

Causes of Treatment Failure in Children With Inflammatory Bowel Disease Treated With Infliximab: A Pharmacokinetic Study

^{*†}Samuele Naviglio, [‡]Doriana Lacorte, [§]Marianna Lucafò, ^{||}Adriana Cifù, [¶]Diego Favretto, [§]Eva Cuzzoni, [#]Tania Silvestri, [§]Martina Pozzi Mucelli, [†]Oriano Radillo, ^{†§}Giuliana Decorti, ^{**}Martina Fabris, [†]Matteo Bramuzzo, ^{†§}Andrea Taddio, [¶]Gabriele Stocco, [‡]Patrizia Alvisi, ^{†§}Alessandro Ventura, and [†]Stefano Martelossi

ABSTRACT

Objectives: Anti-tumor necrosis factor antibodies have led to a revolution in the treatment of inflammatory bowel diseases (IBD); however, a sizable proportion of patients does not respond to therapy. There is increasing evidence suggesting that treatment failure may be classified as mechanistic (pharmacodynamic), pharmacokinetic, or immune-mediated. Data regarding the contribution of these factors in children with IBD treated with infliximab (IFX) are still incomplete. The aim was to assess the causes of treatment failure in a prospective cohort of pediatric patients treated with IFX.

Methods: This observational study considered 49 pediatric (median age 14.4) IBD patients (34 Crohn disease, 15 ulcerative colitis) treated with IFX. Serum samples were collected at 6, 14, 22 and 54 weeks, before IFX infusions. IFX and anti-infliximab antibodies (AIA) were measured using enzyme linked immunosorbent assays. Disease activity was determined by Pediatric Crohn's Disease Activity Index or Pediatric Ulcerative Colitis Activity Index.

Results: Clinical remission, defined as a clinical score <10, was obtained by 76.3% of patients at week 14 and by 73.9% at week 54. Median trough IFX concentration was higher at all time points in patients achieving sustained clinical remission. IFX levels during maintenance correlated also with C-reactive protein, albumin, and fecal calprotectin. After multivariate analysis, IFX concentration at week 14 >3.11 µg/mL emerged as the strongest predictor of sustained clinical remission. AIA concentrations were correlated inversely with IFX concentrations and directly with adverse reactions.

Conclusions: Most cases of therapeutic failure were associated with low serum drug levels. IFX trough levels at the end of induction are associated with sustained long-term response.

Key Words: anti-infliximab antibodies, inflammatory bowel disease, infliximab, therapeutic drug monitoring

What Is Known

- A sizable proportion of pediatric patients with inflammatory bowel disease does not respond or lose response to therapy with infliximab.
- Treatment failure may involve mechanistic (pharmacodynamic), pharmacokinetic, or immune-mediated mechanisms.

What Is New

- Most cases of therapeutic failure were associated with low serum drug levels.
- The occurrence of true so-called mechanistic (pharmacodynamic) failure seems to represent a rare event.
- Infliximab trough levels at the end of induction are associated with sustained long-term response more strongly than other clinical and laboratory parameters.

The use of biologic anti-tumor necrosis factor (TNF) drugs has revolutionized the treatment of inflammatory bowel disease (IBD). In fact, these drugs have been shown to induce clinical and mucosal remission both in adult and pediatric patients, thus possibly modifying the natural history of these conditions. Several studies have demonstrated their efficacy in pediatric patients. In the REACH study, clinical response and remission were achieved respectively by 84% and 58.9% of children with Crohn disease

Accepted July 16, 2018.

From the ^{*}PhD Course in Science of Reproduction and Development, University of Trieste, the [†]Institute for Maternal and Child Health IRCCS Burlo Garofolo, Trieste, the [‡]Pediatric Department, Ospedale Maggiore, Bologna, the [§]Department of Medical and Surgical Sciences, University of Trieste, Trieste, the ^{||}Department of Medical Area, University of Udine, Udine, the [¶]Department of Life Sciences, University of Trieste, Trieste, the [#]Laboratorio Unico Metropolitan, AUSL, Bologna, Bologna, and the ^{**}Department of Laboratory Medicine, Institute of Clinical Pathology, University Hospital of Udine, Udine, Italy.

Address correspondence and reprint requests to Gabriele Stocco, PhD, Department of Life Sciences, University of Trieste, via Fleming 22, I-34127 Trieste, Italy (e-mail: stoccog@units.it).

Samuele Naviglio and Doriana Lacorte contributed equally to the article. Stefano Martelossi is presently working with Dipartimento Materno Infantile, Ospedale Ca' Foncello, Treviso, Italy.

The authors report no conflicts of interest.

(CD) after a 3-dose induction scheme with infliximab (IFX) (1). Similar results were demonstrated in children with ulcerative colitis (UC) treated with IFX, with 73.3% and 40% of patients responding and achieving clinical remission, respectively (2). Nevertheless, a substantial proportion of subjects either do not respond (primary failure, 10%–20%) or lose response during treatment (secondary loss of response: estimated annual risk 10%–20% per patient-year as judged by drug discontinuation, up to 45% per patient-year including also need for therapy intensification) (3–5); or need to discontinue it because of adverse drug reactions. Treatment failure can be generally classified as the result of 3 possible situations: mechanistic (pharmacodynamic) failure, non-immune-mediated pharmacokinetic failure and immune-mediated pharmacokinetic failure (6). Mechanistic failure occurs when drug concentrations are adequate; it may be caused by non-inflammatory conditions or by a switch to non-TNF-driven inflammation. Moreover, some studies indicate that patients with high serum anti-TNF concentration may present active disease because tissue levels of anti-TNF are insufficient to antagonize local TNF (7). Non-immune-mediated pharmacokinetic failure occurs when drug concentrations are suboptimal and no anti-drug antibodies can be detected. Finally, in immune-mediated pharmacokinetic failure, low or undetectable drug concentrations are associated with high titers of anti-drug antibodies.

The relative contribution of each of these situations as a cause of treatment failure in children with IBD is still poorly described. Furthermore, the number of pediatric studies correlating anti-TNF blood levels and anti-drug antibodies with response to therapy is still limited and, overall, the findings are not conclusive. While a recently published meta-analysis of pediatric studies has shown that patients with higher IFX plasma levels more frequently maintained clinical response after induction and after 1 year of therapy (8), another study correlated IFX levels to laboratory response (fecal calprotectin and C-reactive protein, CRP) but not to clinical response (9). Similarly, the role of anti-infliximab antibodies (AIA) and what levels should be considered significant in clinical practice are still incompletely understood.

The main objective of our study was to assess the causes of treatment failure in a cohort of pediatric patients treated with IFX, evaluating serum IFX levels and clinical response to treatment, as well as the role of AIA on IFX serum concentrations, clinical response and adverse effects.

METHODS

Patients

The observational cohort study was approved by the local ethical committees and appropriate informed consent was obtained for all patients from their parents/tutors. Patients with IBD treated at Institute for Maternal and Child health IRCCS “Burlo Garofolo,” Trieste, Italy, and at Maggiore Hospital, Bologna, Italy, were enrolled between March 2012 and June 2016. The inclusion criteria were age between 6 and 18 years, a diagnosis of active IBD (as defined by appropriate clinical scoring—see “Clinical response” section), and treatment with IFX; all patients were biologic naive. IFX was started in case of treatment failure or intolerance to first-line therapies: mesalamine, immunosuppressants and, in the case of CD, enteral nutrition. IFX was used also as first-line treatment in selected patients, as suggested by pediatric guidelines (10). Exclusion criteria were presence of an ileostomy or colostomy, disease needing surgery, infectious complications (including intra-abdominal infections), fulminant UC or toxic megacolon or contemporary presence of other noncontrolled medical conditions (eg, organ failure). Therapy with immunosuppressants (methotrexate, azathioprine) was permitted; therapy with glucocorticoid was permitted if

tapering was undertaken after starting treatment with IFX. The patients enrolled were all the eligible consecutive cases at the participating centers in the time frame of the study. Laboratory tests included CRP, albumin and fecal calprotectin as required by their routine care according to patients’ attending physician. Infliximab concentration measurements were missing for 9, 14, 16, and 23 patients at week 6, 14, 22, and 54, respectively. Measurement of calprotectin was not available for 58 patients visit overall.

The therapeutic protocol consisted of an induction phase with intravenous administration of IFX 5 mg/kg at weeks 0, 2, 6 and of a maintenance phase, when IFX was administered every 8 weeks. All patients with partial or complete response to treatment after induction continued therapy with IFX and were followed till week 54; in selected cases, patients continued treatment even though they were considered nonresponders if clinically indicated according to the treating physician’s opinion. In case of clinical evidence of loss of response, therapy with IFX could be escalated, either by increasing drug dose (up to 10 mg/kg per dose) or by shortening intervals between infusions. Previous therapy with immunosuppressant was permitted but not to treat loss of response. All patients were premedicated before infusion with i.v. methylprednisolone 1 mg/kg and chlorphenamine maleate 0.2 mg/kg, to reduce the risk of adverse reactions during the infusion. Patients developing anaphylactoid reactions were considered in the pharmacokinetic study up to the time when they developed the reaction. (Supplementary methods: statistical analysis, Supplemental Digital Content, <http://links.lww.com/MPG/B471>)

Clinical Response

Clinical disease activity was assessed using Pediatric Crohn’s Disease Activity Index (11) and Pediatric Ulcerative Colitis Activity Index (12) for CD and UC patients, respectively, at baseline and at the time of blood sample collection at weeks 6, 14, 22 and 54. Disease was considered to be in remission if the disease activity index was <10; partial response was defined as a change of at least 15 points from baseline for CD and at least 20 points for UC (12,13). Loss of response was considered either as clinical worsening in a patient who had previously attained clinical response/remission or as need for treatment intensification.

Measurement of Infliximab and Anti-infliximab Antibody

IFX and AIA levels were determined by enzyme linked immunosorbent assay (LISA Tracker Duo IFX, Theradiag, France), on sera collected immediately before the III, IV, V, IX infusion (weeks 6, 14, 22, 54) and, in any case before the last infusion preceding therapy discontinuation. AIA levels were measured when IFX plasma levels were <1.5 µg/mL. The assay results for IFX and AIA levels were obtained retrospectively. In this study there was no intervention, since treating clinicians were not aware of the infliximab pharmacokinetic results, which were analyzed retrospectively.

Statistical Analysis

Statistical analysis was performed using the software R (version 3.4.2). The association between IFX concentrations and therapeutic response was evaluated in a univariate analysis by generalized linear models of the binomial family (logistic regression), using patients response to IFX as the dependent variable and IFX concentration as the independent variable. To identify the best predictor of IFX response, the most significant association between IFX concentrations and response at the various time points

considered was identified on the basis of the logistic regression analysis. Receiver operating characteristic (ROC) curves were then constructed for IFX concentrations, to determine the optimal cutoff to predict patients' clinical response to IFX. Sensitivity, specificity and the positive and negative predictive values of the cutoff point were analyzed.

To test for an association of IFX levels with demographic covariates, generalized linear mixed effects models were used, considering the demographic parameter of interest as the dependent variable and IFX concentration as the independent variable.

To test the association of the identified cutoff value with demographic and clinical covariates (age, sex, IBD type, clinical laboratory parameters including CRP, albumin and calprotectin), univariate logistic regression analysis was performed, considering patients' IFX concentration below or above the cutoff point as the dependent variable and the demographic/clinical covariate as the independent variable.

Multivariate analysis was performed to test the potential confounding effect on the association between therapeutic response and the cutoff identified for IFX concentration by clinical and demographic covariates. The multivariate analysis was done by logistic regression, using therapeutic response as the dependent variable and the cutoff for IFX concentration together with all covariates significantly associated with this cutoff in the univariate analysis, as independent variables.

An analysis on the association between post-induction IFX concentrations and the clinical laboratory parameters was performed also by generalized linear mixed effects models, considering the clinical laboratory parameter of interest as the dependent variable and IFX concentration as the independent variable. For the clinical laboratory parameter, normality of the distribution was evaluated by visual examination of the data histogram and by Shapiro's test and an appropriate transformation was applied to restore normality. The association between AIA concentrations and IFX concentrations was determined by nonparametric Spearman's test.

RESULTS

Clinical Response

Forty-nine patients (Table 1) were enrolled (14). Seven patients were on concomitant immunosuppressive therapy at treatment start (azathioprine) and other 7 patients were receiving systemic corticosteroids. After induction therapy, 9 patients (18.4% of all patients, 3 (8.8%) with CD and 6 (40.0%) with UC, *P* value logistic regression = 0.015) did not respond to therapy, and 2 patients (4.1% total, 1 with CD and 1 with UC) discontinued

IFX due to anaphylactoid reactions during induction infusions. Thirty-eight patients (77.6%, 30 with CD and 8 with UC) responded to induction treatment: 8 patients had a partial response (16.3%, 5 with CD and 3 with UC) and 30 achieved clinical remission (61.2%, 25 with CD and 5 with UC).

At week 54, 24 patients presented sustained clinical response (49.0% of all patients, 58.8% of those with CD and 26.7% of those with UC, *P* value logistic regression = 0.056); of these, 23 presented clinical remission and 1 partial response. Nine patients (18.4% of all patients, 7 CD and 2 with UC) had lost response by 54 weeks, while 5 patients (3 with CD and 2 with UC) discontinued IFX due to anaphylactoid reactions during maintenance therapy. Among patients with secondary loss of response, therapy intensification was used in 6. A total of 134 samples from 49 patients were analyzed at 6, 14, 22 and 54 weeks, for 40, 35, 33 and 26 patients, respectively (Table 2).

Serum Infliximab Levels and Clinical Remission After Induction Treatment

At third infusion (week 6), 24 patients were in clinical remission, while 14 were not. IFX concentrations were different between these 2 groups (median IFX concentration 9.8 µg/mL, IQR 8.4 to 12.6, in patients in clinical remission versus median 7.1 µg/mL, IQR 4.7 to 9.8, in patients not in remission; *P* value logistic regression = 0.044; Fig. 1A). Also at the fourth infusion (week 14), IFX serum concentrations were significantly different (median IFX concentration 5.0 µg/mL, IQR 3.6–9.1, vs 1.0 µg/mL, IQR 0.18–2.7; *P* value logistic regression = 0.00039; Fig. 1B).

Interestingly, at week 14, IFX levels in patients with partial response (median IFX concentration 2.0 µg/mL, IQR 0.74–3.02) were found to be intermediate between those of patients with primary failure (median IFX concentration 0.40 µg/mL, IQR 0.05–0.83) and those in clinical remission (median IFX concentration 5.0 µg/mL, IQR 3.56–6.12, *P*-value logistic regression = 0.00032, Supplementary Figure 1, Supplemental Digital Content, <http://links.lww.com/MPG/B471>).

Serum Infliximab Levels and Clinical Remission at 22 Weeks of Treatment

Considering clinical response at 22 weeks of treatment, significantly different concentrations of serum IFX were observed between patients in clinical remission and those who were not. In particular, median IFX concentrations before III, IV, and V infusion

TABLE 1. Demographic and clinical characteristic of the 49 patients enrolled

	Overall (n = 49)	Crohn disease (n = 34)	Ulcerative colitis (n = 15)
Age, y	14.4 [11.6–16.2]	15.1 [11.7–16.8]	13.6 [11.9–15.3]
Gender (F/M)	20 (40.8%)/29 (59.2%)	12 (35.3%)/22 (64.7%)	8 (53.3%)/7 (46.7%)
Disease location according to Paris classification (14)	—	L1/L2/L3: 4 (11.8%)/4 (11.8%)/26 (76.4%) L4a/L4b: 17 (50%)/5 (14.7%) P: 6 (17.6%) B1/B2/B3: 27 (79.4%)/7 (20.6%)/0	E1/E2/E3/E4: 0/5 (33.3%)/2 (13.3%)/8 (53.3%) S0/S1: 7 (46.7%)/8 (53.3%)
Disease activity index at inclusion*	35 [20–50]	30 [17.5–40]	60 [35–65]
Concomitant immunosuppressive therapy (none/azathioprine/steroids)	35 (71.4%)/7 (14.3%)/7 (14.3%)	26 (76.5%)/7 (20.6%)/1 (2.9%)	9 (60.0%)/0/6 (40.0%)

Median, 1st and 3rd quartile of age and disease activity index at inclusion are reported.

*Clinical disease activity was assessed using Pediatric Crohn's Disease Activity Index (PCDAI) (11) and Pediatric Ulcerative Colitis Activity Index (PUCAI) (12).

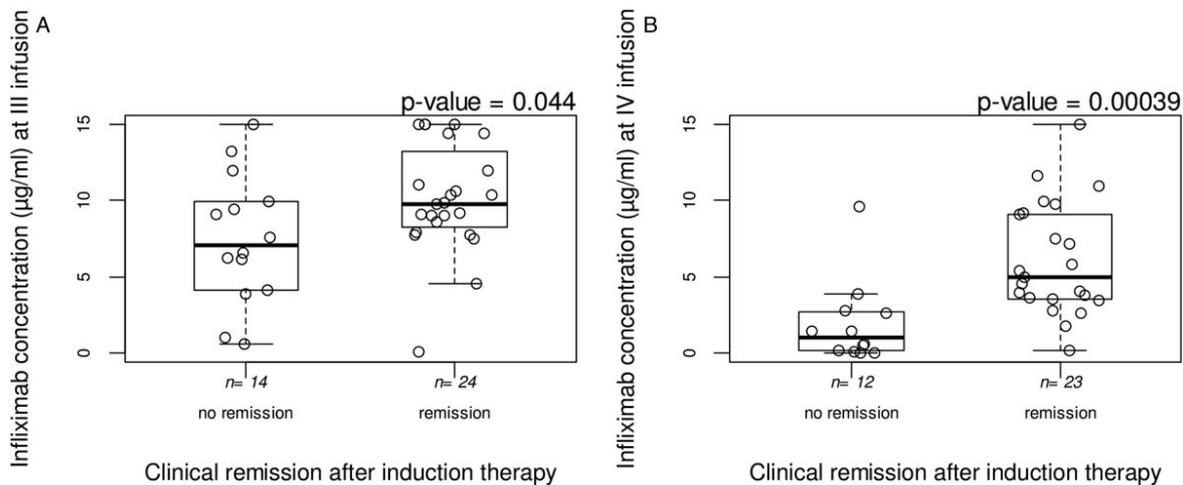


FIGURE 1. Boxplot comparing clinical remission at the end of induction therapy (IV infusion, 14 weeks of treatment) and serum infliximab (IFX) concentration at the III (A) and IV (B) infusion between patients according to remission status. The bold horizontal line represents the median value. Statistical significance was assessed by logistic regression analysis.

were 10.3 $\mu\text{g/mL}$ (IQR 9.0–13.8), 5.0 $\mu\text{g/mL}$ (IQR 3.5–9.1), and 4.4 $\mu\text{g/mL}$ (IQR 2.4–8.7) in patients in clinical remission, and 7.1 $\mu\text{g/mL}$ (IQR 4.7–9.8), 1.0 $\mu\text{g/mL}$ (IQR 0.17–2.7), and 0.6 $\mu\text{g/mL}$ (IQR 0.05–1.3), in patients not in clinical remission (P -value logistic regression <0.01 for all comparisons; Fig. 2).

Serum Infliximab Levels and Clinical Remission at 54 Weeks of Treatment

Clinical remission at 54 weeks of treatment was most significantly correlated with serum IFX levels before the IV infusion (median IFX concentration: 6.1 $\mu\text{g/mL}$, IQR 3.8–9.6 in patients in clinical remission vs 1.4 $\mu\text{g/mL}$, IQR 0.35–2.8 in patients not in clinical remission, P value logistic regression = 0.00038, Fig. 3B). Serum IFX concentrations at the III and V infusion were also significantly associated with sustained clinical remission at 54 weeks of treatment (median IFX concentration: at III infusion 10.4 $\mu\text{g/mL}$, IQR 9.1–14.4, vs 7.8 $\mu\text{g/mL}$, IQR 5.7–10.5, P value logistic regression = 0.0080, Fig. 3A; at V infusion 5.2 $\mu\text{g/mL}$, IQR 2.9–9.0, vs 1.0 $\mu\text{g/mL}$,

IQR 0.34–1.9, P -value logistic regression = 0.0022; Fig. 3C). IFX levels at IX infusion (54 weeks) were also associated with clinical remission, (median IFX concentration 3.8 $\mu\text{g/mL}$, IQR 2.7–6.0, vs 1.2 $\mu\text{g/mL}$, IQR 0.67–1.9, P value logistic regression = 0.025, Fig. 3D).

Receiver operating characteristic (ROC) curves were constructed to assign optimal cutoff values for IFX levels before the IV infusion and clinical response at 54 weeks: an optimal cutoff of 3.11 $\mu\text{g/mL}$ was defined. Area under the ROC curve (AUC) was 85.9% (Supplementary Figure 2, Supplemental Digital Content, <http://links.lww.com/MPG/B471>). The test had a sensitivity of 88.9% and a specificity of 80.0% (positive predictive value 84.2%; negative predictive value 85.7%) for predicting sustained remission. Logistic regression analysis confirmed that patients who reached the cutoff point of 3.11 $\mu\text{g/mL}$ at 14 weeks (19 patients, 16 in sustained remission at 54 weeks) had a higher probability of maintaining sustained remission at 54 weeks compared to those who did not (14 patients, only 2 in sustained remission at 54 weeks), with an odds ratio (OR) of 32.0 (95% confidence interval (CI) 5.5–297.8, $P = 3.0 \times 10^{-5}$).

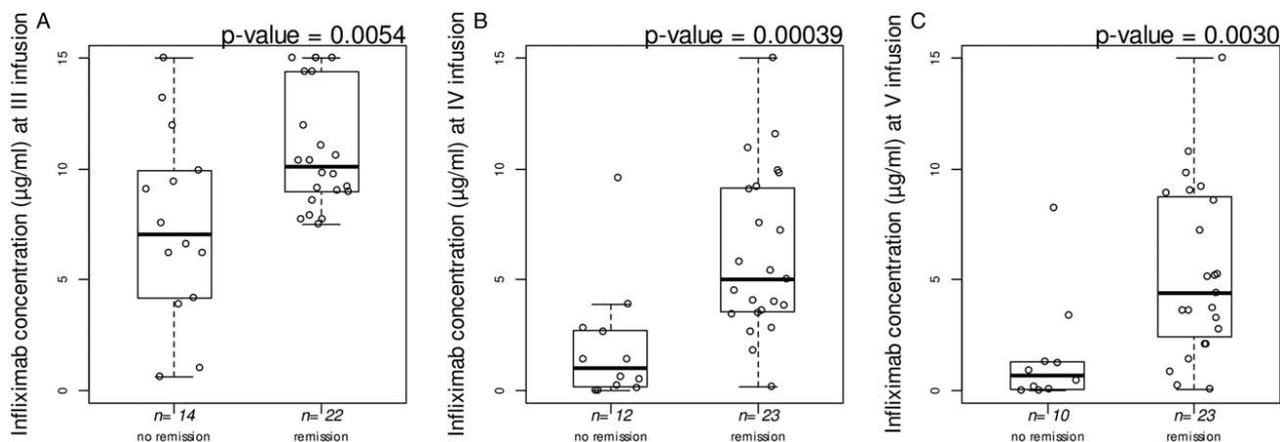


FIGURE 2. Boxplot comparing clinical remission at V infusion (22 weeks of treatment) and serum infliximab (IFX) concentration at the III (A), IV (B), and V (C) infusion between patients according to remission status. The bold horizontal line represents the median value. Statistical significance was assessed by logistic regression analysis.

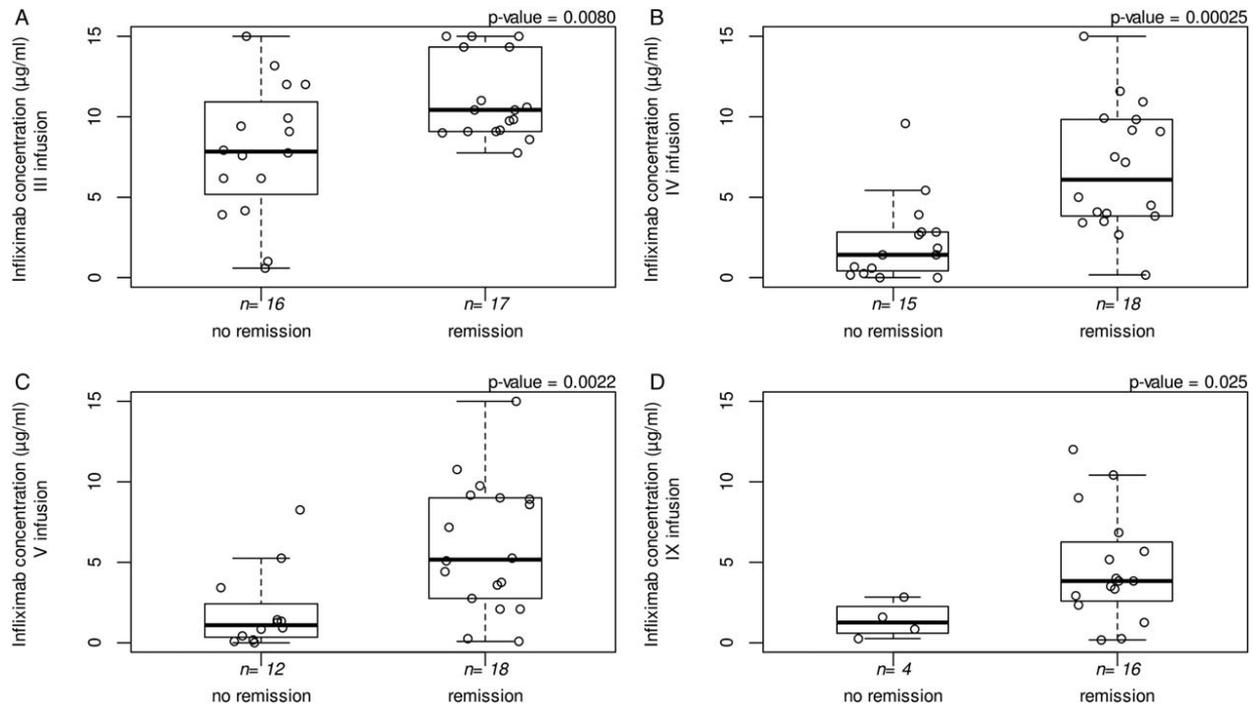


FIGURE 3. Boxplot comparing clinical remission at IX infusion (54 weeks of treatments) and serum infliximab (IFX) concentration at the III (A), IV (B), V (C), and IX (D) infusion between responsive and non-responsive patients. The bold horizontal line represents the median value. Statistical significance was assessed by logistic regression.

Interestingly, considering the 14 patients who lost response during maintenance, most of them (78.6%) presented pharmacokinetic failure, defined as a measurement of infliximab below the cutoff point identified at the end of induction (3.11 µg/mL), in comparison to patients with sustained response (44.0%, *P* value logistic regression = 0.032).

Serum Infliximab Levels and Demographic, Clinical, and Biochemical Variables

Considering demographical variables, neither sex nor age or IBD type were significantly associated with infliximab

concentration or with achieving the IFX cutoff value for sustained remission (ie, 3.11 µg/mL at the IV infusion). The cutoff value was significantly correlated with biochemical parameters measured at IV infusion: patients not achieving the cutoff IFX concentration had significantly higher CRP and calprotectin levels and lower albumin levels compared to others (Fig. 4). CRP (*P* = 0.0031) and calprotectin (*P* = 0.00017), but not albumin (*P* = 0.29), at 14 weeks also showed a significant association in a logistic regression analysis with sustained remission at 54 weeks. Considering all post-induction samples collected (90 in 42 patients), IFX levels were significantly inversely correlated with CRP (*P* linear mixed-effect model *P* = 0.0008) and fecal calprotectin (linear mixed-effect model

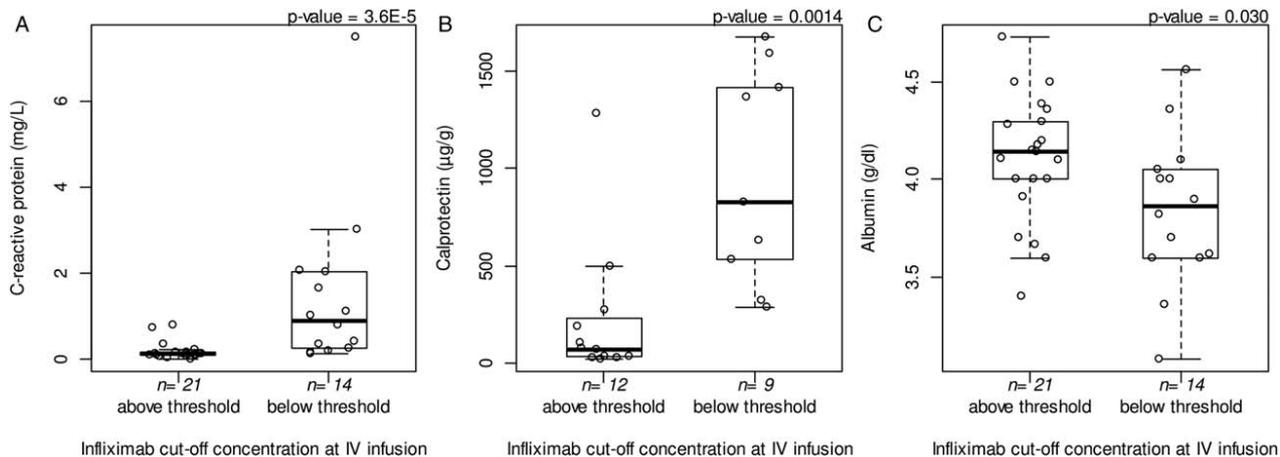


FIGURE 4. Level of clinically relevant laboratory parameters (A) C-reactive protein (CRP), (B) calprotectin, (C) albumin in patients with a concentration of infliximab (IFX) at the IV infusion below or above the threshold level associated with sustained response; *P* values are from logistic regression, considering as infliximab cutoff as the dependent variable and the clinical parameter as the independent variable.

$P=0.025$) and directly correlated with albumin (linear mixed-effect model $P=0.0033$) (Supplementary Figure 3, Supplemental Digital Content, <http://links.lww.com/MPG/B471>).

A multivariate logistic regression model was performed to assess the independence of the association between CRP, calprotectin, IFX concentration cutoff, and sustained clinical response. A model containing all variables did not converge, likely because of the missing values of calprotectin. However, a model with CRP and the IFX concentration cutoff showed a significant effect only for the IFX cutoff (adjusted logistic regression model $P=0.0065$), which therefore was confirmed to be the most robust predictor of sustained clinical response.

Anti-infliximab Antibody Quantification

AIA were measured in all samples that showed serum IFX levels $<1.5 \mu\text{g/mL}$. AIA levels higher than $10 \mu\text{g/mL}$ were considered positive. AIA concentrations were inversely correlated with IFX trough concentration (Spearman test $P=0.00088$; Supplementary Figure 4, Supplemental Digital Content, <http://links.lww.com/MPG/B471>).

Ten patients (20.4%) resulted AIA positive, 2 at the III infusion, 3 at the IV infusion, 2 at the V infusion and 3 at the IX infusion; in all but 1 patient, AIA positivity persisted also at subsequent infusions (Supplementary Figure 5, Supplemental Digital Content, <http://links.lww.com/MPG/B471>).

Serum Anti-infliximab Antibody Levels and Adverse Reaction

A statistical significant association was found between positivity to AIA and anaphylactoid reactions during treatment (logistic regression $P=0.018$, OR 8.00, 95% CI 1.4–50.4): indeed, of the 7 patients with anaphylactoid reactions during the study, 4 were AIA positive (57%), while among the 42 showing no adverse reaction only 6 were AIA positive (14%).

Serum Anti-infliximab Antibody Levels and Therapeutic Response

No statistically significant association was found between positivity to AIA and clinical efficacy. Among the 24 patients showing sustained response to IFX, 3 (12.5%) resulted AIA positive during therapy, while among the 25 patients with unsatisfactory response, 7 (28%) resulted AIA positive (logistic regression $P=0.19$). Considering the 5 patients with a very high AIA concentration ($>100 \mu\text{g/mL}$), a trend was, however, observed toward worse efficacy, with just 1 patient (20%) showing sustained remission, compared to 61.4% of patients not developing AIA or developing AIA at a concentration lower than $100 \mu\text{g/mL}$ (logistic

regression $P=0.073$, OR 2.5, 95% CI 0.85–130.0). Also the 5 patients with intermediate AIA concentration (>10 and $<100 \mu\text{g/mL}$) showed a reduced incidence of sustained remission (40%), which however was not statistically significant. Finally, considering the 14 patients who lost response during maintenance, 3 patients had positive AIA, and the incidence of AIA was not different from those with sustained response (21.4% vs 20.6%) (Table 2).

DISCUSSION

In this work, we evaluated 49 pediatric patients (aged between 6 and 18 years) with IBD treated with IFX. Forty-nine percent of patients achieved sustained response at 54 weeks of therapy, while the incidence of therapeutic inefficacy was 18.4% after induction (primary failure) and 28.6% during maintenance by 54 weeks of therapy (secondary loss of response, including patients needing therapy intensification); moreover, 14.3% of patients had to discontinue treatment because of the occurrence of adverse events (anaphylactoid reactions). Overall, therapeutic efficacy of IFX in our cohort is comparable to previous reports.

IFX trough levels were found to be significantly associated with clinical remission at all time points. Most importantly, IFX concentrations measured at the end of induction therapy (week 14) were predictive of sustained clinical remission without need for treatment intensification at 54 weeks: this is similar to what was previously reported both in adult and pediatric patients. In adult patients, Cornillie et al (15) identified a trough level $\geq 3.5 \mu\text{g/mL}$ at week 14 as the optimal predictor of durable sustained response to maintenance infliximab 5 mg/kg. As in the present study, IFX levels at week 22 were also significantly associated with sustained therapeutic response. Singh et al (16) demonstrated in pediatric patients that week 54 persistent remission was significantly associated with week 14 IFX concentration: in particular, a value of $4 \mu\text{g/mL}$ or above was predictive of sustained response. The cutpoint identified in our study was slightly lower, even if concordant with those reported by other authors (17,18). IFX concentration at the IV infusion was also predictive of response at this same infusion and at 22 weeks: this may be directly reflective of disease status at the time of sample collection. Indeed, at the IV infusion, laboratory parameters associated with disease activity, in particular CRP, calprotectin, and albumin were also significantly different between patients with IFX concentration below or above the long-term efficacy-predicting threshold. CRP and calprotectin at the end of induction were also predictors of sustained IFX efficacy at week 54; however, multivariate analysis indicated that IFX concentration threshold was the best predictor, suggesting a more direct causal role for IFX serum concentrations on treatment failure. Interestingly, a strong correlation was found also between IFX trough levels and biochemical variables (CRP, calprotectin, and albumin) during maintenance (ie, when inflammatory markers tend to reflect response to treatment).

TABLE 2. Therapeutic response to infliximab and pharmacokinetics according to week of therapy

Therapeutic response	n	Infliximab concentrations, $\mu\text{g/mL}$			
		6 weeks	14 weeks	22 weeks	54 weeks
Primary non response	9	9.1 [6.2–9.9]; 9	0.4 [0–1.2]; 6	0.2 [0–0.6]; 4	–
Partial response	7	5.2 [4.1–8.4]; 4	2.0 [0.7–3.6]; 6	1.2 [0.9–3.4]; 5	1.8 [1.3–2.3]; 2
Loss of response during maintenance	3	7.9 [7.8–9.9]; 3	2.8 [2.3–4.1]; 3	1.4 [1.1–3.3]; 3	0.9 [0.5–1.2]; 2
Sustained response	23	10.4 [9.1–14.4]; 17	6.1 [3.9–9.7]; 18	5.2 [2.9–9.0]; 18	3.8 [2.7–6.0]; 16
Reactions	7	4.5 [0.1–7.1]; 7	4.7 [4.2–5.3]; 2	3.3 [1.6–3.4]; 3	0.005 [0–0.007]; 2

Median, 1st and 3rd quartile of infliximab (IFX) concentrations and number of patients according to therapeutic response and week of therapy. n = total number of patients according to therapeutic response.

A relevant result of the present study was the identification of the causes of treatment failure in a population of pediatric IBD patients treated with IFX. It has been suggested that causes of treatment failure in patients treated with anti-TNF biologic agents can be categorized in 3 groups: mechanistic failure, non immune-mediated pharmacokinetic failure and immune-mediated pharmacokinetic failure (6,19). Treatment failure in our cohort was mainly associated with inadequate drug levels or adverse events. In fact, among patients failing to reach clinical remission at the end of induction, only 2 patients had IFX levels $>3 \mu\text{g/mL}$ (Fig. 1B). At 54 weeks, IFX levels measured at IV, V, and IX infusion were $<3 \mu\text{g/mL}$ in most patients not achieving clinical remission, with only few patients maintaining higher IFX levels (Fig. 3). It seems possible to conclude that, among children with IBD treated with IFX, inadequate blood drug levels represent the main cause of treatment failure, along with the occurrence of anaphylactoid reactions, while the occurrence of true so-called mechanistic (pharmacodynamic) failure seems to represent a rare event. The low incidence of mechanistic failure in our cohort may have several explanations. Younger age is associated with less risk of irreversible bowel damage, which may be associated with higher risk of treatment failure (7,20). Furthermore, longer disease duration has been associated to a Th17 pathway-dominated cytokine profile with low levels of TNF, which may impair the efficacy of TNF blockade (21). This eventuality may also be less relevant in children, thus leaving pharmacokinetic failure as the main determinant of treatment inefficacy.

In our study, 20% of patients developed positivity to AIA, and among these 40% developed reactions to IFX, compared to 8% of patients negative to AIA. Incidence of sustained IFX response was, however, not statistically different among patients developing or not AIA in this cohort. Notably, however, we observed a trend towards lower treatment efficacy in patients with very high AIA levels (ie, $>100 \mu\text{g/mL}$).

Our results underscore the importance of therapeutic drug monitoring to guide treatment in pediatric patients with IBD, especially in those who are failing treatment, since therapy intensification has been demonstrated to be able to recapture a part of these patients, especially when no high-level AIAs are detected (22). Greater availability of anti-TNF blood levels laboratory quantification in clinical practice may improve care. Furthermore, point-of-care assays may allow clinicians to immediately adjust or change treatment based on pharmacokinetic results in addition to clinical variables.

Our study had several limits. Not all samples were available for patients at all considered time points. This may have influenced the prospective evaluation of single patients levels of IFX and AIA levels. No endoscopic assessment was available during the study since most patients did not have the clinical indication to undergo endoscopic assessment. Furthermore laboratory data were incomplete, especially for fecal calprotectin levels. AIA were measured only when IFX was low. This may have influenced the possibility of finding a significant association with clinical outcomes, which therefore is not affected by the current results, and should be better evaluated. Measuring AIA by means of assays not affected by the presence of IFX may allow better characterization of the immune anti-IFX response. Moreover, considering the potential difference according to disease subtype, the small number in each category makes it difficult to stratify the optimal cutoff according to disease subtype.

The strong role of IFX concentration at the end of induction therapy as a predictor of sustained efficacy may suggest that monitoring IFX concentration at an earlier stage and adopting a proactive treatment strategy, for example, adding an immune modulatory drug or increasing drug levels in advance, may result

in better long-term success rates. It should be also noted, however, that a minority of patients with sustained remission also had low drug levels, thus possibly implying that intensifying therapy in patients already in remission only because of low drug serum levels may not be always necessary, and that further personalization of therapy may be possible, possibly through identification of other predictors of treatment failure (23,24). Furthermore, prospective studies are needed to determine what is the most effective strategy to guide therapy in pediatric patients treated with IFX (25).

REFERENCES

- Hyams J, Crandall W, Kugathasan S, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. *Gastroenterology* 2007;132:863–73.
- Hyams J, Damaraju L, Blank M, et al. Induction and maintenance therapy with infliximab for children with moderate to severe ulcerative colitis. *Clin Gastroenterol Hepatol* 2012;10:391.e1–9.e1.
- Ben-Horin S, Chowers Y. Review article: loss of response to anti-tnf treatments in Crohn's disease. *Aliment Pharmacol Ther* 2011;33: 987–95.
- Billioud V, Sandborn WJ, Peyrin-Biroulet L. Loss of response and need for adalimumab dose intensification in crohn's disease: a systematic review. *Am J Gastroenterol* 2011;106:674–84.
- Gisbert JP, Panés J. Loss of response and requirement of infliximab dose intensification in crohn's disease: a review. *Am J Gastroenterol* 2009;104:760–7.
- Vande Casteele N, Herfarth H, Katz J, et al. American gastroenterological association institute technical review on the role of therapeutic drug monitoring in the management of inflammatory bowel diseases. *Gastroenterology* 2017;153:835.e6–57.e6.
- Yarur AJ, Jain A, Sussman DA, et al. The association of tissue anti-TNF drug levels with serological and endoscopic disease activity in inflammatory bowel disease: the Atlas study. *Gut* 2016;65:249–55.
- Joose ME, Samsom JN, van der Woude CJ, et al. The role of therapeutic drug monitoring of anti-tumor necrosis factor alpha agents in children and adolescents with inflammatory bowel disease. *Inflamm Bowel Dis* 2015;21:2214–21.
- Hoekman DR, Brandse JF, de Meij TG, et al. The association of infliximab trough levels with disease activity in pediatric inflammatory bowel disease. *Scand J Gastroenterol* 2015;50:1110–7.
- Ruemmele FM, Veres G, Kolho KL, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis* 2014;8:1179–207.
- Hyams JS, Ferry GD, Mandel FS, et al. Development and validation of a pediatric Crohn's disease activity index. *J Pediatr Gastroenterol Nutr* 1991;12:439–47.
- Turner D, Otley AR, Mack D, et al. Development, validation, and evaluation of a pediatric ulcerative colitis activity index: a prospective multicenter study. *Gastroenterology* 2007;133:423–32.
- Turner D, Griffiths AM, Walters TD, et al. Appraisal of the pediatric crohn's disease activity index on four prospectively collected datasets: recommended cutoff values and clinimetric properties. *Am J Gastroenterol* 2010;105:2085–92.
- 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;17:1314–21.
- Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of c-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the accent I trial. *Gut* 2014;63:1721–7.
- Singh N, Rosenthal CJ, Melmed GY, et al. Early infliximab trough levels are associated with persistent remission in pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2014;20:1708–13.
- 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;7:736–43.

18. Vande Castele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology* 2015;148:1320.e3–9e.
19. Ding NS, Hart A, De Cruz P. Systematic review: predicting and optimising response to anti-TNF therapy in Crohn's disease: algorithm for practical management. *Aliment Pharmacol Ther* 2016;43:30–51.
20. Moran GW, Dubeau MF, Kaplan GG, et al. Phenotypic features of Crohn's disease associated with failure of medical treatment. *Clin Gastroenterol Hepatol* 2014;12:434.e1–42.e1.
21. Zorzi F, Monteleone I, Sarra M, et al. Distinct profiles of effector cytokines mark the different phases of Crohn's disease. *PLoS One* 2013;8:e54562.
22. Steenholdt C, Bendtzen K, Brynskov J, et al. Changes in serum trough levels of infliximab during treatment intensification but not in anti-infliximab antibody detection are associated with clinical outcomes after therapeutic failure in Crohn's disease. *J Crohns Colitis* 2015;9:238–45.
23. Lega S, Bramuzzo M, Dubinsky M. Therapeutic drug monitoring in pediatric IBD: current application and future perspectives. *Curr Med Chem* 2018;25:2840–54.
24. Naviglio S, Giuffrida P, Stocco G, et al. How to predict response to anti-tumour necrosis factor agents in inflammatory bowel disease. *Expert Rev Gastroenterol Hepatol* 2018;12:1–14.
25. Ramsey LB, Mizuno T, Vinks AA, et al. Learning health systems as facilitators of precision medicine. *Clin Pharmacol Ther* 2017;101:359–67.