were evaluated according to the maintenance interval, and the adequacy of the 2 experimental kits was evaluated.

The primary outcome of this study was to evaluate whether there was a correlation between the ustekinumab trough concentration measurement, which has not been standardized to date, and the clinical response. The secondary outcome of this study was to determine if there are any differences in the kits we need to know for application in clinical situations.

Measurements

Laboratory data such as ESR, CRP, and albumin levels were obtained from the medical record, clinically available from the hospital laboratories. Fecal calprotectin levels were obtained from a hospital laboratory with Alegria (ORGENTEC, Chicago, IL). Calprotectin is an ELISA-based, automated, and in vitro test system for the quantitative determination of calprotectin in the stool. The results are described in a range of 0–1000 mcg/g, and the cutoff of normal value is less than 50 mcg/g.

Because ustekinumab has only been recently introduced and is not a popular medication for inflammatory bowel disease, 2 commercial ELISA kits [IDKmonitor (Immundiagnostik, Bensheim, Germany) and ImmunoGuide (AybayTech, Ankara, Turkey)] were used for research use only. They were validated for precision, accuracy, and reference range according to the guidelines provided by the manufacturers (see **Table 1**, **Supplemental Digital Content**, http://links.lww.com/TDM/A551). The IDKmonitor ELISA kit and ImmunoGuide ELISA kit are termed kit A and kit B, respectively.

Measuring Trough Concentration and Anti-Ustekinumab Antibody Using ELISA Kit A

For trough concentration measurement, free ustekinumab was bound to a specific monoclonal anti-ustekinumab antibody in the first incubation step. After incubation, plate-bound ustekinumab was detected by adding a peroxidase-labeled antiustekinumab antibody. Tetramethylbenzidine (TMB) was used as a substrate for peroxidase, and the intensity of the color was measured to be directly proportional to the concentration of free ustekinumab in the sample. A dose–response curve of OD versus concentration was generated. The color developed was measured using an x-Mark spectrophotometer (Bio-Rad) and analyzed with the MM6 software (Bio-Rad).

For antibody detection, free antitherapeutic antibodies in the sample were bound to ustekinumab F(ab)2 fragments coated on the plate in the first incubation step. Peroxidase-labeled ustekinumab was then added and incubated again. After each incubation step, washing was performed to remove the unbound substances. The solid phase was incubated with TMB, and then, an acidic stop solution was added. The absorbance of the color compound was determined photometrically (450 nm against 620 nm), and the intensity of the color was directly proportional to the amount of bound antiustekinumab antibody. Samples with an OD higher than the cutoff control (10 AU/mL) were defined as positive.

Measuring Trough Concentration and Anti-Ustekinumab Antibody Using ELISA Kit B

For trough concentration measurement, the samples were incubated in 96-well plates coated with IG-9C7 mAb (Catcher Ab, ImmunoGuide clone 9C7) in the first incubation step. After incubation, a biotinylated antihuman IgG mAb (specific for the Fc part of all human IgG, clone IG-1B5) was added and bound to the Fc part of ustekinumab. Following incubation, horseradish peroxidase (HRP)–conjugated streptavidin was added to bind to the biotinylated 1B5 mAb. The remaining incubation steps were the same as those performed in the IDKmonitor ustekinumab drug level ELISA kit (kit A).

For antibody detection, the peroxidase-labeled drug, F(ab)2 fragment, and TMB used for each washing step to attach free antiustekinumab antibody was the same as kit A. However, the method of determining the cutoff value was different. First, the average OD 450 nm value of calibrator 1–3 was evaluated and twice this value was set as the cutoff value. If the sample OD was equal to or higher than the cutoff value, the sample was regarded as positive for antidrug antibodies (ADAs).

Statistical Analysis

The Mann–Whitney *U* test was performed to compare the median values of each kit and group. Correlations between ustekinumab trough levels and clinical factors (ESR, CRP, albumin, and fecal calprotectin levels) were assessed by calculating Pearson correlation coefficient. Receiver operating characteristic (ROC) curves were constructed to assess the clinical and laboratory responses to ustekinumab trough concentrations during maintenance treatment. Youden J statistic was computed to identify cutoff values. Statistical analyses were performed using SPSS version 27 (IBM Corporation, Armonk, NY). Statistical significance was set at $P < 0.05$.

RESULTS

Patient Characteristics

The characteristics of the patients at the onset of ustekinumab treatment are shown in Table 1. The baseline characteristics are reported as the number of patients for nominal variables and median or interquartile range for continuous variables. Our patient group was younger than the patient groups in other studies with a median age of 23.4 years because they had been diagnosed with Crohn's disease in childhood. The median age at which they were first diagnosed with Crohn's disease was 15.7 years. The median duration from the onset of the disease to the onset of ustekinumab treatment was 8.2 years. The shortest ustekinumab treatment period was 28 weeks, and the longest treatment duration was 80 weeks. The average treatment duration was 46.8 weeks. Every patient had a history of loss of response to treatment with infliximab, adalimumab, or both. Therefore, clinical assessment with the CDAI scores after ustekinumab treatment was high (median value of 227.2). Laboratory findings showed that ESR and CRP, which represent inflammation levels, were higher than normal: the median value was 29.5 mm/h and 1.2 mg/dL for ESR and CRP, respectively. Albumin, which indirectly evaluates diarrhea and nutritional status, was relatively low, with a median value of 4.1. The SES-CD score, which evaluates the endoscopic severity of CD through colonoscopy, was elevated to a median value of 12.

All patients were classified into the A1b and L3 location groups according to the Paris classification[22] at the time of diagnosis. Three patients showed B3 behavior, whereas the other patients showed B1 behavior. Four of them had perianal impairment, and 6 showed features of growth failure. Three patients were using azathioprine for immunomodulation, and none of the patients in the study used corticosteroids at the time of ustekinumab treatment. Four patients had a history of undergoing inflammatory bowel disease–related surgery. Three patients underwent surgery due to perianal problems. One patient underwent small bowel resection and balloon dilatation due to stenosis of the anastomosis site.

Ustekinumab Trough Concentrations and Clinical Correlations

We measured the trough concentration of ustekinumab during maintenance treatment twice in the 32 blood samples obtained from 10 patients using 2 ELISA kits. A flowchart

TABLE 1. Baseline Patient Characteristics at the Beginning of Ustekinumab Treatment

Variables	Values
Age at starting of ustekinumab treatment, years	23.4 (18.9–25.5)
Age at diagnosis, years	15.7 (13.1–16.4)
Disease duration at the start of ustekinumab treatment, years	8.2 (4.6–10.1)
Follow-up time from the initiation of ustekinumab treatment, weeks	44.0 (35.0–58.5)
BMI	23.0 (21.2–24.5)
CDAI*	227.2 (225.2–231.4)
Fecal calprotectin, mcg/g	774.1
Albumin, g/dL	4.1 (3.9–4.4)
ESR, mm/h	29.5 (14.0–43.8)
CRP, mg/dL	1.2 (0.4–3.7)
SES-CD score	12 (7–29)
Paris classification at diagnosis	
Age at diagnosis	
A1a, 0 ≤ 10 yrs old	0
A1b, 10 ≤ 17 yrs old	10
A2, 17–40 yrs old	0
Location	
Lower GI tract involvement	
L1, distal one-third ileum ± limited cecal disease	0
L2, colonic	0
L3, ileocolonic	10
Upper GI tract involvement	
None	8
L4a, upper disease proximal to ligament of Treitz	2
L4b, upper disease distal to ligament of Treitz and proximal to distal one-third ileum	0
L4a + b	0
Behavior	
B1, nonstenosing, nonpenetrating	7
B2, stenosing	0
B3, penetrating	3
B2B3, stenosing and penetrating disease, simultaneously or at different times	0
Perianal impairment	4
Growth	
G0, no evidence of delay in growth	4
G1, delay in growth	6
History of biologics use	
Infliximab	4
Adalimumab	3
Infliximab and adalimumab	3
Concomitant azathioprine use at baseline(Y)	3
Corticosteroid use at baseline(Y)	0
History of IBD-related surgeries	
Fistulotomy or Seton placement	3
Balloon dilatation or bowel resection	1

Values are number or median (interquartile range).
*CDAI score ranges from approximately 0–600, with higher scores indicating worse disease and a 50-point change indicating the minimal clinically important difference.
Y, yes; GI, gastrointestinal; IBD, inflammatory bowel disease.

illustrating how the maintenance treatment interval was altered after ustekinumab induction treatment is described in **Supplemental Digital Content** (see **Figure 1**, http://links.lww.com/TDM/A550). **Supplemental Digital Content** (see **Figure 1**, http://links.lww.com/TDM/A550) shows how the collected blood samples were organized. After the induction treatment, all patients were initially maintained at 12-week intervals according to the guidelines, but 4 of them showed signs of loss of response, and their intervals were changed to 8 weeks.

The median values and interquartile ranges were evaluated and are shown as a box plot in Figure 1. For kit B, the interquartile ranges were narrower than those of kit A. For kit A, a large number of low trough concentrations were undetected and measured as zero. Therefore, the median value of kit B was higher than that of kit A. Nevertheless, each graph of the trough concentrations showed a similar trend over the duration of treatment, as shown in **Supplemental Digital Content** (see **Figure 2**, http://links.lww.com/TDM/A550).

The median trough concentrations of ustekinumab during maintenance treatment, measured using both kits, are described in Table 2. The median trough concentrations measured using kit A was 0.26 mcg/mL, and 0.38 mcg/mL for kit B. The difference between the 2 kits was statistically significant ($P < 0.01$). The interquartile range of kit A was 0–0.66 mcg/mL, and the interquartile range of Kit B was 0.22–0.74 mcg/mL. Because the results of ESR, CRP, albumin, and calprotectin are the values that indicate the disease activity status of Crohn's disease, they were measured using the same blood samples for trough concentration measurements. Therefore, because the values reflect patients' condition at the same time point, correlations between the measured trough concentrations and clinical indicators were evaluated using Pearson correlation. Statistically significant negative correlations with ESR, CRP, and stool calprotectin levels were observed. Low drug concentrations were measured

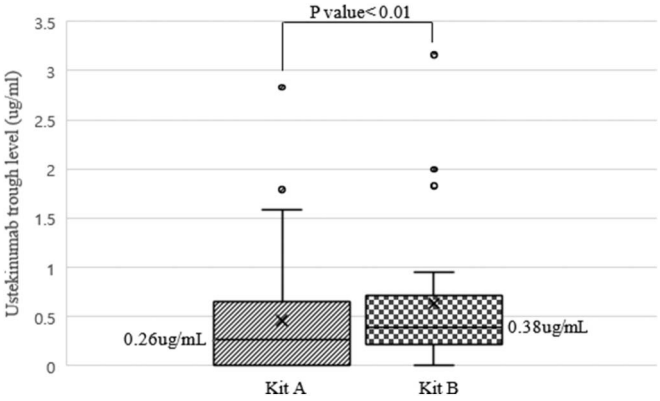

FIGURE 1. A boxplot showing trough concentration of ustekinumab evaluated with 2 experimental kits (sample n = 32). The median trough concentration measured by kit A was 0.26 mcg/mL. The median trough concentration measured by kit B was 0.38 mcg/mL. The first quartile concentration of kit A was 0.00 mcg/mL, and the third quartile concentration was 0.66 mcg/mL. The first quartile concentration of kit B was 0.22 mcg/mL, and the third quartile concentration was 0.74 mcg/mL.

TABLE 2. Trough Levels of Two Experimental Kits and Pearson Correlation Coefficients With *P* Values Between the Clinical Markers, Indicating Activity of Crohn's disease and Ustekinumab Trough Concentrations Obtained During Maintenance Treatment (Sample Number = 32)

	Kit A		Kit B	
Trough concentrations of ustekinumab, median (IQR*)	0.26 mcg/mL (0–0.66)		0.38 mcg/mL (0.22–0.74)	
	Pearson correlation coefficient	***P* Value**	**Pearson correlation coefficient**	***P* Value**
CDAI†	−0.076	0.681	−0.096	0.620
Albumin, g/dL	0.529	0.002	0.582	<0.001
ESR, mm/h	−0.415	0.018	−0.514	0.003
CRP, mg/dL	−0.269	0.137	−0.407	0.021
Fecal calprotectin, mcg/g	−0.727	<0.001	−0.694	0.001

*Interquartile range.
†CDAI score ranges from approximately 0 to 600, with higher scores indicating worse disease and a 50-point change indicating the minimal clinically important difference.

when ESR, CRP, and calprotectin levels were high due to poor clinical status. In the case of kit A, it was confirmed that ESR had a correlation coefficient of −0.415 with a *P* value of 0.018, and a correlation coefficient of −0.269 with a *P* value of 0.135 for CRP. Fecal calprotectin showed the highest absolute value of the correlation coefficient, which was −0.727 ($P < 0.001$). The same trend was observed for kit B. The correlation coefficients of ESR, CRP, and fecal calprotectin levels were −0.514, −0.407, and −0.694 with *P* values of 0.003, 0.021, and 0.001, respectively. In contrast, albumin level had a significant positive correlation with trough concentration. High drug concentration values were measured when the albumin level was high, due to the clinical response to ustekinumab with decreased symptoms of diarrhea. The correlation coefficient of kit A was 0.529 and the *P* value was 0.002, whereas the correlation coefficient of kit B was 0.582, and the *P* value was <0.001. Both kits showed a negative correlation in the CDAI score, which was appropriate for the clinical situation but were not statistically significantly. These correlations are displayed together with the result values through a scatter plot in **Supplemental Digital Content** (see **Figure 3**, http://links.lww.com/TDM/A550).

Although a small population, we also compared the trough concentrations of the 3 patients who were still receiving treatment with azathioprine with those of the other patients. The median values and interquartile ranges were investigated and are presented in **Supplemental Digital Content** (see **Figure 4**, http://links.lww.com/TDM/A550). The median trough concentration of azathioprine group measured by kit A was 0.50 mcg/mL, and the interquartile range was 0.26–0.66 mcg/mL. The median trough concentration of nonazathioprine group measured by kit A was 0.00 mcg/mL, and the interquartile range was 0.00–0.37 mcg/mL. The median trough concentration of azathioprine group measured by kit B was 0.38 mcg/mL and the interquartile range was 0.35–0.54 mcg/mL, whereas the median trough concentration of non-azathioprine group measured by kit B was 0.35 mcg/mL with an interquartile range of 0.19–0.78 mcg/mL.

We also measured the levels of antibodies in all samples using both ELISA kits, and the results indicated that none of the patients developed antibodies against ustekinumab.

Measurement of Optimal Ustekinumab Concentration Targets

The trough concentration at each point and the ROC curve for the evaluation of the therapeutic response are graphically shown in Figure 2. The treatment response was determined by the clinical response, which is mainly based on the CDAI score, and the ESR, CRP, and fecal calprotectin levels were also referenced for the response evaluation. In case of CDAI, the presence of treatment response was determined when the CDAI score decreased by 70 points or more. In the case of ESR, CRP, and calprotectin levels, it was determined that there was a response when it was lowered to the normal range compared with the results of the previous visit.

The area under ROC (AUROC) of kit B was greater than that of kit A. The AUROC value of kit A was 0.744, and the 95% confidence interval of kit A was between 0.572 and 0.916, with a *P* value of 0.023. The optimal cutoff value of kit A was 0.17 mcg/mL. The AUROC value of kit B was 0.921, and the 95% confidence interval was 0.831 and 1.000, with a *P* value of <0.001. The sensitivity and specificity of kit B were 100% and 75%, respectively. The optimal cutoff value of kit B was 0.41 mcg/mL.

Figure 3 also shows the CDAI-70 response as a percentage according to the quartile range of ustekinumab trough concentrations measured with kits A and B.

DISCUSSION

This study aimed to evaluate whether the measured trough concentrations correlated with the clinical disease status and to analyze the potential trough concentration of ustekinumab to predict a clinical response during maintenance treatment. Additionally, 2 commercial ELISA kits were evaluated for future clinical applications.

As a result of measuring the trough concentrations of 32 blood samples using kits A and B, the concentration measurements obtained using kit B were widely dispersed, whereas kit A demonstrated narrowly dispersed measurements and the low concentrations were often measured as zero. Therefore, it may be difficult for clinicians to assess the clinical correlation of the clinical status of patients with a

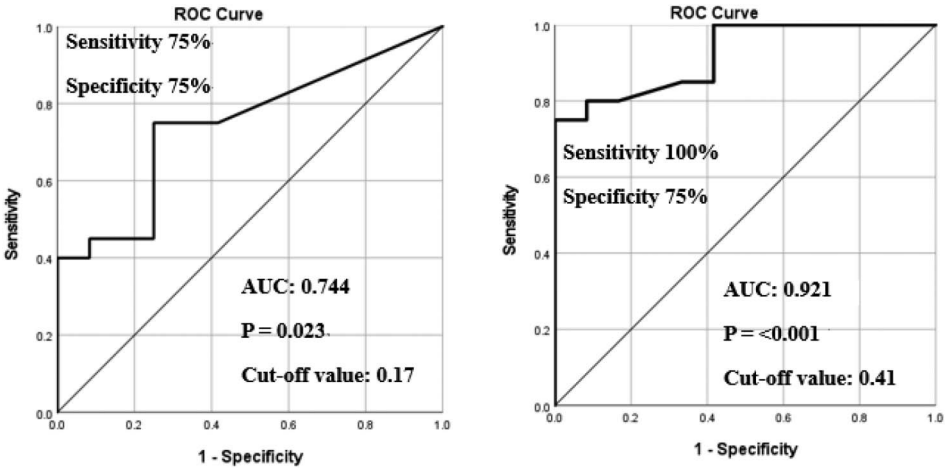

FIGURE 2. ROC curve of each experimental kit showing sensitivity and specificity of ustekinumab trough concentrations at each injection and clinical response in maintenance treatment. Clinical response was based on the CDAI score, ESR, CRP, and calprotectin levels (criteria for positive response: CDAI score decreased by 70 points or more, ESR, CRP, and calprotectin lowered toward the normal range). Left: kit A, right: kit B.

trough concentration of zero when using kit A. After confirming that the trough concentrations measured by both kits correlated with the clinical status using the values of ESR, CRP, and calprotectin, indicating the activity of Crohn's disease, an appropriate cutoff value was determined using the ROC curve. Although the value of kit B was higher than that of kit A, the AUROC value of both kits were greater than 0.5. Both kits showed good predictive values, but the main difference between the kits was that in patients with low ustekinumab concentrations, if the clinician wants to measure the trough concentration to assess the patient's status, kit B can detect even subtle differences in concentration values because it exhibits a lower limit of detection. Knowing these minute differences in concentration values can be helpful in deciding whether to continue using ustekinumab or switch to a different biological agent.

In the comparison between patients who used azathioprine and those who did not, the concentration measured with kit A was higher in the group using azathioprine and the difference in the median trough concentration between the 2 groups was statistically significant, with $P < 0.041$ (see **Figure 4**, **Supplemental Digital Content**, http://links.lww.com/TDM/A550). This difference was possibly because the low trough concentrations in the group that did not use azathioprine was less than the lower limit of quantitation (LLOQ). Contrastingly, in kit B, there was no significant difference in concentration between the 2 groups. Further studies are needed to determine whether azathioprine affects the trough concentration of ustekinumab. Based on these results, we suggest that kit A may provide more meaningful information than kit B in evaluating a significant difference in groups with treatment differences.

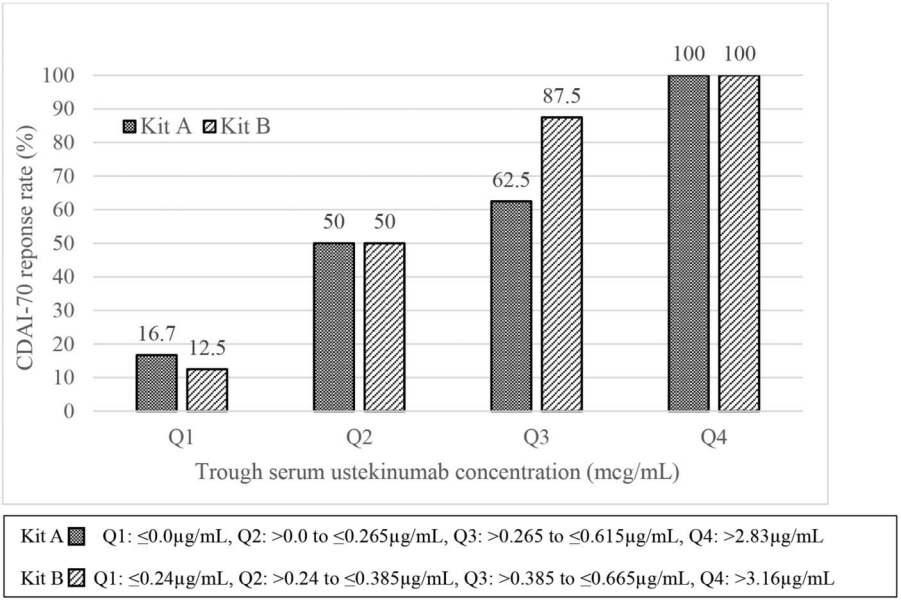

FIGURE 3. Subgroup analyses for CDAI-70 response by quartile serum concentrations of ustekinumab during maintenance treatment. Bar represents CDAI-70 response rate. Quartile ranges of kit A are Q1: ≤0.0 mcg/mL, Q2: >0.0 to ≤0.265 mcg/mL, Q3: >0.265 to ≤0.615 mcg/mL, Q4: >2.83 mcg/mL. Quartile ranges of kit B are Q1: ≤0.24 mcg/mL, Q2: >0.24 to ≤0.385 mcg/mL, Q3: >0.385 to ≤0.665 mcg/mL, Q4: >3.16 mcg/mL.

When comparing the trough concentration between groups that had treatment intervals of 8 and 12 weeks, the 4 patients who received injections at 8-week intervals because of a loss of response showed higher measured concentrations in both kits. The clinical features of the patients also improved from treatments at 8-week intervals compared with those at 12-week intervals, consistent with the trend observed in trough concentration. Based on these results, the clinicians' benefit of checking trough concentrations is that they can predict the presence or absence of a treatment response while monitoring the concentration of ustekinumab, and consider changing the interval of maintenance treatment for patients with low concentration and low response.

We reviewed how other studies and BRIDGeIBD.com measured trough concentrations and observed that their measurement methods varied. Therefore, the trough concentration ranges, units, and cutoff values are described differently. In one study, an ELISA kit with a dilution step similar to kit B was used, but the sample was diluted 20,000-fold and the trough concentrations were 2.5 ± 2.1 mcg/mL (n = 38).[14] In another study, a liquid-phase homogeneous mobility shift assay method was used instead of an ELISA method. The trough concentrations in that study were 4.4 ± 2.0 mcg/mL at 26 weeks and above.[12] Another study used liquid chromatography–tandem mass spectrometry (LC-MS/MS). The clinical response group showed a mean level of 3.58 mg/L, while the nonresponse group showed a mean level of 1.94 mg/L.[16] In these studies, the researchers designed and tested the levels with their own laboratory test methods, so there is a limitation to applying it to testing in other laboratories, as commercially available kits were not used. We used 2 commercial kits for trough concentration measurement and compared them clinically to present more applicable experimental measurements of ustekinumab trough concentrations. In the case of BRIDGeIBD, which provides the most representative reference value, the electrochemiluminescence immunoassay method was used, a more recently developed method than ELISA. They suggested 0.8 mcg/mL as a threshold value for clinical remission at 24 weeks of maintenance treatment.[23] This value is higher than our results. One reason for this difference was the measurement method. Another important reason is that all of our patients had anti-TNF alpha failure. Patients with TNF-alpha failure are more likely to have activated immunity, and this increased immunity may also affect the clearance of ustekinumab, lowering the trough concentration. Therefore, a follow-up study comparing patients with anti-TNF alpha failure and naive treatment with ustekinumab is needed.

We also measured the antibodies in all samples using both ELISA kits, and the results indicated that none of the patients developed antibodies to ustekinumab. Several reasons may explain this finding. First, there is a possibility that the drug itself has a low antibody response rate; second, the observation period may be too short. The longest observation of patients after ustekinumab treatment was 1 year 8 months, which is a short period to produce antibodies, considering the experience with infliximab treatment. According to the PHOENIX-2 trial, although this study was about psoriasis, antibodies against ustekinumab were found in 12.7% of the partial responders compared with 2.0% of the responders at week 52.[24] The rate of antibody formation may have increased if observed for more than 52 weeks. Furthermore, the timing of antibody production in Crohn's disease is yet to be reported. Because there is a possibility that antibodies may be formed in patients with poor clinical symptoms and low trough concentrations, we will continue to evaluate trough concentrations and antibody formation. In addition, it is important to evaluate whether antibody formation is reduced when azathioprine is used in combination with other biological agents such as infliximab.

The limitation of this study may be, first of all, that the number of patients was small. Although the number of patients was small, 32 blood samples were measured twice with different kits, and a sufficient number of results were derived for clinical application to evaluate the usefulness of the 2 kits. Another limitation was that the observation period was relatively short. Therefore, the clinical course, outcome, and complications of patients treated with ustekinumab should be further examined.

CONCLUSIONS

In conclusion, the trough concentrations measured by the 2 commercial ELISA kits were correlated with CRP, ESR, and albumin levels and stool calprotectin levels. Therefore, it can be concluded that trough concentrations can be used to assess clinical conditions during treatments. When comparing the 2 commercial kits, kit B detected even minute changes that were suitable for patients with trough concentrations close to zero. Clinicians can predict the presence or absence of a treatment response while monitoring the concentration of ustekinumab and may consider changing the interval of maintenance treatment for patients with low concentration and low response.

REFERENCES

1. 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.
2. Moschen AR, Tilg H, Raine T. IL-12, IL-23 and IL-17 in IBD: immunobiology and therapeutic targeting. *Nat Rev Gastroenterol Hepatol.* 2019;16:185–196.
3. Luo J, Wu SJ, Lacy ER, et al. Structural basis for the dual recognition of IL-12 and IL-23 by ustekinumab. *J Mol Biol.* 2010;402:797–812.
4. Feagan BG, Sandborn WJ, Gasink C, et al. Ustekinumab as induction and maintenance therapy for Crohn's Disease. *N Engl J Med.* 2016;375:1946–1960.
5. Hanauer SB, Sandborn WJ, Feagan BG, et al. IM-UNITI: three-year efficacy, safety, and immunogenicity of ustekinumab treatment of Crohn's Disease. *J Crohns Colitis*. 2020;14:23–32.
6. Wong ECL, Marshall JK, Reinisch W, et al. Body mass index does not impact clinical efficacy of ustekinumab in Crohn's Disease: a post hoc analysis of the IM-UNITI trial. *Inflamm Bowel Dis.* 2021;27:848–854.
7. Bortlik M, Duricova D, Malickova K, et al. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. *J Crohns Colitis*. 2013;7:736–743.
8. Choi SY, Kang B, Lee JH, et al. Clinical use of measuring trough levels and antibodies against infliximab in patients with pediatric inflammatory bowel disease. *Gut Liver*. 2017;11:55–61.
9. Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are asso-

ciated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut*. 2014;63:1721–1727.
10. Steenholdt C, Bendtzen K, Brynskov J, et al. 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.
11. Adedokun OJ, Xu Z, Gasink C, et al. Pharmacokinetics and exposure response relationships of ustekinumab in patients with Crohn's disease. *Gastroenterology*. 2018;154:1660–1671.
12. Battat R, Kopylov U, Bessissow T, et al. Association between ustekinumab trough concentrations and clinical, biomarker, and endoscopic outcomes in patients with Crohn's disease. *Clin Gastroenterol Hepatol.* 2017;15:1427–1434. e1422.
13. Hanzel J, Zdovc J, Kurent T, et al. Peak concentrations of ustekinumab after intravenous induction therapy identify patients with Crohn's disease likely to achieve endoscopic and biochemical remission. *Clin Gastroenterol Hepatol.* 2021;19:111–118. e110.
14. Morita Y, Imai T, Bamba S, et al. Clinical relevance of innovative immunoassays for serum ustekinumab and anti-ustekinumab antibody levels in Crohn's disease. *J Gastroenterol Hepatol.* 2020;35: 1163–1170.
15. Restellini S, Khanna R, Afif W. Therapeutic drug monitoring with ustekinumab and vedolizumab in inflammatory bowel disease. *Inflamm Bowel Dis.* 2018;24:2165–2172.
16. Thomann AK, Schulte LA, Globig AM, et al. Ustekinumab serum concentrations are associated with clinical outcomes in Crohn's disease—a regional multi-center pilot study. *Z Gastroenterol.* 2020;58:439–444.
17. Soufflet N, Boschetti G, Roblin X, et al. Concentrations of ustekinumab during induction therapy associate with remission in patients with Crohn's disease. *Clin Gastroenterol Hepatol.* 2019;17:2610–2612.
18. Harris KA, Horst S, Gadani A, et al. Patients with refractory Crohn's disease successfully treated with ustekinumab. *Inflamm Bowel Dis.* 2016; 22:397–401.
19. Kopylov U, Afif W, Cohen A, et al. Subcutaneous ustekinumab for the treatment of anti-TNF resistant Crohn's disease–the McGill experience. *J Crohns Colitis*. 2014;8:1516–1522.
20. Brignola C, Campieri M, Bazzocchi G, et al. A laboratory index for predicting relapse in asymptomatic patients with Crohn's disease. *Gastroenterology*. 1986;91:1490–1494.
21. Macfarlane PI, Miller V, Wells F, et al. Laboratory assessment of disease activity in childhood Crohn's disease and ulcerative colitis. *J Pediatr Gastroenterol Nutr.* 1986;5:93–96.
22. Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis.* 2011;17:1314–1321.
23. Papamichael K, Cheifetz AS, Melmed GY, et al. Appropriate therapeutic drug monitoring of biologic agents for patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol.* 2019;17:1655–1668. e1653.
24. Papp KA; PHOENIX 2 Study Investigators. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2). *Lancet*. 2008;371: 1675–1684.