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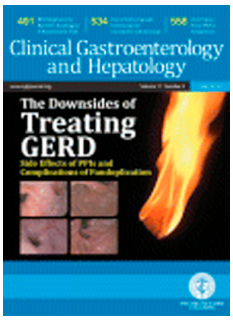

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Peak Concentrations of Ustekinumab After Intravenous Induction Therapy Identify Patients With Crohn's Disease Likely to Achieve Endoscopic and Biochemical Remission

Short title: Early Ustekinumab Exposure-Response in CD

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Abbreviations:

AUC, area under the curve

CD, Crohn's disease

FC, fecal calprotectin

IQR, interquartile range

ROC, receiver operating characteristic

SES-CD, Simple Endoscopic Score for Crohn's Disease

TNF, tumor necrosis factor

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Conflict of interest statement:

The authors have no personal or financial conflicts of interest to declare.

Authors' contributions:

JH, JZ, DD and IG designed the study. DD and IG supervised the study. JH, JZ and NSe analyzed the data. JH, JZ, DD and IG interpreted the data and prepared the manuscript. TK, KJ, KT, MK, NSm, GN, BS, ED, DT and TV acquired the data. All authors critically reviewed the manuscript and approved the final submitted version.

Abstract

Background & Aims: Little is known about the relationship between ustekinumab exposure during the first 2 weeks of treatment and outcomes of patients with Crohn's disease (CD). We investigated the relationship between serum concentrations of ustekinumab during the first 2 weeks of treatment and endoscopic and biochemical remission in patients with CD.

Methods: In a prospective observational study, we measured concentrations of ustekinumab in serum samples from 41 consecutive patients who started treatment with ustekinumab (approximately 6 mg/kg, intravenously, then 90 mg every 8 weeks), due to endoscopic markers of active CD, at a single center from October 2017 through January 2019. We measured ustekinumab exposure parameters during the first 2 weeks (peak concentration measured immediately after intravenous infusion, week 2 concentration, and area under the curve through week 2). We investigated the correlation between these parameters and endoscopic remission (simple endoscopic score for CD scores of 3 or less without ulceration, assessed centrally) and biochemical remission (level of fecal calprotectin below 100 mg/kg) using the Mann-Whitney U test.

Results: Endoscopic remission was achieved in 10 patients (24.4%) at week 24; biochemical remission was achieved in 17 patients (41.5%) at week 8, 17 patients (41.5%) at week 16, and 21 patients (51.2%) at week 24. Peak concentrations associated with endoscopic remission (area under the receiver operating characteristic curve, 0.717; 95% CI, 0.517–0.916); 6/13 patients (46%) with peak concentrations above 105 µg/mL (upper tercile) achieved endoscopic remission, compared to only 1/14 patients (7%) with peak concentrations below 88 µg/mL (lower tercile). All exposure parameters during the first 2 weeks were associated with biochemical remission. There was no significant difference between the associations of peak concentrations, week-2 concentrations, area under the curve through week 2, or later exposure measures (at weeks 4 and 8) with biochemical or endoscopic remission.

Conclusions: In a prospective study, we found that serum concentrations of ustekinumab as early as 1 hour after intravenous infusion might be used to identify patients with CD most likely to achieve endoscopic remission. This early measurement might be used to optimize treatment of CD.

KEY WORDS: pharmacokinetics, therapeutic drug monitoring, inflammatory bowels diseases, prognostic factor

1 Introduction

Crohn's disease (CD) is a debilitating, incurable inflammatory disease. Treatment is focused on achieving clinical and endoscopic remission, with biomarker remission considered an adjunct target.[1]

Ustekinumab is a fully human IgG1κ monoclonal antibody against the p40 subunit, which is shared by IL-12 and IL-23.[2] Its efficacy in inducing and maintaining remission of moderate-to-severe CD has been proven in the UNITI program.[3] Real-world studies of its effectiveness with intravenous induction followed by subcutaneous maintenance have reported clinical remission rates of up to 50%,[4,5] but much lower endoscopic remission rates (7.1–29.7%).[6–8]

Recently, an association of ustekinumab concentrations at weeks 4 and later with treatment outcomes has been observed in trials and real-world cohorts.[8–10] However, the exposure-response relationship at even earlier time points, during the first 2 weeks of treatment, is unclear. Since future optimization protocols (e.g., STARDUST – NCT03107793) include early shortening of the dosing interval to 4 weeks, the identification of patients needing this dosing regimen at an earlier time point than currently studied, i.e., during the first 2 weeks after starting treatment, rather than at weeks 4 or 8, would be clinically informative. This approach would enable early proactive ustekinumab dose optimization for patients who are unlikely to achieve endoscopic remission with the current dosing regimen. It also remains unclear whether alternative measures of ustekinumab exposure would be more informative than concentrations alone.

Our principal aim was thus to prospectively study the relationship of ustekinumab exposure parameters during the first 2 weeks with robust and objective subsequent outcomes: endoscopic and biochemical remission. Furthermore, we also aimed to compare the predictive values of different measures of very early exposure (peak concentrations, week 2 concentrations and area under the curve within the first two weeks [$AUC_{0\text{-}2}$]), with those of ustekinumab exposure at later time points.

2 Methods

2.1 Patients and study design

We performed a prospective observational study at a single tertiary referral center. The study design conforms to the 1975 Declaration of Helsinki and was approved by the National Committee of Medical Ethics (0120-013/2016-2; KME 18 January 2016). All patients provided written informed consent.

All consecutive patients aged 18 years or older with CD who started treatment with ustekinumab between October 2017 and January 2019 were examined for eligibility, allowing a follow-up period of at least 24 weeks. The decision to commence treatment with ustekinumab was made by a multidisciplinary team based on clinical, endoscopic and biochemical evidence of disease activity, as

well as the characteristics of individual patients. We included patients with endoscopically or radiologically proven active luminal disease within 3 months prior to starting ustekinumab.

All patients received an intravenous ustekinumab induction dose of approximately 6 mg/kg (≤55 kg: 260 mg; 55–85 kg: 390 mg; >85 kg: 520 mg) infused over one hour at week 0, followed by a subcutaneous injection of a fixed maintenance dose of 90 mg every 8 weeks. No dosing interval modification or intravenous reinduction was performed in patients with inadequate response. Serum samples were prospectively collected at week 0 (baseline), 1 hour after the intravenous infusion (hereafter referred to as peak), week 2, week 4 and week 8, and stored at -80 °C for subsequent measurement after the completion of the study.

2.2 Outcomes

The primary endpoint of this study was endoscopic remission, which was defined as a Simple Endoscopic Score for CD (SES-CD)[11] ≤3 without mucosal ulceration. Colonoscopies were performed between weeks 24 and 26. The procedures were recorded, the recordings were anonymized and assessed centrally by an expert endoscopist (NSe) who was blinded to the patients' conditions.

The secondary endpoint was biochemical remission defined as a fecal calprotectin (FC) < 100 mg/kg. It was assessed at weeks 8, 16 and 24. The cut-off was chosen based on test characteristics identified by meta-analyses,[12] with a particular emphasis on studies using the same assay as our center.[13]

2.3 Measurements

2.3.1 Ustekinumab exposure

Serum ustekinumab concentrations were measured with a validated enzyme-linked immunosorbent assay (ELISA, ImmunoGuide®, Tani Medikal, Turkey). The assay displayed adequate precision (coefficient of variation < 10%) and accuracy (bias < 10%), and the lower limit of quantification was 0.4 μg/mL. The reliability of the assay was additionally confirmed with a comparison to a reference ELISA (apDia, Belgium, Supplementary figure 1), which was previously shown to be comparable to the assay developed at Janssen (Spring House, USA).[14] Antibodies against ustekinumab were not determined, based on their reported low occurrence rate.[9]

A noncompartmental pharmacokinetic analysis was performed to determine cumulative exposure to ustekinumab, which was reported as the area under the serum concentration-time curve (AUC). The AUC values from treatment initiation to week 2 (AUC_{0-2}), week 4 (AUC_{0-4}) and week 8 (AUC_{0-8}) were calculated using a linear-log trapezoidal method (linear in the ascending phase and logarithmic in the descending phase, Supplementary figure 2).

2.3.2 Biomarkers

Patients collected fecal samples from their first morning bowel movement at home (at baseline, week 8, week 16, and week 24) and transported the cooled samples to the hospital within 24 hours. FC concentrations were measured using the Calprest ELISA assay (Eurospital, Triest, Italy) with a measurement range of 15.6–500 mg/kg.

2.4 Statistical analysis

Descriptive statistics are reported as percentages for nominal variables and as medians and interquartile ranges (IQR) for continuous variables. Ustekinumab exposure was compared between patients who achieved or did not achieve the outcome endpoint using the Mann-Whitney U test. Other variables were compared using the matched pair Wilcoxon signed rank test, χ^2 McNemar test for dependent samples or Fisher's exact test, as appropriate. A one-sided Cochran-Armitage trend test was used to evaluate the presence of a trend in the proportion of patients achieving the outcome across ustekinumab exposure terciles. Correlations were assessed by calculating the Pearson correlation coefficient. Univariable logistic and linear regression analyses were performed to identify independent predictors of outcomes and ustekinumab exposure, respectively. Additionally, the peak ustekinumab concentration and predictors with $P < 0.1$ in the univariable analysis were subjected to a multivariable regression analysis. Receiver operating characteristic (ROC) curves were constructed to assess the diagnostic performance of ustekinumab exposure. Youden's J statistic was computed to identify threshold values.[15] ROC curves were compared using DeLong's method.[16] Data outside the limits of quantification were substituted with limit values (ustekinumab: 0.4 µg/mL; FC: 15 and 500 mg/kg). P <0.05 with no adjustment for multiple comparisons was considered significant. Statistical analyses were performed using SPSS, version 25 (IBM, Chicago, USA). R software, version 3.6.0 (R Development Core Team, Vienna, Austria) and the packages pROC and DescTools were used to compare ROC curves (DeLong test) and for the trend test (Cochran-Armitage test).

3 Results

3.1 Patient characteristics

Fifty-four patients were examined for eligibility and 13 were excluded due to endoscopically inactive disease at baseline (10 patients started treatment with ustekinumab due to psoriasiform skin lesions, three due to frequent infections – all of whom were previously treated with anti-TNF agents), yielding a final cohort of 41 patients. The median disease duration was 16 years (IQR 7–26), and 61% had been exposed to biological therapy (58.5% to anti-TNF agents and 22.0% to vedolizumab (Table 1)).

3.2 Patient outcomes: Endoscopic and biochemical remission

None of the patients discontinued ustekinumab therapy prior to the endoscopic assessment. Ten patients (24.4%) achieved endoscopic remission between weeks 24 and 26, and 12.2% (5/41) had a score of ≤2. After stratification by prior exposure to biologicals, a nonsignificant trend of a higher rate of endoscopic remission was observed in biologically naïve patients (6/16 vs. 4/25; $P = 0.202$).

At baseline, 24.4% (10/41) of patients had FC below 100 mg/kg. Median FC decreased from baseline (160 [IQR 93–265]) to week 8 (122 [IQR 38–212]; $P = 0.029$) and further decreased at week 16 (105 [IQR 45–248]; $P = 0.041$ compared to baseline) and week 24 (82 [IQR 45–202]; $P = 0.022$ compared to baseline).

At week 8, 41.5% (17/41) of patients achieved biochemical remission ($P = 0.039$ compared to the baseline), 41.5% (17/41) of patients achieved remission at week 16 ($P = 0.039$ compared to the baseline) and 51.2% (21/41) of patients achieved remission at week 24 ($P = 0.017$ compared to baseline).

A positive correlation was observed between SES-CD at week 24 and FC at all time points (Supplementary table 1). At week 24, 80% (8/10) of patients in endoscopic remission were also in biochemical remission, while 45.2% (14/31) of patients who did not achieve endoscopic remission were in biochemical remission. FC below 100 mg/kg at week 24 predicted endoscopic remission with a sensitivity, specificity, positive predictive value and negative predictive value of 80, 56, 38 and 89%, respectively. FC below 50 mg/kg at week 24 predicted endoscopic remission with a sensitivity, specificity, positive predictive value and negative predictive value of 40, 79, 40 and 79%, respectively.

3.3 Ustekinumab concentrations and cumulative exposure (AUC)

One hundred fifty-nine serum samples were prospectively collected to measure ustekinumab concentrations. Four samples at week 2 and one sample at week 4 were missing. The median ustekinumab concentrations were 98.3 µg/mL [IQR 83.7–114.2], 27.4 µg/mL [IQR 22.6–32.2], 15.6 µg/mL [IQR 10.3–20.4] and 4.44 µg/mL [IQR 2.78–7.70] at the peak, week 2, week 4 and week 8, respectively. One measurement was below the limit of quantification (week 8).

The median $AUC_{0\text{-}2}$ was 781 µg*day/mL [IQR 646–896], median $AUC_{0\text{-}4}$ was 1063 µg*day/mL [IQR 884–1285] and median $AUC_{0\text{-}8}$ was 1203 µg*day/mL [IQR 953–1455]. Higher ustekinumab exposure was associated with a higher baseline albumin, lower baseline C-reactive protein (CRP), lower baseline FC and the absence of previous biological therapy (Supplementary tables 2 and 3).

3.4 Exposure-response relationship

The three measures of very early ustekinumab exposure were associated with endoscopic remission and biochemical remission at the studied time points (Table 2 and Supplementary table 4). More specifically, peak concentrations predicted both endoscopic and biochemical remission at 24 weeks. Furthermore, peak concentrations were clinically informative, as only 7% (1/14) of patients with a peak concentration below 88 µg/mL achieved endoscopic remission, compared to 46% (6/13) of patients with a concentration above 105 µg/mL (Figure 1, P = 0.010). Importantly, peak concentrations predicted outcomes independently of the ustekinumab dose per kilogram (AUROC for dose per kilogram 0.471–0.573; P = 0.495–0.937; data not shown). The more stringent endpoint of complete mucosal healing (SES-CD 0) yielded similar results (Supplementary table 5). Multivariable logistic regression confirmed the independent predictive value of peak ustekinumab concentrations for endoscopic remission (Supplementary tables 6–8).

Week 2 concentrations and $AUC_{0\text{-}2}$ predicted all biochemical outcomes at the studied time points (Table 2), and the tercile analysis confirmed a higher proportion of patients who achieved remission with higher exposure (Figure 2). Additionally, a nonsignificant trend of higher endoscopic remission was observed in patients with a higher week 2 concentration (Figure 1, P = 0.132) and $AUC_{0\text{-}2}$ (P = 0.052). Although a high negative predictive value for biochemical remission was observed for all three measures of very early exposure (Table 2), $AUC_{0\text{-}2}$ was the best predictor of biochemical remission at the end of the study. Namely, all 10 patients with $AUC_{0\text{-}2}$ > 860 µg*day/mL were in biochemical remission at end of the study at 24 weeks. Quartile analysis confirmed the findings of tercile analysis (Supplementary figures 5 and 6). Apart from ustekinumab exposure, baseline albumin, CRP and FC were additional factors associated with biochemical remission in univariable regression (Supplementary tables 6–8).

Based on the comparison of ROC curves, measures of exposure at later time points (week 4 and 8 concentrations, $AUC_{0\text{-}4}$ and $AUC_{0\text{-}8}$) did not increase the predictive values for the studied outcomes compared to the three measures of very early ustekinumab exposure (peak, week 2 concentration and $AUC_{0\text{-}2}$, data not shown).

4 Discussion

This study is the first with a prospective real-world design to explore the correlation between very early ustekinumab exposure (within 2 weeks of starting treatment) and endoscopic and biochemical outcomes. We confirmed the predictive value of week 4 and 8 drug concentrations identified in previous studies[8–10] for biochemical and endoscopic remission after 6 months of treatment. Additionally, our study is the first to show that very early ustekinumab concentrations, measured within two weeks of treatment, have similar predictive values as concentrations measured at week 4 or later. The most striking observation was that the peak concentration, measured immediately after

the intravenous infusion of ustekinumab, exhibited a similar performance to concentrations measured at the later time points identified. Based on our findings, therapeutic drug monitoring during the first two weeks of initiation of ustekinumab might help stratify patients according to the probability of achieving important treatment outcomes with the currently approved dosing regimen.

The high negative predictive value of peak ustekinumab concentrations, measured immediately after the intravenous infusion, enables the accurate and timely identification of patients who are unlikely to achieve endoscopic remission at 6 months. This finding might help guide ustekinumab treatment optimization – either through the earlier administration of the first subcutaneous dose or using maintenance dosing every 4 weeks. The latter strategy is being increasingly reported in real-world studies,[5,6] although data from a prospective trial supporting this approach are still awaited (STARDUST NCT03107793).

Our study expands on previous studies reporting an exposure-response relationship[8–10] by focusing on even earlier time points, i.e., before week 4. This very early time window has not yet been studied in a real-world cohort. Adedokun et al.[9] analyzed the data from the UNITI trials, where peak concentrations were measured after the intravenous infusion, but a detailed analysis of an exposure-response relationship was not provided for these very early measurements. Although minor discrepancies in outcome definitions preclude a direct comparison, the predictive value of ustekinumab concentrations measured up to week 2 was at least as good, if not better, than that of concentrations measured at weeks 4 and 8 in two previous studies.[8,10] The identified cut-off values at later time points in our study were in the range of those identified in previous studies, which further supports the validity of our results.

Our study revealed a strong relationship between patient outcomes and peak ustekinumab concentrations, which depends on the ustekinumab volume of distribution. Patients with active disease have higher serum concentrations of the proinflammatory cytokines IL-12 and IL-23.[17] A higher target concentration might lead to increased binding of ustekinumab to these cytokines, which would result in a higher apparent volume of distribution and consequently a lower peak concentration. Thus, the peak ustekinumab concentration could be used to stratify patients according to disease activity. Consistent with our findings, a recent study of rituximab in diffuse large B-cell lymphoma identified a positive association between the rituximab volume of distribution and baseline total metabolic tumor volume: the higher the tumor burden, the higher the volume of distribution and the lower the exposure.[18] .

Researchers have not clearly determined the best parameter to predict the initial response to biologics: peak drug concentration, trough concentration or cumulative exposure.[19] In our study, the differences in the predictive value between these three measures were minor and the correlations between them were very strong. We therefore recommend measuring peak ustekinumab concentrations immediately after the intravenous infusion: a single measurement that provides as much information as multiple serial measurements and enables very early therapeutic intervention.

In contrast to previous studies, which reported endoscopic remission rates ranging from 7.1% to 10.9%,[8,20] we observed a higher endoscopic remission rate of 24.4% with a more liberal definition of SES-CD ≤3 without mucosal ulceration. In our study, 12.2% of patients had a SES-CD score ≤2. . Compared to other studies of ustekinumab we observed a higher endoscopic remission rate, which might be associated with the higher proportion of bio-naïve patients.

The progressive decrease in median FC in our cohort suggested an improvement in disease control. Despite using a stringent threshold to define biochemical remission, approximately half of the patients without endoscopic remission achieved biochemical remission and the test characteristics of FC cut-off values were poorer than those reported in meta-analyses.[12,21] The accuracy of FC to predict endoscopic remission strongly depends on the context, with important differences observed between different disease locations in patients with CD. Low levels of FC have been reported, despite the presence of endoscopically active isolated ileal disease,[22] which was present in approximately one-third of patients in our cohort and may at least partially explain the observed divergence between biochemical and endoscopic remission.

The strength of our study was its prospective design with objective and robust endpoints. We acknowledge that the relatively small sample size and the single center design were limitations. Given the rare occurrence of antibodies, they were not measured.[3] Moreover, despite the lower percentage of patients who were previously exposed to biologicals than in other studies, our findings cannot be readily extrapolated to bio-naïve patients. The lower baseline FC may partially be explained by interassay differences,[23] as the assays used in other studies reported consistently higher values than our assay, as well as a higher proportion of patients with ileal disease. Finally, we are unable to exclude the possibility that our cohort was different from previously studied cohorts, although all our patients had confirmed endoscopically active disease. Unfortunately, the baseline endoscopy performed in our study was not externally read, and we were therefore unable to provide a baseline SES-CD to facilitate comparisons with previous studies.

In conclusion, this prospective real-world study is the first to report an exposure-response relationship between ustekinumab concentrations measured during the first two weeks of treatment and robust endpoints of endoscopic remission and FC normalization at 6 months. These findings provide a unique opportunity for very early proactive treatment optimization, supported by therapeutic drug monitoring.

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Figure and table captions

Table 1. Patients' characteristics at baseline (n = 41).

Table 2. Analysis of the receiver operating characteristic (ROC) curves for ustekinumab exposure parameters (serum ustekinumab concentrations at peak, week 2, week 4, week 8 and the cumulative exposure parameters AUC_{0-2}, AUC_{0-4}, and AUC_{0-8}), biochemical remission (a fecal calprotectin < 100 mg/kg at week 8, week 16, and week 24) and endoscopic remission (SES-CD ≤ 3 without ulceration).

Figure 1. Analysis of the proportion of patients who achieved endoscopic remission at week 24 for different terciles of the peak ustekinumab concentration (A), ustekinumab concentration at week 2 (B) and cumulative exposure up to week 2 (AUC_{0-2}, C).

Figure 2. Analysis of the proportion of patients who achieved biochemical remission at week 8, week 16 and week 24 for different terciles of peak ustekinumab concentration (A), ustekinumab concentration at week 2 (B) and cumulative exposure up to week 2 (AUC_{0-2}, C).

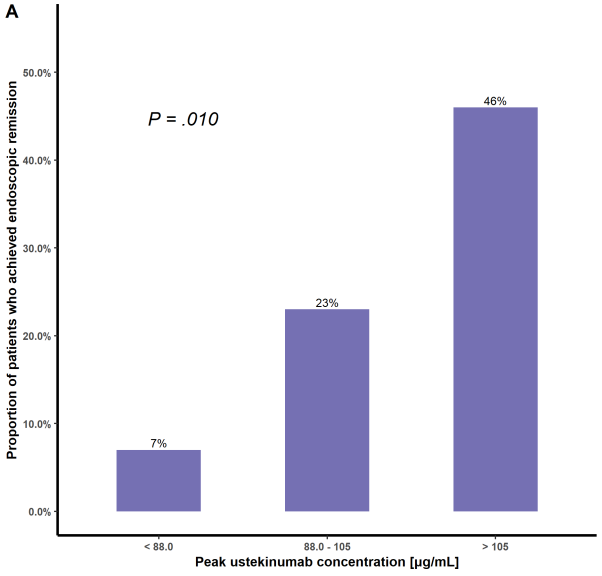
Table 2. Analysis of the receiver operating characteristic (ROC) curves for ustekinumab exposure parameters (serum ustekinumab concentrations at peak, week 2, week 4, week 8 and the cumulative exposure parameters AUC_{0-2}, AUC_{0-4}, and AUC_{0-8}), biochemical remission (a fecal calprotectin < 100 mg/kg at week 8, week 16, and week 24) and endoscopic remission (Simple Endoscopic Score for Crohn's disease ≤ 3 without ulceration).

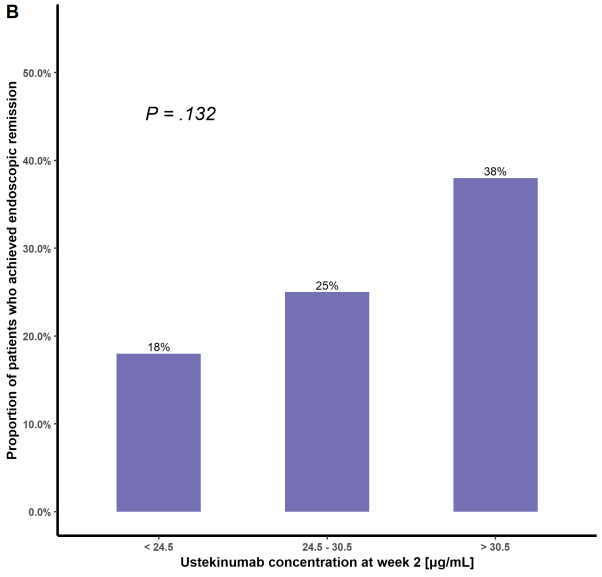
	Median exposure parameter (IQR)		*P*-value	AUROC	95% CI	Optimal cut-off	Sensitivity, %	Specificity, %	PPV, %	NPV, %
	Responders	Non-responders								
Biochemical remission at week 8										
Peak (µg/mL)	107.3 (95.7, 123.3)	88.3 (79.3, 103.5)	0.004	0.768	0.612-0.923	96.0	82	71	70	83
Ustekinumab w2 (µg/mL)	30.9 (26.1, 35.9)	24.1 (21.4, 29.3)	0.004	0.778	0.617-0.938	24.7	88	67	71	86
Ustekinumab w4 (µg/mL)	20.4 (15.8, 23.6)	11.7 (7.7, 16.3)	< 0.001	0.840	0.709-0.972	15.0	88	76	75	89
Ustekinumab w8 (µg/mL)	7.4 (4.4, 11.0)	3.3 (2.5, 5.9)	0.001	0.815	0.679-0.952	6.85	65	86	79	75
AUC_{0-2} (µg*day/mL)	868 (642, 1093)	643 (503, 784)	0.002	0.804	0.657-0.951	714	88	67	71	86
AUC_{0-4} (µg*day/mL)	1230 (1073, 1405)	893 (833, 1077)	0.001	0.804	0.660-0.947	1063	82	76	74	84
AUC_{0-8} (µg*day/mL)	1450 (1208, 1596)	1008 (903, 1242)	< 0.001	0.824	0.688-0.959	1091	88	71	71	88
Biochemical remission at week 16										
Ustekinumab w2 (µg/mL)	31.1 (25.6, 35.9)	24.3 (20.4, 28.7)	0.007	0.761	0.602-0.921	24.7	82	61	67	79
Ustekinumab w4 (µg/mL)	20.3 (15.6, 23.7)	11.7 (7.9, 17.3)	0.002	0.787	0.643-0.932	15.0	82	67	67	82
Ustekinumab w8 (µg/mL)	7.2 (4.6, 11.3)	3.3 (2.3, 6.2)	0.003	0.782	0.635-0.928	4.37	82	67	67	82
AUC_{0-2} (µg*day/mL)	844 (762, 987)	667 (637, 785)	0.013	0.745	0.572-0.918	747	82	72	74	81
AUC_{0-4} (µg*day/mL)	1202 (1036, 1405)	909 (836, 1099)	0.006	0.756	0.596-0.916	989	82	67	67	82
AUC_{0-8} (µg*day/mL)	1410 (1147, 1596)	1008 (908, 1242)	0.003	0.779	0.627-0.931	1085	88	67	68	88
Biochemical remission at week 24										
Peak (µg/mL)	108.8 (94.1, 123.4)	85.5 (77.6, 100.3)	0.003	0.783	0.632-0.934	104	62	94	93	65
Ustekinumab w2 (µg/mL)	30.5 (25.2, 35.6)	23.2 (19.0, 25.9)	0.003	0.800	0.647-0.953	27.2	70	85	88	65
Ustekinumab w4 (µg/mL)	19.4 (13.9, 22.7)	11.6 (8.4, 14.9)	0.002	0.803	0.660-0.946	15.0	71	80	83	67
Ustekinumab w8 (µg/mL)	7.1 (4.0, 10.7)	3.3 (2.2, 5.0)	0.018	0.729	0.566-0.892	6.85	52	94	92	60
AUC_{0-2} (µg*day/mL)	856 (758, 983)	643 (606, 745)	0.001	0.835	0.694-0.975	714	85	77	85	77
AUC_{0-4} (µg*day/mL)	1216 (1023, 1383)	884 (827, 1013)	0.002	0.794	0.643-0.945	924	86	67	78	77
AUC_{0-8} (µg*day/mL)	1401 (1119, 1576)	975 (907, 1108)	0.001	0.818	0.679-0.958	1085	81	75	81	75
Endoscopic remission at week 24										
Peak (µg/mL)	113.6 (96.3, 130.0)	89.9 (81.9, 106.6)	0.043	0.717	0.517-0.916	111	60	83	55	86

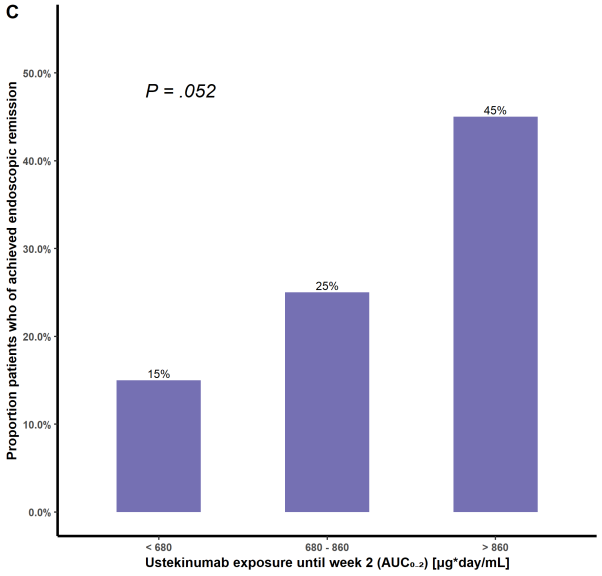
IQR, interquartile range; AUROC, area under the ROC curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the ustekinumab serum concentration-time curve; w2, week 2; w4, week 4; w8, week 8

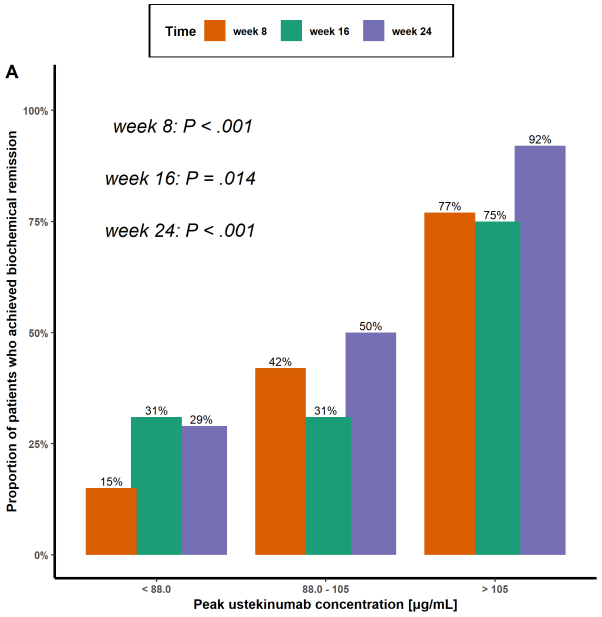
Table 1. Patients' characteristics at baseline (n = 41). Abbreviations: CD – Crohn's disease; IQR – interquartile range; TNF – tumor necrosis factor; UST – ustekinumab

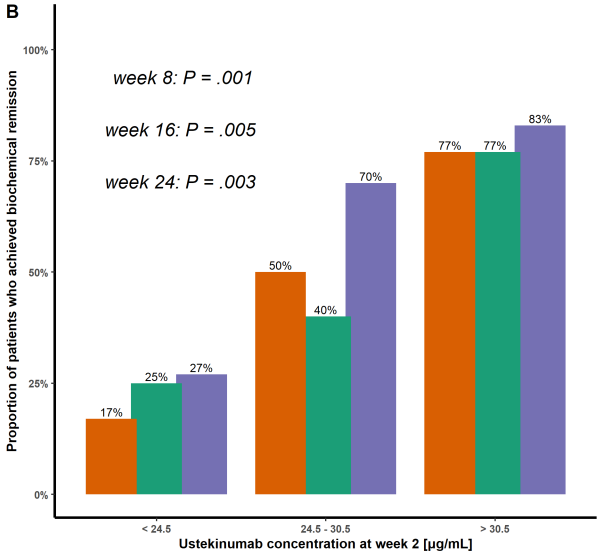
Women, n (%)	21 (51)
Age at UST initiation, years, median (IQR)	48 (31–55)
Weight, kg, median (IQR)	70 (59–83)
Height, cm, median (IQR)	170 (163–180)
Intravenous ustekinumab dose, n (%)	
260 mg	6 (15)
390 mg	26 (63)
520 mg	9 (22)
Disease duration, years, median (IQR)	16 (7–26)
Disease location, n (%)	
ileal (L1)	12 (29.3)
colonic (L2)	3 (7.3)
ileocolonic (L3)	26 (63.4)
upper gastrointestinal involvement (L4)	3 (7.3)
Fistulizing perianal disease, n (%)	6 (14.6)
History of CD-related surgery, n (%)	26 (63.4)
Smoking status, n (%)	
active smoking	5 (12.2)
previously smoking	9 (22.0)
never smoked	27 (65.9)
Previous biological therapy, n (%)	25 (61)
previous anti-TNF exposure	24 (58.5)
previous vedolizumab exposure	9 (22.0)
previous anti-TNF and vedolizumab exposure	8 (19.5)
Systemic steroids at baseline, n (%)	6 (14.6)
Topical steroids at baseline, n (%)	2 (4.9)
Immunomodulators at baseline, n (%)	4 (9.7)
azathioprine	3 (7.3)
methotrexate	1 (2.4)
Harvey-Bradshaw score, median (IQR)	7 (4–10)
Fecal calprotectin, mg/kg, median (IQR)	160 (91–279)
C-reactive protein, mg/L, median (IQR)	3 (3–13)
Albumin, g/L, median (IQR)	43 (41–44)

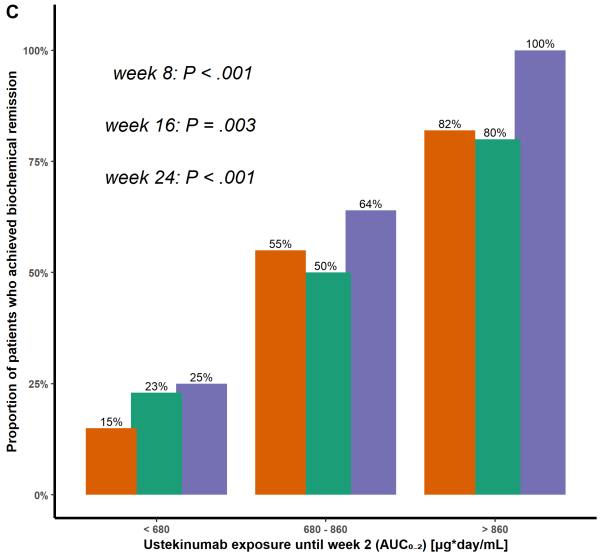

A
P = .010
Proportion of patients who achieved endoscopic remission
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
7%
23%
46%
< 88.0
88.0 - 105
> 105
Peak ustekinumab concentration [µg/mL]


B
P = .132
Proportion of patients who achieved endoscopic remission
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
18%
25%
38%
< 24.5
24.5 - 30.5
> 30.5
Ustekinumab concentration at week 2 [µg/mL]


C
P = .052
Proportion patients who of achieved endoscopic remission
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
15%
25%
45%
< 680
680 - 860
> 860
Ustekinumab exposure until week 2 (AUC0-2) [µg*day/mL]


Time
week 8
week 16
week 24
A
week 8: P < .001
week 16: P = .014
week 24: P < .001
Proportion of patients who achieved biochemical remission
100%
75%
50%
25%
0%
15%
31%
29%
42%
31%
50%
77%
75%
92%
< 88.0
88.0 - 105
> 105
Peak ustekinumab concentration [µg/mL]


B
week 8: P = .001
week 16: P = .005
week 24: P = .003
Proportion of patients who achieved biochemical remission
100%
75%
50%
25%
0%
17%
25%
27%
50%
40%
70%
77%
77%
83%
< 24.5
24.5 - 30.5
> 30.5
Ustekinumab concentration at week 2 [µg/mL]


C
week 8: P < .001
week 16: P = .003
week 24: P < .001
Proportion of patients who achieved biochemical remission
100%
75%
50%
25%
0%
15%
23%
25%
55%
50%
64%
82%
80%
100%
< 680
680 - 860
> 860
Ustekinumab exposure until week 2 (AUC0-2) [µg*day/mL]

Supplementary table 1. Correlations between ustekinumab exposure measures and outcomes, presented as Pearson's correlation coefficients.

	Peak ustekinumab	Ustekinumab w2	Ustekinumab w4	Ustekinumab w8	AUC_{0-2}	AUC_{0-4}	AUC_{0-8}	FC week 8	FC week 16	FC week 24
Ustekinumab w2	0.627***									
Ustekinumab w4	0.462**	0.754***								
Ustekinumab w8	0.370*	0.611***	0.931***							
AUC_{0-2}	0.911***	0.892***	0.704***	0.547***						
AUC_{0-4}	0.786***	0.937***	0.824***	0.700***	0.980***					
AUC_{0-8}	0.738***	0.923***	0.894***	0.797***	0.944***	0.989***				
FC week 8	-0.284	-0.485**	-0.514***	-0.468**	-0.463**	-0.384*	-0.425**			
FC week 16	-0.364*	-0.448**	-0.530***	-0.466**	-0.507**	-0.401*	-0.439**	0.709**		
FC week 24	-0.328*	-0.520**	-0.509**	-0.461**	-0.474**	-0.407*	-0.457**	0.581**	0.641**	
SES-CD week 24	-0.404**	-0.333*	-0.441**	-0.480**	-0.398*	-0.410**	-0.451**	0.565**	0.402*	0.560**

FC, fecal calprotectin; SES-CD, Simple Endoscopic Score for Crohn's disease; AUC, area under the ustekinumab serum concentration-time curve; w2, week 2; w4, week 4; w8, week 8; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Supplementary table 2. Univariable linear regression analysis of predictors of ustekinumab exposure.

	Peak (µg/mL)		Ustekinumab w2 (µg/mL)		Ustekinumab w4 (µg/mL)		Ustekinumab w8 (µg/mL)		AUC_{0-2} (µg*day/mL)		AUC_{0-4} (µg*day/mL)		AUC_{0-8} (µg*day/mL)	
	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value
Baseline serum albumin (g/L)	1.91 (0.97)	0.057	1.20 (0.41)	0.006	1.06 (0.29)	0.001	0.56 (0.17)	0.002	25.34 (9.04)	0.008	34.83 (11.68)	0.005	45.29 (14.09)	0.003
Baseline CRP (mg/L)	-0.37 (0.22)	0.098	-0.20 (0.08)	0.023	-0.21 (0.07)	0.003	-0.11 (0.04)	0.007	-4.07 (1.79)	0.029	-7.27 (2.59)	0.008	-9.19 (3.14)	0.006
Baseline FC (mg/kg)	-0.03 (0.02)	0.106	-0.01 (0.01)	0.186	-0.018 (0.01)	0.009	-0.008 (0.004)	0.031	-0.31 (0.19)	0.108	-0.58 (0.25)	0.027	-0.77 (0.29)	0.014
No previous biological therapy	13.42 (5.96)	0.030	8.22 (2.16)	0.001	5.12 (1.96)	0.013	3.45 (1.07)	0.002	156.74 (48.92)	0.003	208.97 (73.61)	0.007	274.87 (87.95)	0.003
Body weight (kg)	0.34 (0.17)	0.056	0.004 (0.078)	0.960	-0.0002 (0.06)	0.997	0.02 (0.03)	0.481	1.87 (1.66)	0.267	1.11 (2.31)	0.480	1.26 (2.82)	0.658
Sex	7.32 (6.07)	0.235	3.24 (2.47)	0.197	1.44 (2.08)	0.494	0.17 (1.17)	0.884	72.98 (53.40)	0.180	93.06 (78.05)	0.241	110.20 (94.34)	0.250
Disease duration at baseline (years)	0.18 (0.29)	0.525	-0.15 (0.11)	0.191	-0.06 (0.09)	0.517	-0.01 (0.05)	0.802	-1.15 (2.51)	0.65	-2.03 (3.62)	0.578	-1.94 (4.33)	0.656

SE, standard error; FC, fecal calprotectin; AUC, area under the ustekinumab serum concentration-time curve; w2, week 2; w4, week 4; w8, week 8; Reference class: Sex, Male

Supplementary table 3. Multivariable linear regression analysis of predictors of ustekinumab exposure.

	Peak (µg/mL)		Ustekinumab w2 (µg/mL)		Ustekinumab w4 (µg/mL)		Ustekinumab w8 (µg/mL)		AUC_{0-2} (µg*day/mL)		AUC_{0-4} (µg*day/mL)		AUC_{0-8} (µg*day/mL)	
	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value	Coefficient (SE)	*P*-value
Baseline serum albumin (g/L)	0.82 (1.06)	0.442	0.77 (0.38)	0.052	0.59 (0.31)	0.064	0.29 (0.17)	0.097	16.73 (8.68)	0.063	13.66 (11.99)	0.263	19.31 (13.78)	0.171
Baseline CRP (mg/L)	-0.20 (0.22)	0.367	-0.11 (0.07)	0.140	-0.08 (0.07)	0.235	-0.04 (0.04)	0.249	-2.33 (1.65)	0.167	-2.86 (2.65)	0.290	-3.60 (3.02)	0.242
Baseline FC (mg/kg)	/	/	/	/	-0.01 (0.01)	0.105	-0.005 (0.003)	0.178	/	/	-0.38 (0.26)	0.145	-0.51 (0.28)	0.079
No previous biological therapy	9.83 (6.08)	0.115	6.70 (2.04)	0.002	3.50 (1.90)	0.075	2.61 (1.04)	0.017	123.81 (46.83)	0.012	172.10 (73.74)	0.026	219.94 (83.84)	0.013
Body weight (kg)	0.21 (0.18)	0.246	/	/	/	/	/	/	/	/	/	/	/	/

SE, standard error; FC, fecal calprotectin; AUC, area under the ustekinumab serum concentration-time curve; w2, week 2; w4, week 4; w8, week 8; Reference class: Sex, Male. Only variables with $P < 0.1$ in the univariable analysis were included in the multivariable analysis.

Supplementary table 4. Analysis of the receiver operating characteristic (ROC) curves for ustekinumab concentration at peak, week 2, week 4, and week 8, cumulative ustekinumab exposure up to week 2, week 4 and week 8 (AUC_{0-2}, AUC_{0-4}, and AUC_{0-8}) and biochemical responses at week 8, week 16 and week 24 (fecal calprotectin < 100 mg/kg).

	Median exposure parameter (IQR)		*P*-value	AUROC	95% CI	Optimal cut-off	Sensitivity, %	Specificity, %	PPV, %	NPV, %
	Responders	Non-responders								
Biochemical remission at week 16										
Peak (µg/mL)	108.8 (88.7, 119.9)	91.2 (81.9, 103.1)	0.095	0.661	0.475-0.847	107	53	91	82	70
Endoscopic remission at week 24										
Ustekinumab w2 (µg/mL)	30.7 (24.8 – 35.9)	25.7 (22.6, 31.3)	0.256	0.627	0.408-0.846	27.2	80	58	42	88
Ustekinumab w4 (µg/mL)	18.4 (13.7, 25.0)	15.0 (9.5, 20.3)	0.064	0.700	0.510-0.890	23.7	40	97	80	82
Ustekinumab w8 (µg/mL)	5.7 (2.8, 12.8)	4.5 (3.2, 7.5)	0.221	0.633	0.413-0.854	11.1	40	97	80	83
AUC_{0-2} (µg*day/mL)	886 (747, 1003)	736 (643, 855)	0.089	0.673	0.459-0.887	776	80	62	44	89
AUC_{0-4} (µg*day/mL)	1249 (1019, 1402)	1013 (872, 1213)	0.128	0.666	0.452-0.879	1073	80	62	42	90
AUC_{0-8} (µg*day/mL)	1421 (1137, 1611)	1114 (966, 1391)	0.117	0.683	0.477-0.890	1208	80	60	40	90

IQR, interquartile range; AUROC, area under the ROC curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the ustekinumab serum concentration-time curve; w2, week 2; w4, week 4; w8, week 8

Supplementary table 5. Analysis of the receiver operating characteristic (ROC) curves for ustekinumab concentrations at peak, week 2, week 4, and week 8, cumulative ustekinumab exposure up to week 2, week 4 and week 8 (AUC_{0-2}, AUC_{0-4}, and AUC_{0-8}) and complete mucosal healing (Simple Endoscopic Score for Crohn's disease of 0).

	Median exposure parameter (IQR)		*P*-value	AUROC	95% CI	Optimal cut-off	Sensitivity, %	Specificity, %	PPV, %	NPV, %
	Responders (n = 2)	Non-responders (n = 39)								
Endoscopic remission at week 24										
Peak (µg/mL)	120.8 (/)	96.1 (83.1, 110.2)	0.126	0.842	0.690–0.994	110.7	100	76	17	100
Ustekinumab w2 (µg/mL)	32.0 (/)	27.4 (22.5, 32.2)	0.384	0.706	0.395-1.000	35.9	50	85	17	96
Ustekinumab w4 (µg/mL)	24.8 (/)	15.2 (9.9, 20.0)	0.024	0.946	0.869-1.000	23.7	100	91	33	100
Ustekinumab w8 (µg/mL)	12.1 (/)	4.4 (2.9, 7.5)	0.032	0.932	0.852-1.000	11.1	100	91	40	100
AUC_{0-2} (µg*day/mL)	910 (/)	742 (635, 869)	0.219	0.784	0.564-1.000	792	100	65	13	100
AUC_{0-4} (µg*day/mL)	1313 (/)	1060 (838, 1276)	0.185	0.803	0.576-1.000	1144	100	66	13	100
AUC_{0-8} (µg*day/mL)	1546 (/)	1170 (944, 1452)	0.144	0.829	0.606-1.000	1376	100	68	14	100

IQR, interquartile range; AUROC, area under the ROC curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the ustekinumab serum concentration-time curve; w2, week 2; w4, week 4; w8, week 8

Supplementary table 6. Univariable logistic regression analysis of predictors of treatment outcomes.

	Endoscopic remission at week 24		Biochemical remission at week 8		Biochemical remission at week 16		Biochemical remission at week 24	
	OR	*P*-value	OR	*P*-value	OR	*P*-value	OR	*P*-value
Baseline serum albumin (g/L)	1.113	0.426	1.372	0.034	1.235	0.107	1.443	0.026
Baseline CRP (mg/L)	0.908	0.215	0.859	0.036	0.931	0.100	0.929	0.064
Baseline FC (mg/kg)	0.993	0.088	0.975	0.007	0.986	0.014	0.992	0.023
No previous biological therapy	3.00	0.144	3.571	0.065	3.572	0.065	4.768	0.044
Body weight (kg)	1.013	0.537	1.016	0.413	1.006	0.759	1.006	0.771
Sex	2.667	0.209	2.016	0.295	1.571	0.493	3.575	0.070
Disease duration at baseline (years)	1.004	0.906	1.002	0.952	1.025	0.437	1.044	0.177
Peak (µg/mL)	1.043	0.046	1.055	0.012	1.034	0.092	1.064	0.008
Ustekinumab w2 (µg/mL)	1.034	0.503	1.178	0.013	1.166	0.016	1.221	0.011
Ustekinumab w4 (µg/mL)	1.142	0.049	1.292	0.002	1.221	0.005	1.221	0.008
Ustekinumab w8 (µg/mL)	1.198	0.072	1.462	0.004	1.388	0.007	1.390	0.017
AUC_{0-2} (µg*day/mL)	1.004	0.140	1.008	0.007	1.006	0.023	1.010	0.007
AUC_{0-4} (µg*day/mL)	1.002	0.118	1.005	0.003	1.004	0.011	1.005	0.010
AUC_{0-8} (µg*day/mL)	1.002	0.079	1.005	0.002	1.004	0.007	1.004	0.006
Peak (dichotomous)	7.501	0.013	5.33	0.034	8.449	0.016	13.64	0.020

FC, fecal calprotectin; AUC, area under the ustekinumab serum concentration-time curve; w2, week 2; w4, week 4; w8, week 8; OR odds' ratio; Reference classes: Sex, Male; Peak (dichotomous): below 111 µg/mL

Supplementary table 7. Multivariable logistic regression of predictors of treatment outcomes. Ustekinumab peak concentration is considered a dichotomous categoric variable – reference class below 111 µg/mL.

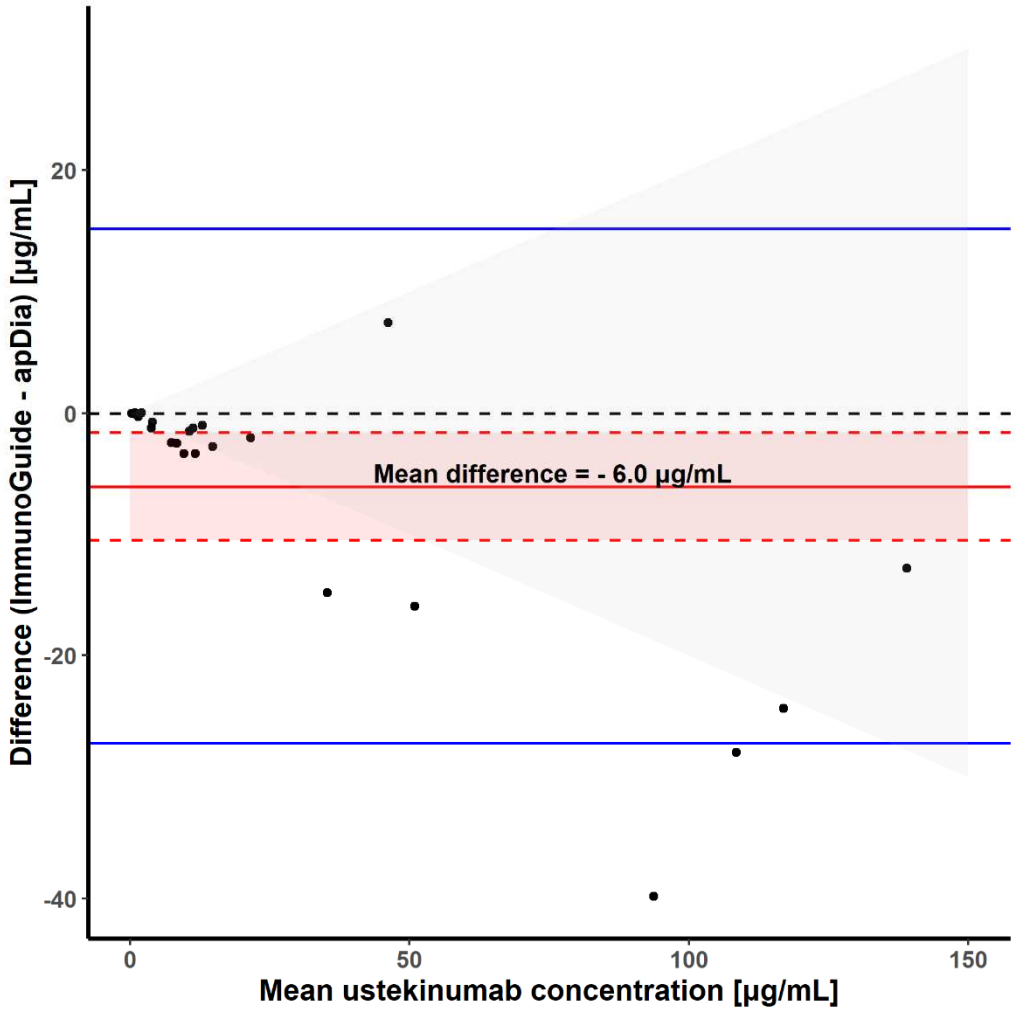
	Endoscopic remission at week 24		Biochemical remission at week 8		Biochemical remission at week 16		Biochemical remission at week 24	
	OR	*P*-value	OR	*P*-value	OR	*P*-value	OR	*P*-value
Baseline serum albumin (g/L)	/	/	1.057	0.857	/	/	1.577	0.073
Baseline CRP (mg/L)	/	/	0.774	0.347	/	/	0.961	0.504
Baseline FC (mg/kg)	0.995	0.147	0.968	0.062	0.980	0.027	0.997	0.499
No previous biological therapy	/	/	5.867	0.246	7.192	0.141	4.132	0.235
Sex	/	/	/	/	/	/	6.073	0.124
Peak (dichotomous)	7.966	0.031	1.847	0.796	118.1	0.100	12.55	0.091

CRP, C-reactive protein; FC, fecal calprotectin; OR odds' ratio; Reference classes: Sex, Male; Peak (dichotomous): below 111 µg/mL. Only variables with $P < 0.1$ in univariable analysis were included in the multivariable analysis.

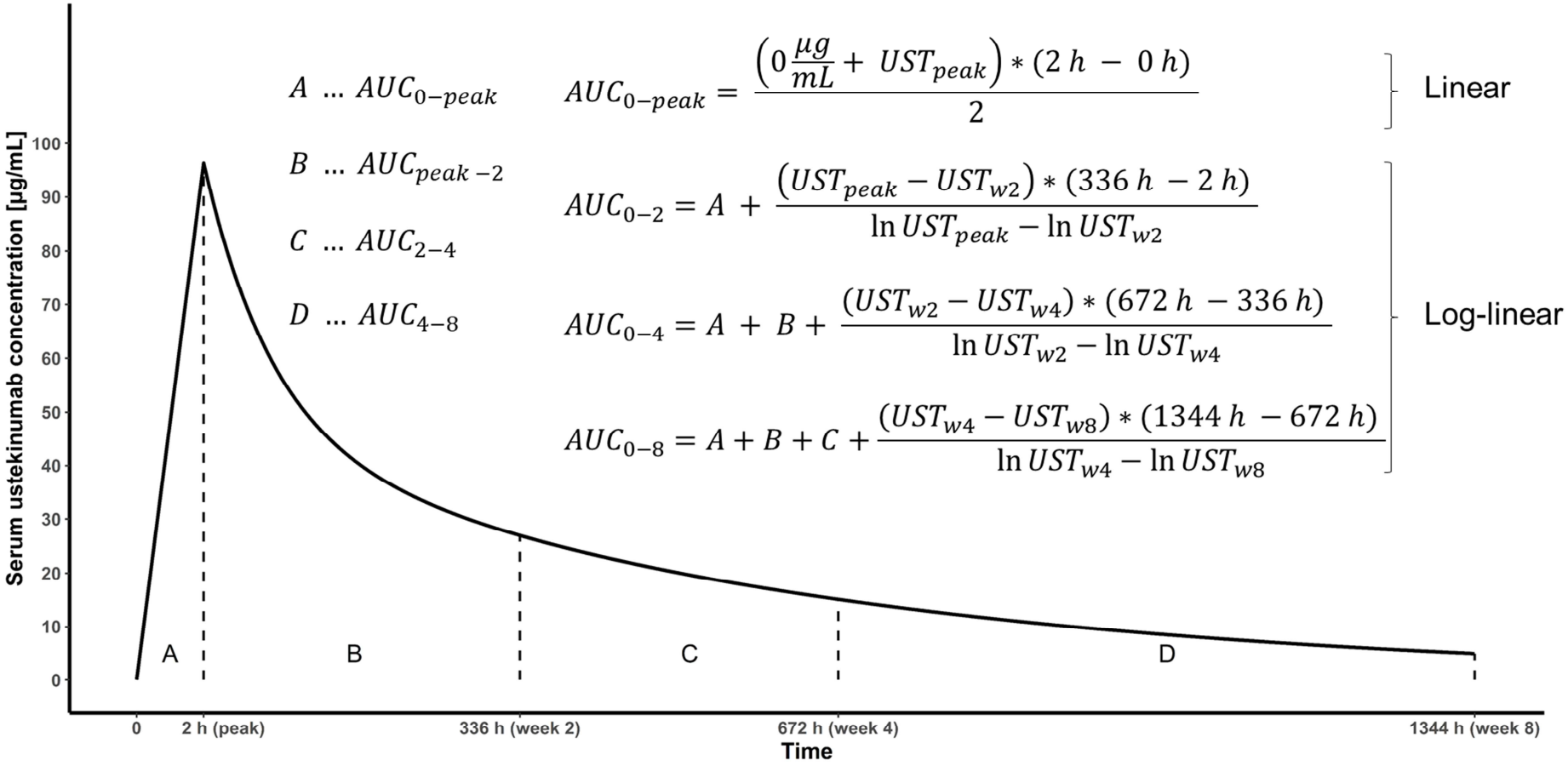
Supplementary table 8. Multivariable logistic regression of predictors of treatment outcomes. Ustekinumab peak concentration is considered a continuous variable.

	Endoscopic remission at week 24		Biochemical remission at week 8		Biochemical remission at week 16		Biochemical remission at week 24	
	OR	*P*-value	OR	*P*-value	OR	*P*-value	OR	*P*-value
Baseline serum albumin (g/L)	/	/	1.225	0.509	/	/	1.412	0.146
Baseline CRP (mg/L)	/	/	0.695	0.398	/	/	0.965	0.509
Baseline FC (mg/kg)	0.995	0.176	0.975	0.085	0.985	0.022	0.997	0.561
No previous biological therapy	/	/	5.165	0.260	4.108	0.169	2.529	0.389
Sex	/	/	/	/	/	/	4.660	0.183
Peak (µg/mL)	1.040	0.101	1.055	0.210	1.008	0.798	1.038	0.170

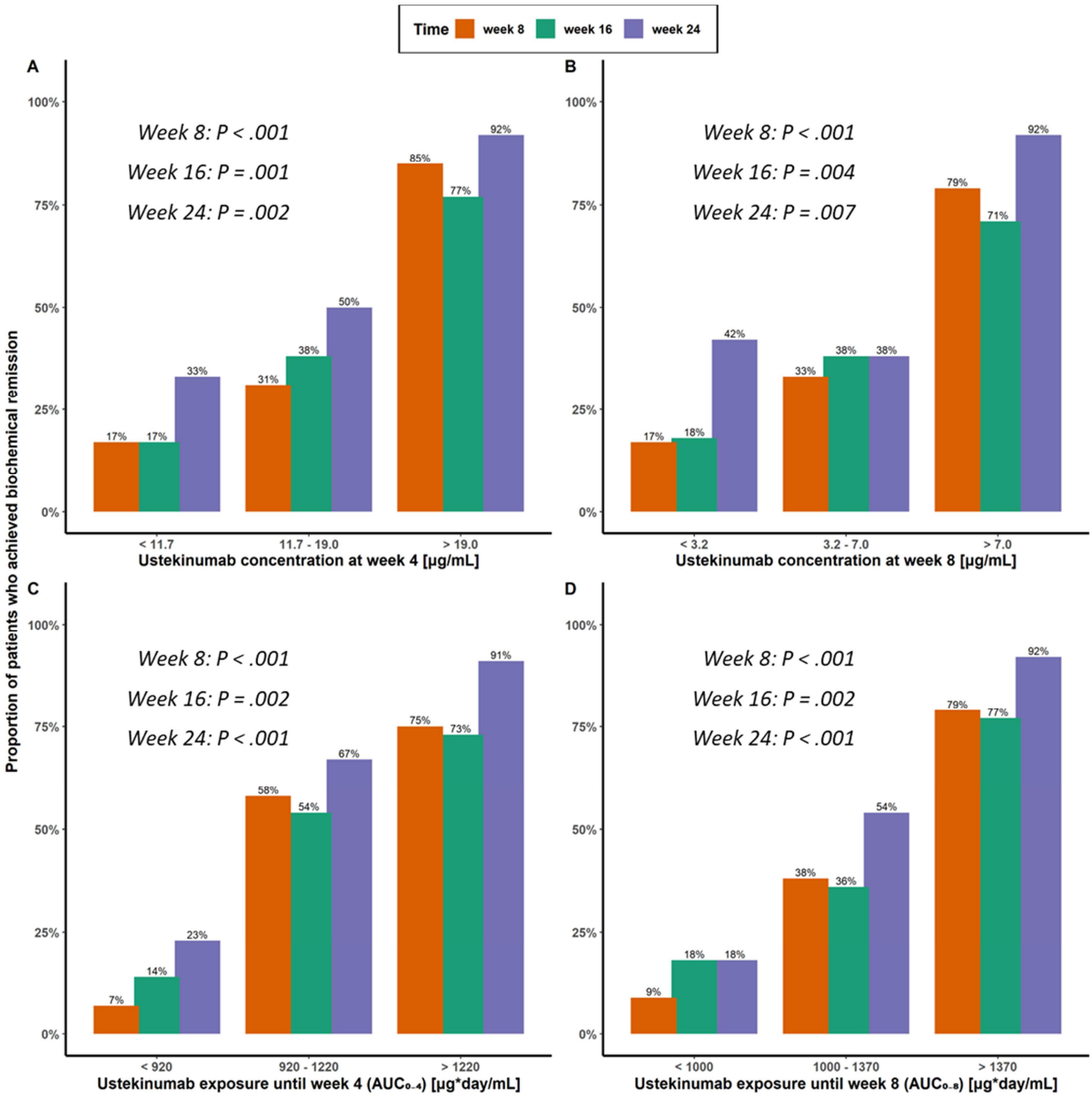
CRP, C-reactive protein; FC, fecal calprotectin; OR odds' ratio; Reference classes: Sex, Male; Peak (dichotomous): below 111 µg/mL. Only variables with $P < 0.1$ in univariable analysis were included in the multivariable analysis.



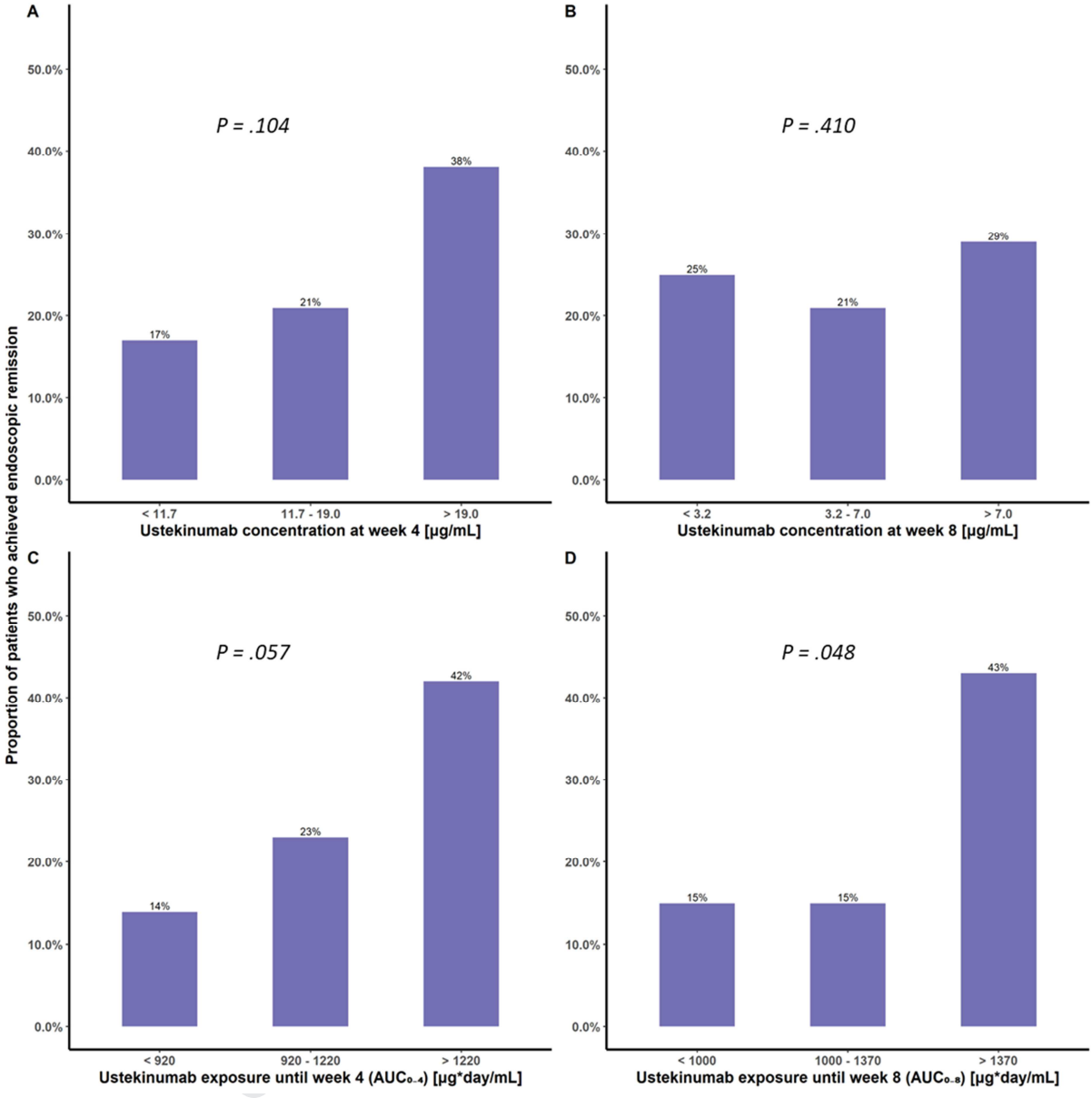
Supplementary figure 1. Bland-Altman diagram showing the mean difference (red line), 95 % interval of agreement (blue lines) and 95% confidence interval for the mean difference (red dashed lines, red shaded area). The grey area represents a relative difference of 20%.



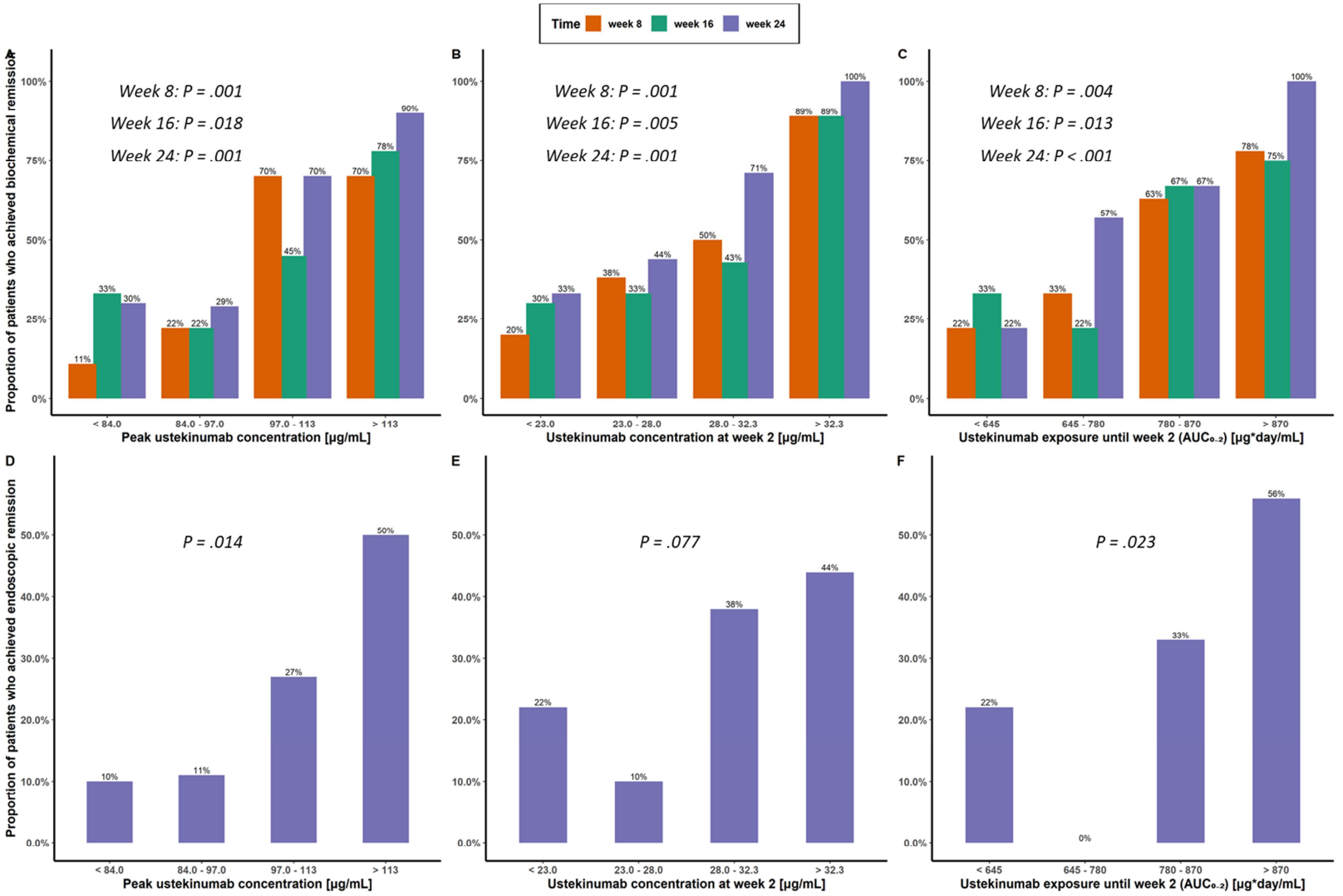
Supplementary figure 2. Typical serum ustekinumab concentrations (bold line) and representation of the cumulative exposure calculation as area under the serum concentration curve.



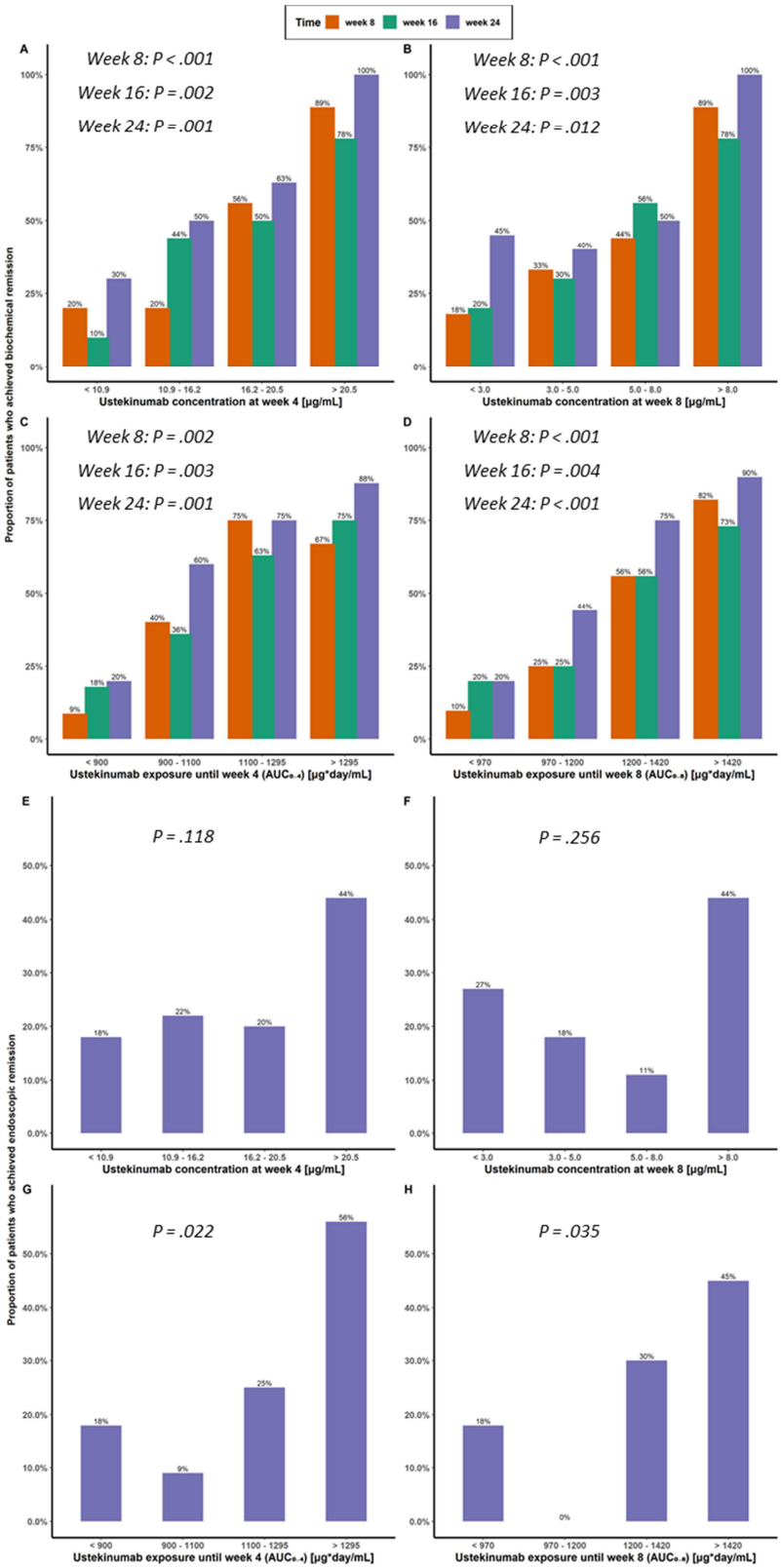
Supplementary figure 3. Analysis of the proportions of patients who achieved biochemical remission at week 8, week 16 and week 24 for different terciles of ustekinumab concentrations at week 4 (A), week 8 (B), and cumulative exposure up to week 4 (AUC_{0-4}, C) and up to week 8 (AUC_{0-8}, D).



Supplementary figure 4. Analysis of the proportions of patients who achieved endoscopic remission at week 24 for different terciles of ustekinumab concentrations at week 4 (A), week 8 (B), and cumulative exposure up to week 4 (AUC_{0-4}, C) and up to week 8 (AUC_{0-8}, D).



Supplementary figure 5. Analysis of the proportions of patients who achieved biochemical remission at week 8, week 16 and week 24 (A-C) for quartiles of peak ustekinumab concentrations (A), ustekinumab concentrations at week 2 (B) and cumulative exposure up to week 2 ($AUC_{0\text{-}2}$, C); and the proportions of patients who achieved endoscopic remission at week 24 (D-F) for quartiles of peak ustekinumab concentrations (D), ustekinumab concentrations at week 2 (E) and $AUC_{0\text{-}2}$ (F).



Supplementary figure 6. Analysis of the proportions of patients who achieved biochemical remission at week 8, week 16 and week 24 (A-D) for quartiles of ustekinumab concentrations at week 4 (A), week 8 (B), cumulative exposure up to week 4 (AUC_{0-4}, C) and up to week 8 (AUC_{0-8}, D); and proportions of patients who achieved endoscopic remission at week 24 (E-H) for quartiles of ustekinumab concentrations at week 4 (E) and week 8 (F), AUC_{0-4} (G) and AUC_{0-8} (H).