Actual Anti-TNF Trough Levels Relate to Serum IL-10 in Drug-Responding Patients With Crohn's Disease

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Background: Patients with Crohn's disease (CD) responding to anti–tumor necrosis factor (anti-TNF) show great variability in serum drug levels, even within the therapeutic range. We aimed at exploring the role of inflammatory, genetic, and bacterial variables in relation to anti-TNF through levels in CD patients.

Methods: Consecutive CD patients receiving stable doses of infliximab or adalimumab were included. Clinical and analytical parameters were recorded. Cytokine response, bacterial DNA translocation, and several immune-related genes' genotypes were evaluated, along with serum through anti-TNF drug levels. A linear regression analysis controlled by weight and drug regimen was performed.

Results: One hundred nineteen patients were initially considered. Five patients on infliximab and 2 on adalimumab showed antidrug antibodies in serum and were excluded. One hundred twelve patients were finally included (62 on infliximab, 50 on adalimumab). Fourteen patients on infliximab and 15 on adalimumab (22.6% vs 30%, $P = 0.37$) were receiving an intensified drug regimen. C-reactive protein (CRP), fecal calprotectin, Crohn's Disease Activity Index, leukocyte count, and albumin levels in plasma were not significantly associated with infliximab or adalimumab levels in the multivariate analysis. Serum interleukin-10 (IL-10) levels were directly related to infliximab (Beta = 0.097, $P < 0.0001$) and adalimumab levels (Beta = 0.069, $P = 0.0241$). The best multivariate regression model explaining the variability of serum infliximab and adalimumab levels included IL-10. Predicted drug levels by this model robustly fitted with actual drug levels ($R^2 = 0.841$ for infliximab, $R^2 = 0.733$ for adalimumab).

Conclusion: Serum IL-10 is significantly related to serum anti-TNF levels in CD patients, showing how the disposition of anti-TNF drugs is significantly influenced by the degree of immunological activation.

Key Words: Crohn's disease, infliximab, adalimumab, interleukin 10, inflammation

INTRODUCTION

Anti–tumor necrosis factor (anti-TNF) therapy has been a major advance of the last 2 decades in the treatment of patients with Crohn's disease (CD). Anti-TNF induces and maintains remission in patients with moderate to severe luminal or fistulizing Crohn's disease that is refractory to conventional immunosuppressive therapy.[1–3]

Along with the use of this drug, safety and efficacy data from a vast number of patients have been recorded. From this experience, safety issues have emerged related to the increased risk of infections or cancer in some cohorts.[4–6] In terms of efficacy, therapy with anti-TNF is useful in approximately two-thirds of CD patients, whereas 13%–40% of patients show primary loss of response and 10%–20% show secondary loss of response.[7–10]

The mechanisms underlying loss of response are multifactorial and include disease characteristics (phenotype, location, severity), drug metabolism (pharmacokinetics, pharmacodynamic, immunogenicity), and treatment strategy (dosing regimen).[11–15] These factors, among others, have pushed the need for evaluating serum drug levels in IBD patients[16–18] as an effort toward finding an objective tool useful to guide a safer and more efficient anti-TNF therapy. However, the pharmacokinetics variability observed in many different studies keeps this topic under constant discussion.[19, 20]

Our group has described the frequent presence of bacterial translocation in CD patients, along with the value of bacterial DNA (bactDNA) detection as an independent risk

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factor of flare in the short term.[21] The translocation of bacterial products is more frequent among patients with *NOD2* and *ATG16L1* variant genotypes or in active disease conditions. The inflammatory status around these bacteria antigen translocation-related events includes a significantly increased pro-inflammatory cytokine response, altered phagocytic and bactericidal activities in blood neutrophils, and faster anti-TNF consumption in CD patients on biologic therapy.[22] These results identify a subgroup of patients with a different inflammatory status who are likely to show decreased efficacy of anti-TNF therapy in controlling the risk of flare-up. The aim of this study has been to provide evidence of the extent to which this inflammatory state, along with common genetic variants and bacterial DNA translocation, influences the disposition and plasma concentrations of anti-TNF drugs.

METHODS

Patients

Consecutive patients diagnosed with Crohn's disease and controlled at 3 hospitals in the area of Alicante, Spain, who were receiving stable anti-TNF dosing in the previous 14 weeks were included in this prospective, observational, and multicenter study. The diagnosis of CD was established according to standard clinical, endoscopic, histological, and radiographical criteria.[23] Patients treated with antibiotics in the previous 4 weeks, patients with signs of active infection, and those who refused to sign informed consent to participate in the study were excluded. The Ethics Committee from Hospital General Universitario de Alicante approved the study protocol.

The usual clinical and analytical variables in the management of CD patients, including fecal calprotectin, were recorded in all patients. All patients were Caucasian of Mediterranean ethnicity and were classified according to the Montreal classification.[24] All included patients received diaries to record symptoms 1 week before inclusion and sample collection. Patients were included if they were treated with infliximab or adalimumab at stable doses at least for 3 months of 5 mg/kg 8-weekly or 40 mg every other week, respectively. Also, patients with anti-TNF intensified therapy, defined either by increased dose or increase in the frequency of infusions vs dosing or schedule, upon start of treatment were included (infliximab 5 mg/kg 6-weekly or 10 mg/kg 8-weekly; adalimumab 40 mg each week or every 10 days). Optimization in these patients was performed as reactive therapeutic drug monitoring after treatment failure.

At inclusion, blood samples taken just before anti-TNF infusions were used for haematological and biochemical studies and for infliximab and adalimumab through serum level determination. A part of serum samples was inoculated in aerobic and anaerobic blood culture bottles, 10 mL each. Simultaneously, 2 separate blood samples were inoculated under aseptic conditions in rubber-sealed sterile Vacutainer SST II and K3E tubes, respectively (BD Diagnostics, Erembodegem, Belgium), that were never exposed to free air.

Serum Cytokine and Free Anti-TNF-α Levels; Presence of Antidrug Antibodies

Serum levels of TNF-α, interferon-γ, and interleukin (IL)-12p40, as mediators of immunogenic dendritic cell (DC) activation, and the subsequent proinflammatory adaptive response, IL-10, as responsible for tolerogenic DC activation and the counterbalancing anti-inflammatory response, and IL-26, which binds to bacterial DNA and participates in bacterial antigen recognition and processing, were determined by enzyme-linked immunosorbent assays (ELISAs) using Human Quantikine kits from R&D Sytems (Minneapolis, MN, USA). ELISAs were also carried out to measure free infliximab and adalimumab levels and to detect antidrug antibodies (Matriks Biotek, Ankara, Turkey) according to the manufacturer's instructions. All samples were tested in triplicate and read in a Sunrise Microplate Reader (Tecan, Männedorf, Switzerland). The detection limit for each cytokine assay varied between 2 and 5 pg/mL and between 0.1 and 0.3 ug/mL in the case of free anti-TNF-α kits. Standard curves were generated for every plate, and the average 0 standard optical densities were subtracted from the rest of the standards and samples to obtain a corrected concentration for all parameters. The presence of antidrug antibodies was evaluated by a cutoff value estimated by multiplying the optical density (OD) of the 0 standard by 3, as indicated by the manufacturers. Samples were considered positive when the ratio sample OD/0 standard OD was higher than 3.

Identification of BactDNA Fragments and NOD2 Genotyping

Genomic DNA was isolated from 5×10^6 cells with the QIAmp DNA Blood Minikit (Qiagen, Hilden, Germany). BactDNA was identified by running a broad-range polymerase chain reaction (PCR) with 5'-AGAGTTTGATCATGGCTCAG-3' as forward and 5'-ACCGCGACTGCTGCTGGCAC-3' as reverse universal eubacterial primers of a conserved region of 16SrRNA gene, followed by partial nucleotide sequencing. Full methodology descriptions including specificity and sensitivity are described elsewhere.[21] The threshold for bactDNA detection was 10 pg, and patients above this limit were considered bactDNA-positive.

The 3 common NOD2/CARD15 allelic variants at single nucleotide polymorphism–8 (SNP-8; R702W, rs2066844), SNP-12 (G908R, rs2066845), and SNP-13 (L1007finsC, rs2066847), the ATG16L1 variation rs2241880, the IRGM variation rs4958847, the TLR5 variation rs5744168, and the PTPN2 variations rs2542151 and rs1893217 were genotyped by TaqMan technology (Applied Biosystems, Carlsbad, CA, USA) using commercially available TaqMan SNP Genotyping Assays (for

NOD2, ATG16L1, TLR5, and PTPN2) or self-designed primers (IRGM) and TaqMan Genotyping Master Mix on a 7900HT Fast Real-Time PCR System using SDS 2.4 Software (Applied Biosystems), as previously described.[22] A variant genotype was defined as carrying any of the variations either in homozygosis or heterozygosis. All genotyping results were assessed twice. The evaluators were not aware of either the patient's disease status or each other's genotype results. A Hardy-Weinberg test was performed as a quality control measure in controls. No missing genotypes were present.

Statistical Analysis

Descriptive statistics were provided with means and standard deviations for continuous variables following a normal distribution or medians and interquartile ranges (IQRs) for noncontinuous variables. Categorical variables were described by frequencies and percentages. Comparisons between patient groups (according to drug or induction/maintenance treatment) were carried out using the chi-square test for categorical variables. Differences in quantitative variables were analyzed with a *t* test or a Mann-Whitney *U* test, depending on the normality of the distribution of data. Normality was evaluated with the Shapiro-Wilk test.

A univariate linear regression analysis controlled by weight and regimen (induction/maintenance) was conducted to assess the association of clinical and experimental variables with trough drug levels (Table 3 and 4). Variables achieving statistical significance ($P < 0.05$) were considered in a multivariate linear regression model. The fit of the linear regression models was determined by the coefficient of determination (R^2). The Kolmogorov-Smirnov test, probability–probability plot, and the scatter plot of residuals vs predicted values were performed to check that parametric assumptions of the linear regression model could be assumed.

All tests for significance will be conducted using a 2-sided approach with a 5% significance level. Bonferroni correction was performed for multiple comparisons. All statistical analyses were performed using R software (R Core Team, Vienna, Austria; https://www.R-project.org/).

RESULTS

Patients' Characteristics

One hundred nineteen patients were initially included. Sixty-seven patients were on infliximab, and 52 patients were on adalimumab. Seven patients, 5 patients on infliximab and 2 patients on adalimumab, showed the presence of antidrug antibodies in serum and were excluded from the study. A final series of 112 patients were included, 62 of them on infliximab and 50 on adalimumab.

The clinical and analytical characteristics of patients, distributed by drug, are shown in Table 1. All clinical variables were similar between groups, except for concomitant treatment with azathioprine, which was more frequent in the infliximab group than in the adalimumab group (35.5% vs 14%, $P = 0.01$). Twenty patients on infliximab and 10 patients on adalimumab showed a Crohn's Disease Activity Index (CDAI) >150 (32.3% vs 20%, $P = 0.14$). The concomitant use of steroids was also similar between patients on infliximab (n = 8, 13%) and patients on adalimumab (n = 9, 18%; $P = 0.477$). Fourteen patients on infliximab and 15 on adalimumab (22.6% vs 30%, $P = 0.37$) were receiving an intensified drug regimen. Supplementary Table 1 shows the clinical and analytical characteristics of patients distributed not only by drug but also by their established drug schedules. Fecal calprotectin levels were significantly higher in patients on intensified infliximab compared with the rest of the groups. This result may be related to the increased rate of CDAI-determined disease activity in patients receiving an intensified regimen of infliximab. The concomitant use of steroids was similar between patients in all 4 groups.

Table 2 resumes all experimentally determined variables of the study. No significant differences were found for any of the serum cytokine levels or genetic polymorphisms evaluated. The presence of bactDNA, as a surrogate marker of bacterial translocation, in the serum of patients was also similar between groups (40% vs 44%, $P = 0.27$). Supplementary Table 2 resumes all experimentally determined variables in patients distributed not only by drug but also by their established drug schedules. Serum IL-26 levels were significantly higher in patients receiving intensified infliximab regimes. The rates of bactDNA translocation were significantly different in patients receiving intensified vs regular adalimumab schedules. The presence of a variant NOD2 genotype was more frequent among patients on intensified vs regular infliximab regimes. No significant differences were found for any serum cytokine levels or genetic polymorphisms evaluated when comparing patients on intensified schedules of infliximab vs adalimumab.

Serum Trough Levels of Anti-TNF in the Study Cohort

Figure 1 shows serum trough levels of both infliximab and adalimumab according to their regimen. Interestingly, despite higher doses or shorter intervals of use, patients on intensified schedules showed similar trough levels of anti-TNF than patients on regular schedules (5.2 ± 2.4 μg/mL vs 5.6 ± 2.1 μg/mL, $P = 0.36$).

Linear regression was performed to identify whether any of the experimentally determined variables might influence infliximab and adalimumab trough levels. Table 3 shows the results of univariate and multivariate analyses controlled by weight and regimen for patients on infliximab. Common clinical and analytical parameters related to inflammatory state, such as CRP, fecal calprotectin, CDAI index, leukocyte count, and albumin levels in plasma, were not significantly associated

TABLE 1. Clinical and Analytical Characteristics of Patients Distributed by Established Drug

	Infliximab (62)	Adalimumab (50)	*P*
Male, No. (%)	32 (51.6)	25 (50)	0.87
Age, mean (SD), y	40.10 (14.01)	38.27 (12.48)	0.47
Weight, mean (SD), kg	72.61 (19.22)	71.17 (18.92)	0.77
Smoke, no/yes/ex, No. (%)	No: 23 (41.8)	No: 17 (24)	0.35
	Yes: 21 (38.2)	Yes: 26 (52)	
	Ex: 11 (20)	Ex: 7 (14)	
Montreal (age of onset), No. (%)	A1: 7 (11.5)	A1: 2 (4)	0.12
	A2: 44 (72.1)	A2: 44 (88)	
	A3: 10 (16.4)	A3: 4 (8)	
Montreal (location), No. (%)	L1: 18 (29.5)	L1: 16 (32)	0.27
	L2: 19 (31.1)	L2: 8 (16)	
	L3: 20 (32.8)	L3: 23 (46)	
	L4: 4 (6.6)	L4: 3 (6)	
Montreal (behavior), No. (%)	B1: 40 (66.7)	B1: 26 (52)	0.73
	B2: 8 (13.3)	B2: 8 (16)	
	B3: 12 (20)	B3: 16 (32)	
CDAI >150, No. (%)	20 (32.3)	10 (20)	0.14
Perianal disease, No. (%)	17 (27.4)	21 (42)	0.10
Previous surgery, No. (%)	13 (22.8)	19 (38)	0.087
Disease duration, mean (SD), mo	110.21 (83.29)	120.57 (86.90)	0.55
Azathioprin, No. (%)	22 (35.5)	7 (14)	0.01
Steroids, No. (%)	8 (13.1)	9 (18)	0.48
Anti-TNF intensification, No. (%)	14 (22.6)	15 (30)	0.37
Anti-TNF trough level, mean (SD), ng/mL	5414.41 (2336.60)	5612.93 (2116.87)	0.64
Total WBCs, mean (SD), mm^3	7089.67 (2611.66)	8475.31 (3537.03)	0.051
Hematocrit, mean (SD), %	47.40 (46.50)	41.85 (6.46)	0.66
CRP, mean (SD), mg/dL	1.01 (1.76)	0.89 (1.15)	0.95
Albumin, mean (SD), g/dL	3.97 (0.5)	4.00 (0.5)	0.90
Fecal calprotectin, mean (SD), ug/g	299.32 (613.90)	181.59 (357.84)	0.14

TABLE 2. BactDNA, Cytokines, and Genotypes Evaluated in Patients Distributed by Established Drug

	Infliximab (n = 62)	Adalimumab (n = 50)	*P*
BactDNA+, No. (%)	21 (33.9)	22 (44)	0.27
IL10, mean (SD), pg/mL	38.67 (24.11)	38.85 (21.06)	0.63
IL12, mean (SD), pg/mL	672.64 (304.16)	701.17 (355.32)	0.79
IL26, mean (SD), pg/mL	39.83 (37.28)	51.61 (38.13)	0.10
IFN-gamma, mean (SD), pg/mL	461.28 (180.29)	475.60 (196.98)	0.87
TNF-alpha, mean (SD), pg/mL	71.23 (29.48)	71.60 (29.18)	0.95
varATG16L1, No. (%)	36 (58)	32 (64)	0.52
varNOD2, No. (%)	42 (67.7)	33 (66)	0.85
varPTPN2, No. (%)	17 (27.4)	14 (28)	0.95
varTLR5, No. (%)	2 (3.2)	3 (6)	0.65
varIRGM, No. (%)	23 (37.1)	24 (48)	0.25

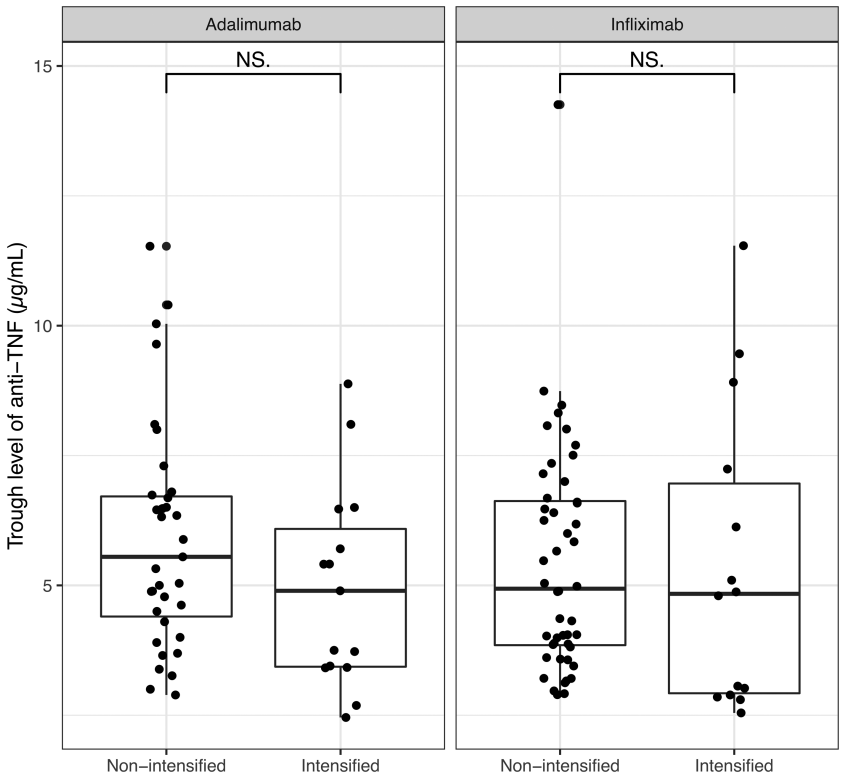

FIGURE 1. Serum trough levels of infliximab and adalimumab in all included patients according to their anti-TNF drug regimen. Anti-TNF values are expressed in µg/mL. Median and percentiles (P_{25}–P_{75}) are represented. *$P < 0.05$.

with infliximab levels in the study cohort in either the univariate analysis or multivariate analysis. Among cytokines and genotypes evaluated, only serum IL-10 levels were directly related to infliximab levels (Beta = 0.097, $P < 0.0001$).

The same analyses were performed for patients on adalimumab (Table 4). Similar to patients on infliximab, none of clinical and analytical parameters were significantly associated with adalimumab levels in the multivariate 2-factor controlled analysis, whereas IL-10 remained independently associated with adalimumab levels in our study cohort (Beta = 0.069, $P = 0.0241$). The correlation between serum IL-10 and anti-TNF levels is represented in Figure 2. These correlations were persistent when comparing patients according to their disease Montreal behavior (Supplementary Fig. 1). Regarding therapeutic outcomes such as CDAI score or fecal calprotectin levels, no significant correlations were found between them and trough levels of anti-TNF drugs in our series of patients (CDAI vs infliximab: $r = 0.0015$, $P = 0.82$; CDAI vs adalimumab: $r = 0.0096$, $P = 0.54$; fecal calprotectin vs infliximab: $r = 0.061$, $P = 0.14$; fecal calprotectin vs adalimumab: $r = 0.0037$, $P = 0.70$).

Figure 3A and B shows that infliximab and adalimumab levels predicted by IL-10 robustly fitted with actual drug levels ($R^2 = 0.841$ for infliximab, $R^2 = 0.733$ for adalimumab), suggesting a role for IL-10 in explaining anti-TNF levels' variability in the blood of CD patients. Figure 4A and B represents IL-10 levels, considering infliximab and adalimumab levels as quartiles.

DISCUSSION

In the present study, we have evaluated a set of inflammatory, genetic, and bacterial variables in relation to anti-TNF serum through levels in a cohort of CD patients on stable anti-TNF therapy. Our results show that anti-TNF levels' variability among responding patients, most within therapeutic range, can be related to serum IL-10, which strengthens the role of inflammatory status when studying anti-TNF disposition in CD patients.

The introduction of anti-TNF therapy has improved IBD care in the last 2 decades. Nevertheless, important drawbacks remain present. The rates of primary nonresponse and secondary loss of response are considerable.[25,26] The measurement of anti-TNF levels, as well as antidrug antibodies, has been claimed to help in managing patients either to elucidate reasons for loss of response or to set up drug regimes. Different studies have shown that high serum levels of anti-TNF are associated with favorable therapeutic outcomes whereas low anti-TNF levels are associated with therapeutic failure.[26–29] However, these measurements show a number of limitations as well, recently summarized by Govani et al.,[19] related to the wide variability in concentrations found in most studies.

TABLE 3. Univariate and Multivariate Analyses of Variables Influencing Infliximab Levels, Controlled by Weight and Drug Regimen

	Univariate Analysis			Multivariate Analysis		
	β	*P*	R^2	β	*P*	R^2
Sex (female)	–17.177	0.014	0.1094	0.549	0.4470	
Age	0.030	0.2406	0.027			
Smoke (no)	Yes: 0.341	Yes: 0.6758	0.0101			
	Ex: 0.048	Ex: 0.9627				
Montreal A (A1)	A2: 1.992	A2: 0.0617	0.0679			
	A3: 2.029	A3: 0.1379				
Montreal L (L1)	L2: –1.241	L2: 0.1332	0.0682			
	L3: –0.093	L3: 0.9147				
	L4: 1.059	L4: 0.5671				
Montreal B (B1)	B2: 2.371	B2: 0.0155	0.1168	0.437	0.3850	
	B3: 0.891	B3: 0.2875		0.205	0.7108	
Perianal disease (yes)	0.493	0.4894	0.0101			
Previous surgery (no)	1.304	0.0965	0.06			
Disease duration	0.001	0.7271	0.0034			
Azathioprine (no)	0.366	0.6041	0.0062			
Steroids (no)	–1.729	0.0634	0.0663			
Leukocytes	–0.00019	0.15	0.0408			
Hematocrit	0.000053	0.9940	0.0016			
CRP	–0.044	0.8090	0.0036			
Albumin	0.4369	0.52	0.0149			
FCT	–0.0008	0.1856	0.0768			
CDAI >150 (no)	0.235	0.7390	0.0032			
Bacterial DNA (no)	–1.376	0.0462	0.0739	1.544	0.0634	
IL10	0.094	<0.0001	0.8592	0.097	<0.0001	0.9392
IL12	–0.0033	0.0010	0.1881	–0.004	0.0509	
IL26	–0.0037	0.7602	0.0544			
IFN-gamma	–0.007	<0.0001	0.2753	0.003	0.3265	
TNF-alpha	–0.041	0.0004	0.2147	–0.00008	0.9969	
ATG16L1 (wt)	–2.771	0.0043	0.3089	0.087	0.8431	
PTPN2_1 (wt)	0.240	0.8725	0.0082			
NOD2 (wt)	–1.633	0.0237	0.0938	–0.209	0.7720	
TLR5 (wt)	1.700	0.5531	0.0496			
IRGM (wt)	1.230	0.2479	0.0869			

All analyses were controlled by weight and regimen.

Interpatient variability in anti-TNF serum levels depends largely on differences in drug clearance and distribution in the body.[30] Anti-TNF clearance changes are partly explained by differences in antigenic burden or by antidrug antibodies, which accelerate anti-TNF drugs' elimination.[13, 31] In multiple studies, the presence and level of antibodies to anti-TNF correlated with lower serum levels, loss of clinical efficacy, and increased risk of infusion reactions.[32] However, the existence of long-term responders with low anti-TNF levels and undetectable antibodies to anti-TNF has been also described,[33] suggesting that factors other than immunogenicity influence the pharmacokinetics of anti-TNF drugs. These factors include sex, body size, concomitant use of immunosuppressive agents, disease type, serum albumin concentration, and degree of systemic inflammation.[31] A real-life population pharmacokinetic study revealed that body weight, serum albumin, and titers of antibodies to anti-TNF (0–53,000 AU/mL) were the main factors influencing clearance infliximab variability.[34]

It is important to point out that the results presented herein are controlled by weight and drug dosage and that

TABLE 4. Univariate and Multivariate Analyses of Variables Influencing Adalimumab Levels, Controlled by Weight and Drug Regimen

	Univariate Analysis			Multivariate Analysis		
	Beta	*P*	R^2	Beta	*P*	R^2
Sex (female)	–0.504	0.43	0.0930			
Age	–0.014.68	0.54	0.0881			
Smoke (no)	Yes: 0.403	Yes: 0.55	0.09			
	Ex: –0.011	Ex: 0.99				
Montreal A (A1)	A2: 1.802	A2: 0.2427	0.1086			
	A3: 1.706	A3: 0.3548				
Montreal L (L1)	L2: –0.378	L2: 0.6739	0.0601			
	L3: –1.278	L3: 0.0628				
	L4: –1.632	L4: 0.2114				
Montreal B (B1)	B2: –0.915	B2: 0.28	0.11			
	B3: –0.474	B3: 0.49				
Perianal disease (yes)	1.147	0.0538	0.1529			
Previous surgery (no)	0.154	0.752	0.0315			
Disease duration	–0.003	0.4435	0.09			
Azathioprine (no)	0.962	0.2949	0.1027			
Steroids (no)	–0.281	0.72	0.0834			
Leukocytes	–0.000095	0.26	0.1034			
Hematocrit	–0.045	0.3422	0.0965			
CRP	–0.0072	0.98	0.078			
Albumin	–0.3823	0.5177	0.0933			
FCT	–0.0006	0.53	0.0718			
CDAI >150 (no)	–0.206	0.78	0.0823			
Bacterial DNA (no)	–1.097	0.10	0.1337			
IL10	0.085	<0.0001****	0.7386	0.069	0.0241*	0.8092
IL12	–0.0023	0.0050**	0.227	0.0005	0.9171	
IL26	–0.021	0.0224*	0.178	0.0033	0.8524	
IFN-gamma	–0.004	0.00634**	0.2196	–0.00025	0.9699	
TNF-alpha	–0.043	<0.0001****	0.385	0.014	0.6486	
ATG16L1 (wt)	–3.389	0.00208**	0.4902	–1.654	0.1544	
PTPN2_1 (wt)	1.216	0.472	0.1732			
NOD2 (wt)	–2.227	0.00015***	0.3299	–0.943	0.5884	
TLR5 (wt)	–3.161	0.5560	0.1417			
IRGM (wt)	0.836	0.4687	0.1504			

All analyses were controlled by weight and regimen.

patients with detectable antibodies to anti-TNF were excluded, which allows a more precise analysis of the influence of inflammation on the anti-TNF serum levels. It is also important to stress that the infliximab and adalimumab levels in our cohort of patients are in, or close to, therapeutic range.[35, 36] Two conditions may explain this. First, all included patients were stable in their drug schedules for at least 3 months. Second, in our hospitals, anti-TNF levels are monitored as a reactive strategy in patients without clinical response, and this may explain that patients on intensified drug schedules do not show higher anti-TNF levels. These issues may also explain the low or "normalized" values for CRP, fecal calprotectin, and albumin in our series of patients and the lack of correlations between anti-TNF and these variables in patients who, on the other hand, still show some anti-TNF variability.

A significant association was observed between baseline CRP levels and maintained remission after infliximab therapy in the ACCENT I trial.[37] Posterior studies have shown that patients with a baseline concentration of CRP >50 mg/L had lower serum concentrations of infliximab after 6 weeks of treatment, suggesting the existence of a relationship between pretreatment inflammatory status, anti-TNF clearance, and

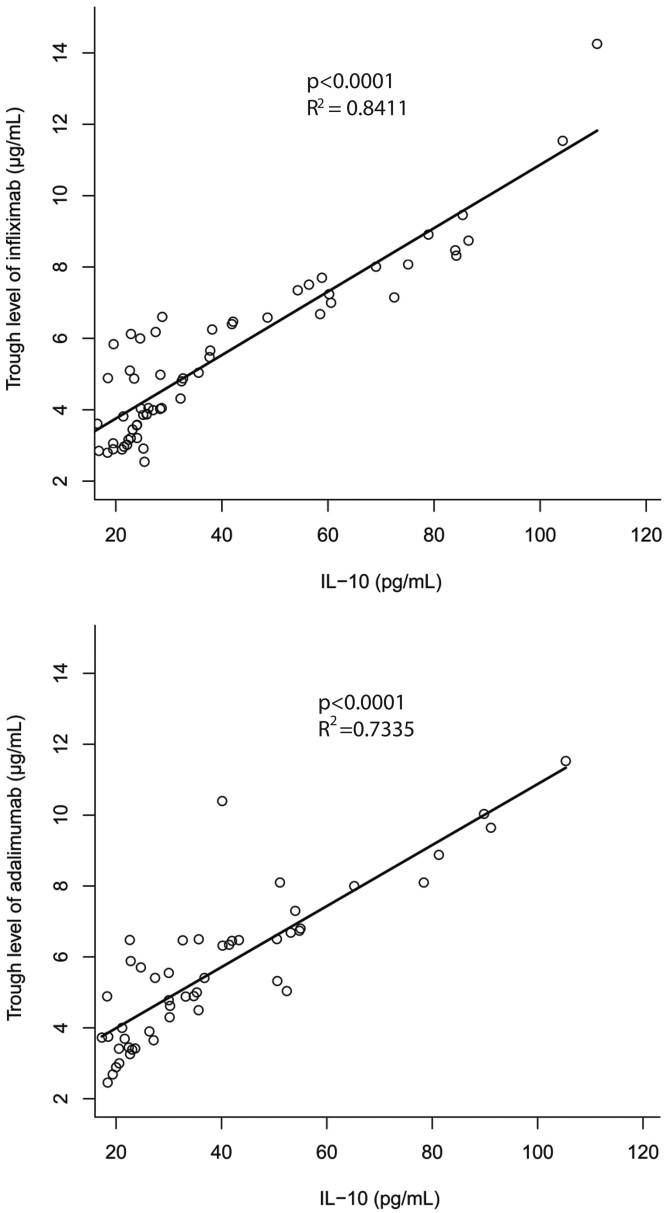

FIGURE 2. Correlation between serum trough levels of anti-TNF drugs (µg/mL) and serum IL-10 levels (pg/mL) in all included patients.

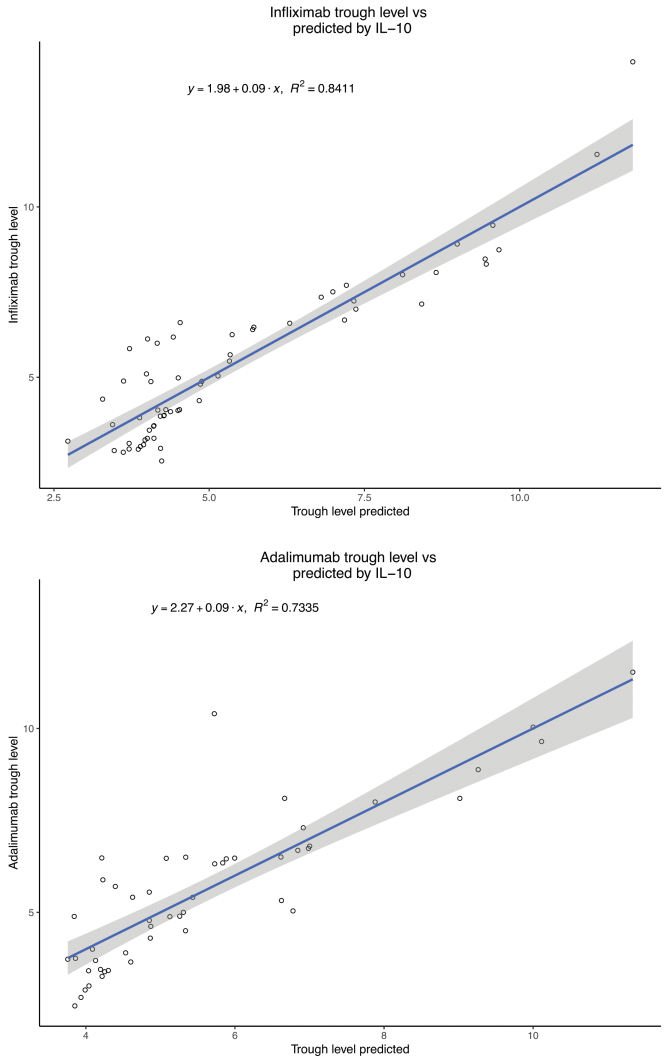

FIGURE 3. Prediction models of (A) infliximab and (B) adalimumab levels by IL-10. Anti-TNF values are expressed in µg/mL.

response to treatment.[37] Similarly, subtherapeutic adalimumab concentrations have been associated with higher fecal calprotectin and CRP concentrations in CD patients.[38] The infliximab concentration–dependent effect on CRP concentrations described in the past[39] is not present in our study. However, in our series, this relationship was observed with IL-10. In addition to the previously noted characteristics of our series of patients, we have seen in the past that serum levels of anti-TNF correlate with the percentage of FoxP3$^+$ regulatory T cells.[40] This population importantly contributes to the tolerogenic response through IL-10 production, and it may explain the strong association between anti-TNF levels and IL-10 observed in our series. In fact, others have shown an increase in IL-10 and TGF-B when the regulatory T-cell population is restored in cell cocultures of anti-TNF responder patients.[41] Our results suggest, therefore, a significant contribution to anti-TNF drugs' disposition of the secreted cytokine environment beyond other inflammatory markers such as CRP or fecal calprotectin.

Although a mechanistic study should be specifically designed to decipher the close relationship observed between IL-10 and anti-TNF levels in the serum of CD patients, at least 2 ideas can be discussed. On the one hand, clearance of anti-TNF drugs occurs via the reticulo-endothelial system (RES),[42] and it has been described that chronic inflammation, in which endogenous B-cell IgG is produced, may saturate the antibody recirculation system at the RES, leading to increased clearance.[7] Therefore, the characteristic pro-inflammatory environment

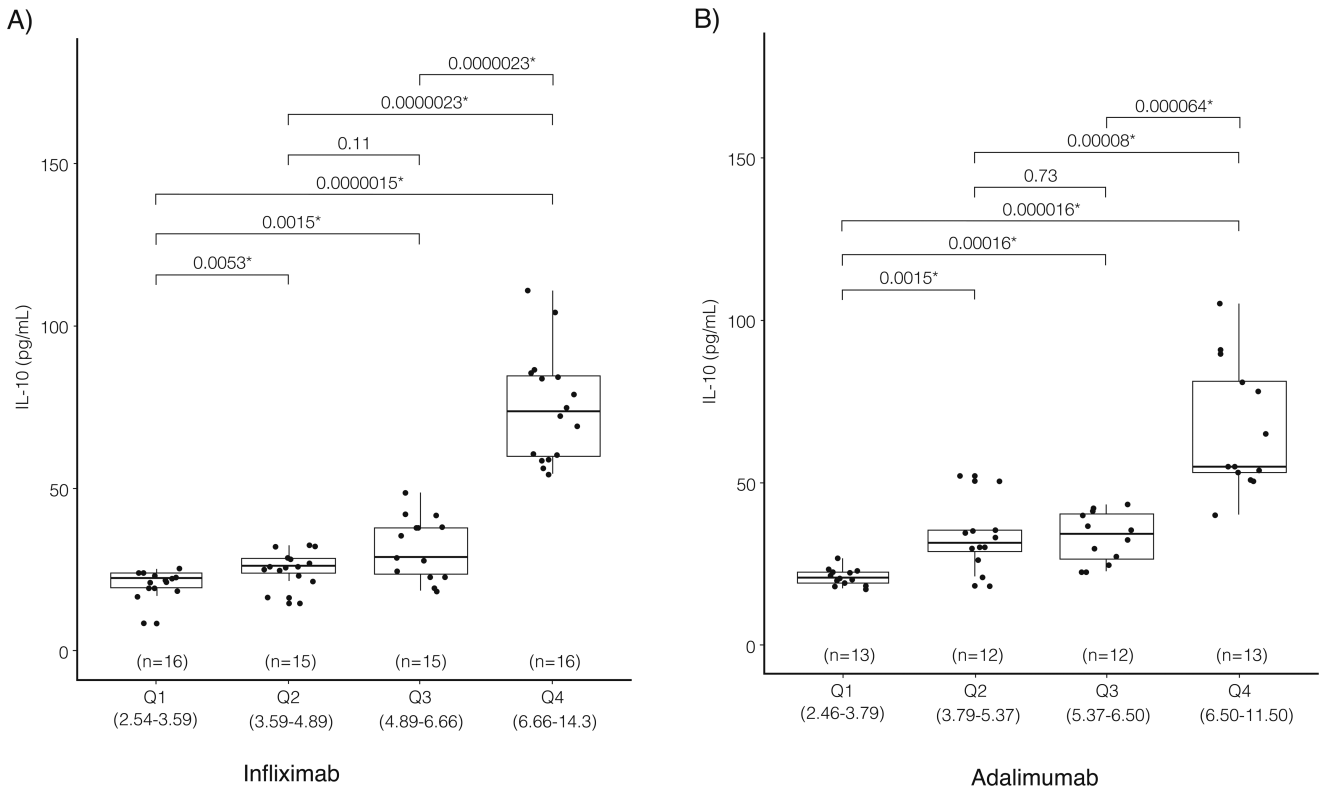

FIGURE 4. Serum IL-10 levels according to quartiles of infliximab (A) and adalimumab (B) levels.

present in CD patients, which also promotes a counterbalancing IL-10 production, might partially explain the association between IL-10 and anti-TNF levels. On the other hand, we could hypothesize about the direct effect of these drugs on transmembrane TNF-α and, therefore, on monocyte cytokine secretion through their apoptotic activity. Transmembrane TNF-α expression has been documented in macrophages and activated T cells from CD patients.[43, 44] In fact, infliximab and adalimumab have been shown to induce apoptosis in monocytes from CD patients in a caspase-dependent manner.[44, 45] In addition, infliximab has shown to revert mucosal T-cell death through this pathway in CD.[46] These mechanisms would suggest a close modulation of the cytokine profile beyond TNF-α blockade. In fact, both infliximab and adalimumab have been reported to affect monocyte-derived IL-10 and IL-12 compared with etanercept in cell cultures from CD patients,[45] and the maintenance of an IL-10$^+$ phenotype in human effector T cells by TNF-α blockade has been recently described.[47]

In summary, we show that the serum levels of IL-10 are significantly and linearly related to serum infliximab and adalimumab levels in a series of CD patients controlled by weight and drug regimen. Considering that the inflammatory status of CD patients may be affected by genetic and microbiological factors, this study points toward an easy-to-measure tool that may help in explaining anti-TNF levels. Future studies should address whether patients with anti-TNF levels in the therapeutic range but low levels of IL-10 have a worse clinical evolution.

SUPPLEMENTARY DATA

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

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