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ORIGINAL ARTICLE

Infliximab serum concentrations in luminal Crohn's disease and its relationship with disease activity: A multicentric cross-sectional study

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Abstract
Objectives: In Latin America, experience with monitoring serum Infliximab (IFX) concentrations is scarce. Our study aimed to compare IFX serum concentrations between patients with active disease or in remission.
Patients and methods: A cross-sectional study was performed in patients with luminal Crohn's disease (CD) during maintenance treatment with IFX. Patients were classified as in remission or disease activity according to clinical scores and endoscopic, radiological, and laboratory markers. A comparison of IFX trough levels between the two groups was performed.
Results: 80 CD patients were included [41 (51%) in remission and 39 (49%) with active disease]. In the analysis of general disease activity, the median serum levels of IFX in patients with remission and with active CD were 5.63 [0.03–14.40] vs. 3.84 [0.03–14.40] ($p = 0.287$). Furthermore, there was no difference in serum IFX concentrations in endoscopic, radiological, and laboratory activities. Only in the clinical evaluation there was a significant difference in the median serum IFX levels between patients in remission and disease activity, 5.63 [0.03–14.40] vs. 2.14 [0.32–10.54] ($p = 0.042$).
Conclusions: IFX serum concentrations during maintenance treatment were similar in patients with luminal CD in remission and general, endoscopic, radiological, and laboratory disease activity. Patients with clinically active disease had lower IFX concentrations than patients in remission.

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PALABRAS CLAVE
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Enfermedad inflamatoria intestinal;
Infliximab;
Dosis;
Nivel mínimo;
Monitoreo terapéutico de medicamentos

Concentraciones séricas de infliximab en la enfermedad de Crohn luminal y su relación con la actividad de la enfermedad: un estudio transversal multicéntrico

Resumen
Objetivos: En Latinoamérica, la experiencia en el monitoreo de las concentraciones séricas de infliximab (IFX) es escasa. Nuestro estudio tuvo como objetivo comparar las concentraciones séricas de IFX entre pacientes con enfermedad activa y remisión.
Pacientes y métodos: Se realizó un estudio transversal en pacientes con enfermedad de Crohn (EC) luminal durante el tratamiento de mantenimiento con IFX. Según una combinación de puntuaciones clínicas, marcadores endoscópicos, radiológicos y de laboratorio, los pacientes se clasificaron en remisión o actividad. Los niveles mínimos de IFX fueran determinados y comparados entre los dos grupos.
Resultados: Se incluyeron 80 pacientes con EC [41 (51%) en remisión y 39 (49%) con actividad]. En el análisis de la actividad general de la enfermedad, la mediana de los niveles séricos de IFX en pacientes con remisión y con EC activa fue 5,63 [0,03 - 14,40] vs. 3,84 [0,03 - 14,40] (p = 0,287). Además, no hubo diferencias en las concentraciones séricas de IFX en actividades endoscópicas, radiológicas y de laboratorio. Solo en la evaluación clínica hubo una diferencia significativa en los niveles séricos medios de IFX entre pacientes en remisión y actividad de la enfermedad, 5,63 [0,03 - 14,40] vs. 2,14 [0,32 - 10,54] (p =0,042).
Conclusiones: Las concentraciones séricas de IFX durante el tratamiento de mantenimiento fueron similares en pacientes con EC luminal en remisión y actividad general, endoscópica, radiológica y de laboratorio de la enfermedad. Los pacientes con enfermedad clínicamente activa tenían concentraciones de IFX más bajas que los pacientes en remisión.

Introduction

Inflammatory bowel diseases (IBD), namely Crohn's disease (CD) and ulcerative colitis (UC), are chronic diseases that usually affect young patients, causing limitations in their quality of life and productive capacity.[1] The introduction of biological agents in recent years has dramatically benefited IBD patients, significantly reducing hospitalization and surgery rates.[2] However, there are still some limitations to their use. It is estimated that 10–40% of patients exposed for the first time to any immunobiological will not respond to treatment (primary non-response – PNR). Furthermore, among patients who initially benefit from a specific drug, up to 50% may lose response over time (secondary loss of response – SLR). For IFX, SLR rates have been estimated to be 12% per year.[3] Some reasons for these pitfalls are insufficient drug serum level, a consequence of an inadequate dosage or the emergence of anti-drug antibodies (pharmacokinetic failure), or failure in the choice of the mechanism of action in which the drug is not appropriate for the patient's disease profile (pharmacodynamic failure).[4]

Monitoring levels of immunobiologicals in IBD (therapeutic drug monitoring – TDM) has emerged as an alternative to improve the performance of therapies. It assumes a positive correlation between drug concentrations and therapeutic outcomes. Moreover, the lower the concentration, the greater the chance of PNR and SLR and the development of antibodies to IFX (ATI), especially in the maintenance phase.[5,6]

The idea of routinely checking serum levels is attractive to physicians as it provides a sense of better control over treatment, as is reinforced by positive association studies. However, two recently published meta-analyses that compared strategies of TDM identified divergent results.[7,8]

There is scarce data from Latin America regarding TDM with IFX in CD. Only two studies correlated serum IFX concentrations with clinical outcomes but with fewer patients and limited analyses of objective markers.[9,10] This study aimed to compare serum IFX concentrations in patients with luminal CD presenting with remission or disease activity using a combination of clinical scores and endoscopic, radiological, and laboratory markers.

Material and methods

Study design and data source

A cross-sectional study was conducted in patients with luminal CD who were treated with IFX during the maintenance phase from two tertiary referral centers in south Brazil between August 2019 and September 2021. Patients were selected sequentially and consecutively by a non-random convenience sample. The inclusion criteria were: patients over 18 years old; diagnosis of CD for at least three months with luminal involvement and inflammatory, structuring, or penetrating disease behavior confirmed by clinical and endoscopic, radiological, and histological criteria; patients taking intravenous IFX for more than 14 weeks (maintenance phase) and who have undergone induction therapy 5 mg/kg infusions at weeks 0, 2 and 6. Patients were excluded if they had undetermined IBD, an isolated fistulizing perineal CD

involvement without clinically significant luminal disease, and no sufficient data available on their medical records. When these patients were undergoing routine clinical, endoscopic, radiological, and laboratory monitoring, they were offered the possibility of collecting IFX serum concentrations independently of their disease activity status.

Outcome parameters

Demographic variables were collected. When all available clinical scores assessments and endoscopic, radiological, and laboratory markers described a state of disease remission, the patient was assumed to be in remission of general disease activity. When at least one or more of these assessments described a state of disease activity, the patient was characterized as having general disease activity. Complementary tests were considered between a window of 8 weeks before or after the date of blood collection for IFX concentrations.

The clinical activity of luminal CD was evaluated by the Harvey–Bradshaw Index (HBI), analyzing the variables collected in the last 24 h before the consultation. The patient was considered in remission if the HBI was $\leq$4 points.[11] For the endoscopic activity, the patient was considered in remission if there were no ulcers (small or large) during colonoscopy and if there was no inflammatory stricture in all segments of the terminal ileum and colon. When CD location could not be reached by colonoscopy, the definition of remission or disease activity was performed by radiological markers and analyzed during CT or MRI enterography. Patients were considered in remission if there were none of the following findings: intestinal wall thickening, segmental hyperenhancement of the intestinal wall after intravenous contrast administration, abscesses, fistulas, or inflammatory stenosis.[12] Laboratory activity was evaluated by C-reactive protein (CRP) and fecal calprotectin. Patients were considered in remission if CRP was below the upper limit of normality of the assay or the fecal calprotectin was $\leq$250 μg/g.[13]

The Promonitor® ELISA immunoassay kit (Proteomika, Progenika Biopharma, Bizkaia, Spain) was used to measure serum IFX concentrations. Blood samples for this analysis were collected at trough levels, for a maximum of 48 h before each patient's next infusion. The analyses were centrally performed at the Clinical Laboratory of one center. Between the day of collection and analysis, blood samples were stored at −20 °C. The absolute value of the dosage in μg/mL was used for correlation with the outcomes. The serum IFX level detection limits were 0.035–14.4 μg/mL. This assay is sensitive to the presence of ATI, allowing quantification of only the excess drug-free circulating drug-antibody complexes.[14]

Serum IFX concentrations were compared between patients in remission and active disease in combination (general disease activity) and separately in clinical, endoscopic, radiological, and laboratory activities. Further, the proportion of patients in remission and active disease were compared according to different ranges of serum IFX levels, chosen between 3–7 μg/mL and 5–10 μg/mL.

Statistical analysis

Data were collected and stored in a Microsoft Excel spreadsheet, and IBM SPSS version 21.0 software was used for database preparation, statistical analysis, and graph editing. Quantitative variables were presented as to their distribution pattern: mean and standard deviation, or median, minimum, and maximum values. The differences between the two groups were studied by a parametric test (Student's *t*-test) for the normal distribution variables and a nonparametric test (Mann–Whitney) for the variables without normal distribution. Qualitative variables were presented as percentages. The Chi-square test was used to compare two proportions (of independent samples), and Fisher's exact test was used for a small number of expected frequencies (when the expected number of cases was less than 20). The Kolmogorov–Smirnov test was used to investigate whether serum IFX levels followed a normal Gaussian distribution ($p < 0.001$). Hence, this data was analyzed using the Mann–Whitney nonparametric test. Boxplot graphics were created to describe the variation of median serum IFX concentrations observed utilizing quartiles at remission and activity among distinct types of general, clinical, endoscopic, radiological, and laboratory activities. These graphics show the median, the upper and lower quartiles, minimum, maximum, and outliers' values. The significance level adopted for the statistical tests was 5%.

Results

Initially, 112 patients with IBD using IFX during the maintenance phase of treatment were identified. After excluding twenty-two patients with UC, five patients who did not agree to undergo monitoring tests, two patients under 18 years old, and three patients for lack of data available in medical charts, eighty patients with luminal CD were finally included (Fig. 1). 41 (51%) were in remission and 39 (49%) had some disease activity. The baseline demographic characteristics of these patients are detailed in Table 1.

As observed, there were no differences in most variables between the groups. BMI (in kg/m^2) was significantly higher in remission patients than those with general disease activity (26.15 ± 5.48 vs. 24.39 ± 3.53, $p = 0.008$). Fecal calprotectin levels were higher in patients with active disease as compared to those in remission (35.00 [8.00–235.00] vs. 249.00 [30.00–2118.00], $p = 0.001$).

All patients used the originator medication; none used an IFX biosimilar. Clinical evaluation was performed in 80 (100%) patients, colonoscopy was performed in 74 (92%), radiological examination was performed in 33 (41%), calprotectin was evaluated in 30 (35%), C-reactive protein in 61 (76%) and serum albumin in 50 (62%) patients. In 2 (2%) patients, the characterization of remission or general disease activity was done by clinical assessment exclusively. Still, it was performed in 78 (98%) patients using a combination of clinical scores and available endoscopic, radiological, and laboratory markers.

Boxplots illustrating median serum IFX concentrations between patients in remission and active disease among

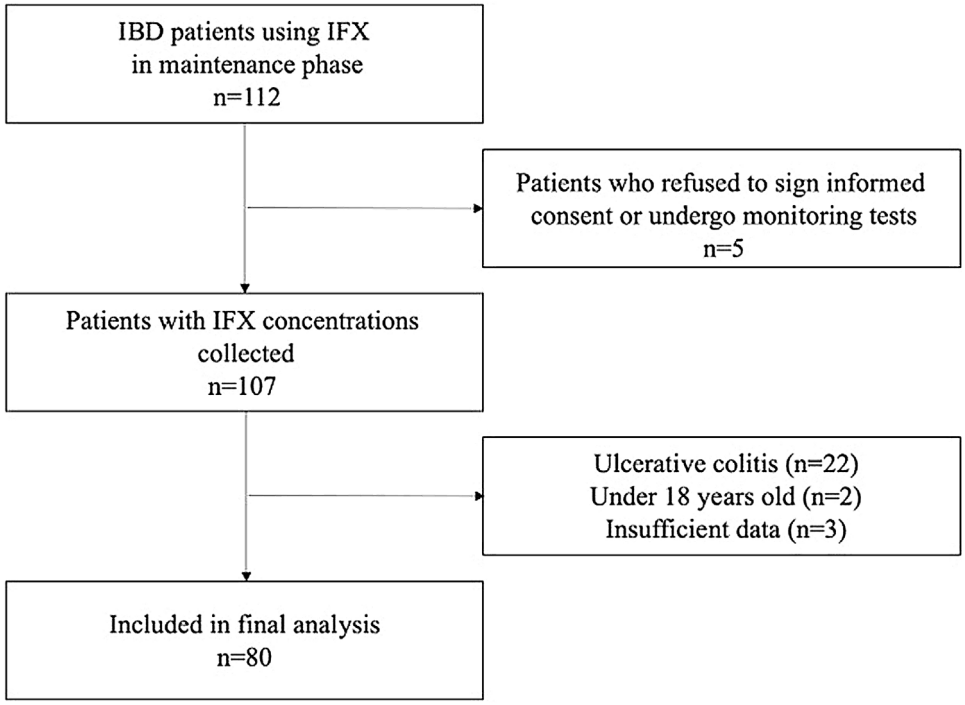

Figure 1 Patient flowchart with excluded patients and reasons for exclusion.

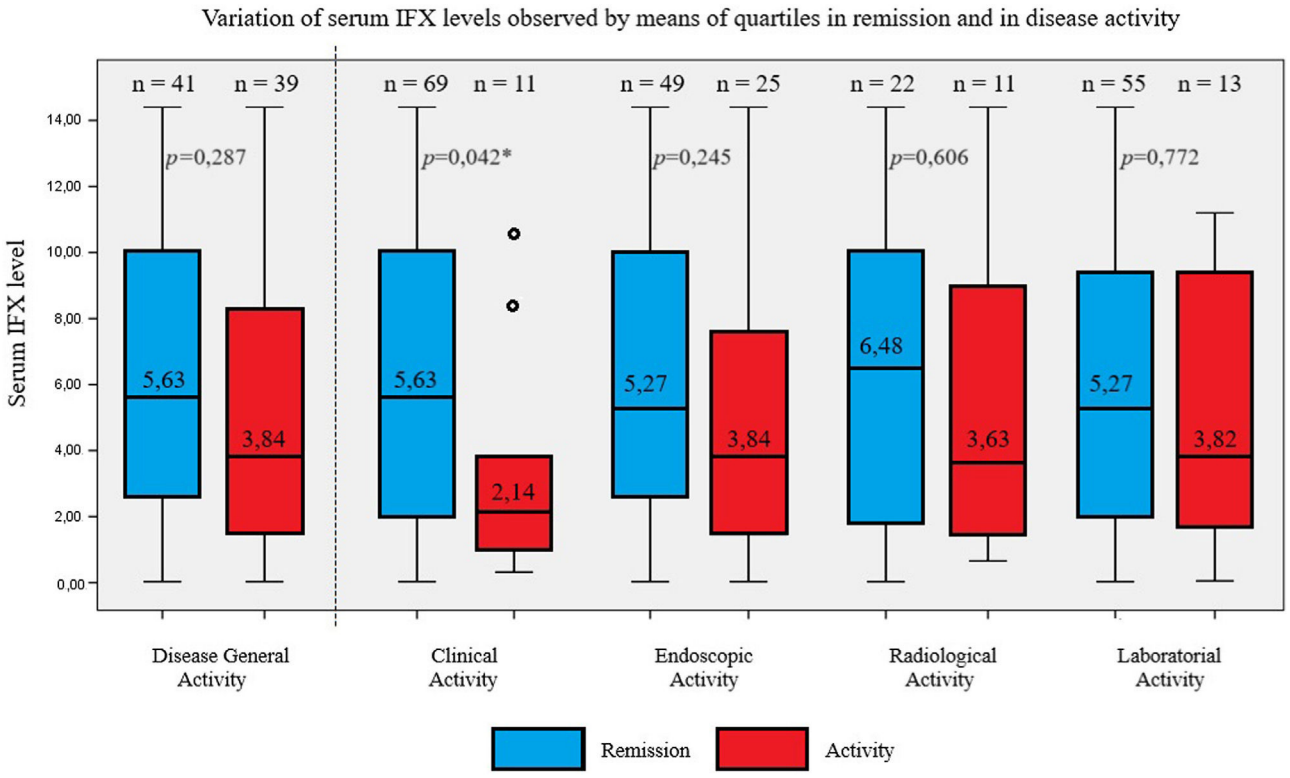

Figure 2 Boxplot graphics with variation of serum IFX concentrations observed by medians and interquartile ranges between patients in remission and different types of disease activity.

general, clinical, endoscopic, radiological, and laboratory activities are detailed in Fig. 2 and Supplementary Table A. There were no differences between median serum IFX concentrations between patients in remission and general activity of the disease (5.63 μg/mL [0.03–14.40] vs. 3.84 [0.03–14.40], $p = 0.287$); in endoscopic activity (5.27 μg/mL [0.03–14.40] vs. 3.84 μg/mL [0.03–14.40], $p = 0.245$); in radiological activity (6.48 μg/mL [0.03–1

Table 1 Clinical and demographic characteristics of patients with luminal Crohn's disease in remission and general disease activity.

Variables	Disease status		
	Remission ($n = 41$)	General activity ($n = 39$)	*p value*
Male, n (%)	22 (54)	21 (54)	1.000$^{\Phi}$
Age in years (mean ± SD)	39.30 ± 13.37	38.54 ± 13.76	0.773¶
BMI (mean ± SD)	26.15 ± 5.48	24.39 ± 3.53	0.008¶
Disease duration (months) until serum IFX collection (mean ± SD)	127.98 ± 108.96	144.58 ± 118.16	0.316¶
Montreal – age at diagnosis			
A1 (<17 years), *n* (%)	3 (7)	3 (8)	
A2 (17–40 years), *n* (%)	34 (83)	32 (82)	1.000$^{\Phi}$
A3 (>40 years), *n* (%)	4 (10)	4 (10)	
Montreal – disease location			
L1 (terminal ileum), *n* (%)	5 (12)	9 (23)	
L2 (colon), *n* (%)	13 (31)	7 (18)	0.228$^{\Phi}$
L3 (ileocolonic), *n* (%)	23 (56)	23 (59)	
L4 (upper GI), *n* (%)	4 (10)	4 (10)	1.000$^{\Phi}$
Montreal – disease behavior			
B1 (inflammatory), *n* (%)	20 (49)	21 (54)	
B2 (stricturing), *n* (%)	13 (31)	15 (38)	0.334$^{\Phi}$
B3 (penetrating), *n* (%)	8 (20)	3 (8)	
Perianal disease – p, n (%)	23 (56)	15 (38)	0.125$^{\Phi}$
Smoking, n (%)	6 (15)	4 (10)	0.738$^{\Phi}$
Previous CD-related surgery, n (%)	27 (66)	22 (56)	0.492$^{\Phi}$
Serum IFX concentration (median, min–max)	5.63 (0.03–14.40)	3.84 (0.03–14.40)	0.287$^{\Delta}$
Optimized therapy, n (%)	12 (30)	18 (46)	0.168$^{\Phi}$
Combination therapy			
Azathioprine, *n* (%)	27 (66)	27 (69)	0.799$^{\Phi}$
Metotrexate, *n* (%)	6 (15)	7 (18)	
Number of previous biologicals			
1, *n* (%)	37 (90)	31 (80)	
2, *n* (%)	4 (10)	7 (18)	0.262$^{\Phi}$
3, *n* (%)	0 (0)	1 (2)	
Calprotectin, n (%)	15 (36)	15 (36)	1.000$^{\Phi}$
Calprotectin level (median, min–max)	35.00 (8.00–235.00)	249.00 (30.00–2118.00)	0.001$^{\Delta}$
Albumin level (mean ± SD)	4.40 ± 0.26	4.26 ± 0.33	0.171¶

Legend: mean ± standard deviation = mean ± SD, Fisher's exact test = Φ, Student's *t* test = ¶, Mann–Whitney test = Δ, min = minimum value, max = maximum value.

4.40] vs. 3.63 μg/mL [0.66–14.40], $p = 0.606$); and in laboratory activity (5.27 μg/mL [0.03–14.40] vs. 3.82 μg/mL [0.50–11.20], $p = 0.772$). We identified a significant difference between patients in remission as compared with those with clinically active CD (5.63 μg/mL [0.03–14.40] vs. 2.14 μg/mL [0.32–10.54], $p = 0.042$).

When comparing proportions of patients in remission with those with active CD (general, clinical, endoscopic, radiological, and laboratory activities) according to different ranges of serum IFX concentrations (3–7 μg/mL and 5–10 μg/mL), there was a significant difference only in endoscopic activity in the range of ≥3 μg/mL and ≤7 μg/mL (76% vs. 24%, $p = 0.047$). These data are described in detail in Table 2. No correlation was found between fecal calprotectin and infliximab serum levels ($r = 0.161$, $p = 0.394$).

Discussion

TDM with biologics was recently introduced in Latin America. As with any clinical application tool, a learning curve is required to understand the real benefit of its use. Kampa et al. reported the initial experience of monitoring serum IFX levels in forty-five Brazilian patients. From the sixty-three measurements described, 39 (61.9%) were done regardless of disease activity. Dose optimization was performed in 22 (34.92%) cases to adjust the serum IFX concentrations to a therapeutic range and in 8 (12.69%) cases, according to clinical criteria.[15] It is essential to highlight that when TDM is used, the attending physician may face some clinical scenarios in which the decision to maintain or modify the treatment is not well established. The most typical example is when the

Table 2 Comparison between the proportions of patients in remission and different types of disease activity, according to ranges of serum IFX concentrations between 3–7 μg/mL and 5–10 μg/mL.

Serum IFX concentration	*n*	Remission	Activity	*p*	Serum IFX concentration	*n*	Remission	Activity	*p*
General disease activity (n = 80)									
>7	31 (39)	17 (55)	14 (45)	0.710$^{\Phi}$	>10	20 (25)	12 (60)	8 (40)	0.503$^{\Phi}$
≥3 and ≤7	18 (22)	9 (50)	9 (50)	1.000$^{\Phi}$	≥5 and ≤10	20 (25)	11 (55)	9 (45)	0.826$^{\Phi}$
<3	31 (39)	15 (48)	16 (52)	1.000$^{\Phi}$	<5	40 (50)	18 (45)	22 (55)	0.637$^{\Phi}$
Clinical activity (n = 80)									
>7	31 (39)	29 (94)	2 (6)	<0.001$^{\Phi}$	>10	20 (25)	19 (95)	1 (5)	<0.001$^{\Phi}$
≥3 and ≤7	18 (22)	16 (89)	2 (11)	0.002$^{\Phi}$	≥5 and ≤10	20 (25)	19 (95)	1 (5)	<0.001$^{\Phi}$
<3	31 (39)	24 (77)	7 (23)	0.004$^{\Phi}$	<5	40 (50)	31 (77)	9 (23)	<0.001$^{\Phi}$
Endoscopic activity (n = 74)									
>7	23 (39)	20 (69)	3 (31)	0.580$^{\Phi}$	>10	19 (26)	13 (68)	6 (32)	0.164$^{\Phi}$
≥3 and ≤7	17 (23)	13 (76)	4 (24)	0.047$^{\Phi}$	≥5 and ≤10	19 (26)	13 (68)	6 (32)	0.164$^{\Phi}$
<3	28 (38)	16 (57)	12 (43)	0.569$^{\Phi}$	<5	36 (48)	23 (64)	13 (36)	0.139$^{\Phi}$
Radiological activity (n = 33)									
>7	15 (46)	11 (73)	4 (27)	0.118$^{\Phi}$	>10	9 (27)	6 (66)	3 (33)	0.502$^{\Phi}$
≥3 and ≤7	5 (15)	3 (60)	2 (40)	1.000$^{\Phi}$	≥5 and ≤10	7 (21)	6 (85)	1 (15)	0.123$^{\Phi}$
<3	13 (39)	8 (62)	5 (28)	0.578$^{\Phi}$	<5	17 (52)	10 (58)	7 (42)	0.632$^{\Phi}$
Laboratorial activity (n = 68)									
>7	26 (38)	21 (81)	5 (19)	0.002	>10	16 (24)	13 (81)	3 (19)	0.022$^{\Phi}$
≥3 and ≤7	15 (22)	12 (80)	3 (20)	0.034	≥5 and ≤10	19 (28)	17 (89)	2 (11)	0.001$^{\Phi}$
<3	27 (40)	22 (81)	5 (19)	0.002	<5	33 (48)	25 (76)	8 (24)	0.006$^{\Phi}$

Legend: Fisher's exact test = Φ.

patient is in disease remission despite serum levels considered inadequate, in which switching therapy based only on serum concentrations carries a potential risk of inconvenient repeated tests and infusions, overtreatment with biologics, increased treatment cost without necessarily achieving the target.[7] Furthermore, tailoring treatments to achieve a predetermined serum level is not considered a therapeutic goal in the management of IBD to date.[16]

When measuring serum concentrations of biologics, some features should be considered: the method by which the measurement was performed, the association between serum concentrations and therapeutic outcomes, and the possibility of individual variability.

The lack of standardization of methods is a significant challenge for TDM. Several techniques allow measurements of the serum concentrations, such as solid phase enzyme-linked immunosorbent assay (ELISA), cell-based reporter gene assay (RGA), fluid-phase radioimmunoassay (RIA), homogeneous mobility shift assay (HMSA), and the point-of-care tests (POCT), which have already been compared with other methods and demonstrated divergent results. Most studies indicated a good correlation between different tests, but some described significant analytical differences, which may compromise the interchangeability of results between other assays. To minimize this problem, experts recommend using the same test over time to facilitate comparative assessments.[6] Our study used only the same ELISA test, which was validated according to technical recommendations and regulatory agencies. This test was chosen for convenience since it was available at our site.

The main reason that supports TDM with biologics is the assumption that there is a positive association between the serum concentration of the drug and a specific therapeutic outcome. Several studies have already described this positive association between serum IFX concentrations and patients' chances of achieving a particular outcome. This has already been demonstrated for all biologics and in different scenarios, mainly in patients using IFX. Supplementary Table B summarizes the findings of the most important studies in regard to this association.[6,17]

On the other hand, some studies during maintenance therapy described a negative association between serum concentrations and patients' chances of reaching a specific outcome, as observed in our study. Edlund et al. performed a post hoc analysis of two studies that evaluated 47 CD patients with SLR who underwent IFX dose optimization. The authors found no association between IFX concentrations and specific clinical and laboratory outcomes.[18] Gomes et al., in a cross-sectional study with forty patients with CD, did not identify differences between serum IFX concentrations in the groups in remission and disease activity, defined by clinical, endoscopic, and radiological outcomes, in a similar methodology used in our study. However, the population was comprised of a small number of patients, and the values of serum IFX concentrations in their sample were surprisingly high (80% of the samples had serum IFX > 10 μg/mL).[10] Dreesen et al. observed that in 116 CD patients treated with IFX, there was a positive association between serum concentrations and endoscopic outcomes only in a specific subgroup of patients who had their dose optimized to 10 mg/kg of IFX, with no association in patients with regular dosing.[19]

Results from prospective randomized and controlled studies that evaluated serum concentrations of anti-TNF agents proactively or reactively are worth highlighting. Despite the comparison of these two strategies being out of the scope of this study, it is interesting to point out that a clear relationship between serum concentrations of anti-TNFs with different outcomes was not uniformly observed. In the TAXIT trial, more patients undergoing the proactive strategy remained with sustained IFX concentrations within the therapeutic range throughout the maintenance phase (74% vs. 57%, $p < 0.001$). However, there was no difference between the two groups regarding the study's primary objective (clinical and laboratory outcomes – 69% vs. 66%, $p = 0.686$).[20] In the TAILORIX trial, a similar proportion of the three groups (two groups undergoing proactive monitoring and one group undergoing reactive monitoring) had sustained IFX levels > 3 μg/mL throughout the maintenance phase (47% vs. 46% vs. 60%, $p = 0.38$). Still, there was no difference between the three groups regarding clinical and endoscopic outcomes (33% vs. 27% vs. 40%, $p = 0.50$).[21]

The studies with negative associations suggest that reaching a target serum concentration for the patient to achieve clinical outcomes is insufficient. In our study, serum IFX levels in the maintenance phase were similar in the assessment of general disease activity for patients with luminal CD (median of 5.63 [0.03–14.40] in remission vs. 3.84 [0.03–14.40] with active disease, $p = 0.287$). Similarly, de Souza et al. correlated serum ADA concentrations in patients in remission with clinical and endoscopic disease activity and found no differences between the groups.[22]

When types of disease activity were evaluated separately, there was a significant difference in IFX serum concentrations only in the evaluation of clinically active disease (median of 5.63 [0.03–14.40] in remission vs. 2.14 [0.32–10.54] in active disease, $p = 0.042$). It is possible that for this subgroup of patients, measuring serum IFX levels during maintenance therapy makes more sense. However, it is essential to note that clinical disease scores are poorly correlated with endoscopic and radiological disease activity.[16] Still, no differences were identified in the evaluation of endoscopic, radiological, and laboratory activities, with no discriminatory power between patients in remission and active disease. Eventually, with a larger sample of patients, differences could be observed.

Although increased BMI can be associated with a greater chance of adverse outcomes, such as PNR and SLR,[4] patients in remission had a significantly higher BMI than those with active disease (26.15 ± 5.48 vs. 24.39 ± 3.53, $p = 0.008$). Weight gain after initiation of treatment has been previously documented. An explanation for this seems to be the better control of disease activity after the use of IFX, with consequent reversal of the effects of systemic TNF-α on body composition, such as the reduction of proteolysis, in addition to the increase in adipogenesis and appetite-induced using anti-TNF agents.[23]

The definition of cutoff points for serum IFX concentrations is challenging. The TAXIT trial considered the therapeutic range of serum IFX levels in the maintenance phase between 3 and 7 μg/mL.[20] The TAILORIX trial evaluated the therapeutic range of serum IFX concentrations in the maintenance phase > 3 μg/mL.[21] A technical review

by the American Association of Gastroenterology described that if serum levels of IFX in the maintenance phase are $\geq$3 μg/mL, it is expected that 15% of patients should have active disease if they are $\geq$5 μg/mL, proportion is reduced to 8% and if it is $\geq$7 μg/mL or $\geq$10 μg/mL 4% of patients are expected to present active disease. Furthermore, increasing serum IFX levels above $\geq$7 μg/mL or $\geq$10 μg/mL did not appear to significantly increase the proportion of patients in remission.[24] In 2021, a literature review followed by expert consensus suggested that this therapeutic range should be 5–10 μg/mL.[6] In our study, the proportions of patients in remission and disease activity were not influenced by any range of serum IFX concentration, whether between 3 and 7 μg/mL or 5 and 10 μg/mL. The only exception was in the evaluation of endoscopic activity that between serum levels of 3–7 μg/mL, the proportion of patients in remission was higher than that of patients with active disease (76% vs. 24% respectively, $p = 0.047$).

An explanation for these negative correlations between serum levels of IFX and the different outcomes can be found in the study by Fernandes et al. After prospectively following 135 patients with IBD for up to 2 years using a proactive model of treatment adjustment, targeting serum levels between 5 and 10 μg/mL, authors observed a positive association between serum IFX levels at week 14 with clinical outcomes and laboratory tests (calprotectin level < 250 μg/g). In this specific group of patients who achieved laboratory remission at week 14, the serum level of IFX increased progressively from week 14 to the end of follow-up (2.71 vs. 8.54 μg/ml, $p < 0.001$).[25] A similar finding was observed in the post hoc analysis of the TAILORIX trial, in which the reduction in calprotectin levels occurred in parallel with the progressive increase in serum IFX levels in patients with endoscopic remission.[19] This suggests that reducing the inflammatory burden may precede and allow the progressive increase in serum IFX concentrations, as mentioned by Nguyen.[7] By accepting this hypothesis, serum concentrations could represent a consequence of a favorable biological response to the treatment instead of being the reason for the targeted endpoints, as suggested by all positive association studies.

Finally, the influence of individual variability must be considered. IBD is a complex disease with heterogeneous presentations, but so far, with limited treatment options usually chosen without precision medicine strategies. Treatment adjustments could be made according to serum concentrations, even without knowing whether the patient's biological profile is favorable toward response to the agent (pharmacodynamic failure). Some markers have already been described to anticipate the lack of response to anti-TNFs.[26] In an ideal scenario, serum concentrations should be measured after these markers to minimize the risk of inadequate dosage (pharmacokinetic failure).

This study presents some inherent limitations: (a) it was a cross-sectional study performed in a single time interval, with no longitudinal analysis; (b) a convenience sample was used, which may have determined a selection bias (only patients with the adequate response could be captured during the study inclusion period); (c) endoscopic disease activity was not analyzed centrally and as a consequence it was not possible to use endoscopic IBD scores, which could cause bias; (d) the analysis of radiological and laboratory activities through calprotectin measurement were carried out in a reduced number of patients, which makes it difficult to reach a solid conclusion regarding the correlation between infliximab levels during remission and disease activity; (d) due to the COVID-19 pandemic, some patients were unable to perform monitoring tests, what resulted in limited number of patients. On the other hand, the strengths of our study are worth mentioning. This was one of the most significant samples of Latin American patients with IBD measuring IFX concentrations to date and the first that used objective markers of remission and disease activity. In addition, the remission and disease activity criteria represented positive outcomes for patients with a reduced risk of clinical relapses, intestinal structural damage, and disability. Finally, it demonstrates that in the maintenance phase, the definition of remission and disease activity may not be associated with serum IFX levels. Hence, using TDM in this patient profile seems to have little clinical utility. This fact may significantly impact cost reduction by limiting the indications for TDM and avoiding unnecessary optimization.

Conclusion

There were no differences in IFX concentrations in patients with luminal CD who met the criteria for remission or general disease activity. When different forms of disease activity were considered separately, there was also no difference in endoscopic, radiological, and laboratory disease activities. Only patients with clinical disease activity had a significant difference in drug levels. The results of this study allow us to question the positive association between IFX concentrations and the achievement of different therapeutic outcomes. We could speculate an inversion of the associations since adequate serum concentrations of IFX may be a consequence of the reliable performance of the treatment itself and not its cause.

Ethical considerations

The study was performed according to the Declaration of Helsinki, and the study protocol was approved by the Institutional Review Boards of the Pontifical Catholic University of Paraná, under reference number CAAE 12450919.8.1001.0020. All patients provided written informed consent.

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Conflicts of interest

RB Nones has nothing to disclose. EF Miranda has nothing to disclose. GN Marçal has nothing to disclose. FSB Baraúna has nothing to disclose. MR Loures has nothing to disclose. PC Senger has nothing to disclose. DO Magro has nothing to disclose. PG Kotze has been a consulter and speaker for Abbvie, Janssen, Pfizer, and Takeda.

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Appendix A. Supplementary data

Supplementary material associated with this article can be found in the online version available at https://doi.org/10.1016/j.gastrohep.2023.12.011

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