Endoscopic Activity and Serum TNF-α Level at Baseline Are Associated With Clinical Response to Ustekinumab in Crohn's Disease Patients

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Background and Aims: The therapeutic efficacy and safety of ustekinumab for Crohn's disease (CD) have been reported from randomized controlled trials and real-world data. However, there are few studies describing the identification of patients most suitable for ustekinumab therapy. The aim of this study was to prospectively evaluate the patients receiving ustekinumab and identify predictors of the treatment efficacy.

Methods: Patients with moderate to severe active CD scheduled to receive ustekinumab were enrolled. The responders and nonresponders were compared at weeks 0, 8, 24, and 48 by evaluating patient demographics, simple endoscopic scores (SES-CD), ustekinumab and cytokine concentrations, and cellular fractions.

Results: The clinical response and clinical remission rates in the 22 enrolled patients were 59.1% and 31. 8% at week 8, 68.2% and 45.5% at week 24, and 54.4% and 40.9% at week 48, respectively. There were no significant differences in patients' demographic and disease characteristics at baseline between responders and nonresponders. A combination of low SES-CD and high serum TNF-α concentration at baseline showed a good correlation with the clinical response. Serum TNF-α concentration was decreased because of the therapy. The ratio of $CD4^+$TNF-α cells at baseline was significantly higher in responders than in nonresponders; however, the ratios of $CD45^+CD11b^+$TNF-α and $CD45^+CD11c^+$TNF-α cells were not different. The ratio of $CD4^+$ TNF-α cells decreased with the treatment in the responders but not in the nonresponders.

Conclusions: The combination of 2 factors, namely higher serum TNF-α concentration and lower SES-CD at baseline, may assist clinicians in selecting the appropriate therapy for patients with moderate to severe CD.

Key Words: biomarkers, clinical trials, cytokine, endoscopy, small intestine

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Abbreviations: CD, Crohn's disease; TNF, Tumor necrosis factor; IL-12, interleukin 12; IL-23, interleukin 23; IL-17, interleukin 17; SES-CD, simple endoscopic score for Crohn's disease; HBI, Harvey-Bradshaw index; CDAI, Crohn's disease activity index; CRP, C-reactive protein.

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INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disease of the gastrointestinal tract and is characterized by relapses and remissions.[1, 2] The incidence and prevalence of CD are increasing, and the disease has become a cause for global health concern.[3, 4] The main goals in the management of CD patients are to achieve clinical and endoscopic remission and to avoid complications, such as fistula and stenosis. The treatment options for CD have expanded during the past years; conventional drugs, such as corticosteroids, azathioprine, and especially tumor necrosis factor alpha (TNF-α) antagonists have dramatically transformed the treatment of moderate to severe active CD.[5–8] Tumor necrosis factor alpha antagonists can induce and maintain clinical remission, but approximately half of the patients experience loss of response to TNF-α antagonists. A lot of effort has been made in this regard, but there are still serious problems in the management of CD patients.[9–11]

Ustekinumab is a monoclonal antibody against the p40 subunit of interleukin-12 (IL-12) and interleukin-23 (IL-23).[12] It is currently approved as a treatment for moderate

to severe active CD in the United States, Europe, and other countries. The therapeutic effect of ustekinumab for CD patients has been demonstrated in a phase 2 study (CERTIFI), phase 3 induction studies (UNITI-1 and UNITI-2), and in a maintenance study (IM-UNITI). In the phase 2 study, patients administered the ustekinumab therapy showed higher clinical response and remission rates than those in the placebo group at week 6 and 20.[13] In the phase 3 studies, ustekinumab therapy showed higher clinical response rate at week 8.[14] In IM-UNITI, a clinical trial for a maintenance therapy, the remission rate was 30%–40% at week 44.[15] Several observational studies have shown that ustekinumab induces short- and long-term clinical responses.[16, 17]

Several studies have also indicated that patients with ileocolonic disease at induction, a history of previous intestinal resection, and a clinical score (Harvey-Bradshaw index [HBI]) ≥7 were less likely to achieve clinical response.[18, 19] Another study also indicated that an HBI score ≥7 at induction was associated with high risk for the loss of response. We investigated whether the disease activity at induction was associated with the clinical response by analyzing the serum C-reactive protein (CRP) levels and the simple endoscopic score for Crohn's disease (SES-CD) at induction. In addition, ustekinumab binds to the p40 unit of IL-12 and IL-23 and prevents their interaction with the natural killer or T cells. Interleukin-12 is involved in the induction of differentiation of interferon-γ and TNF-α producing Th1 cells, and IL-23 reinforces the Th17 cell response, which induces interleukin 17, interleukin 6, and TNF-α.[20] Therefore, we hypothesized that Th1 and Th17 cytokines modulate the clinical response to ustekinumab, and their concentrations may be associated with the response. The optimal setting for ustekinumab induction in CD treatment is not clear, and there are few studies on factors that are useful for predicting the clinical response to ustekinumab. Such predictors would help clinicians to strategize personalized treatments for CD patients.

The aim of this study was to identify the CD patient subgroups that are most likely to benefit from ustekinumab therapy by identifying predictors of response to ustekinumab.

MATERIALS AND METHODS

Ethical Considerations

This study was conducted with the approval of the ethics committee of Nagoya University Hospital, Japan. The study was registered in the University Hospital Medical Information Network, a clinical trial registry (UMIN000028506). All the participants provided written informed consent.

Patients and Study Design

Patients with moderate to severe active CD (CDAI score between 220 and 450) who were administered ustekinumab at Nagoya University Hospital from October 2017 to May 2019 were prospectively enrolled. Patients received a weight range–based dose that approximated 6 mg of ustekinumab per kilogram of body weight (patients weighing ≤55 kg received 260 mg, those weighing >55 kg and ≤85 kg received 390 mg, and those weighing >85 kg received 520 mg of ustekinumab) every 8 weeks. Each patient was evaluated based on the CDAI score and provided blood samples for determination of the ustekinumab and cytokine concentrations and fractions of cytokines in lymphocytes, macrophages, and dendritic cells. The evaluation of these parameters was done before induction (baseline) and at weeks 8, 24, and 48 after induction. The clinical response was defined as a reduction from the baseline CDAI score of 100 or more points or below 150. Clinical remission was defined as a CDAI score below 150. The patients were categorized as responders and nonresponders. The evaluated parameters were patient characteristics, simple endoscopic score, ustekinumab and cytokine concentrations, and fractions of cytokines. The responders were defined as those with a reduction from baseline CDAI score of 100 or more or those with CDAI score below 150 at 24 weeks.

SES-CD and Modified SES-CD

The endoscopies were performed for all the patients within 2 months of the introduction of ustekinumab and were assessed by the SES-CD score. The SES-CD score was evaluated in 5 predefined ileocolic segments. The SES-CD score was decided by a single reader who was blind to the clinical results.

The majority of the enrolled patients underwent double balloon endoscopy (DBE) to examine small intestinal lesions before the induction of ustekinumab. To evaluate the small intestinal lesions with DBE, we divided the small intestine into 3 sections referring to the previous report[21] and evaluated each section using the modified SES-CD. We performed DBE before induction and at week 24 to 32 after induction and examined the therapeutic effect on the small intestine.

We categorized the 22 patients into an improved group and an unimproved group using their SES-CD or modified SES-CD. The improved group was defined as the group of individuals with a reduction in the SES-CD or modified SES-CD of 3 or more, or more than 50% improvement from the baseline or remission at week 24 according to the previous reports.[22–24]

Serum Ustekinumab Concentration

Serum ustekinumab concentration was determined using the ustekinumab ELISA kit (Eagle Biosciences Inc., Columbia, USA) according to the manufacturer's instructions. The absorbance of each sample was measured at 450 nm/570 nm using a PowerScan4 microplate reader (DS Pharma Medical Co., Osaka, Japan). The concentration of ustekinumab was calculated on the basis of a standard curve. The lowest quantifiable concentration of ustekinumab in the serum was 1.5 ng/mL.

Serum Concentrations of Cytokines

The serum levels of TNF-α, IL-17A, and IFN-γ were assessed using the Quantikine ELISA kits (R and D systems, Minneapolis, MN, USA), according to the manufacturer's instructions. The absorbance of each sample was measured at 450 nm/570 nm using a PowerScan4 microplate reader (DS Pharma Medical Co.). The concentrations of cytokines were calculated on the basis of a standard curve. The lowest quantifiable concentration of TNF-α in the serum was 6.23 ng/mL.

Immunohistochemistry

For immunohistochemical studies, formaldehyde-fixed, paraffin-embedded biopsy samples obtained by colonoscopy were deparaffinized, and the antigens were retrieved by boiling the samples in a target retrieval solution at pH 6 (Dako, Santa Clara, CA, USA) for 30 minutes. The tissue sections were washed with phosphate-buffered saline (PBS), blocked with a blocking reagent (Dako), and incubated overnight at 4°C with anti-TNF-α antibody (Abcam, Cambridge, UK) diluted in PBS. The sections were then washed, treated with 5% hydrogen peroxide/ethanol solution for 30 minutes at room temperature, and incubated at room temperature for 60 minutes with horseradish peroxidase–conjugated antirabbit IgG secondary antibody (EnVision System; Dako), which was followed by the detection of signal using diaminobenzidine (DAB) solution.

Isolation of Peripheral Blood Mononuclear Cells

A mixture of 10 mL of patient's blood sample and 10 mL of Roswell Park Memorial Institute medium (Sigma, St. Louis, MO) was placed on 10 mL of Ficoll (GE Healthcare) and centrifuged at 20°C for 40 minutes at 400 × *g*. The supernatant was discarded, and the middle layer was collected and centrifuged at 20°C for 10 minutes at 400 × *g*. Thereafter, the supernatant was discarded, and the cells were stored using cellbanker (ZENOAQ) at −80°C.

Flow Cytometry of Peripheral Blood Mononuclear Cells ($CD4^+$)

The peripheral blood mononuclear cells (PBMCs) were suspended in Roswell Park Memorial Institute medium supplemented with 10% FBS. Next, 100 µL of the suspension was plated in individual wells of a 96-well plate. Subsequently, 0.1 µL of phorbol 12-myristate 13-acetate (PMA), 0.1 µL of ionomycin, and 0.2 µL of golgistop were added to the wells, and the plates were incubated at 37°C for 4 hours. A fixable viability dye, eFluor 506 (Thermo Fisher, Waltham, MA, USA), anti-CD3-PerCPCy5.5 (Biolegend, San Diego, CA, USA), anti-CD4-APC-Cy7 (Biolegend, SAN, USA), and anti-CD8-BV785 (Biolegend) were added, and the cells were allowed to stain for 15 minutes in a dark room at 4°C. Thereafter, 150 µL of fluorescence-activated cell sorting buffer was added and the suspension was centrifuged at 400 × g for 3 minutes at 4°C. The supernatant was discarded, and 200 µL of FOXP3 FIX solution (Thermo Fisher) was added to the cell pellet. The cells were allowed to stain for 30 minutes in the dark at 4°C. Subsequently, 200 mL of perm/wash buffer was added, and the suspension was centrifuged at 600 × *g* for 3 minutes at 4°C. Anti-Foxp3-PE (Thermo Fisher), anti-TNF-α-AF 488 (Biolegend), anti-IL-4-BV421 (BD Biosciences San Jose, CA, USA), anti-IFN-γ-PE-Cy7 (Biolegend, USA), and anti-IL-17A-AF 647 (BD Biosciences) were added to 50 µL of perm/wash buffer, and staining was done for 20 minutes in a dark room at 4°C. Thereafter, 150 µL of perm/wash buffer was added, and the suspension was centrifuged at 600 × *g* for 3 minutes at 4°C. Flow cytometric analysis was performed using a BD LSR Fortessa X-20 (BD Biosciences), and data was analyzed with FlowJo software (Tree Star, Ashland, OR, USA).

Flow Cytometry of PBMCs ($CD45^+CD11b^+$ and $CD45^+CD11c^+$)

The procedure was similar to that for $CD4^+$, but the cells were stimulated with lipopolysaccharide for 12 hours at 37°C. The fixable viability dye, eFluor 506 (Thermo Fisher), anti-CD45-eFlour 450 (eBioscience, San Diego, CA, USA), anti-CD11b-PerCP/Cy5.5 (Biolegend), anti-CD11c-PE/Cy7 (Biolegend) of HLA-DR-APC/Fire 750 (Biolegend), IL-12R-PE (BD Biosciences), and IL-23R Allophycocyanin Mab (R and D Systems) were added, and the cells were stained for 15 minutes in a dark room at 4°C. Thereafter, 200 µL of FOXP3 FIX solution (Thermo Fisher) was added and incubated for 30 minutes in the dark at 4°C. Then, 200 µL of perm/wash buffer was added and the suspension was centrifuged at 600 × *g* for 3 minutes at 4°C. Anti-TNF-α-AF 488 (Biolegend) was added, and the cells were stained for 20 minutes in a dark room at 4°C. Thereafter, 150 µL of perm/wash buffer was added, and the suspension was centrifuged at 600 × *g* for 3 minutes at 4°C. Flow cytometric analysis was performed using a BD LSR Fortessa X-20 (BD Biosciences), and data were analyzed with FlowJo software (Tree Star).

Statistical Analysis

The statistical software package SPSS (SPSS Inc., Chicago, IL, USA) was used for data analyses. The data from the responders and nonresponders were compared using the Mann–Whitney *U* test or χ^2 test. In all the analyses, $P < 0.05$ was considered to be statistically significant.

ETHICAL CONSIDERATIONS

The contents of this article have not been published elsewhere. There are no ethical problems or conflicts of interest with regard to this article.

RESULTS

Ustekinumab Is Safe and Effective for Moderate to Severely Active CD Patients

A total of 60 patients were administered ustekinumab in our hospital from October 2017 to May 2019, 22 of whom were

found to have moderate to severe active CD (with CDAI scores between 220 and 450). We analyzed these 22 patients who all continued ustekinumab until week 48 (Fig. 1A). The demographic and baseline disease characteristics of the 22 patients are shown in Supplemental Table 1. Fourteen patients were male, and 8 were female; the median age was 42.5 years. (range 20–85 years.), and the median body weight was 52.2 kg (29.0–78.6 kg). In all, 81.8% of the patients suffered from ileocolonic disease, 54.5% had nonstructuring and nonpenetrating disease, and 36.4% were receiving concomitant immunomodulators at

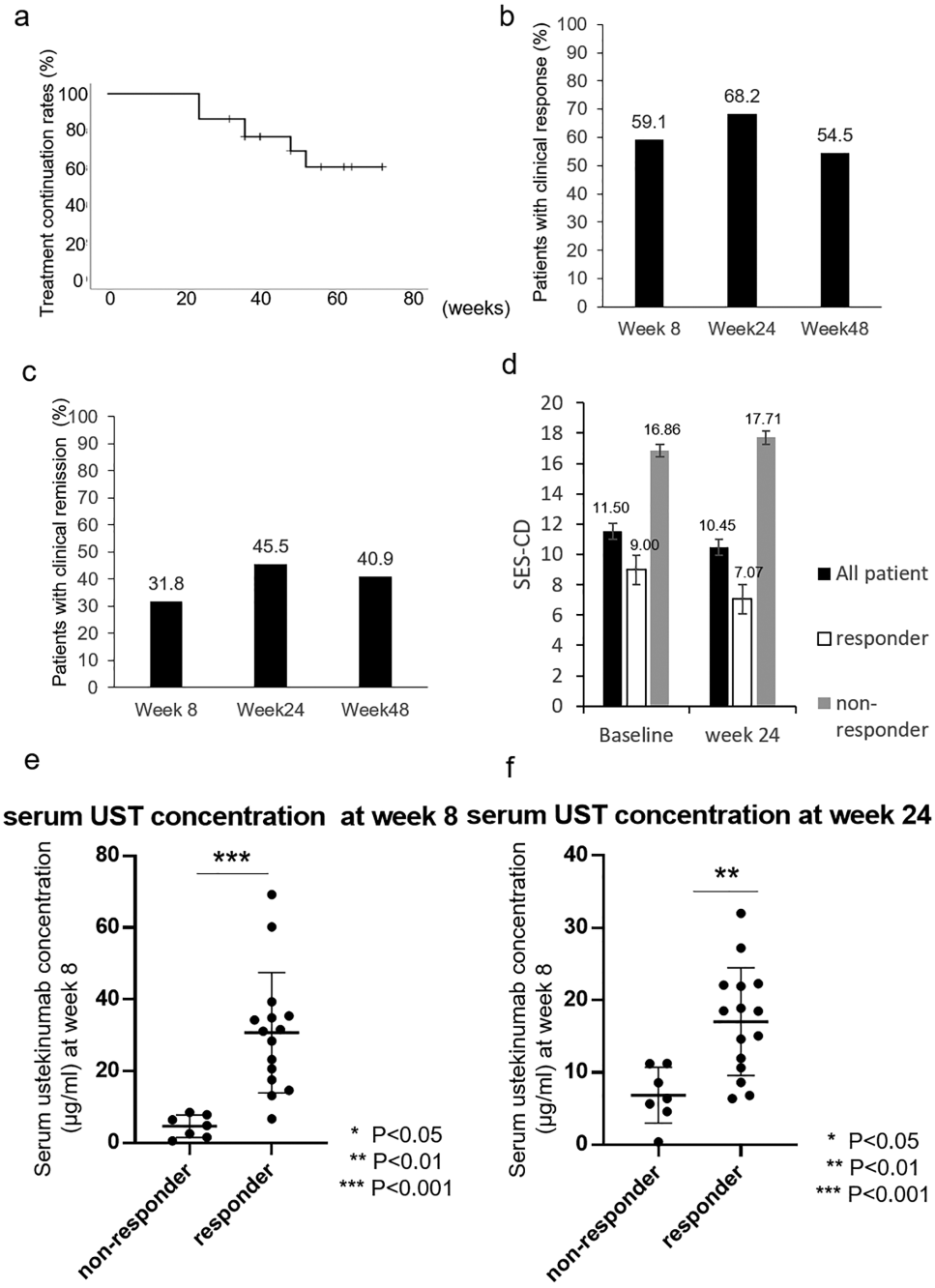

FIGURE 1. Proportions of patients who achieved clinical response and remission at weeks 8 and 24. A, Kaplan–Meier analysis of the continuation rate of ustekinumab. B, Clinical response rate at weeks 8, 24, and 48. C, Clinical remission rate at weeks 8, 24, and 48. D, Changes in mean SES-CD values (baseline and 24 weeks later), all patients (N = 22; 11.50 to 10.45), responders (N = 15; 9.00 to 7.07), nonresponders (N = 7; 16.86 to 17.71). Serum concentration of ustekinumab at week 8 is significantly higher in the responders than in the nonresponders. E, Serum ustekinumab concentration at week 8. F, Serum ustekinumab concentration at week 24.

baseline. A total of 72.7% of the patients had been treated with at least 1 TNF-α antagonist, and 50% had been treated with 2 or more TNF-α antagonists; 27.3% of the patients had not received any TNF-α antagonist.

The percentage of patients who showed a clinical response was 59.1% at week 8, 68.2% at week 24, and 54.5% at week 48 (Fig. 1B). The percentage of patients who achieved clinical remission was 31.8% at week 8, 45.5% at week 24, and 40.9% at week 48 (Fig. 1C); 59.1% of the patients showed a clinical response up to week 8, and about 10% of the remaining patients showed a clinical response between week 8 and week 24. None of the patients showed a clinical response after week 24. In addition, in patients who showed a clinical response at week 8, the therapeutic effect was not diminished between week 8 and week 24. The average of SES-CD was 11.50 in all patients (9.0 in responders and 16.86 in nonresponders). The average of SES-CD in responders decreased at week 24 but did not decrease in nonresponders (Fig. 1D).

Serum Ustekinumab Concentration Is Associated With Treatment Efficacy

For detecting the potential predictors of the response to ustekinumab, we categorized the 22 patients into responders and nonresponders using their CDAI scores. The responders were defined as those with a reduction in the CDAI score of 100 or more from the baseline or below 150 at week 24. The baseline characteristics in the responders and nonresponders are shown in Table 1. There were no significant differences in the demographic characteristics or disease characteristics between both groups. Likewise, the baseline concomitant medications and history of TNF-α antagonist treatment did not differ significantly between the 2 groups. There was no significant difference in patient baseline characteristics and clinical findings between responders and nonresponders (Supplemental Table 2).

Recently, several studies have shown that the serum ustekinumab concentration is associated with treatment efficacy.[25, 26] Consistent with previous studies, the serum ustekinumab concentration in the responders was significantly higher than that in the nonresponders (Fig. 1E). Similar results were obtained at week 24 (Fig. 1F).

Low SES-CD at Baseline Is Associated With the Response to Ustekinumab

We aimed to identify the predictors of the response to ustekinumab at baseline. Previous studies have shown that the disease activity at baseline was associated with the response to ustekinumab.[27] We did not find any significant difference in the CDAI score or CRP concentration at baseline between the responders and nonresponders, but the SES-CD scores in the responders were significantly lower than those in the nonresponders (Fig. 2A). We also analyzed the receiver operating characteristic curve of SES-CD at baseline; the cut-off value of SES-CD for the clinical response was determined to be 13 (area under the receiver operating characteristics was 0.757) (Fig. 2B). The SES-CD at week 24 tended to decline from the baseline and was lower in responders compared with nonresponders (Figs. 2C and 2D).

TABLE 1. Comparison of Responder (n = 15) and Nonresponder (n = 7) in Patient Background and Laboratory Data at Baseline

Characteristics	Responder (N = 15)	Nonresponder (N = 7)	*P*
Male, %	66.7	57.1	0.187**
Age, median, yr	43	42	1.000*
Weight, kg	55.39 ± 14.18	50.57 ± 14.74	0.630*
Median disease duration, yr	14.4 ± 9.5	16.3 ± 13.5	0.680*
CDAI	281.07 ± 114.46	246.28 ± 31.94	0.123*
Median CRP, mg/L	1.02 ± 1.34	0.97 ± 1.50	0.630*
Median Albumin, g/dl	3.18 ± 0.72	3.69 ± 0.61	0.142*
GI areas involved, % (Montreal classification)			
L1 ileal (%)	6	28.5	0.227**
L2 colonic (%)	0	14.2	0.318**
L3 ileocolonic (%)	94	57.1	0.227**
p perianal disease (%)	40	71.4	0.181**
Baseline concomitant medications, %			
Corticosteroid	13.3	57.1	0.054**
Immunomodulator	40	28.5	0.604**
History of TNF antagonist treatment (%)	46.6	57.1	1.000**
Patients who received 2 or 3 drugs (%)	33.3	14.2	0.616**

*Mann–Whitney *U* test, ** χ^2 test

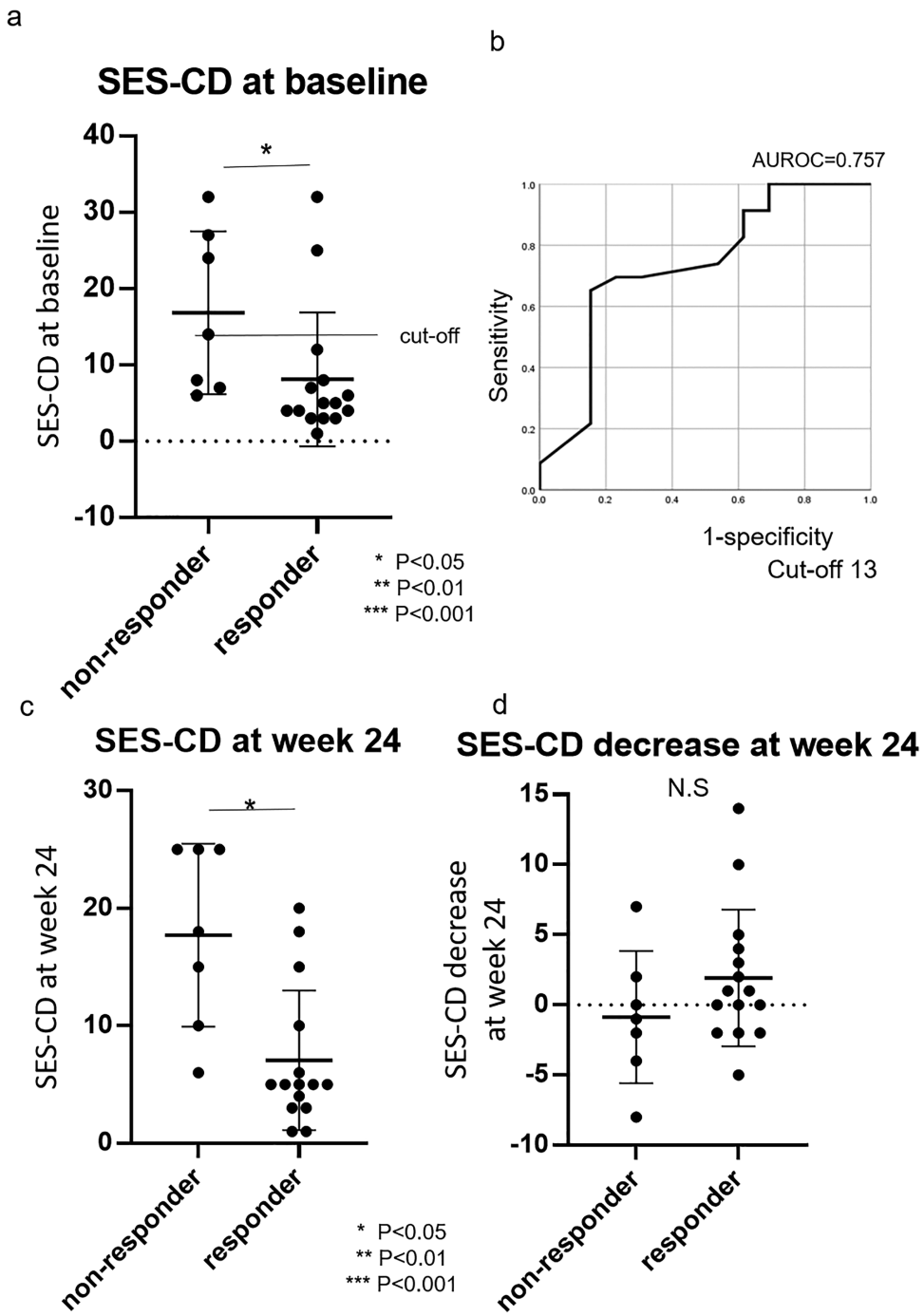

FIGURE 2. SES-CD at baseline is lower in responders than in nonresponders. A, SES-CD at baseline in responders (n = 15) and nonresponders (n = 7). B, ROC curve has a cut-off value of 13.00 at AUROC 0.757. C, SES-CD 24 weeks later in responders and nonresponders. D, Decrease in SES-CD after 24 weeks in responders and nonresponders.

Transoral and transanal DBE were performed in 18 patients, and there was no significant difference in baseline, modified SES-CD between the responders and nonresponders (Supplemental Fig. 1A). Modified SES-CD decreased in the responders (Supplemental Fig. 1B–D).

Serum TNF-α Concentration at baseline Is Associated With the Response to Ustekinumab

Ustekinumab blocks the IL-12 and IL-23 bioactivity by preventing their interaction with the cell receptors on T-cells, natural killer cells, and antigen-presenting cells.[12, 28]

Interleukin-12 and IL-23 induce the differentiation from naïve T cells to Th1 and Th17 cells.[29] We hypothesized that cytokine concentrations at baseline would be associated with the response to ustekinumab. We measured the serum IFN-γ, IL-17A, and TNF-α concentrations. There was no significant difference in the IL-17A and IFN-γ concentrations between the 2 groups (Supplemental Figs. 2A–F). The serum TNF-α concentration in the responders at baseline was significantly higher than that in the nonresponders (Fig. 3A). We also confirmed that the serum TNF-α concentration in the responders was significantly decreased at week 8 (Fig. 3C). At 24 and 48 weeks, the TNF-α concentration in the responders tended to decline, and there was no significant difference between the responders and nonresponders (Figs. 3D and 3E). The cut-off values for TNF-α were 19.58 pg/mL and 0.819 pg/mL for area under the receiver operating characteristics (Fig. 3B).

We categorized the 22 patients into improved and unimproved groups using their SES-CD or modified SES-CD. The improved group was defined as the group of individuals with a reduction in the SES-CD or modified SES-CD of 3 or more, or more than 50% improvement from the baseline or remission at week 24, according to the previous reports.[22–24] We examined CDAI score, SES-CD score, serum TNF-α concentration, CRP, and albumin concentration at baseline in the improved group and the unimproved group. Serum TNF-α concentration was higher in the improved group than in the unimproved group. We did not find any significant difference in the CDAI score, serum TNF-α concentration, CRP, or albumin concentration at baseline between the improved group and the unimproved group when we categorized patients using SES-CD (Supplemental Fig. 9). When we categorized patients using modified SES-CD, serum TNF-α concentration was higher in the improved group compared with the unimproved group. We did not find any significant difference in the CDAI score, SES-CD, CRP, or albumin concentration at baseline between the improved group and the unimproved group (Supplemental Fig. 10).

$CD4^+TNF\text{-}\alpha^+$ Cell Population Are Higher in Responders than in Nonresponders

TNF-α is a critical mediator of intestinal inflammation and is produced by various cells, mainly macrophages and T cells. To identify the cells that are induced by ustekinumab for the production of TNF-α, we analyzed the PBMCs in the responders and nonresponders. The $CD4^+$ $TNF\text{-}\alpha^+$ and $CD4^+$ $IFN\text{-}\gamma^+$ $TNF\text{-}\alpha^+$ cell populations in the responders were significantly higher than in the nonresponders at baseline (Figs. 4A–C). In addition, the $CD4^+$ $IL\text{-}17^+$ $TNF\text{-}\alpha^+$ cell populations were significantly higher than in the nonresponders (Figs. 4D and E). In contrast, there was no difference in the $IL\text{-}4^+$ $TNF\text{-}\alpha^+$ cell populations between the 2 groups (Figs. 4F and G). We also analyzed TNF-α from monocytes and dendritic cells. The $CD45^+CD11b^+CD11c^+TNF\text{-}\alpha^+$, $CD45^+CD11b^-CD11c^+TNF\text{-}\alpha+$, $CD45+CD11b+CD11c^-TNF\text{-}\alpha^+$, and $CD45^+CD11b^-CD11c^-TNF\text{-}\alpha^+$ cells were combined into TNF-α cells derived from macrophages and dendritic cells. There was no difference in the $CD45^+CD11b^+CD11c^+TNF\text{-}\alpha^+$, $CD45^+CD11b^-CD11c^+TNF\text{-}\alpha^+$, $CD45^+CD11b^+CD11c^-TNF\text{-}\alpha^+$, and $CD45^+CD11b^-CD11c^-TNF\text{-}\alpha^+$ cell populations between the 2 groups at baseline (Supplemental Figs. 3A–H). In addition, the $CD4^+$ $TNF\text{-}\alpha^+$ cell populations in the responders decreased after the induction by ustekinumab (Figs. 5A and C). These cell populations in the nonresponders did not alter after the induction by ustekinumab (Figs. 5B and D). Furthermore, $CD45^+CD11b^+CD11c^+TNF\text{-}\alpha^+$cell populations did not decrease in the responders and nonresponders after the induction of ustekinumab (Supplemental Figs. 4A–D). The same result was obtained for all combinations of CD11b and CD11c. (Supplemental Figs. 5–7). The production of TNF-α from $CD11b^+$ and $CD11c^+$ cells was low, but the production of TNF-α significantly increased after lipopolysaccharide stimulation (Supplemental Fig. 11). These results suggest that TNF-α derived from Th1 cells and Th17 cells is associated with the response to ustekinumab.

We analyzed populations of $CD4^+$ $TNF\text{-}\alpha^+$, $CD4^+$ $IFN\text{-}\gamma^+$, and $CD4^+$ $IL\text{-}17A^+$ in the responders and nonresponders. There was no significant difference in the populations of $CD4^+$ $IFN\text{-}\gamma^+$ and $CD4^+$ $IL\text{-}17A^+$ between the responders and nonresponders. The populations of $CD4^+$ $TNF\text{-}\alpha^+$ in the responders at baseline was significantly higher than that in the nonresponders (Supplemental Fig. 12).

Serum TNF-α Concentration Does Not Correlate With Disease Activity

To test if the serum TNF-α level correlated with the disease activity, we analyzed the TNF-α expression in intestinal specimens, the CDAI score, and the CRP level. Tumor necrosis factor alpha was highly expressed in intestinal specimens in the responders (ie, the serum TNF-α concentration was high) compared with its expression in the nonresponders (ie, the serum TNF-α was low; Supplemental Fig. 8A). There was no correlation between the serum TNF-α concentration, CDAI score, and CRP level (Supplemental Figs. 8B and C).

Combination of SES-CD and Serum TNF-α Concentration Is Associated With Response to Ustekinumab

We identified 2 predictors for the response to ustekinumab, viz, a lower SES-CD score and a higher serum TNF-α concentration at baseline. The patients were divided into 4 groups based on the SES-CD score and serum TNF-α concentration. The patients with a low SES-CD score and a high serum TNF-α concentration showed the highest response rate(92.3%), and those with a high SES-CD score and a low serum TNF-α concentration showed the lowest response rate (25%; Fig. 6).

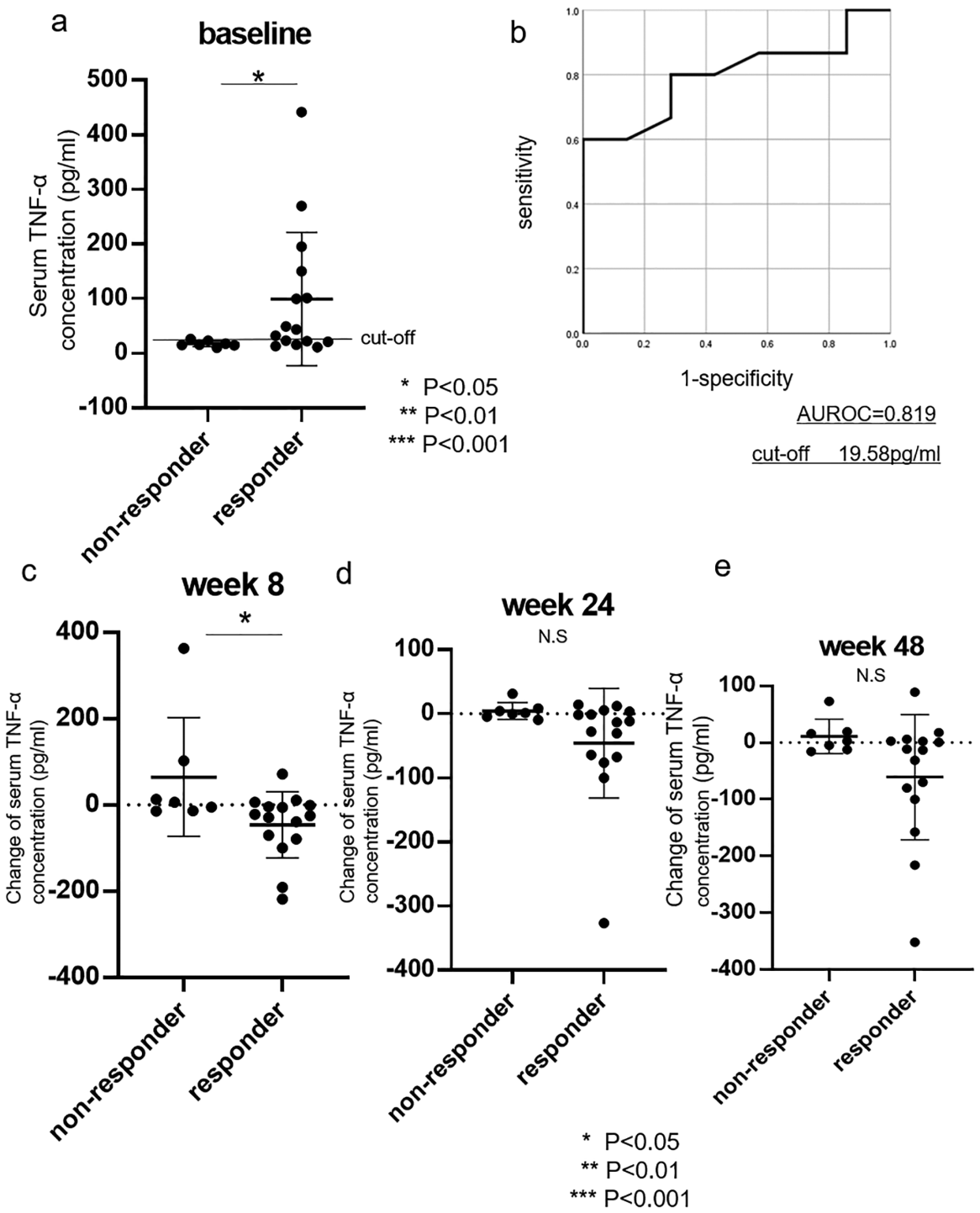

FIGURE 3. Serum TNF-α concentration at baseline is significantly higher in responders (n = 15) than in nonresponders (n = 7). A, Serum TNF-α concentration at baseline. B, ROC curve has a cut-off value of 19.58 pg/mL at AUROC 0.819. C, Change in serum TNF-α concentration from the baseline at week 8. D, Change in serum TNF-α concentration from the baseline at week 24. E, Change in serum TNF-α concentration from the baseline at week 48.

DISCUSSION

We prospectively observed the patients who received ustekinumab and found that 68.2% of them showed a clinical response at week 24. Based on our results, most of the effects of the treatment were obtained at week 24. Moreover, week 24 was a suitable time for evaluating the early response to ustekinumab

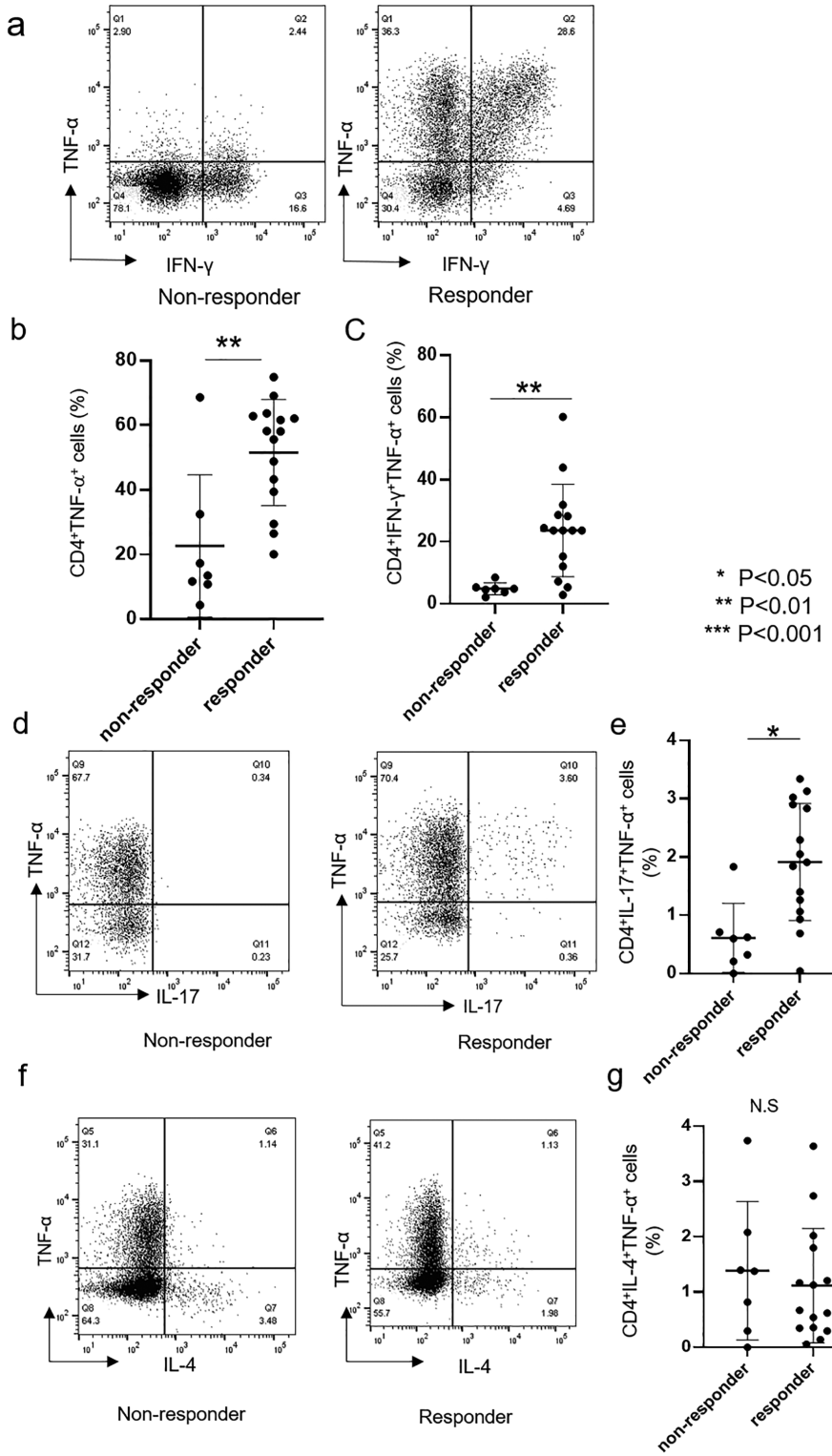

FIGURE 4. Th1- and Th17-derived TNF-α-positive cells are higher in responders (n = 15) than in nonresponders (n = 7) at baseline. A, Flow cytometric profile of PBMCs stained for CD4, IFN-γ, and TNF-α in responders and nonresponders. B, Statistical comparison of $CD4^+$ $TNF\text{-}\alpha^+$ cell (%) in responders and nonresponders. C, Statistical comparison of $CD4^+$ $IFN\text{-}\gamma^+$ $TNF\text{-}\alpha^+$ cell (%) in responders and nonresponders. D, Flow cytometric profile of PBMCs stained for CD4, IL-17, and TNF-α in responders and nonresponders. E, Statistical comparison of $CD4^+$ $IL\text{-}17^+$ $TNF\text{-}\alpha^+$ cell (%) in responders and nonresponders. F, Flow cytometric profile of PBMCs stained for CD4, IL-4, and TNF-α in responders and nonresponders. G, Statistical comparison of $CD4^+$ $IL\text{-}4^+$ $TNF\text{-}\alpha^+$ cell (%) in responders and nonresponders.

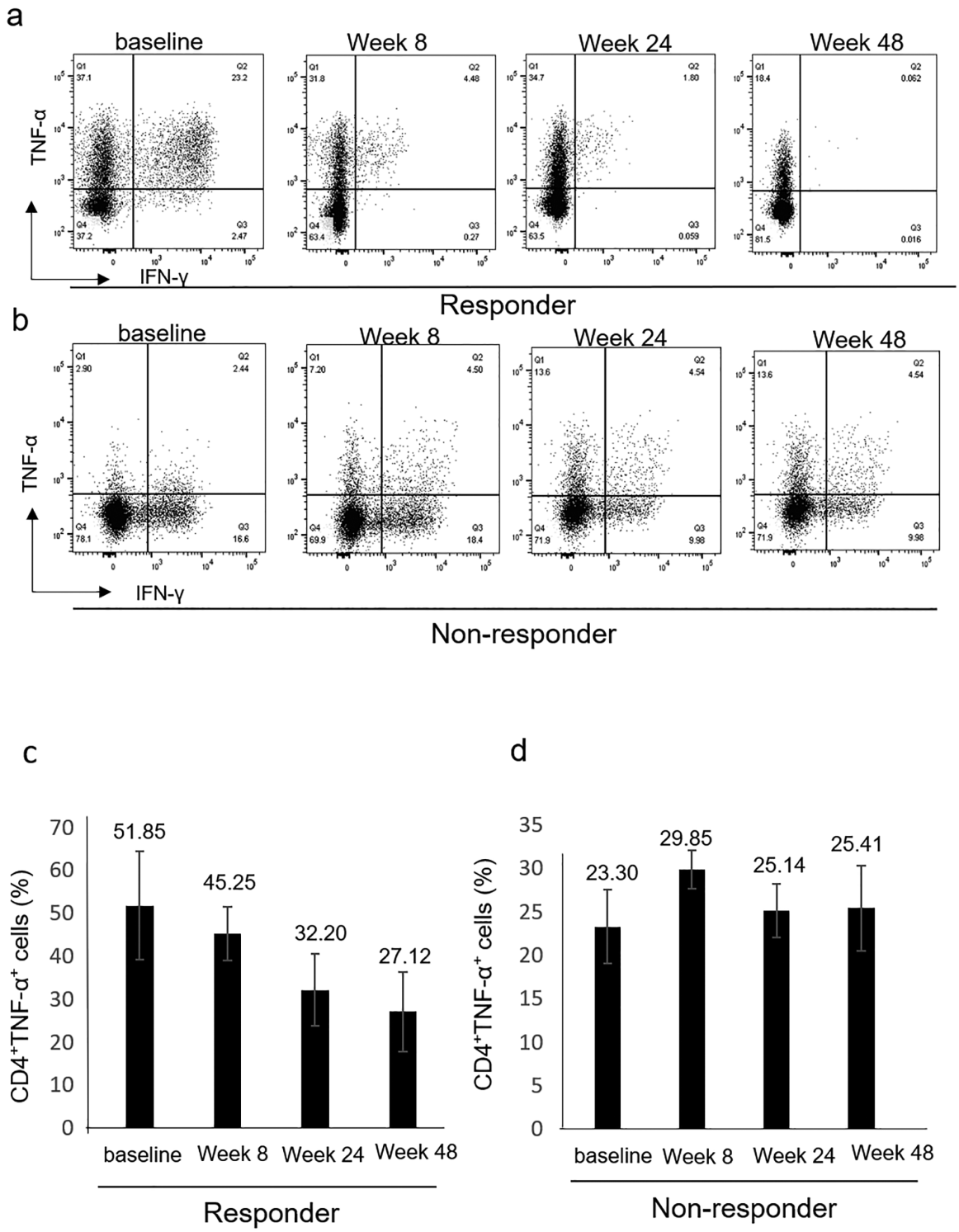

FIGURE 5. Decrease in TNF-α$^+$ IFN-γ$^+$ cell populations in responders (n = 15). A, Representative flow cytometric profile of PBMCs stained for CD4, IFN-γ, and TNF-α in responders; it was the same patient. B, Representative flow cytometric profile of PBMCs stained for CD4, IFN-γ, and TNF-α in nonresponders (n = 7); it was the same patient. C, Change in the IFN-γ$^+$ TNF-α$^+$ cell populations in responders. D, Change in IFN-γ$^+$ TNF-α$^+$ cell populations in nonresponders.

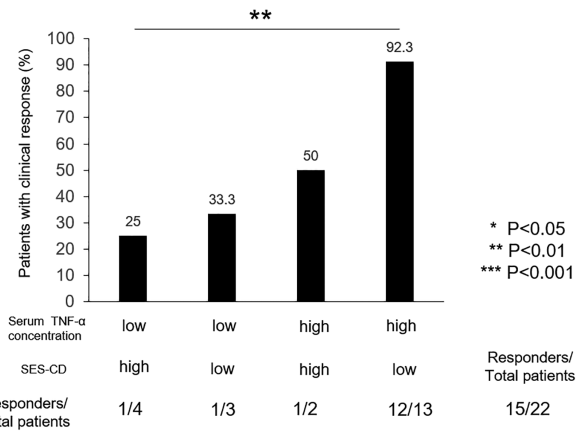

FIGURE 6. Combination of serum TNF-α concentration and SES-CD is more predictive of the response to ustekinumab.

because at this time, the clinical remission rate was higher than at week 8, and no patient showed clinical response after week 24.

We confirmed the efficacy and safety of ustekinumab in moderate to severe active CD patients. Approximately 30% of the patients did not respond to ustekinumab. Currently, several new biological therapies targeting different pathways are approved, and it is important to personalize the treatments. To our knowledge, validated biomarkers predicting the response to ustekinumab are lacking. Therefore, we aimed to identify treatment effect predictors.

Serum ustekinumab concentrations in our study were higher compared with previous reports. We think this may be due to differences in reagents and measurement methods. In addition, antidrug antibodies and CRP concentrations have been reported to affect serum ustekinumab concentration.[27] C-reactive protein concentrations in our patients were lower compared with the previous report,[27] and this may affect serum ustekinumab concentration.

We identified high serum TNF-α concentration and low SES-CD score at baseline to be associated with the response to ustekinumab. Tumor necrosis factor alpha is a driver of inflammation and is produced as a transmembrane protein (mTNF-α). Its extracellular domain is cleaved by TNF-α converting enzyme (TACE) to become soluble TNF-α (sTNF-α). Both the forms of TNF-α are biologically active and transmit signals through 2 distinct receptors.[30, 31] We observed that the level of soluble TNF-α was higher in the responders than in the nonresponders at baseline. An elevated level of TNF-α is present in the serum and intestinal specimens of CD patients.[32] In our study, the TNF-α levels in the intestinal specimens correlated with the serum TNF-α concentration. However, the serum TNF-α concentration did not correlate with the CDAI score or CRP levels.

Tumor necrosis factor alpha is produced by a variety of cells, mainly macrophages and T cells. We also found that the $CD4^+$ $TNF\text{-}\alpha^+$ cell population, derived from Th1 and Th17 cells, was higher in the responders than in the nonresponders, and the population was decreased during the treatment. The $CD45^+CD11b^+CD11c^+TNF\text{-}\alpha^+$, $CD45^+CD11b^-CD11c^+TNF\text{-}\alpha^+$, $CD45^+CD11b^+CD11c^-TNF^-\alpha^+$, and $CD45^+CD11b^-CD11c^-TNF\text{-}\alpha^+$ cell populations at baseline did not differ between the responders and the nonresponders. This indicates that there was no difference between the groups with regard to TNF-α derived from macrophages and dendritic cells at baseline. Furthermore, ustekinumab reduced $TNF\text{-}\alpha^+$ derived from $CD4^+$ T cells, but it did not reduce TNF-α derived from macrophages and dendritic cells, which were the main producers of TNF-α. These results can be explained based on the mechanism in which ustekinumab blocks IL-12 and IL-23 by inhibiting the interaction with the receptors on the T cells modulating the expression of inflammatory cytokines.[33, 34] On the other hand, anti-TNF-α antibodies have been reported to block both sTNF-α and mTNF-α and induce apoptosis in macrophages and $CD4^+$ T cells.[35] Ustekinumab regulates the TNF-α levels by a mechanism different from that of anti-TNF-α antagonists; this may help in patient selection where ustekinumab would be expected to be more effective.

It has been suggested in several studies that IL-12 production is in part regulated by TNF-α, and conversely, IL-12 stimulates TNF-α production from T cells.[36, 37] A previous study has indicated that administration of the antibody to the p40 subunit of IL-12 in patients with psoriasis downregulated the TNF-α levels on T cells, and high TNF-α levels at baseline correlated with the clinical response to the antibody to the p40 subunit of IL-12 at week 16.[38] Other studies have indicated that IL-23 production is upregulated on T cells in patients with CD unresponsive to anti-TNF therapy.[39, 40] Our results suggest control of IL-12 and IL-23 pathways and TNF-α production on T cells are important to regulate CD activity.

It has been suggested in several studies that clinical score, disease location, and structuring disease are related to the response to ustekinumab.[41, 42] Endoscopic evaluation is an efficient tool to assess these factors. We performed endoscopies and assessed disease activities and characteristics using SES-CD scores, commonly used in clinical trials to evaluate the endoscopic disease activity. Consistent with previous studies, a low SES-CD score was associated with the response to ustekinumab. Additionally, 81.8% of the patients enrolled in this study were ileocolonic type, and we performed DBE and evaluated small intestinal lesions using modified SES-CD. Modified SES-CD decreased due to the therapeutic effect in responders, but there was no difference between the responders and nonresponders before induction of ustekinumab. From these results, we think SES-CD is better for predicting the therapeutic response to ustekinumab. The SES-CD score correlates with the disease activity, but the serum TNF-α concentration has little relationship with the activity. Based on

the results of our study, it is recommended to evaluate the SES-CD activity and narrow down patients with serum TNF-α concentration.

Even if only 1 of these 2 factors is useful, the therapeutic effect can be predicted to some extent. However, stronger correlation is achieved using both factors. If the SES-CD score at baseline is low, the therapeutic effect cannot be predicted accurately, and the same is true even if the serum TNF-α concentration at baseline is high. Therefore, endoscopy is important in selecting patients for ustekinumab treatment, and serum TNF-α concentration, a newly discovered predictor, improves the accuracy of response predictions.

These findings could help clinicians to choose an appropriate therapy for moderate to severe active CD patients. With advances in biologic drugs for CD patients, the treatment strategy for deciding the best medication to be used at a particular time becomes very important. This is expected to improve the response and also influence the duration of remission. In this regard, any factor that predicts the treatment effect is important, and it is desirable to identify an accurate predictor reflective of multiple variables. Although the sample sizes in this study were small and this was a single-center study, the evidence suggests that high TNF-α expression and low SES-CD score at baseline could predict the response to ustekinumab. It appears to be more useful to combine these 2 predictors.

In conclusion, the combination of 2 predictors obtained in this study could be a clinically useful tool and assist clinicians in choosing the most appropriate therapy for CD patients.

SUPPLEMENTARY DATA

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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