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Original Article

The Influence of Subclinical Active Inflammation on IFX Pharmacokinetic Modeling and Disease Progression Assessment: Findings from a Prospective Real-World Study in Inflammatory Bowel Disease Patients

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Abstract

Background and aims: Effective management of inflammatory bowel disease (IBD) relies on a comprehensive understanding of infliximab (IFX) pharmacokinetics (PK). This study's primary goal was to develop a robust PK model, identifying key covariates influencing IFX clearance (CL), while concurrently evaluating the risk of disease progression during the maintenance phase of IBD treatment.

Methods: The multicenter, prospective, real-world DIRECT study was conducted in several care centers, which included 369 IBD patients in the maintenance phase of IFX therapy. A two-compartment population PK model was used to determine IFX CL and covariates. Logistic and Cox regressions were applied to elucidate the associations between disease progression and covariates embedded in the PK model.

Results: The PK model included the contributions of weight, albumin, antidrug antibody (ADA), and fecal calprotectin (FC). On average, higher ADA, FC concentration and weight, and lower albumin concentration resulted in higher IFX CL. In the multivariate regression analyses, FC levels influenced the odds of disease progression in the majority of its definitions, when adjusted for several confounding factors. Additionally, alongside FC, both IFX and CL demonstrated a significant impact on the temporal aspect of disease progression.

Conclusion: In this 2-year real-world study, readily available clinical covariates, notably FC, significantly impacted IFX availability in IBD patients. We demonstrated that subclinical active inflammation, as mirrored by FC or CRP, substantially influenced IFX clearance. Importantly, FC emerged as a pivotal determinant, not only of IFX pharmacokinetics but also of disease progression. These findings underscore the need to integrate FC into forthcoming IFX pharmacokinetic models, amplifying its clinical significance.

Key Words: inflammatory bowel disease; pharmacokinetic model; infliximab

1. Introduction

Inflammatory bowel diseases (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), refer to the chronic inflammatory disorders of the gastrointestinal tract, driven by a dysregulation of the immune response, resulting in increased secretion of pro-inflammatory cytokines.[1]

The central role of tumor necrosis factor-α (TNF-α) in IBD has led to the widespread use of TNF-α inhibitors for treatment. However, many patients do not respond adequately to these drugs, prompting interest in therapeutic drug monitoring to improve their effectiveness.[2–5] Despite ongoing research into serum infliximab (IFX) trough concentrations and clearance (CL), challenges such as immunogenicity and variable treatment responses persist.[6–9]

The association between serum IFX trough concentrations and specific disease outcomes has also been explored, but the analyses did not contemplate the disease as a whole.[7,10–15] Considering this scenario and the current concerns regarding IBD treatment, the development of models that can predict the outcome of IFX treatment, with higher precision and reliability, has opened new perspectives to ascertain disease progression and improve the well-being of patients.[16–19] However, most of the available studies have short follow-up periods, lacking consistency.

In parallel, disease monitoring can also rely on the assessment of clinical activity through non-invasive biomarkers. In fact, the identification of reliable biomarkers that predict therapeutic outcomes can influence the control of disease progression and patient well-being. Among these, fecal calprotectin (FC) has been identified as a key biomarker to distinguish IBD from functional disorders, monitor disease activity and response to therapy, and predict clinical relapse.[20,21] Despite its recognized importance as a reference biomarker, FC has only recently been included as a covariate in pharmacokinetic (PK) models for IFX in IBD.[17]

Considering the exposed unmet needs, this prospective observational study aimed to: 1) identify the factors that affect IFX CL; 2) develop a PK model to explain IFX CL in an IBD population in the maintenance phase of treatment; 3) assess the importance of FC in IFX clearance models; and 4) determine the relationship between the covariates of the PK model, including FC, and disease progression in IBD patients treated with IFX during the maintenance phase.

2. Materials and methods

2.1. Study design and participants

The DIRECT study was a multicenter, prospective, real-world study conducted at eight Portuguese hospital centers, between May 2016 and October 2019. The inclusion criteria were 1) more than 18 years of age; 2) registration in the Portuguese IBD group (GEDII) registry; 3) diagnosis of moderate-to-severe active CD or UC; and 4) IFX treatment. Clinical activity was defined by: 1) abdominal pain score > 1 or liquid-to-very soft stool frequency > 1.5, in the case of CD; or 2) rectal bleeding > 0 or stool frequency > 1, in UC patients; in two consecutive or at least three not necessarily consecutive visits during the follow-up period in patient-reported outcome 2 (PRO-2) questionnaire answers.[22]

This analysis contemplated all DIRECT study patients diagnosed with CD or UC who received IFX treatment at least 14 weeks before study entry (maintenance phase). IFX was administered in a 5 or 10 mg/kg dose, as a 3-hour intravenous infusion at 4, 6, or 8-week intervals for a follow-up period of 2 years, according to routine practice. Blood samples were collected just before drug administration to assess IFX concentration in addition to all biochemical parameters. Stool samples were also collected at each time point to evaluate FC levels. Additional details regarding the methodology behind the DIRECT study can be found in the Supplementary Material section.

2.2. Ethical considerations

All patients were informed about the purpose of the study and signed a written informed consent before enrolment. The study was centrally monitored, approved by all local ethics committees and by the Portuguese Data Protection Authority, and conducted according to the principles of the Declaration of Helsinki.

2.3. Population pharmacokinetic (PK) modeling

PK analysis was conducted using a nonlinear mixed effects modeling approach with NONMEM (version 7.4, ICON, Ellicott City, Maryland) in conjunction with a g95 (64-bit) compiler using Perl-Speaks NONMEM (PsN, version 4.7.0) as an interface to manage NONMEM runs and perform computational tasks. R (version 4.0.1) and various packages (including dplyr, ggplot2, xpose) in RStudio (version 1.1.447) were used for exploratory analysis and post-processing of NONMEM outputs.

As previously published, the PK profile of IFX was described by a first-order distribution and elimination model.[7] The structural model was fitted to IFX concentration-time data while fixing one or more parameters to data reported in previous studies, assuming eventual variability between patients.

Finally, we employed the frequentist prior informative approach during PK parameter estimation.[7,23] All runs were evaluated, and consequently, the base IFX model was obtained.

Then, we tested potential predictors of the IFX CL from a comprehensive set of available data. If clinical plausibility and predefined statistical criteria were met, the covariates were retained in the model. Continuous covariates were weight, age, antidrug antibody (ADA), C-reactive protein (CRP), erythrocyte sedimentation rate, albumin, hemoglobin, leukocytes, neutrophils, lymphocytes, iron, ferritin, transferrin, and FC levels. Categorical covariates were IBD type, sex, presence of ADA, and concomitant treatment with immunomodulating drugs.

Final model appropriateness was performed using both numerical and graphical diagnostics, such as goodness-of-fit plots. Further details regarding the population PK analysis methodology are available in the Supplementary Material section.

2.4. Disease progression outcomes

Five different composite outcomes (CO) reflective of disease progression were applied. The global composite outcome with symptoms included clinical-related (first occurrence of IBD-related surgery or hospitalization; new fistulae, abscess or stricture; or isolated symptomatology) and drug-related items (first occurrence of prescription with at least one course of oral corticosteroids or more than 10 mg of prednisolone per day, or prescription with *de novo* azathioprine or methotrexate,

Table 1. Summary of clinical and socio-demographic characteristics of patients.

n (%) or range	CD (n = 288)*	UC (n = 81)*	Total (n = 369)*	*p*-value**
Baseline data				
Age (years), mean (SD)	38.75 (12.74)	45.00 (15.37)	40.12 (13.59)	**<0.01**
Age at diagnosis (years), mean (SD)	27.88 (11.15)	35.62 (14.18)	29.58 (12.29)	**<0.01**
Gender				
Male	148 (51%)	32 (40%)	180 (49%)	0.06
Female	140 (49%)	49 (60%)	189 (51%)	
Smoking habits (cigarettes per day)				
Never smoked	148 (52%)	53 (65%)	201 (55%)	**0.04**
< 10	42 (15%)	4 (5%)	46 (12%)	
≥ 10 and < 20	21 (7%)	2 (3%)	23 (6%)	
> 20	7 (2%)	1 (1%)	8 (2%)	
Ex-smoker	70 (24%)	21 (26%)	91 (25%)	
Weight (kg), median (P25-P75)	69 (59-78)	66 (58-78)	69 (59-78)	0.40
Body mass index (kg/m^2), median (P25-P75)	24 (21-27)	24 (22-26)	24 (22-27)	0.78
Disease location of CD				
L1	112 (39%)	–	–	–
L2	49 (17%)	–	–	–
L3	127 (44%)	–	–	–
Upper GI involvement				
No	243 (84%)	–	–	–
Yes	45 (16%)	–	–	–
Disease classification of CD				
B1	144 (50%)	–	–	–
B2	56 (19%)	–	–	–
B3	89 (31%)	–	–	–
Disease extension of UC				
E1	–	1 (1%)	–	–
E2	–	37 (46%)	–	–
E3	–	42 (52%)	–	–
Perianal disease				
No	190 (66%)	81 (100%)	271 (73%)	**<0.01**
Yes	98 (34%)	0 (0%)	98 (27%)	
Corticodependant				
No	191 (66%)	42 (52%)	233 (63%)	**0.02**
Yes	97 (34%)	39 (48%)	136 (37%)	
Corticoresistant				
No	282 (98%)	78 (96%)	360 (98%)	0.40
Yes	6 (2%)	3 (4%)	9 (2%)	
History of previous surgery				
No	131 (46%)	77 (95%)	208 (56%)	**<0.01**
Yes	157 (54%)	4 (5%)	161 (44%)	
Duration of IFX therapy until study enrolment (months), mean (SD)	50.69 (39.42)	43.02 (31.18)	49.01 (37.86)	0.28
Infliximab drug				
Originator	239 (83%)	68 (84%)	307 (83%)	0.15
Biosimilar	49 (17%)	13 (16%)	62 (17%)	
IFX dose (mg/kg)				
5	213 (74%)	55 (68%)	268 (73%)	0.28
10	75 (26%)	26 (32%)	101 (27%)	
IFX treatment frequency (weeks)				
4	2 (1%)	2 (2%)	4 (1%)	0.19
6	79 (27%)	27 (34%)	106 (29%)	
8	207 (72%)	51 (64%)	258 (70%)	
Biomarkers Data				
Anti-drug antibody concentration (µg/mL), n (%)				

Table 1. Continued

n (%) or range	CD (n = 288)*	UC (n = 81)*	Total (n = 369)*	*p*-value**
< 1.7	247 (87%)	67 (86%)	314 (87%)	0.86
≥ 1.7	38 (13%)	11 (14%)	49 (13%)	
CRP concentration (mg/L), median (P25-P75)	3.13 (1.00-7.55)	1.94 (0.78-4.90)	2.64 (1.00-6.80)	**0.04**
Ferritin concentration (ng/L), median (P25-P75)	66.90 (38.10-118.85)	84.40 (38.85-184.55)	71.80 (38.70-129.52)	0.07
Iron concentration (µg/L), median (P25-P75)	82.00 (62.00-108.70)	84.00 (65.20-107.90)	82.50 (63.25-108.40)	0.58
Transferrin concentration (mg/dL), median (P25-P75)	254.00 (224.00-289.50)	247.50 (213.00-287.25)	253.00 (222.00-289.00)	0.25
Albumin concentration (g/L), median (P25-P75)	41.60 (39.48-43.80)	41.50 (39.73-43.40)	41.60 (39.60-43.68)	0.61
FC concentration (µg/g), median (P25-P75)	243.00 (78.00-545.00)	322.50 (99.50-613.00)	253.00 (80.00-569.00)	0.50
Data collected at all time-points				
Treatment data				
Mesalazine				
No	257 (90%)	44 (54%)	301 (82%)	**<0.01**
Yes	30 (10%)	37 (46%)	67 (18%)	
Corticosteroids				
No	275 (96%)	73 (90%)	348 (95%)	0.05
Yes	12 (4%)	8 (10%)	20 (5%)	
Thiopurines				
No	156 (55%)	46 (57%)	202 (55%)	0.72
Yes	130 (45%)	35 (43%)	165 (45%)	
Methotrexate				
No	276 (96%)	77 (95%)	353 (96%)	0.66
Yes	11 (4%)	4 (5%)	15 (4%)	
IFX concentration (µg/mL), median (P25-P75)***	4.50 (2.40-7.60)	5.00 (1.87-9.00)	4.60 (2.30-8.00)	0.14
Anti-drug antibody concentration (µg/mL), median (P25-P75)***	0.60 (0.40-1.10)	0.70 (0.40-1.20)	0.60 (0.40-1.10)	**<0.01**
Events data				
Occurrence of abscesses/fistulas				
No	185 (65%)	76 (94%)	261 (71%)	**<0.01**
Yes	102 (35%)	5 (6%)	107 (29%)	
Surgery				
No	131 (46%)	77 (95%)	208 (56%)	**<0.01**
Yes	157 (54%)	4 (5%)	161 (44%)	

–, not applicable or data not collected;.
*Total percentages may not sum up to 100% due to missing data; ** Chi-square or Mann-Whitney tests results with significant *p*-values in bold;
***Corresponding to a total of 4402 measurements of 369 patients (CD:3422; UC: 980).
CD: Crohn's Disease; CRP: C-reactive protein; FC: fecal calprotectin; GI: gastrointestinal; IFX: infliximab; P: percentile; SD: standard deviation; UC: ulcerative colitis.

or swap of biologic therapy [to adalimumab, golimumab, vedolizumab, or ustekinumab], or azathioprine dose increase other than by weight fluctuation, or IFX dose escalation or interval reduction). The global composite outcome without symptoms did not include the isolated symptomatology item. The global composite outcome with symptoms without IFX did not take into account IFX dose escalation or interval reductions. The clinical-related composite outcome was composed solely of clinical-related items, while the drug-related one focused on drug-related items. These endpoints were used in previous studies.[22,24,25]

2.5. Statistical analysis

Categorical variables were summarized by absolute and relative frequencies, while continuous variables were described by their median and interquartile range (P25-P75). Associations between disease type and continuous variables were assessed using Mann-Whitney U tests. Categorical variables were compared using either the Chi-square or Fisher's exact test.

The assessment of the probability of disease progression was conducted both at a specific moment and within the context of temporal evolution. We explored the univariate and multivariate effects of the median of each variable encompassed within the PK model on each composite outcome using logistic regressions, Cox proportional hazard models, and Kaplan-Meier curves. Multivariate analyses were adjusted to several confounding factors (gender, age, IBD type, treatment dosage and frequency, and time to initiate IFX). Odds ratios (OR) and hazard ratios (HR) were estimated with 95% confidence intervals (CI). Receiver operating characteristic (ROC) curve analyses were also performed for the multivariate logistic regression models and areas under the curve (AUC)

were estimated. All p-values were two-sided, with the significance level set at 5%. Data were processed using RStudio (version 1.1.447).

3. Results

3.1. Demographic and clinical characteristics

A total of 369 patients (CD: 288; UC: 81) were included in the present analysis of the DIRECT cohort (Supplementary Figure 1). Overall, patients presented a mean age of 40.12 (SD: 13.59) years and were diagnosed at a mean age of 29.58 (SD: 12.29); 51% were female (Table 1). At baseline, patients had been treated with IFX for a mean of 49 (SD: 37.86) months; 83% received the originator drug. Most participants received IFX at 5 mg/kg every eight weeks (Table 1). Median (interquartile range [IQR]) CRP and FC concentrations were 2.64 (1.00-6.80) mg/L and 253.00 (80.00-569.00) µg/g, respectively (Table 1). In the CD group, 44% of patients presented an ileocolonic location and 47% had an inflammatory behavior. On the other hand, most UC patients (52%) had pancolitis (Table 1).

3.2. Development of the pharmacokinetic (PK) model

For the development of the PK model for IFX CL, the whole study population (369 patients) contributed with 4402 measurements. A two-compartment PK model was fitted to the data; CL was estimated as only trough concentrations were available.

The covariates to be included in the model were identified based on exploratory graphics and scientific interest. Strong associations were found between IFX CL and weight, albumin, ADA, and FC concentrations (Figure 1). On the other hand, covariates such as age, IFX dosage and frequency, thiopurine usage, hemoglobin, iron concentrations, gender, or IBD type did not present an effect on CL (Supplementary Figure 2). Moreover, CRP concentration was not included in the model, given its correlation with FC concentration.

PK parameter estimates and 95% CI from the final population PK model are shown in Supplementary Table 1. All parameters were estimated with good precision, as indicated by the relative standard error (RSE) percentage of the estimates (lower than 25%). The interindividual variability in CL was 18.1%, indicative of moderate unexplained variability. On the other hand, the residual error (unexplained random residual variability in the model) was 6.3%, which is considered small (Supplementary Table 1).

Furthermore, goodness-of-fit plots showed consistency between the observed and predicted data, with no observation of abnormal trends in the conditional weighted residuals versus time and individual- or population-predicted serum IFX concentrations (Supplementary Figures 3 and 4).

The final PK model for IFX CL can be described by the following equation:

$$CL\left(\frac{L}{day}\right) = 0.275 \cdot e^{0.0461\cdot(ADA-0.5)} \cdot \left(1+\frac{5.63}{100}\right)\cdot ALB_{<36}\cdot\left(\frac{WT}{70}\right)^{0.411}\cdot \left(1+0.0215\cdot\ln\left(\frac{FC}{50}\right)\right)$$

For a typical patient (Weight [WT] = 70 kg; Antidrug antibody [ADA] ≤ 0.5 µg/mL; Albumin [ALB] > 36 g/L; Fecal calprotectin [FC] ≤ 50 µg/g), IFX CL was 0.275 (SE: 0.0045) L/day.

All considered variables were continuous, except for albumin. Our model demonstrated that when patients presented with an ADA concentration of 8 µg/mL, a weight of 80 kg, albumin levels of 34 g/L, and FC levels at 700 µg/g, their CL increased by 66.61% compared to the reference. Similarly, for a typical patient presenting solely with elevated FC levels of 1000 µg/g, CL exhibited a 12.43% increase.

3.3. Disease progression assessment

To elucidate the association between IFX treatment and disease progression, we assessed the distribution of IFX concentration (median and inter-quartile range) for each definition of disease progression through a box-and-whisker plot (Supplementary Figure 5). Notably, the median IFX concentrations were lower in patients who achieved the composite outcomes. This finding is further supported by the observation that the 25th and 75th percentiles were also generally lower in these patients. Collectively, these results suggest that IFX treatment may be associated with a lower risk of disease progression.

3.4. Disease progression assessment—logistic regression analyses

Considering the variables used in the PK analysis, univariable logistic regressions revealed that median CL, IFX, FC, and CRP concentrations displayed a significant association with most composite outcomes (Supplementary Table 2). These variables were then inputted into multivariate models (either IFX + FC + CRP or CL + FC + CRP), in which FC levels remained statistically significant when adjusted to several confounding factors (gender, age, IBD type, treatment dosage and frequency, and time to initiate IFX). Conversely, IFX trough concentrations below 3 µg/mL showed significant associations with three out of five composite outcomes, while CRP lost statistical significance (Figure 2A and Supplementary Table 3A). Additionally, when CL was substituted for IFX concentration in the multivariate model, it demonstrated a significant association with only one of the five composite outcomes (Figure 2B and Supplementary Table 3B).

ROC curve analyses were also performed for both multivariate models in association with each composite outcome (Figure 3). The models demonstrated good performance in differentiating patients who developed disease progression from those who did not. The AUC values for the IFX + FC + CRP model ranged from 0.705 to 0.830, while those for the CL + FC + CRP model varied between 0.794 to 0.826. Higher AUC values indicate greater discrimination between the two groups.

3.5. Disease progression assessment—time-to-event analyses

In time-to-event analyses, we adopted a similar approach to our previous assessment, focusing on the influence of PK model variables on the timing of disease progression. CL, IFX, FC, and CRP emerged as significant factors (Supplementary Figures 6-10 and Supplementary Table 4).

When included in a multivariate Cox regression model alongside potential confounders, FC's impact on the timing of composite outcomes became evident. Patients with FC

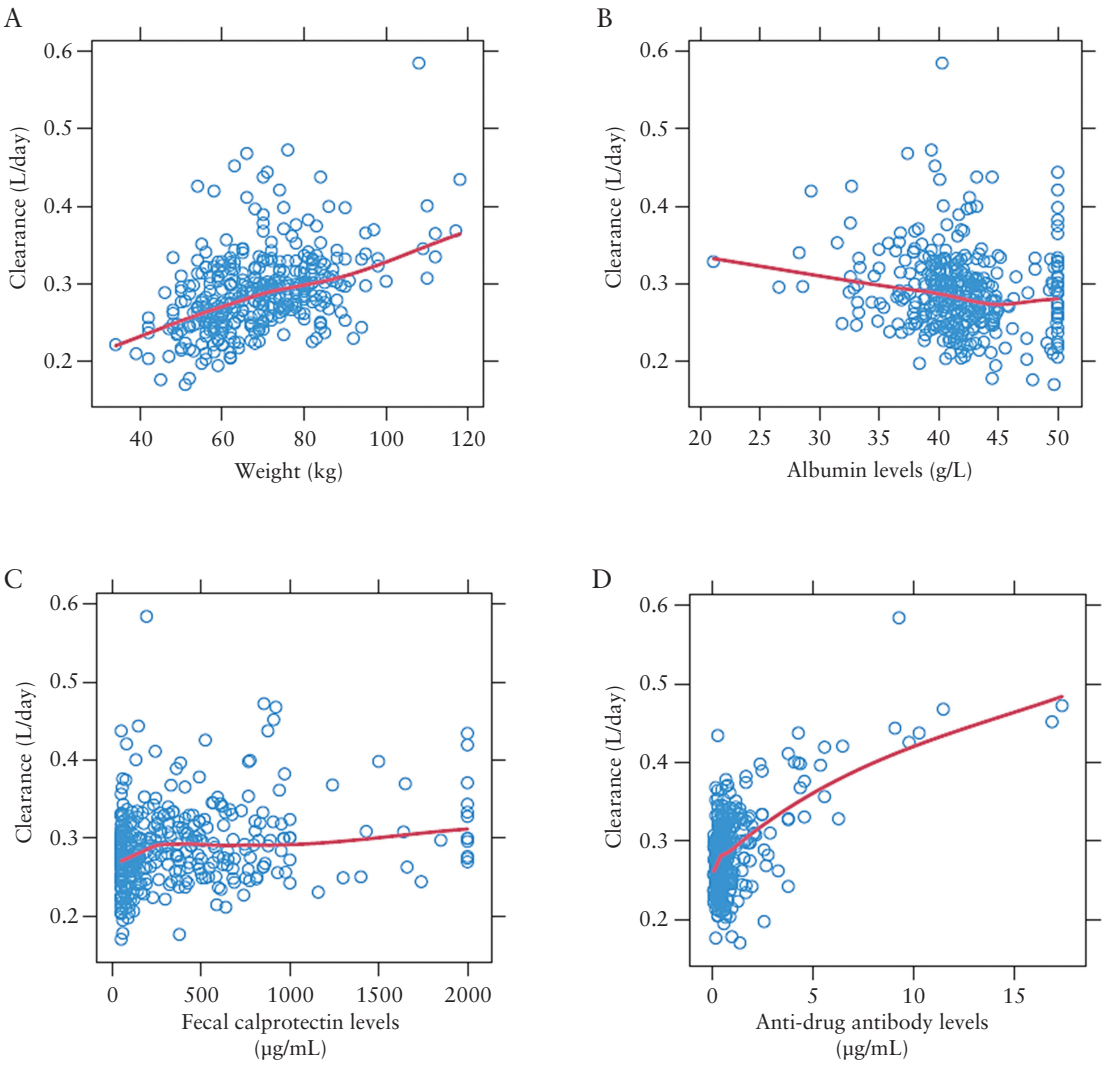


Figure 1. Empirical Bayesian estimates of individual IFX clearance (L/day) and covariates presenting strong associations. (A) Weight (kg); (B) Albumin levels (g/L); (C) Fecal calprotectin levels (µg/g); (D) Anti-drug antibody levels (µg/mL). ADA: anti-drug antibody; FC: fecal calprotectin; IFX: infliximab; IFX CL: infliximab clearance.

levels above 250 µg/g experienced disease progression sooner in both IFX + FC + CRP and CL + FC + CRP models. In contrast, IFX concentrations below 3 µg/mL were associated with an earlier occurrence of three out of the five composite outcomes (Figure 4A and Supplementary Table 5A). Higher CL levels were also significantly associated with one of the outcomes (Figure 4B and Supplementary Table 5B). On the other hand, CRP lost statistical significance in this time-dependent context.

4. Discussion

In this study, we developed a population PK model for IFX administered to IBD patients during the maintenance phase, using data from the multicenter prospective DIRECT study. The model herein established allowed for the PK characterization of the population over time while considering the variability between patients and clinically relevant covariates. The results showed that higher ADA, FC concentrations and weight, and lower albumin concentration resulted in higher IFX CL. Additionally, other baseline variables such as age, gender, IBD type, IFX dosage and frequency, thiopurine usage, hemoglobin, and iron levels did not show any influence on IFX CL. Consistency in goodness-of-fit plots indicated that the developed population PK model described our real-world data.

While the effect of other covariates has been deeply investigated in IBD patients,[7,15,16,18,26–29] the role of FC levels in IFX PK during the maintenance phase has not been fully explored. In fact, to our knowledge, FC has only been included as a covariate in a PK model developed in the scope of a previous phase 4 dose-escalation study involving patients with CD.[17] Our study evidenced for the first time a positive association between FC levels and IFX CL and consequently with IFX levels. These results are in line with those of the referred phase 4 study and evidenced the importance of including FC as a covariate in PK models, in the setting of IBD.

Regarding other covariates, the decrease of IFX CL with albumin levels is also in agreement with the results of previous reports.[7,15,16,18,26–29] On the other hand, concerning the effect of weight on IFX CL, past research has retrieved contradictory results.[7,26,27] In addition, even though co-therapy

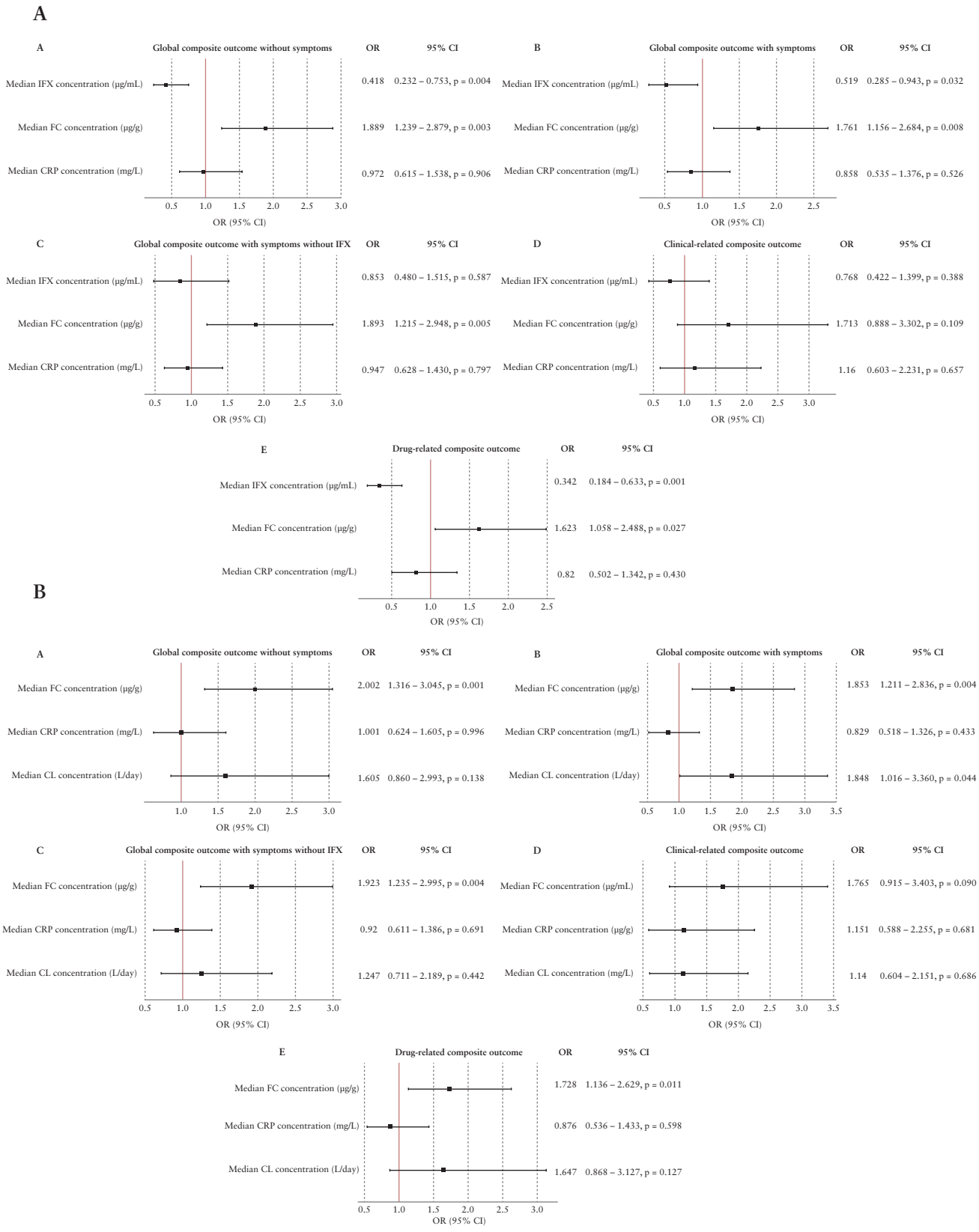
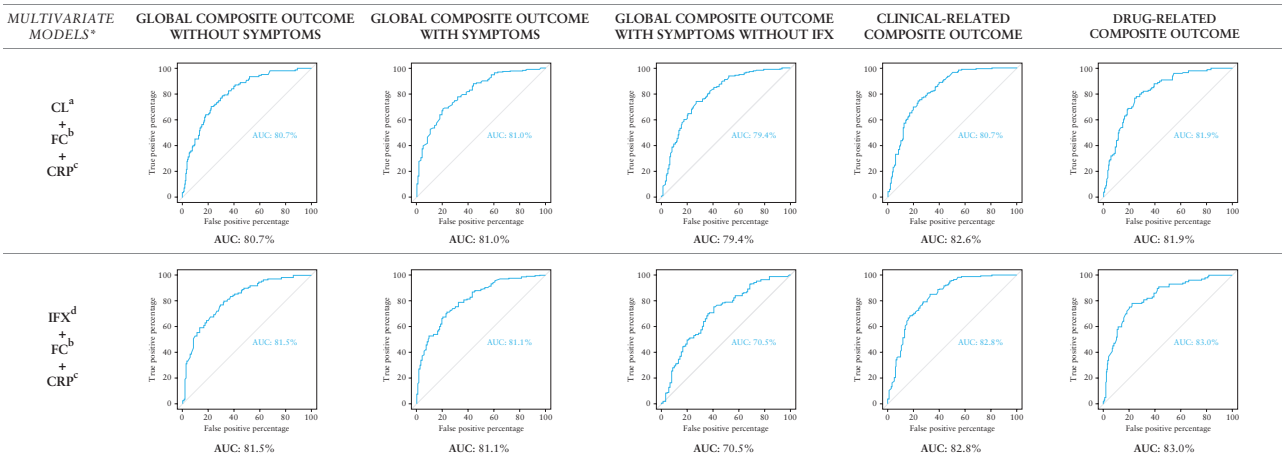


Figure 2. A. Forest plot showing multivariate logistic regression (IFX + FC + CRP) analysis of the effect of different parameters on: (A) Global composite outcome without symptoms, (B) Global composite outcome with symptoms, (C) Global composite outcome with symptoms without IFX, (D) Clinical-related composite outcome, and (E) drug-related composite outcome. B. Forest plot showing multivariate logistic regression (CL + FC + CRP) analysis of the effect of different parameters on: (A) Global composite outcome without symptoms, (B) Global composite outcome with symptoms, (C) Global composite outcome with symptoms without IFX, (D) Clinical-related composite outcome, and (E) drug-related composite outcome. CI: confidence interval; CL: clearance; CRP: C-reactive protein; FC: fecal calprotectin; IFX: infliximab; OR: odds ratio.



*Multivariate models were adjusted to the following confounder factors: gender, age, IBD type, treatment dosage and frequency, and time to initiate IFX.
[a]CL levels are categorized into the following: ≤ 0.3, >0.3 L/day.
[b]FC concentration is categorized into the following: < 250, 250-750, ≥ 750 µg/g.
[c]**CRP concentration is categorized into the following: ≤ 3, 3-10, ≥10 mg/L.**
[d]**IFX concentration is categorized into the following: < 3, ≥ 3 µg/mL.**

Figure 3. ROC curves of the multivariate logistic regression models for each composite outcome. AUC: Area under the curve; CL: clearance; CRP: C-reactive protein; FC: fecal calprotectin; IFX: infliximab.

with immunomodulators has been previously described as a covariate with impact on IFX PK, we did not observe a direct association between this covariate and IFX CL.[7,15,27]

In our model, the IFX CL value obtained for a typical patient (0.275 L/day) is in line with data from previous research. However, higher IFX CL values can also be found in the literature.[7,27,29] These discrepancies may be related to the type of model (one-compartment or two-compartments) but also to the covariates used in each PK model and to the inclusion of time as a variable. The volume of peripheral and central compartments was also in concordance with those reported in the literature for IFX and monoclonal antibodies.[26,27,30]

During the development process, CRP was excluded as a covariate from the PK model, since the existing correlation between CRP and FC levels could hide a possible effect of the latter. However, we opted to explore CRP in the subsequent analyses due to its clinical relevance and association with IFX CL.

Logistic regression and time-to-event analyses were carried out to assess the impact of the PK model covariates on IBD progression. Multivariate logistic regression models were used, adjusted for potential confounding factors including gender, age, IBD type treatment dosage and frequency, as well as the time taken to initiate IFX, to account for their influence on the outcomes. FC concentration was found to be a significant predictor for most definitions of disease progression, while CRP did not show a similar effect. Additionally, IFX and CL levels lost statistical significance for composite outcomes when not considering IFX dose adjustments or interval reductions. This trend might be attributed in part to the use of median IFX concentration values, potentially diminishing sensitivity to punctual variations and recent measurements. Furthermore, FC is a more direct measure of gut inflammation than low levels of IFX.

Time-to-event analyses showed that IFX and FC levels were positively associated with the earlier occurrence of the majority of the composite outcomes, while CL was found to be a predictor of one of the outcomes. Notably, the statistical significance of IFX levels in relation to these outcomes only emerged when considering variations in the frequency of administration and dosage of the drug over time. Consequently, our data suggests that FC levels may serve as a more robust and earlier biomarker for monitoring the progression of IBD. These results are in line with previous reports that evidenced the applicability of FC levels in predicting disease relapse and monitoring disease activity and therapy effectiveness.[21] For instance, FC levels have been shown to predict relapse in IBD patients under maintenance IFX therapy.[31] In the case of UC patients in the maintenance phase, it has been proposed that two consecutive FC level measurements above 300 µg/g may predict relapse.[32]

This study has some limitations that shall be discussed. First, though this is a prospective study, its observational nature increased the risk of bias and posed limitations to the development of the model. In fact, due to the observational nature of the study, we could not estimate all model parameters while obtaining an adequate range of parameter values, their uncertainties, successful minimization, and the number of gradients in the final estimation step. Therefore, we tried fitting the structural model to IFX concentration-time data while fixing one or more model parameters to values reported in previous publications, assuming the presence/absence of variability between patients in distribution parameters.

Overall, the relevance of the PK model developed in the scope of this study is based on the inclusion of key biomarkers such as FC, which was shown to have an impact on all composite outcomes, and on the use of prospective data obtained from the systematic observation of a large cohort of IBD patients in the maintenance phase of treatment, over a period of 2 years. In fact, previous studies included data from small cohorts of patients (in the induction or at specific points of the maintenance phase of treatment) followed by short periods (maximum 1 year).

Our findings showcased the substantial impact of inflammatory burden, reflected by FC levels (which are correlated with CRP), on IFX clearance. This underscores FC's pivotal role as a non-invasive biomarker in IBD patients receiving IFX maintenance therapy and the imperative need for its integration into PK models for this drug. As the development of composite outcomes depends on the combined effect of

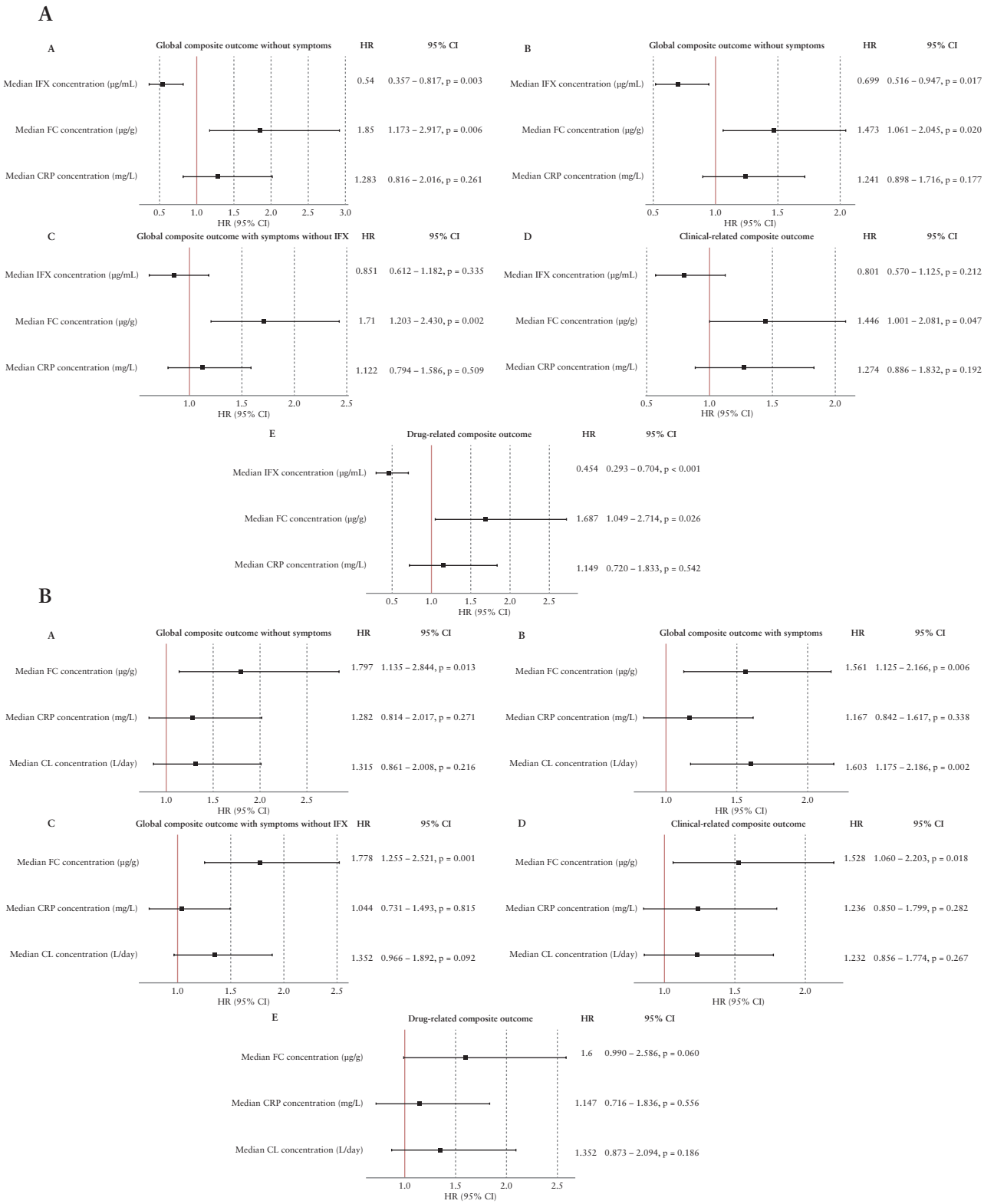


Figure 4. A. Forest plot showing multivariate Cox regression (IFX + FC + CRP) analysis of the effect of different parameters on: (A) Global composite outcome without symptoms, (B) Global composite outcome with symptoms, (C) Global composite outcome with symptoms without IFX, (D) Clinical-related composite outcome, and (E) drug-related composite outcome. B. Forest plot showing multivariate Cox regression (CL + FC + CRP) analysis of the effect of different parameters on: (A) Global composite outcome without symptoms, (B) Global composite outcome with symptoms, (C) Global composite outcome with symptoms without IFX, (D) Clinical-related composite outcome, and (E) drug-related composite outcome. CI: confidence interval; CL: clearance; CRP: C-reactive protein; FC: fecal calprotectin; HR: hazard ratio; IFX: infliximab.

different variables, our results sustain the monitoring of combined disease activity measures, including several different variables, and may support more informed and sustained clinical decision-making processes. In this process, healthcare professionals may have conditions to define more effective treatment plans for patients in which FC levels are misaligned with the reference values.

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Disclosures

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Author contributions

FM: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project Administration, Supervision, Writing (original draft, review, and editing).
SF: Investigation, Writing (review and editing)
MME: Investigation, Writing (review and editing)
MP: Investigation, Writing (review and editing)
BA: Investigation, Writing (review and editing)
PL: Investigation, Writing (review and editing)
IR: Investigation, Writing (review and editing)
HTS: Investigation, Writing (review and editing)
PM: Investigation, Writing (review and editing)
IM: Investigation, Writing (review and editing)
AV: Investigation, Writing (review and editing)
JC: Investigation, Writing (review and editing)
JM: Investigation, Writing (review and editing)
JR: Investigation, Writing (review and editing)
EC: Investigation, Writing (review and editing)
PS: Investigation, Writing (review and editing)
FP: Investigation, Writing (review and editing)
LC: Investigation, Writing (review and editing)
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SDi: Data Curation, Project Administration, Supervision, Writing (review and editing)
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Data availability

Individual participant data will not be shared. Data are available upon reasonable request. Qualified researchers should contact the Portuguese IBD Study Group (GEDII) at geral@gedii.pt.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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