Infliximab Trough Levels at Induction to Predict Treatment Failure During Maintenance

Claire Liefferinckx, MD, Charlotte Minsart, MSc,* Jean-François Toubeau, MSc,† Anneline Cremer, MD,*,‡ Leila Amininejad, MD,*,‡ Eric Quertinmont,* Jacques Devière, MD,*,‡ Ann Gils, PhD,§ André van Gossum, MD, PhD,‡ and Denis Franchimont, MD, PhD*,‡*

Background: Infliximab (IFX) is indicated for the treatment of inflammatory bowel diseases (IBD). Nevertheless, loss of response (LOR) to IFX is reported in up to 10% to 30% of patients within the first year of treatment. Our objective was to evaluate the impact of the pharmacokinetics of IFX at induction on treatment failure.

Methods: This is a longitudinal cohort study on 269 patients with IBD treated with IFX in a single center. A total of 2331 blood samples were prospectively collected from 2007 until March 2015 with a retrospective analysis of clinical data. IFX trough levels (TLs) were measured by enzyme-linked immunosorbent assay. Antibodies to IFX were measured by drug-sensitive bridging assay.

Results: During follow-up, patients were defined according to treatment outcome. At week 6, median IFX TL in patients requiring a switch to another treatment due to LOR (*LOR switched group*) (2.32 μg/mL [0.12–19.93 μg/mL]) was lower than in patients with long-term response (*long-term responders*) (8.66 μg/mL [0.12–12.09 μg/mL], $P = 0.007$) and in patients responding to optimization (*LOR optimized group*) (7.28 μg/mL [0.17–14.91 μg/mL], $P = 0.021$). At week 2, median IFX TL was lower in the *LOR switched group* (5.7 μg/mL [0.15–12.09 μg/mL]) compared with the *long-term responders* (11.92 μg/mL [0.14–19.93 μg/mL], $P = 0.041$) but no significant difference was reached with the *LOR optimized group* (11.91 μg/mL [0.23–12.09 μg/mL], $P = 0.065$). In the *LOR switched group*, median IFX TL at induction (weeks 2 and 6) was significantly lower when patients had been previously exposed to anti–tumor necrosis factor compared with naive patients (0.91 μg/mL [0.12–4.4 μg/mL] versus 6.6 μg/mL [0.15–19.93 μg/mL], $P = 0.044$).

Conclusions: This study suggests that patients who do not respond to any optimization strategy have lower IFX TLs during induction at week 6. IFX TLs measured early on at induction might predict treatment failure to IFX during maintenance.

(Inflamm Bowel Dis 2017;23:1371–1381)

Key Words: infliximab, pharmacokinetics, inflammatory bowel disease

Infliximab (IFX), a chimeric monoclonal immunoglobulin G1 (IgG1) targeting tumor necrosis factor (TNF), has dramatically improved the therapeutic management of Crohn's disease (CD) and ulcerative colitis (UC). Despite the efficacy of IFX in inducing and maintaining remission in CD and UC,[1,2] loss of response (LOR) represents a therapeutic challenge for many patients. LOR, also defined as secondary nonresponse, refers to patients who have initially responded to treatment at induction but have subsequently experienced a flare-up of the disease during treatment maintenance. Its estimated rate varies from 10% to 30% over 12 months of treatment,[3,4] although there is no consensus on the accurate rate.[5]

Immunogenicity, that is the generation of antibodies to IFX (ATIs), induced by the chimeric monoclonal IgG, seems to enhance drug clearance[6] and may partially explain LOR.[7–9] Indeed, the amount of circulating ATIs is correlated with lower IFX trough levels (TLs) as well as lower duration of response.[6,8] Consequently, to optimize IFX treatment, several studies were conducted to decipher the pharmacokinetics of IFX during maintenance, with focus on TLs,[10,11] ATI measurements,[12] or impact of immunosuppressants (IMM).[13,14] An interval of 3 to 7 μg/mL IFX TL was shown to be correlated with sustained clinical response or remission.[3,15–17] A therapeutic decision algorithm based on IFX TLs, referred as therapeutic concentration monitoring (TCM), was proposed in patients with inflammatory bowel diseases with LOR and active disease.[3,15–18] On the one hand, patients with active

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

Received for publication November 15, 2016; Accepted March 2, 2017.

From the *Laboratory of Experimental Gastroenterology, Université libre de Bruxelles, Brussels, Belgium; †Electrical Power Engineering Unit, Université de Mons, Mons, Belgium; ‡Department of Gastroenterology, Erasme Hospital, Brussels, Belgium; and §Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Belgium.

C. Liefferinckx is supported by FNRS (Belgian National Fund of Scientific Research) and D. Franchimont is research director of FNRS.

The authors have no conflict of interest to disclose.

Address correspondence to: Denis Franchimont, MD, PhD, Department of Gastroenterology, Erasme Hospital and Laboratory of Experimental Gastroenterology, Université Libre de Bruxelles, Route de Lennik 808, 1070 Bruxelles, Belgium (e-mail: Denis.franchimont@erasme.ulb.ac.be).

DOI 10.1097/MIB.0000000000001120

Published online 11 May 2017.

disease with low IFX TLs should be optimized during maintenance either by increasing the dose or shortening the interval in case of low ATI levels but might need to be switched in case of high ATI levels. On the other hand, patients with active disease and optimal range of IFX TLs (3–7 μg/mL) should be considered for switching to another treatment.[18] Nevertheless, this approach is in essence *reactive*. Inversely, TCM was then implemented following a *proactive* approach in which IFX TLs are measured upfront to titrate the next IFX infusion dose.[19] In line with this proactive approach, randomized controlled trials such as the TAXIT or TAILORIX trials have been conducted. Patients were randomly assigned to proactive dose optimization based on TCM or standard of care, namely optimization of IFX based on clinical criteria, serum C-reactive protein (CRP) levels, and endoscopy findings. The TAXIT trial,[20] a Leuven single center trial, suggested a better clinical outcome in CD (but not in UC) patients when TCM was proactively targeted within the optimal interval of 3 to 7 μg/mL IFX TL. However, the TAXIT trial did not achieve its primary endpoint, defined as a significant difference in the proportion of patients in clinical and biological remission at 1 year after optimization between the 2 arms. Nonetheless, proactive TCM was associated with lower drug costs. The TAILORIX trial, a multinational and multicenter trial,[21] had a slightly different design and only enrolled patients with CD. Similar to the TAXIT trial, the primary outcome, that was sustained steroid-free clinical remission from week 22 to week 54, was not reached suggesting that proactive TCM was not superior to standard of care for IFX optimization during treatment maintenance.

Currently, all strategies to optimize IFX treatment in patients with LOR have considered only the maintenance phase of the treatment. By contrast, very few data are available on IFX TLs at induction and their impact on long-term response in maintenance. Recently, week 14 TLs and ATIs were shown to predict success to IFX therapy when reinitiating IFX in patients previously exposed to IFX.[22] This retrospective study suggests that TL measurement before the first maintenance dosing (week 14) may indeed help in predicting long-term response in patients previously treated with IFX. The aim of our study was to look at the IFX pharmacokinetics at induction (weeks 2 and 6) and evaluate its impact on treatment failure during maintenance.

PATIENTS AND METHODS

Study Design

This study was conducted in a single center, at Erasme Hospital, Brussels, Belgium. The samples were collected prospectively from 2007 to March 2015 with a retrospective analysis of clinical data. It should be noted that the treating physicians were not aware of IFX TL and ATI measurements at the time of treatment optimization that was therefore based on clinical criteria only (i.e., standard of care). All the samples were prospectively collected blindly as all the samples were only measured on March 2015. Thus, the measurements data could only be interpreted after the retrospective analysis of clinical data.

Study Population

A total of 269 patients received IFX treatment. Thirty-two patients were excluded from analyses for the following reasons: 1 patient died and 31 patients were lost during follow-up. Seven patients were switched toward another biotherapy due to side effects (severe infusion reactions [n = 2], vasculitis [n = 1], severe psoriatiform cutaneous reaction [n = 1], lupus [n = 1], or others [n = 2]). Finally, 20 patients were primary nonresponders. In this longitudinal retrospective follow-up, 3 outcomes were characterized and analyzed: the *LOR switched group* included 28 patients who required switch to another treatment because of LOR. The *LOR optimized group* included 60 patients who experienced LOR but responded to IFX optimization (by shortening the interval or increasing the dose). The *long-term responders group* included 64 patients who were treated with IFX without requiring optimization. Supplementary Table 1, Supplemental Digital Content 1, http://links.lww.com/IBD/B494 shows the criteria defining the different groups. A fourth group, the *Stop group*, was also analyzed, composed of 58 patients who stopped treatment because of deep remission, defined by no symptoms, normal CRP level, and partial or complete mucosal healing at endoscopy. Finally, 207 patients were considered in the different groups for IFX TL analyses.

Data Collection

Clinical information was retrospectively collected from the hospital electronic patient chart that allows chronological listing of all events. In addition to simple demographic data, the following data were collected in details: dates of first induction and end/abortion of IFX treatment, dates of the 3 induction regimen infusions, infusion dates during maintenance, dates of changes in the interval of administration, dates of dose optimization, use of concomitant IMM, dates of any changes in escalation or deescalation of IMM (starting or stopping IMM), type I immediate hypersensitivity, delayed type III hypersensitivity, reasons for stopping IFX (secondary nonresponder, pregnancy, loss of follow-up, need for surgery, deep remission, the occurrence of adverse events [e.g., infections] or switch to other biologics).

Active disease was defined on clinical, biological, and endoscopic criteria. Disease activity was evaluated retrospectively by recollection of clinical criteria from the electronic patient chart. Biological activity was assessed when available using serum CRP levels. A CRP below 10 mg/L was classified as biological response in patients with elevated CRP at baseline. Endoscopic activity was assessed when available within the month before IFX infusion. In this retrospective study, dates of all clinical, biological, and endoscopy data were entered to take into account all disease activity parameters over time in a comprehensive manner. Primary nonresponder was defined

as lack of response to IFX within 10 weeks after the first infusion requiring treatment modification (e.g., steroids, surgery, …). Secondary nonresponder was defined by the need for IFX optimization and/or to switch to other biologics because of active disease (see above). Data collection was stopped on September 30, 2015. The different outcomes described in the flowchart were reported up to this date.

Blood Samples

Blood samples were prospectively collected and stored in the Biobank of the Laboratory of Experimental Gastroenterology. This Biobank was approved by Erasme Hospital Ethics Committee and each patient has signed an informed consent (EC number B2011/005).

We analyzed 2331 samples issued from 269 patients with inflammatory bowel diseases treated with IFX.

All patients underwent routine 14 mL blood sample collection at the infusion unit before each new infusion, defined as IFX trough level (IFX TL). These samples were centrifuged and the plasma was divided into 1000 μL aliquots and stored at −20°C.

Laboratory Methods

All plasma samples were analyzed for IFX TL using an enzyme-linked immunosorbent assay. This kit (ApDia, Turnhout, Belgium) was based on microtiter strips coated with TNF-α and an enzyme horseradish peroxidase–conjugated monoclonal antibody (clone 6B7) recognizing IFX specifically. All samples were tested by a standard dilution of 1/100. A calibration curve was obtained by plotting the absorbance values versus the corresponding calibrator values and the concentration of IFX was determined by interpolation from the calibration curve. The sample processing was automatized by DS2 (Dynex Technologies, Chantilly, Virginia). IFX TLs are expressed as micrograms per milliliter (μg/mL).

From the 2331 samples, 42% (72/172), 23% (134/585), and 24% (255/1044) were measured below 1 μg/mL in the *LOR switched*, *long-term responders* and *LOR optimized groups*, respectively. Ninety-two samples were therefore analyzed for IFX ATI using drug-sensitive bridging enzyme-linked immunosorbent assay. This assay can only detect ATI when IFX concentration is below quantification limit. IFX was used as coating and tagging antibody and an anti-IFX antibody (MA-IFX-10F9) was used as standard. All samples were tested by a standard dilution of 1/25. Samples with a titer above 100 were subsequently tested using a 1/200 dilution and samples with a titer above 1000 were subsequently diluted 1/1600. For patients not requiring optimization, the first sample with TL below quantification limit was chosen. For patients requiring optimization, the sample just before optimization was selected if TL was below quantification limit. For patients requiring switch because of LOR, any sample with TL below quantification limit at induction (week 2 or 6) was selected. ATI are expressed as nanograms per milliliter (ng/mL) MA-IFX10F9 equivalents. ATI were reported as negative when the concentration was less than 2.5 ng/mL.[23]

Statistical Analysis

To compare the different outcomes, Kolmogorov–Smirnov test was used. This nonparametric test was indeed privileged after having identified the nonnormality of distribution, thanks to χ^2 goodness-of-fit test. Kruskal-Wallis test was used when more than 2 outcomes were compared. Results were therefore expressed as median with confidence interval at 95%. χ^2 test was used to compare categorical variables. Significant difference between outcomes was set for $P < 0.05$. All data were gathered in a central database using Excel (Microsoft, Redmond, WA) and analyzed using SPSS 23.

RESULTS

Study Population

Baseline demographics and detailed characteristics of the different outcomes are summarized in Table 1. No difference was observed between the groups excepted for CD/UC ratio, disease location according to Montreal classification for CD, and surgery. The follow-up is discussed below.

Patients were treated with IFX alone defined as monotherapy in 40.9% (n = 110/269) and cotreated with IFX and immunosuppressant (IMM) defined as combotherapy in 31.6% (n = 85/269). About 27.5% were sequentially treated with monotherapy and combotherapy (n = 74/269) with the subsequent repartition: 24 patients treated first by monotherapy and then combotherapy, 31 patients treated by combotherapy and then monotherapy and 19 patients had been treated with both sequences (mono → combo → mono or combo → mono → combo).

The chart review was stopped in September 2015. A flowchart of the study population is presented in Figure 1. Thirty-nine patients were excluded from analyses (see in Patients and Methods). To analyze the IFX pharmacokinetics, the study population was divided into 3 groups according to treatment outcome during maintenance as described in Patients and Methods.

During maintenance, 32.7% of the patients (n = 88/269) experienced LOR corresponding to secondary nonresponders, requiring treatment optimization by either shortening the interval of administration and/or by increasing the dose.

1. 31.8% of these patients (n = 28/88) with persistent clinical and/or endoscopic activity and/or elevated CRP, did not respond to any optimization strategy and were therefore switched to another biotherapy (20/28) or underwent surgery (8/28), and were defined as the *LOR switched group*.
2. 68.2% of patients (n= 60/88) responded to optimization, corresponding to secondary responders to optimization and were defined as the *LOR optimized group.* Optimization was based on clinical criteria only in 57.6% and together with elevated CRP and endoscopic activity in 20% and 22.4%, respectively.

The *Stop group* was composed of patients who stopped treatment because of deep remission, as defined by no symptoms,

TABLE 1. Demographic Data

	All Patients (n = 269)	Stop IFX Group (n = 66)	LOR Optimized Group (n = 60)	Long-term Responders Group (n = 64)	LOR Switched Group (n = 28)	P^a
Gender, n (%)						
Females	149 (55.4)	41 (62.1)	35 (58.3)	30 (46.9)	15 (53.6)	
Males	120 (44.6)	25 (37.9)	25 (41.7)	34 (53.1)	13 (46.4)	0.28
Age, yr	42 (16–98)	46 (21–98)	42 (17–75)	39 (16–80)	44 (19–89)	0.45
Disease features						
CD	194 (72.1)	47 (71.2)	48 (80)	50 (78.1)	14 (50)	
UC	75 (27.9)	19 (28.8)	12 (20)	14 (21.9)	14 (50)	**0.02**
Age at diagnosis						
A1 (<17 yr)	42 (15.6)	6 (9.1)	11 (18.3)	10 (15.7)	5 (17.9)	0.25
A2 (17–40 yr)	158 (58.7)	39 (59.1)	31 (51.7)	40 (62.5)	15 (53.6)	
A3 (>40 yr)	53 (19.7)	13 (19.7)	13 (21.7)	13 (20.3)	8 (28.5)	
Unknown	16 (6)	8 (12.1)	5 (8.3)	1 (1.5)	0 (0)	
CD						
Location						
L1	89 (45.9)	13 (27.7)	5 (10.4)	16 (32)	4 (28.6)	
L2	57 (29.4)	15 (31.9)	15 (31.3)	14 (28)	3 (21.4)	**0.02**
L3	46 (23.7)	19 (40.4)	28 (58.3)	18 (36)	7 (50)	
+L4	23	5	3	7	2	
Unknown	2 (1)	0 (0)	0 (0)	2 (4)	0 (0)	
Behavior						
B1	91 (46.9)	21 (44.7)	18	23 (46)	10 (68.7)	
B2	34 (17.5)	10 (21.3)	9	8 (16)	2 (12.5)	0.42
B3	68 (35.1)	16 (34)	21	17 (34)	2 (18.8)	
Unknown	1 (0.5)	0	0	1 (2)	0	
Anoperineal disease	75 (38.7)	19 (40.4)	21 (43.8)	19 (38.8)	3 (21.4)	0.51
UC						
Location						
E1	1 (1.3)	1 (5.3)	0 (0)	0 (0)	0 (0)	
E2	31 (41.3)	9 (47.4)	6 (50)	7 (50)	3 (21.4)	0.51
E3	39 (52)	8 (42.1)	6 (50)	6 (42.9)	10 (71.5)	
Unknown	4 (5.3)	1 (5.3)	0 (0)	1 (7.1)	1 (7.1)	
Current smoker	59 (21.7)	16 (24.2)	12 (20)	15 (23.4)	5 (19.3)	0.83
Concomitant medications						
Monotherapy	110 (40.9)	35 (53)	20 (33.3)	23 (35.9)	12 (45.2)	
Combotherapy	85 (31.6)	19 (28.8)	14 (23.3)	19 (29.7)	10 (35.5)	0.055
Both	74 (27.5)	12 (18.2)	26 (43.3)	22 (34.4)	6 (19.3)	
Previous biotherapy						
No	182 (67.7)	53 (80.3)	36 (60)	50 (78.1)	20 (67.7)	
Yes	87 (32.3)	13 (19.7)	24 (40)	14 (21.9)	8 (32.3)	
IFX	40	11	15	6	7	0.06
Adalimumab	19	5	12	8	3	
Others	28	0	2	2	0	
Follow-up						
Median duration, mo	35 (0–192)	14 (1–67)	52 (6–192)	30 (5–104)	18 (5–70)	$\mathbf{5.57 \times 10^{-5}}$
Need for surgery	58	9 (13.6)	15 (25)	7 (10.9)	9 (29)	**0.034**

[a]*P* value comparing different outcomes.
Bold values are significant.

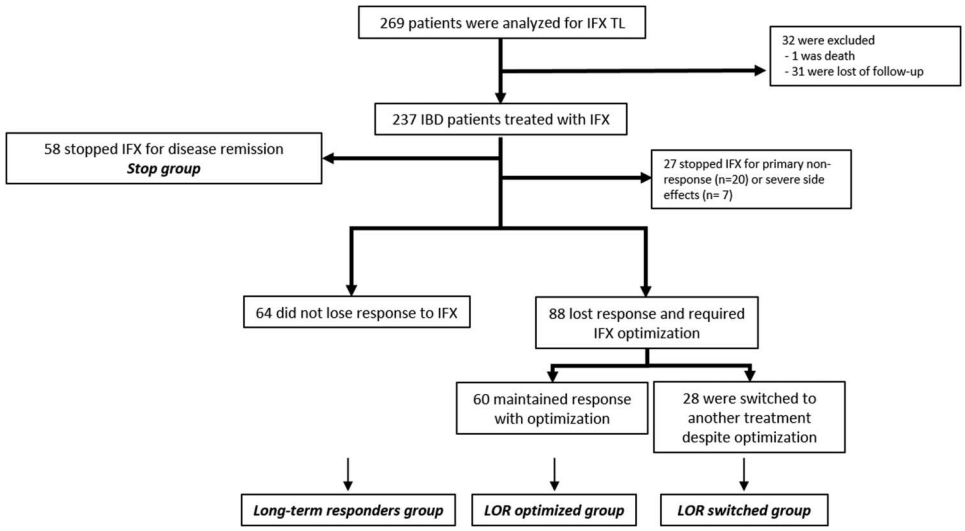

FIGURE 1. Flowchart of the study population. Description of study population according to the different treatment outcomes as defined at the end of the study time period.

normal CRP, and partial or complete mucosal healing. Some of these patients were initially treated with combo and then deescalated to IMM alone (bridge therapy) (n = 26/58) and others were discontinued from both IFX and IMM (30/58). It should be noted that among these 30 patients, at 1 year of follow-up, 14 required a retreatment with a biologics. At the end of the data review on September 2015, the 3 groups had different median follow-ups ($P = 4.46 \times 10^{-9}$). The *LOR optimized group* had the longer median duration of treatment, 52 months (6–192 mo). The *long-term responders group* had a median duration of 30 months (5–104 mo). Finally, the *LOR switched group* had a median duration of 18 months (5–70 mo).

Trough Levels During Maintenance

Trough Levels Are Different Between Monotherapy and Combotherapy During Maintenance

During maintenance, median IFX TL was significantly higher in patients always on combotherapy (n = 85) compared with patients always on monotherapy (n = 110) (2.11 μg/mL [0.1–11.98 μg/mL] versus 1.23 μg/mL [0.09–12.09 μg/mL], $P = 2 \times 10^{-7}$). Figure 2A–D displays IFX TLs in patients treated with the 2 following regimens, monotherapy and combotherapy. Overall, median IFX TLs were significantly higher in patients with combotherapy when patients were directly initiated with combotherapy as described on Figure 2A, C. In contrast, no differences of IFX TLs were observed between the 2 regimens, monotherapy and combotherapy, when patients were first initiated with monotherapy as described on Figure 2B, D.

Trough Levels Are Different Among the 3 Outcome Groups During Maintenance

The different groups had different profiles of median IFX TL during maintenance: the *LOR switched group* had lower median IFX TL (1.43 μg/mL [0.7–11.98 μg/mL]) compared with the *long-term responders* (2.15 μg/mL [0.9–12.1 μg/mL], $P = 3.46 \times 10^{-7}$) and the *LOR optimized group* (2.59 μg/mL [0.84–12.1 μg/mL], $P = 1.6 \times 10^{-5}$). Regardless of the different outcomes, median IFX TL was significantly lower in UC than CD patients (1.8 μg/mL [0.08–12.09 μg/mL] versus 2.15 μg/mL [0.08–12.09 μg/mL], $P = 0.0003$).

Trough Levels at Induction

There was no significant difference in median IFX TL between long-term responders and patients experiencing LOR regardless of the 2 outcomes, the *LOR switched* or *LOR optimized groups*. Therefore, we considered the analyses at induction separately among the 3 different outcomes.

Trough Levels Are Different Among the 3 Outcome Groups at Induction

Looking at induction (weeks 2 and 6 combined), median IFX TL was not significantly different between the *long-term responders* (11.89 μg/mL [0.12–19.93 μg/mL]) and the *LOR optimized group* (9.85 μg/mL [0.17–14.91 μg/mL]), $P = 0.88$. However, median IFX TL at induction was significantly lower in the *LOR switched group* compared with the 2 other groups (4.4 μg/mL [0.12–19.93 μg/mL], $P = 0.023$) (Fig. 3A). To rule out the influence of UC/CD ratio on these aforementioned findings, TLs were separately analyzed in CD and UC patients without any observed difference: 3.3 μg/mL (0.12–19.93 μg/mL) for CD versus 4.4 μg/mL (0.15–12.09 μg/mL) for UC in the *LOR switched group* ($P = 0.8$), 10.3 μg/mL (3.36–13.97 μg/mL) for CD versus 9.5 μg/mL (0.17–14.91 μg/mL) for UC in the *LOR optimized group* ($P = 0.14$) and 11.8 μg/mL (1.44–11.96 μg/mL) for CD versus 11.9 μg/mL (0.12–19.93 μg/mL) for UC in the *long-term responders group* ($P = 0.7$).

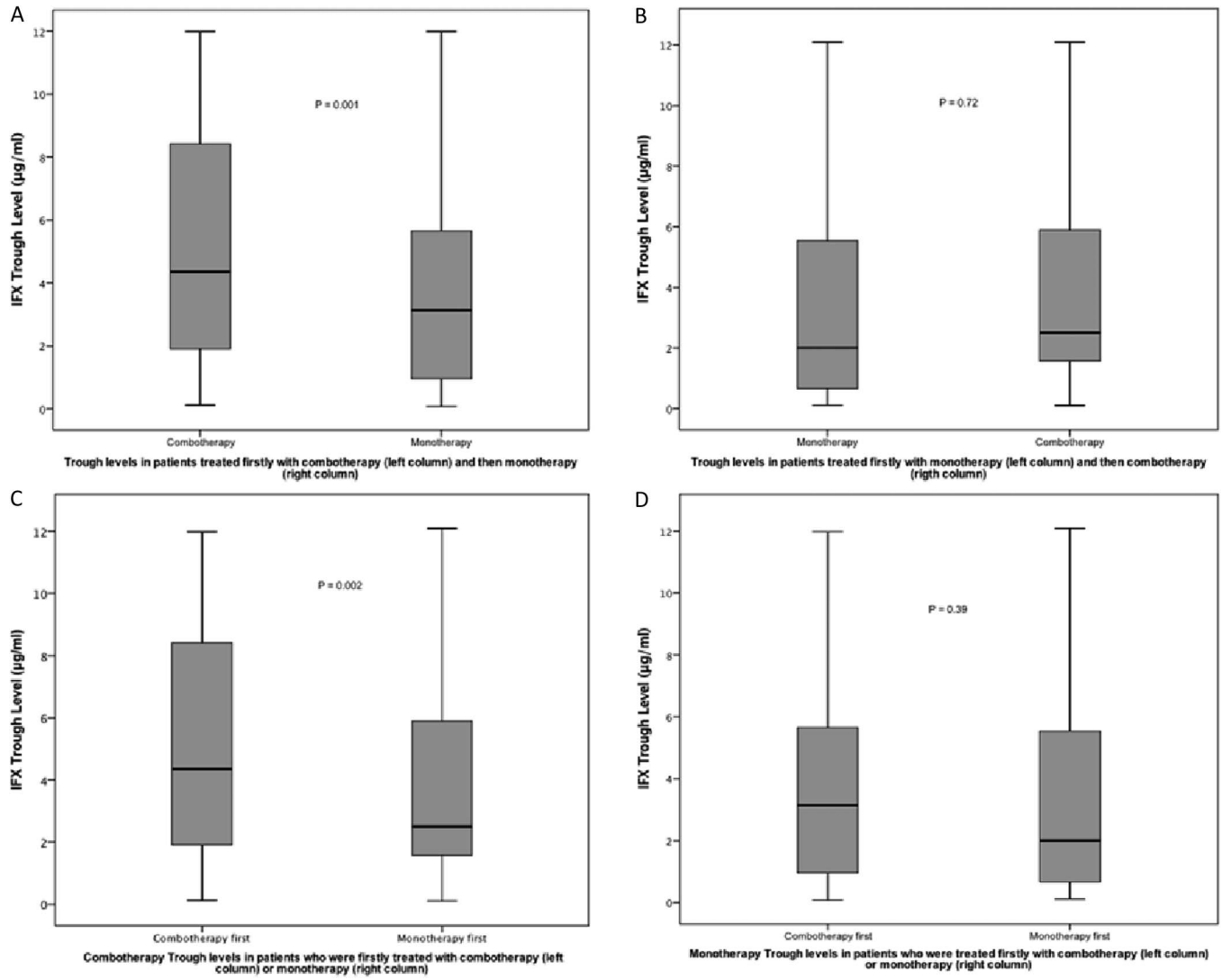

FIGURE 2. A, Median IFX TLs during maintenance in monotherapy and combotherapy patients. Median IFX TLs are significantly different between patients under combotherapy (4.35 μg/mL [0.12–11.98 μg/mL]) and patients under monotherapy (3.14 μg/mL [0.08–11.98 μg/mL]) ($P = 0.001$). B, Median IFX TLs during maintenance in the monotherapy to combotherapy sequence. Median IFX TLs in patients under monotherapy (2 μg/mL [0.11–12.09 μg/mL]) and under combotherapy (2.51 μg/mL [0.11–12.09 μg/mL]) ($P = 0.71$). C, Median IFX TLs under combotherapy in the 2 treatment sequences. Median IFX TLs in patients treated with the sequence "combotherapy first then monotherapy" (4.35 μg/mL [0.12–11.98 μg/mL]) are different than in patients treated with the sequence "monotherapy first then combotherapy" (2.51 μg/mL [0.11–12.09 μg/mL]) ($P = 0.002$). D, Median IFX TLs under monotherapy in the 2 treatment sequences. Median IFX TLs in patients treated with the sequence "monotherapy first then combotherapy" (2 μg/mL [0.11–12.09 μg/mL]) and patients treated with the sequence "combotherapy first then monotherapy" (3.14 μg/mL [0.08–11.98 μg/mL]) ($P = 0.39$).

During the induction phase, the second (week 2) and third infusion (week 6) TLs were separately analyzed to evaluate which time point would be the most indicative and clinically useful as predictive marker of treatment failure. Figure 3B shows median IFX TL of the 3 groups at the third infusion time point (week 6). Median IFX TL at week 6 in the *LOR switched group* (2.32 μg/mL [0.12–19.93 μg/mL]) was lower than in the *long-term responders* (8.66 μg/mL [0.12–12.09 μg/mL], $P = 0.007$) and the *LOR optimized group* (7.28 μg/mL [0.17–14.91 μg/mL], $P = 0.021$). At the second infusion (week 2), median IFX TL was lower in the *LOR switched group* (5.7 μg/mL [0.15–12.09 μg/mL]) compared with the *long-term responders* (11.92 μg/mL [0.14–19.93 μg/mL], $P = 0.041$) but no significant difference was reached with the *LOR optimized group* (11.91 μg/mL [0.23–12.09 μg/mL], $P = 0.065$) (see Supplementary Figure 1, Supplemental Digital Content 2, http://links.lww.com/IBD/B495).

Figure 4 describes the distribution of IFX TLs in each group at induction with an arbitrary subdivision in 4 ranges of IFX TL values: below 3 μg/mL, between 3 and 9 μg/mL, between 9 and 12 μg/mL, and above 12 μg/mL. More than

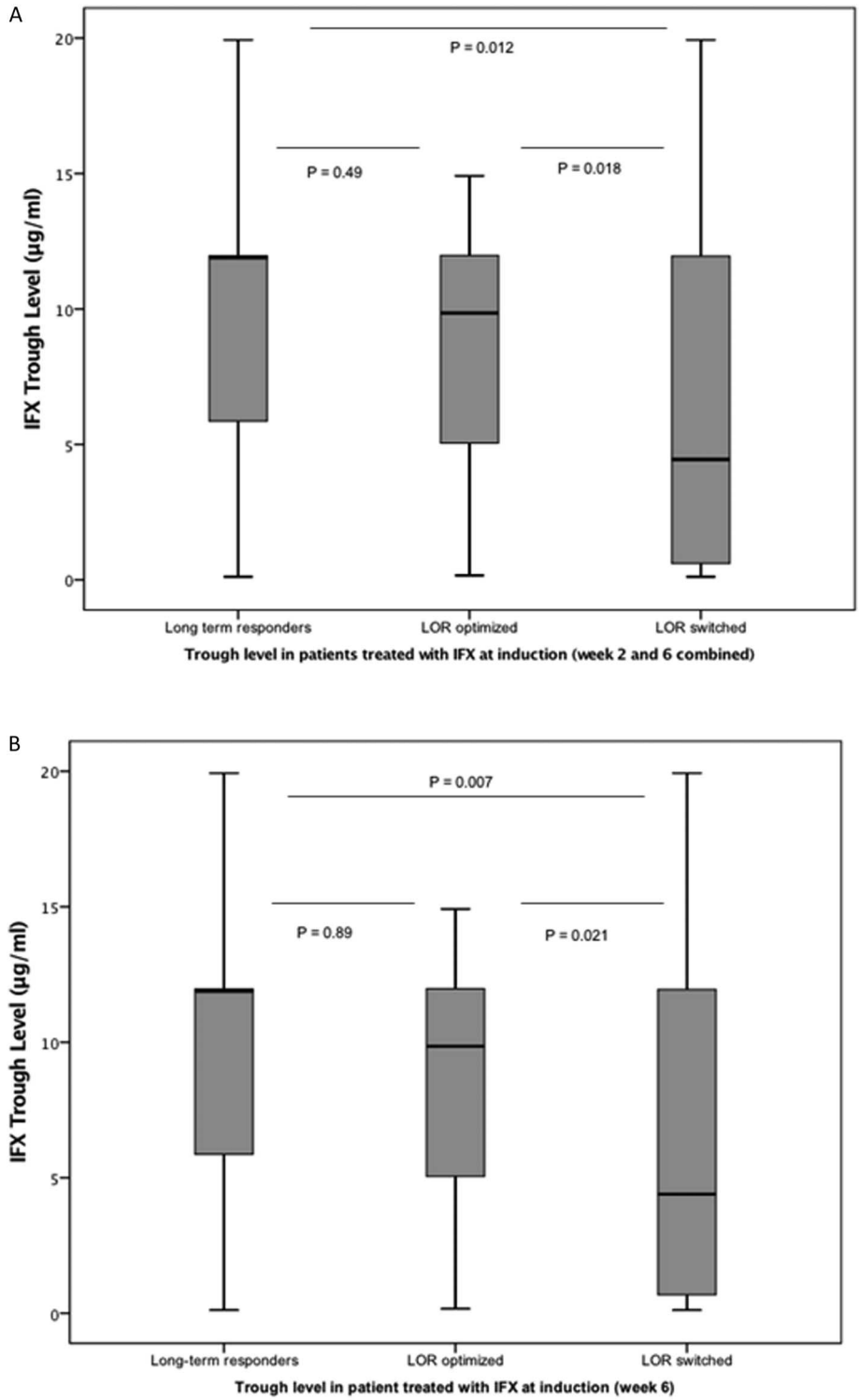

FIGURE 3. A, Median IFX TLs during induction adding weeks 2 and 6. Median IFX TLs are 11.89 μg/mL (0.12–19.93 μg/mL) in *long-term responders*, 9.85 μg/mL (0.17–14.91 μg/mL) in *LOR optimized* and 4.4 μg/mL (0.12–19.93 μg/mL) in the *LOR switched groups*. Median IFX TLs are significantly lower in the LOR switched group than in *long-term responders* ($P = 0.012$) and the *LOR optimized group* ($P = 0.018$). B, Median IFX TLs during induction at week 6. Median IFX TLs are 8.66 μg/mL (0.12–12.09 μg/mL) in *long-term responders*, 7.28 μg/mL (0.17–14.91 μg/mL) in the *LOR optimized* and 2.32 μg/mL (0.12–19.93 μg/mL) in the *LOR switched groups*. Median IFX TLs are significantly lower in LOR switched group than in *long-term responders* ($P = 0.007$) and the *LOR optimized group* ($P = 0.02$).

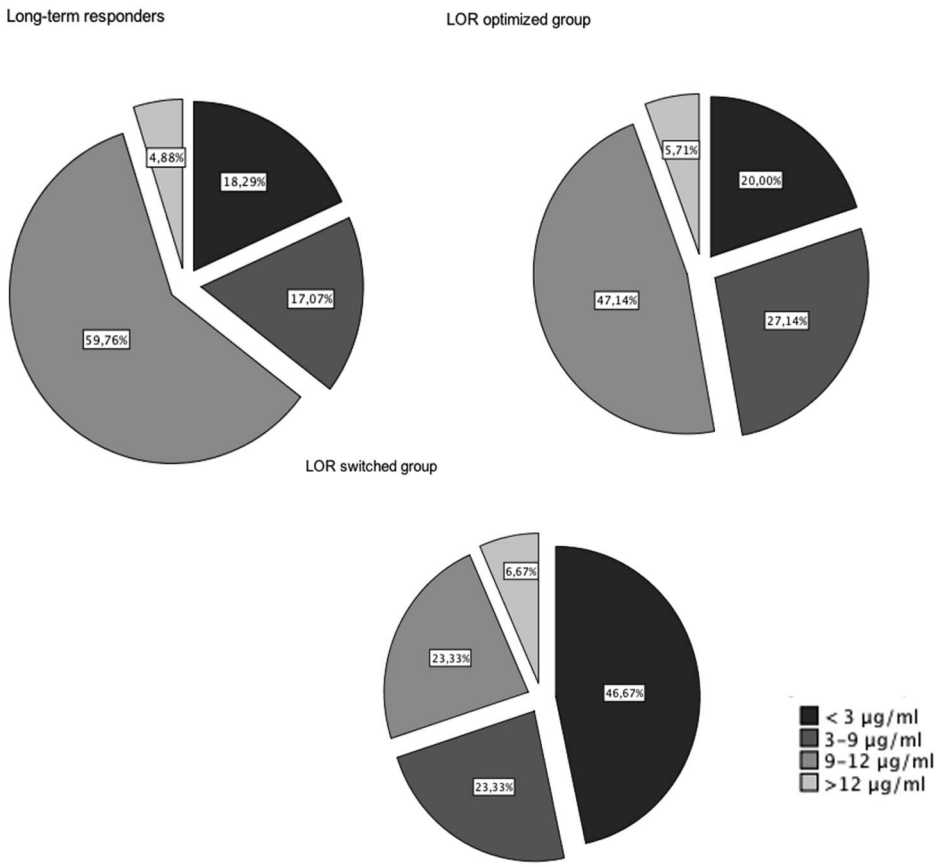

FIGURE 4. Distribution of IFX TLs according to different ranges in induction. Pie distribution of IFX TLs in each outcome during induction with an arbitrary subdivision in 4 ranges of IFX serum values: <3 μg/mL, between 3 and 9 μg/mL, between 9 and 12 μg/mL and >12 μg/mL.

46% of patients had IFX TLs measured below 3 μg/mL in the *LOR switched group*. Likewise, the pie representing the *LOR optimized group* showed a larger proportion of low IFX TLs, below 3 μg/mL and between 3 and 9μg/mL, than in the *long-term responders group.*

Induction Trough Levels Are Different According to Previous Treatment Status

Each group was subdivided according to naive or previous treatment with anti-TNF (IFX or adalimumab) status. In the *LOR switched group*, median IFX TL was significantly lower in previously exposed patients (10/28) than in naive patients (18/28) (0.92 μg/mL [0.12–4.4 μg/mL] versus 6.6 μg/mL [0.15–19.93 μg/mL], P= 0.044) (Fig. 5A). Inversely, there was no significant difference between median TL in the *LOR optimized group* between naive (36/60) or previously exposed patients (24/60) (9.38 μg/mL [0.17–14.91 μg/mL] versus 11.82 μg/mL [0.17–14.91 μg/mL], P = 0.52) as well as in naive (50/64) or previously exposed (14/64) *long-term responders* (9.57 μg/mL [1.44–11.97 μg/mL] versus 11.91 μg/mL [0.12–19.93 μg/mL], P = 0.92).

Subsequently, the 3 groups were separately analyzed based on naive or previously exposed status.

In the naive population, despite a trend for a lower median TL in the *LOR switched group* (6.6 μg/mL [0.15–19.93 μg/mL] compared with the *LOR optimized group* 9.38 μg/mL [0.17–14.91 μg/mL]) and *long-term responders* (9.57 μg/mL [1.44–11.97 μg/mL]), no significant difference was observed between the groups (P = 0.146). Considering the analysis discriminating week 2 and 6 TLs separately, no significant differences were observed between the 3 groups in the naive population (see Supplementary Figure 2A, B, Supplemental Digital Content 2, http://links.lww.com/IBD/B495).

In the previously exposed patients, the *LOR switched group* had a lower median IFX TL (0.92 μg/mL [0.12–4.40 μg/mL]) compared with the *long-term responders* (9.57 μg/mL [0.44–11.97 μg/mL], P = 0.015) and *LOR optimized group* (11.82 μg/mL [0.23–12.09 μg/mL], P = 0.005). Similarly, considering the analysis discriminating weeks 2 and 6 TLs separately, no significant differences were observed between the 3 groups in the previously exposed population (see Supplementary Figure 2C, D, Supplemental Digital Content 2, http://links.lww.com/IBD/B495).

Presence of ATI in the 3 Outcome Groups

A total of 92 samples were analyzed for ATI measurement based on IFX TLs at induction and maintenance. Sample

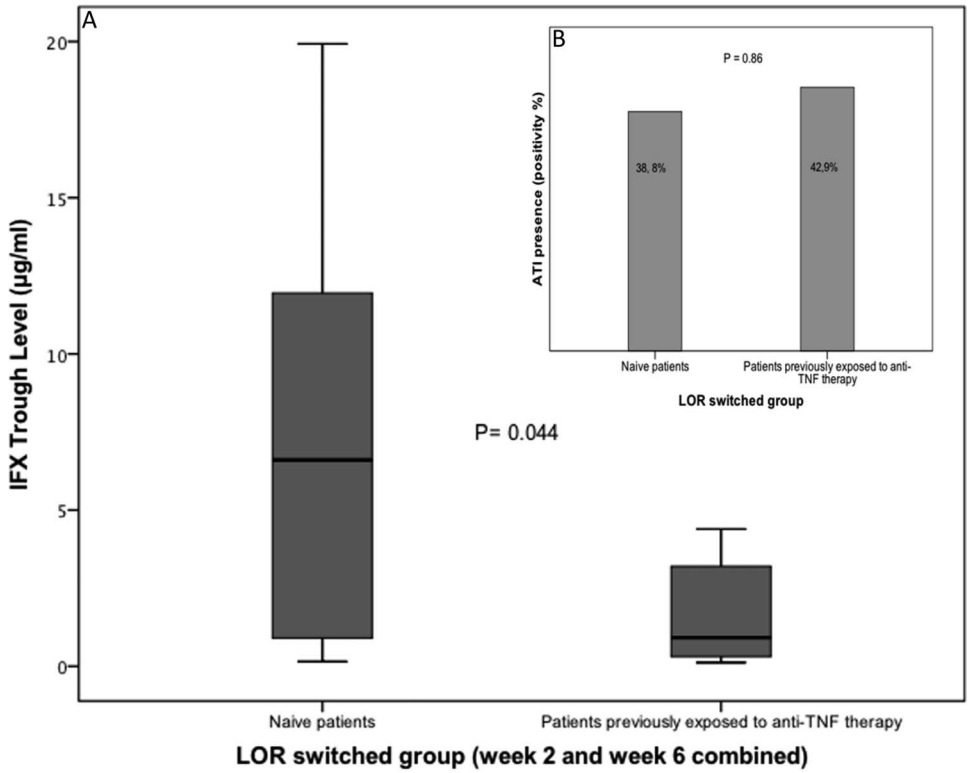

FIGURE 5. A, Median IFX TL in LOR switched group according to naive or previously exposed to anti-TNF status. Median IFX TLs are 0.92 µg/mL (0.12–4.4 µg/mL) in the previously exposed patients (10/28) and 6.6 µg/mL (0.15–19.93 µg/mL) in naive patients (18/28) with a significant difference ($P = 0.044$). B, Presence of ATI (%) according to naive status or previously exposed to anti-TNF. The percentage of ATI occurrence are 38.8% and 42.9% in naive patients and previously exposed to anti-TNF patients, respectively, without significant difference ($P = 0.86$).

selection among the different outcome groups was described in Patients and Methods. A total of 32, 35, and 25 samples were analyzed in the *LOR optimized*, *long-term response*, and *LOR switched groups*, respectively. The percentage of ATI occurrence was significantly lower in the *long-term responders* (5.7% [n = 2/35]) than in the *LOR optimized* (37.5% [n = 12/32], $P = 0.002$) and *LOR switched groups* (40% [n = 10/25], $P = 0.002$). Interestingly, among the *LOR switched group*, the percentage of ATI occurrence was similar in patients whether naive or previously exposed to anti-TNF (38.8%, n = 7/18 versus 42.9%, n = 3/7, $P = 0.86$) (Fig. 5B). The same observation was found in the *LOR optimized group* (25%, n = 3/12 versus 45%, n = 9/20, $P = 0.45$). No comparison was possible within the *long-term responders group* due to the low presence of ATI.

DISCUSSION

The aim of our study was to look at the pharmacokinetics of IFX at induction and evaluate its impact on long-term response. We observed that patients who were switched to another treatment because of LOR during maintenance (*LOR switched group*) had significantly lower median TLs at induction than patients with sustained response with or without optimization. This difference was even more obvious in previously exposed patients to anti-TNF, whether Adalimumab or IFX. Looking carefully at induction, week 6 IFX TLs appeared to be the most indicative and clinically useful as predictive marker of treatment failure. Unsurprisingly, the presence of ATI was correlated with the need of optimization during maintenance. However, the presence of ATI in patients whether naive or previously exposed to anti-TNF was similar in the 2 groups experiencing LOR (*LOR switched* and *LOR optimized groups*), which suggests that lower TLs may not be related only to immunogenicity to IFX.

Despite several studies on IFX pharmacokinetics aspects[3,10–17] or prospective trials evaluating proactive monitoring during treatment maintenance,[20,21] the understanding of LOR remains challenging.[3,4] We aimed at examining IFX TLs early at induction, as it seems to offer an even more predictive approach of LOR than proactive monitoring of IFX TLs during maintenance. Baert et al[22] had already reported that IFX dosage at week 14 could be an earlier predictor of long-term IFX response when reinitiating IFX therapy in patients who had been previously treated with IFX. Remarkably, in our study, TLs at earlier time point at week 6 appeared to be predictive of LOR in patients who required a switch to another treatment. This appeared to be at least true for patients previously exposed to anti-TNFs, not only to IFX but also to adalimumab. Early TLs at induction in naive patients could also be predictive of long-term response during maintenance, but would

need to be confirmed in a larger patient population (due to the limited size of our *LOR switched group* [n = 28]). The difference is small in median IFX TLs of the 3 outcome groups during maintenance, as it represents the median value of all TLs in each of the 3 groups and, therefore only partially highlights the difference in TLs between the groups. Pharmacokinetics measurements during maintenance may not be as indicative as initially expected. On the one hand, TLs measured just before optimization or switch may be, but not always, below the therapeutic dose range. On the other hand, a progressive decrease of TLs occur in all patients during maintenance; even a significant proportion of patients with long-term remission may have eventually low TLs, as previously reported by Buurman et al[11] This further supports that induction may a more discriminative window of opportunity to detect patients who will maintain response or fail to IFX in the long term.

Furthermore, we confirmed some IFX pharmacokinetics characteristics already reported during maintenance. First, TLs during maintenance were higher when patients were treated with combotherapy than monotherapy.[14,24] Also TLs during maintenance were higher when patients were initially treated with combotherapy compared with patients secondarily treated with combotherapy, heralding the importance of starting patients on combotherapy to lessen immunogenicity against IFX.[25] Second, a negative correlation between CRP and TLs was observed as described in other studies[3,26,27] (data not shown).

To evaluate the impact of antibodies to IFX (ATI), antibodies were measured in patients with low TLs at induction or just before optimization during maintenance according to indications in Patients and Methods. The percentage of ATI detection was similar in the different groups as reported in other studies[8,28] except in the *long-term responders* where ATI detection was very low. This low presence of ATI in the *long-term responders* suggests that ATI presence is correlated to the need of optimization, which had been already demonstrated in previous studies.[6] Interestingly, the occurrence of ATI was comparable in both naive patients or previously exposed patients who experienced LOR (*LOR switched and LOR optimized groups*). These observations suggest other mechanisms than immunogenicity, such as nonimmune drug clearance, scavenging process, or active disease depleting anti-TNF drugs,[9,11,18,29–31] to be involved in patients with LOR and low IFX TLs. Our study has a few limitations. Despite prospective collection of blood samples, missing values/data could impact the robustness of this study. However, this study is based on a large cohort, which partially offsets the incomplete blood collection. Finally, the clinical data were analyzed retrospectively, and clinical evaluation and work-up were not strictly performed at the exact same time.

In conclusion, this study suggests that the induction may be a momentum to discriminate patients who will maintain response or fail to IFX during treatment maintenance, especially in patients with previous exposure to anti-TNFs. New prospective studies on IFX pharmacokinetics and on all new biologics should focus on induction to personalize and optimize the dosing early on directly at induction to improve long-term response and remission.

ACKNOWLEDGMENTS

The authors thank Apdia group for their commercial efforts in the purchase of enzyme-linked immunosorbent assay kits.

REFERENCES

1. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet.* 2002;359:1541–1549.
2. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* 2005;353:2462–2476.
3. 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;63:1721–1727.
4. Roblin X, Marotte H, Leclerc M, et al. Combination of C-reactive protein, infliximab trough levels, and stable but not transient antibodies to infliximab are associated with loss of response to infliximab in inflammatory bowel disease. *J Crohns Colitis.* 2015;9:525–531.
5. Roda G, Jharap B, Neeraj N, et al. Loss of response to anti-TNFs: definition, epidemiology, and management. *Clin Transl Gastroenterol.* 2016;7:e135.
6. Rojas JR, Taylor RP, Cunningham MR, et al. Formation, distribution, and elimination of infliximab and anti-infliximab immune complexes in cynomolgus monkeys. *J Pharmacol Exp Ther.* 2005;313:578–585.
7. Vande Casteele N, Gils A. Pharmacokinetics of anti-TNF monoclonal antibodies in inflammatory bowel disease: adding value to current practice. *J Clin Pharmacol.* 2015;55(suppl 3):S39–S50.
8. Nanda KS, Cheifetz AS, Moss AC. Impact of antibodies to infliximab on clinical outcomes and serum infliximab levels in patients with inflammatory bowel disease (IBD): a meta-analysis. *Am J Gastroenterol.* 2013;108:40–47; quiz 8.
9. Ben-Horin S, Kopylov U, Chowers Y. Optimizing anti-TNF treatments in inflammatory bowel disease. *Autoimmun Rev.* 2014;13:24–30.
10. Dotan I, Ron Y, Yanai H, et al. Patient factors that increase infliximab clearance and shorten half-life in inflammatory bowel disease: a population pharmacokinetic study. *Inflamm Bowel Dis.* 2014;20:2247–2259.
11. Buurman DJ, Maurer JM, Keizer RJ, et al. Population pharmacokinetics of infliximab in patients with inflammatory bowel disease: potential implications for dosing in clinical practice. *Aliment Pharmacol Ther.* 2015;42:529–539.
12. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med.* 2003;348:601–608.
13. Lemann M, Mary JY, Duclos B, et al. Infliximab plus azathioprine for steroid-dependent Crohn's disease patients: a randomized placebo-controlled trial. *Gastroenterology.* 2006;130:1054–1061.
14. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med.* 2010;362:1383–1395.
15. Adedokun OJ, Sandborn WJ, Feagan BG, et al. Association between serum concentration of infliximab and efficacy in adult patients with ulcerative colitis. *Gastroenterology.* 2014;147:1296–1307.e5.
16. Bortlik M, Duricova D, Malickova K, et al. Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. *J Crohns Colitis.* 2013;7:736–743.
17. Drobne D, Bossuyt P, Breynaert C, et al. Withdrawal of immunomodulators after co-treatment does not reduce trough level of infliximab in patients with Crohn's disease. *Clin Gastroenterol Hepatol.* 2015;13:514–521.e4.
18. Ordas I, Feagan BG, Sandborn WJ. Therapeutic drug monitoring of tumor necrosis factor antagonists in inflammatory bowel disease. *Clin Gastroenterol Hepatol.* 2012;10:1079–1087; quiz e85–e86.
19. Vaughn BP, Sandborn WJ, Cheifetz AS. Biologic concentration testing in inflammatory bowel disease. *Inflamm Bowel Dis.* 2015;21:1435–1442.
20. Vande Casteele N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology.* 2015;148:1320–1329.e3.

21. D'Haens SV G, Lambrecht G, Baert F, et al. OP029 drug-concentration versus symptom-driven dose adaptation of infliximab in patients with active Crohn's disease: a prospective, randomised, multicentre trial (Tailorix). *J Crohns Colitis.* 2016.
22. Baert F, Drobne D, Gils A, et al. Early trough levels and antibodies to infliximab predict safety and success of reinitiation of infliximab therapy. *Clin Gastroenterol Hepatol.* 2014;12:1474–1481.e2; quiz e91.
23. Van Stappen T, Billiet T, Vande Casteele N, et al. An optimized anti-infliximab bridging enzyme-linked immunosorbent assay for harmonization of anti-infliximab antibody titers in patients with inflammatory bowel diseases. *Inflamm Bowel Dis.* 2015;21:2172–2177.
24. Dulai PS, Siegel CA, Colombel JF, et al. Systematic review: monotherapy with antitumour necrosis factor alpha agents versus combination therapy with an immunosuppressive for IBD. *Gut*. 2014;63: 1843–1853.
25. Van Assche G, Magdelaine-Beuzelin C, D'Haens G, et al. Withdrawal of immunosuppression in Crohn's disease treated with scheduled infliximab maintenance: a randomized trial. *Gastroenterology*. 2008;134: 1861–1868.
26. Levesque BG, Greenberg GR, Zou G, et al. A prospective cohort study to determine the relationship between serum infliximab concentration and efficacy in patients with luminal Crohn's disease. *Aliment Pharmacol Ther.* 2014;39:1126–1135.
27. Hibi T, Sakuraba A, Watanabe M, et al. C-reactive protein is an indicator of serum infliximab level in predicting loss of response in patients with Crohn's disease. *J Gastroenterol.* 2014;49:254–262.
28. Vande Casteele N, Gils A, Singh S, et al. Antibody response to infliximab and its impact on pharmacokinetics can be transient. *Am J Gastroenterol.* 2013;108:962–971.
29. Nguyen DL, Flores S, Sassi K, et al. Optimizing the use of anti-tumor necrosis factor in the management of patients with Crohn's disease. *Ther Adv chronic Dis.* 2015;6:147–154.
30. Vos AC, Wildenberg ME, Duijvestein M, et al. Anti-tumor necrosis factor-alpha antibodies induce regulatory macrophages in an Fc region-dependent manner. *Gastroenterology*. 2011;140:221–230.
31. Atreya R, Zimmer M, Bartsch B, et al. Antibodies against tumor necrosis factor (TNF) induce T-cell apoptosis in patients with inflammatory bowel diseases via TNF receptor 2 and intestinal CD14(+) macrophages. *Gastroenterology*. 2011;141:2026–2038.