EBioMedicine 21 (2017) 123–130

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Calprotectin and the Magnitude of Antibodies to Infliximab in Clinically-stable Ulcerative Colitis Patients are More Relevant Than Infliximab Trough Levels and Pharmacokinetics for Therapeutic Escalation

Fernando Magro [a,b,c,*], Joana Afonso [b], Susana Lopes [c], Rosa Coelho [c], Raquel Gonçalves [d], Paulo Caldeira [e], Paula Lago [f], Helena Tavares de Sousa [g,h,u], Jaime Ramos [i], Ana Rita Gonçalves [j], Paula Ministro [k], Isadora Rosa [l], Ana Isabel Vieira [m], Patrícia Andrade [c], João-Bruno Soares [d], Diana Carvalho [i], Paula Sousa [k], Tânia Meira [m], Joanne Lopes [n], Joana Moleiro [l], Cláudia Camila Dias [o,p], Amílcar Falcão [q,r], Karel Geboes [s], Fatima Carneiro [n,t], on behalf of the Portuguese IBD Study Group (GEDII)

[a] Department of Biomedicine, Unity of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, Porto, Portugal
[b] MedInUP, Centre for Drug Discovery and Innovative Medicines, University of Porto, 4200 Porto, Portugal
[c] Gastroenterology Department, Centro Hospitalar São João, Porto, Portugal
[d] Gastroenterology Department, Hospital de Braga, Braga, Portugal
[e] Gastroenterology Department, Centro Hospitalar do Algarve, Faro, Portugal
[f] Gastroenterology Department, Centro Hospitalar do Porto, Porto, Portugal
[g] Gastroenterology Department, Centro Hospitalar do Algarve, Portimão, Portugal
[h] Biomedical Sciences and Medicine Department, University of Algarve, Faro, Portugal
[i] Gastroenterology Department, Centro Hospitalar de Lisboa, Lisboa, Portugal
[j] Gastroenterology Department, Centro Hospitalar Lisboa Norte, Lisboa, Portugal
[k] Gastroenterology Department, Hospital de S. Teotónio, Viseu, Portugal
[l] Gastroenterology Department, Instituto Português de Oncologia de Lisboa, Lisboa, Portugal
[m] Gastroenterology Department, Hospital Garcia de Orta, Almada, Portugal
[n] Department of Pathology, Centro Hospitalar São João, Porto, Portugal
[o] Department of Community Medicine, Information and Health Decision Sciences, Faculty of Medicine, University of Porto, Porto, Portugal
[p] CINTESIS - Centre for Health Technology and Services Research, Porto, Portugal
[q] Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal
[r] CNC – Centre for Neuroscience and Cellular Biology, Coimbra, Portugal
[s] Department of Pathology, University Hospital of KU Leuven and UZ Gent, Leuven, Belgium
[t] Institute of Molecular Pathology and Immunology of the University of Porto [Ipatimup], University of Porto, Porto, Portugal
[u] ABC-Algarve Biomedical Center, University of Algarve, Faro, Portugal

ARTICLE INFO

Article history:
Received 28 April 2017
Received in revised form 23 May 2017
Accepted 3 June 2017
Available online 7 June 2017

Keywords:
Ulcerative colitis
Antibodies to infliximab
Fecal calprotectin
Therapeutic escalation

ABSTRACT

Although infliximab (IFX) is an efficient therapy for ulcerative colitis (UC) patients, a considerably high rate of therapeutic failures still occurs. This study aimed at a better understanding of IFX pharmacokinetics and pharmacodynamics among clinically-asymptomatic UC patients. This was a multicentric and prospective study involving 65 UC patients in the maintenance phase of IFX therapy. There were no significant differences between patients with positive and negative clinical, endoscopic and histological outcomes concerning their IFX trough levels (TLs), area under the IFX concentration *vs.* time curve (AUC), clearance and antibodies to infliximab (ATI) levels. However, the need to undergo therapeutic escalation later in disease development was significantly associated with higher ATI levels (2.62 μg/mL *vs.* 1.15 μg/mL, $p = 0.028$). Moreover, and after adjusting for disease severity, the HR (hazard ratio) for therapeutic escalation was significantly decreased for patients with an ATI concentration below 3 μg/mL (HR = 0.119, $p = 0.010$), and increased for patients with fecal calprotectin (FC) level above 250 μg/g (HR = 9.309, $p = 0.018$). In clinically-stable UC patients, IFX pharmacokinetic features cannot predict therapeutic response on a short-term basis. However, high levels of ATIs or FC may be indicative of a future therapeutic escalation.

* Corresponding author at: Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 420-319 Porto, Portugal.
E-mail address: fm@med.up.pt (F. Magro).

http://dx.doi.org/10.1016/j.ebiom.2017.06.004

1. Introduction

The knowledge of the crucial role played by the tumor necrosis factor α (TNFα) on the pathophysiology of auto-immune inflammatory disorders, such as inflammatory bowel diseases (IBD), led to the development of a class of biological drugs that target this cytokine. Infliximab (IFX) was the first anti-TNFα approved for the treatment of IBD (Danese et al., 2015). Since its introduction, IBD patients experienced an improvement in their quality of life, a decrease on the number of bowel-related surgeries and hospitalizations, and an increase in steroid-free remission and mucosal healing rates (Gecse et al., 2016; Strik et al., 2016). Notwithstanding, and despite the therapeutic success of these biological drugs, some patients fail to respond to anti-TNFα in the induction period (primary non-responders), whereas others initially benefit from the treatment but eventually loose response (secondary non-responders) (Mould et al., 2016). Immunogenicity, *i.e.*, the development of anti-drug antibodies, is an unavoidable drawback of biological treatments and a possible explanation for the lack or loss of response. Antibodies to infliximab (ATIs) can directly neutralize the IFX effects by interfering with the TNFα-binding domain, or can affect the drug's clearance rate by forming immune complexes with IFX, thereby promoting its removal from the circulating system (Gecse et al., 2016).

Therapeutic drug monitoring (TDM)-based dosing is an interesting and efficient strategy to overcome IFX lack or loss of response. In order to establish an accurate algorithm to support the decision-making process on a TDM approach, many studies have attempted to elucidate IFX pharmacokinetics and to define therapeutic thresholds for IFX exposure (often using serum trough levels [TLs] as a proxy) and for ATI levels that can guide dose adjustments (Strik et al., 2016; Moore et al., 2016; Williet et al., 2016; Vande Casteele et al., 2015; Warman et al., 2015; Paul et al., 2013; Cornillie et al., 2014).

In parallel with drug monitoring, disease monitoring through non-invasive biomarkers plays an important role in IBD patients, as it allows an assessment of the inflammatory burden without the risks involved in colonoscopy-related procedures. Calprotectin constitutes up to 60% of the cytosolic protein content in granulocytes, and its presence in feces reflects the migration of neutrophils through the inflamed bowel wall to the mucosa (Gisbert and McNicholl, 2009). Recent evidences suggest that fecal calprotectin (FC) levels can be used to discriminate organic from functional disease, to assess disease activity and response to therapy, and to predict relapses (Benítez and García-Sánchez, 2015).

This study aimed to explore IFX pharmacodynamics and to assess the utility of monitoring drug and FC levels among a specific population of ulcerative colitis (UC) patients: those that are asymptomatic and considered to be in remission according to the Montreal classification. The main goal of this study was thus to define how useful – from a clinical point of view – is the monitoring drug, anti-drug antibodies and disease biomarker's levels in clinically-stable patients.

2. Material and Methods

2.1. Patients

UC patients in the maintenance phase of IFX therapy - 5 mg/kg infusions every six or eight weeks - were prospectively and consecutively recruited from 10 different hospitals. Only patients older than 18 years, with at least 14 weeks of IFX treatment and in remission according to the Montreal classification (at baseline and at least in the immediately previous consultation) were invited to participate. Moreover, all patients were in their regimens (6 or 8 weeks-interval infusions) for at least three infusions, to ensure stability. The decision of enrolling these patients in biological therapy had been done previously by the attending physician, following an inadequate response to AZA (azathioprine) or 6-MP (6-mercaptopurine) after a period of treatment equal or superior to three months, intolerance to these agents, or a severe acute relapse. Patients in the 6-weeks infusion interval had been initially allocated to the 8-weeks regimen, but were empirically placed in the shorter interval due to loss of response (LOR). Previously defined concomitant medication was maintained (dose and regimen) throughout the entire study. Exclusion criteria included patients with proctitis only; history of malignancy in the previous five years, opportunistic infections or demyelinating diseases; existence of adenomatous polyps or known viral infections; pregnancy and breastfeeding; and use of topical treatment (5-ASA or steroids) during the study period or in the previous month.

This study was approved by the ethic committee of all hospitals involved and by the Portuguese Data Protection Authority (Comissão Nacional de Protecção de Dados). All patients enrolled did so voluntarily and after signing a written informed consent. The national coordinator of the Portuguese IBD group (GEDII – Grupo de Estudo de Doenças Inflamatórias Intestinais) monitored the study.

2.2. Study Design

This was a multicentric and prospective observational study. All patients were closely monitored for six or eight weeks after an IFX infusion. Demographic and baseline characteristics were collected before the infusion (T = 0), whereas histological, endoscopic and clinical outcomes were assessed immediately before the following infusion (T = 42 or 56 days). IFX and ATIs were quantified 2 h and 14 days after the initial infusion, as well as immediately before the following one (T = 42 or 56 days). The different assessments and their timings are depicted in Fig. 1.

2.2.1. IFX and ATI Quantification

The levels of IFX were quantified using an *in-house* ELISA assay, as previously described by Ben-Horin et al. (Ben-Horin et al., 2011). The presence and amount of ATIs were assessed using the anti-human lambda chain assay (AHLC), an *in-house* ELISA procedure also described by Ben-Horin et al. (Ben-Horin et al., 2011). The ATI concentrations are expressed in μg/mL-equivalent, hereafter referred to as μg/mL for the purpose of brevity. The concentration of IFX at each time point was used to construct a concentration *vs.* time curve. The area under the curve (AUC) was calculated for each individual using the Linear Up/Log Down Trapezoidal method, whereas clearance was computed as the total IFX dose per patient divided by the correspondent AUC.

2.2.2. Endoscopic Activity

Endoscopic activity was evaluated using Ulcerative Colitis Endoscopic Index of Severity (UCEIS) (Travis et al., 2012), and the presence of macroscopic lesions was assessed with the Mayo endoscopic subscore (Schroeder et al., 1987). Patients were considered to be in endoscopic remission whenever UCEIS was below 2, whereas mucosal healing was defined as a Mayo endoscopic sub-score either equal to 0 or lower than 2.

2.2.3. Histological Activity

To assess the presence of histological inflammation, an average of two samples per localization was collected from the sigmoid and rectum. Histological activity was evaluated following the Geboes score (Geboes et al., 2000), and histological remission was defined as a Geboes index lower than 3.1. All samples were the subject of a central reading by two independent pathologists blinded to the patients' disease status and endoscopic results. Disagreements between pathologists were resolved by a review including a third pathologist (K. Geboes) and using a multiheaded microscope, defining the final score.

2.2.4. Clinical Remission

Clinical remission was evaluated according to the Global Mayo score. Patients were considered to be in clinical remission if their global Mayo score was below or equal to 2 and no individual sub-score was above 1.

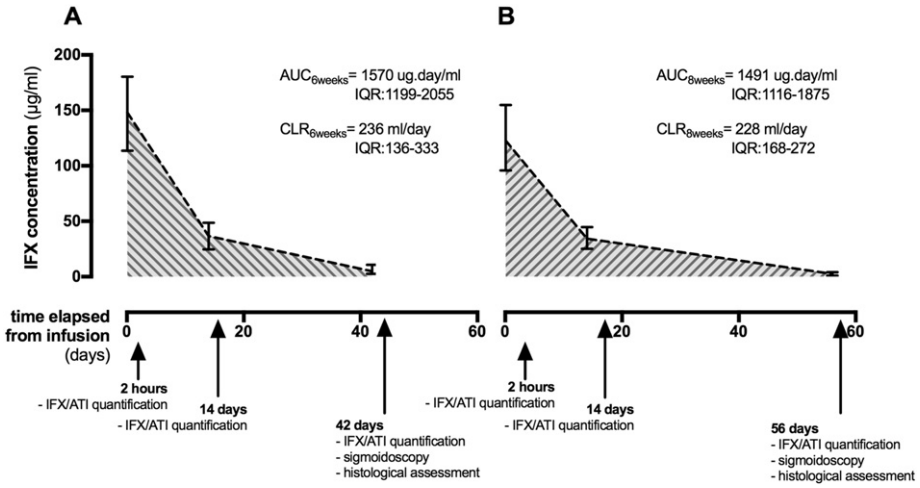

Fig. 1. IFX levels variation throughout time in patients on the 6-weeks (A) or the 8-weeks (B) schedule. The different assessments made during this study and their timing is indicated in the time bar.

2.2.5. FC Quantification

Stool samples were collected and kept at 4 °C (for a maximum of 48 h) until shipment to the central laboratory (Department of Pharmacology and Therapeutics, Faculty of Medicine of University of Porto). FC was extracted from stools within a maximum of seven days after collection using the 'Fecal sample preparation kit' (Roche Diagnostics, Germany) according to the instructions provided by the manufacturer and stored at −80 °C until quantification. FC samples were quantified using a fluoroenzyme immunoassay (EliA Calprotectin®, Thermo Fisher Scientific, Germany) according with manufacturers' instructions.

2.2.6. Assessment of Therapeutic Escalation

The files of all patients included in this study were later assessed in order to evaluate how many required therapeutic escalation. Therapeutic escalation was defined as has been previously suggested (Kalla et al., 2016), and included the presence of at least one of the following events: starting a new immunomodulator or biological drug; switching immunomodulator; increasing biological dosage or shortening infusion interval; switching biological drug due to LOR; need to undergo bowel-related surgery.

2.3. Statistical Analysis

Categorical variables were described through absolute (n) and relative (%) frequencies and continuous variables were described as mean and standard deviation, median, interquartile range (IQR), and minimum/maximum values, whenever appropriate. When testing hypothesis concerning continuous variables, nonparametric Kruskall Wallis tests were used as appropriate, taking into account normality assumptions and the number of groups compared. In order to have a more thorough understanding of the factors associated with clearance, univariate and multivariate logistic regression modelling were used. The time elapsed from assessment to therapeutic escalation was evaluated using survival analysis. To determine the factors associated to therapeutic escalation, Cox regression was used. The cumulative probabilities of event-free survival were estimated with the Kaplan-Meier method using log-rank and Breslow tests. The reported p values were two-sided, and p values below 0.05 were considered to be statistically significant. The cut-offs used to stratify the outcomes concerning IFX trough levels, clearance, and ATI levels were chosen based on the literature (Afonso et al., 2016; Vande Casteele et al., 2015). All data was arranged, processed and analyzed with SPSS® v.20.0 data (Statistical Package for Social Sciences), whereas graphs were designed using Prism 6.

3. Results

3.1. Characterization of the Cohort

The cohort analyzed in this study included 65 UC patients in remission being treated with 5 mg/kg IFX every six weeks (n = 21, 32.3%) or every eight weeks (n = 44, 67.7%) (Table 1). Overall, most patients were female (56.9%) and had never smoked (68.9%). The location of the disease was distributed as follows: 50.8% of the patients had left-side and 49.2% patients had extensive colitis. Concerning concomitant therapies, 67.7% of the patients were or had been on AZA, whereas 10.8% were or had been taking steroids. There were no significant differences between the baseline characteristics of the patients doing the 6-weeks' and the 8-weeks' regimen (data not shown).

3.2. Pharmacokinetics

Patients were followed during one IFX infusion cycle, and the assessments made throughout time are illustrated in Fig. 1. The IFX trough levels (TLs) were significantly higher in the patients enrolled in the 6-weeks' regimen when compared to those in the 8-weeks' one: median $TL_{6\ weeks}$ = 5.00 μg/mL, IQR: 2.68–9.60 *vs.* median $TL_{8\ weeks}$ = 2.43 μg/mL, IQR: 0.91–3.70, p = 0.006. However, there was no significant difference between the two regimens concerning the ATI concentration (median $ATI_{6\ weeks}$ = 1.15 μg/mL, IQR: 0.88–2.48 *vs.* median $ATI_{8\ weeks}$ = 1.51 μg/mL, IQR: 0.80–2.18, p = 0.592),

Table 1
Cohort characterization.

	n	%
Gender		
Male	28	43.1
Female	37	56.9
Smoking status		
Never smoked	42	68.9
Former smoker	15	24.6
Smoker	4	6.6
Location of disease		
Left-side colitis	33	50.8
Extensive colitis	32	49.2
Extra-intestinal manifestations	16	26.2
Azathioprine	44	67.7
Azathioprine intolerant	10	16.1
Steroids	7	10.8
Corticodependent	39	60.9
Corticoresistent	12	18.5

the AUC ($p = 0.768$) or the clearance ($p = 0.941$). The concentration of ATIs at the 6th/8th week was inversely correlated with IFX levels 14 days after the infusion (Spearman correlation coefficient = −0.295, $p = 0.022$) and IFX TLs (Spearman correlation coefficient = −0.480, $p < 0.001$).

To address the importance and interaction between ATIs and IFX-TLs concerning clearance and AUC, these parameters were analyzed in patients stratified according to their status (positive or negative) regarding clinical cut-offs of IFX TLs (3 μg/mL) and ATI concentration at the 6th/8th week (1.7 μg/mL) (Afonso et al., 2016; Vande Casteele et al., 2015) (Table 2). Both clearance and AUC of the 8 weeks-regimen patients varied in a significant fashion according to the ATI/IFX-defined patient group. These parameters were clearly associated with the presence of ATIs, as ATI positive patients had a higher clearance and consequently a lower AUC. Concerning only ATI-negative patients, those that were positive for IFX trough levels had a lower clearance and a higher AUC.

As expected, clearance and ATI levels were correlated in a significant fashion (Spearman's coefficient: 0.391, $p = 0.005$). A multiple regression analysis was made using clearance as the dependent variable and considering patients' height, weight, albumin, UCEIS (as a proxy for inflammatory burden) and ATI concentration. The multivariate model is depicted in Table 3 and shows that ATI concentration is the only independent predictor of clearance in these patients. Moreover, when UCEIS was replaced by either the endoscopic Mayo score (stratified by 0 *vs.* ≥1 or ≤1 *vs.* >1) or by the Geboes index (stratified by <3.1 *vs.* ≥3.1), the results were similar (Supplementary Tables 1, 2 and 3).

3.3. Pharmacodynamics

The patients' outcomes after the infusion cycle were evaluated in an inclusive way, including the Mayo Global score assessment, the presence of endoscopic activity and histological inflammation, and the FC levels (Table 4). Most patients (71.5%) had a global Mayo score equal to or below 2, and 70.8% were considered to be in clinical remission (defined as global Mayo score below or equal to 2 and no individual subscore above 1). Endoscopic activity according to the UCEIS was absent in 76.2% of the patients, whereas 60.3% and 82.5% did not exhibit macroscopic lesions when the Mayo endoscopic score threshold was set at 0 and 1, respectively. Histological inflammation was present in 31.3% of the patients, and 22.2 and 11.1% were above the FC threshold when that was set at 150 and 250 μg/g, respectively. There were no significant differences between the outcomes of the patients under the 6-weeks' and the 8-weeks' regimen (data not shown).

To test whether the IFX pharmacokinetic features were related to patients' response in the cohort under study, patients were stratified according their outcomes, and IFX TLs (Supplementary Table 4), ATIs (Supplementary Table 5), AUC (Supplementary Tables 6 and 7) and clearance (Supplementary Table 8) were compared between positive and negative outcomes. However, there were no significant differences to report.

Table 3
Regression analyses of the clearance rate (mL/day).

Variables	OR	95% CI	p-Value
Height (m)	413.589	−127.938; 955.115	0.130
Weight (kg)	1.028	−1.483; 3.539	0.411
Albumin	0.758	−6.114; 7.630	0.824
[ATI] μg/mL	**12.210**	**2.381; 22.040**	**0.016**
UCEIS			
≤1	Ref		
>1	0.849	−66.549; 68.247	0.980

All variables were included using the "enter" method; $R^2 = 0.293$; OR-Odds Ratio 95% CI – 95% confidence interval.

3.4. Therapeutic Escalation

A total of 60 patients were re-evaluated to detect whether a therapeutic escalation was required later in their follow-up (five patients of the initial cohort were lost to follow up). Overall, 10 patients escalated, and the time spent from initial assessment to escalation was, in median, 15.00 months (IQR: 8.00–20.00). To test whether the IFX pharmacokinetic features assessed previously were related to patients' escalation, values of IFX TLs, ATIs, AUC and clearance were compared between patients with or without the need to escalate their therapy (Fig. 2). Patients are undistinguishable based on IFX TLs, clearance and AUC. However, there is a clear trend for higher ATIs among patients who later require therapeutic escalation.

Moreover, a Kaplan-Meyer analysis showed that patients with ATIs levels above 1.7 μg/mL (Fig. 3A) and above 3 μg/mL (Fig. 3B) escalate faster than those with lower levels, although only the 3 μg/mL cut-off has statistical significance. This analysis was expanded in order to include the biomarker FC, and the results show that patients with higher levels of FC also escalate faster than their counterparts (Fig. 3C and D), although significant results are only present for the 250 μg/g cut-off. Furthermore, the escalation was also faster when any of these conditions (or both) were present (*i.e.*, ATI above 3 μg/mL or FC above 250 μg/g), as compared to those patients whom had both values below the cut-offs (Fig. 3E). Finally, this faster escalation is unrelated to the disease severity from an histological and endoscopic perspective (Supplementary Fig. 1). In fact, a Cox regression considering all these parameters shows that only ATI and FC levels are significant for therapeutic escalation: whereas an FC level above 250 μg/g has an HR (hazard ratio) of escalating of 9.309, an ATI level below 3 μg/mL has an HR of 0.119 (Table 5). These values are maintained irrespective of whether the endoscopic Mayo score cut-off was placed at 1 or 2.

4. Discussion

The success of IFX in the treatment of many UC patients, materialized in a decrease of the number of surgeries and hospitalizations and an increase in these patients' quality of life, is overshadowed by the no-

Table 2
Median and IQRs for clearance rates and AUC values stratified by ATI and IFX trough levels.

[ATI] cut-off = 1.7 [IFX] cut-off = 3	ATI − IFX −	ATI − IFX +	ATI + IFX −	ATI + IFX +	p-Value[a]
Clearance (mL/day)	228 [212.5–280.0]	164 [120–236]	323.5 [252–360]	228.5 [168–327.5]	<**0.001**
N	8	23	14	4	
AUC 6/6 weeks (μg·day/mL)	1388 [1206–1570]	1789 [1328–2389]	826 [468–1199]	1448 [1186–2055]	0.071
N	2	11	3	3	
AUC 8/8 weeks (μg·day/mL)	1532 [1310–1617]	1821 [1603–2302]	1090 [991–1479]	1426 [1426–1426]	**0.004**
N	6	14	12	1	

[a] Kruskall Wallis test.

Table 4
Outcomes at 6/8 weeks post-infusion.

	n	%
Global mayo score		
1	36	55.4
2	10	16.1
3	6	9.7
4	2	3.2
5	5	4.8
6	2	3.2
7	1	1.6
8	1	1.6
11	1	1.6
Remission = no	19	29.2
Remission = yes	46	70.8
Endoscopic mayo score		
0	38	60.3
≥1	25	39.7
≤1	52	82.5
>1	11	17.5
UCEIS		
≤1	48	76.2
>1	15	23.8
Histology (Geboes score)		
<3.1	44	68.8
≥3.1	20	31.3
FC (at 6/8 weeks) (μg/g)		
<150	49	77.8
≥150	14	22.2
<250	56	88.9
≥250	7	11.1

ticeable number of treatment failures. In order to address this issue, IFX pharmacokinetics, pharmacodynamics and disease outcomes – including the biomarker FC – were closely monitored during one IFX infusion cycle in a population of clinically-stable UC patients.

The values of IFX TLs, AUC and clearance reported in this study were within the range of those previously described in different studies and clinical trials (Fasanmade et al., 2009; Brandse et al., 2016; Paserchia, 1999; Anon, n.d.). Interestingly, the different IFX regimens had similar AUCs and clearance values, but could be distinguished based on their IFX TLs, which were significantly higher in the shorter regimen. Shortening the infusion interval is a commonly used strategy to intensify IFX therapy, shown to be superior or, at least, equivalent to the increase of IFX dosage in LOR (Katz et al., 2012; St Clair et al., 2002). As in the ATTRACT study, our results show that a shorter interval is associated with higher IFX TLs (St Clair et al., 2002). Moreover, this increase in IFX TLs - even without a concomitant increase in the IFX AUC - was sufficient for patients who suffered a LOR in the 8-weeks regimen regain response to IFX. In fact, their outcomes were similar to those experienced by the patients that remained in the 8-weeks' regimen.

Interestingly, ATIs levels were the only significant factor affecting IFX clearance in this cohort. One can hypothesize that variables that affect clearance in more severely ill patients (such as albumin, height, weight and inflammatory burden) are not significant in the population addressed in this study, which was constituted by patients in a stable condition. Therefore, the presence of ATIs seems to be the main factor affecting IFX availability, although these results worth a confirmation on a larger population.

The analysis of the cohort outcomes and disease indicators show that, despite being classified as in remission according to the Montreal classification, a considerable proportion of patients still has endoscopic lesions and a relatively high inflammatory burden. Interestingly, neither IFX TLs, AUC, clearance nor ATI concentrations were able to differentiate patients with positive and negative outcomes. IFX TLs are considered to be particularly useful for this: in fact, a search through the literature shows that IFX TLs are many times used to monitor this drug on a therapeutic scenario, and different authors have found significant differences between IFX TLs in responders and non-responders, many times using cut-off values close to the one used in this study (3 μg/mL), as described by Silva-Ferreira et al. and references included (Silva-Ferreira et al., 2016). Our analysis, however, suggest that these differences are absent or undiscernible when assessing clinically-stable and asymptomatic patients.

We have then analyzed whether the pharmacokinetic profile of these patients could be used to evaluate their long-term risk of requiring therapeutic escalation. Interestingly, there was a clear and significant trend for patients with higher ATI levels to need therapeutic escalation later on their lives. Moreover, a cut-off of 3 μg/mL could be statistically associated with the requirement of therapeutic escalation.

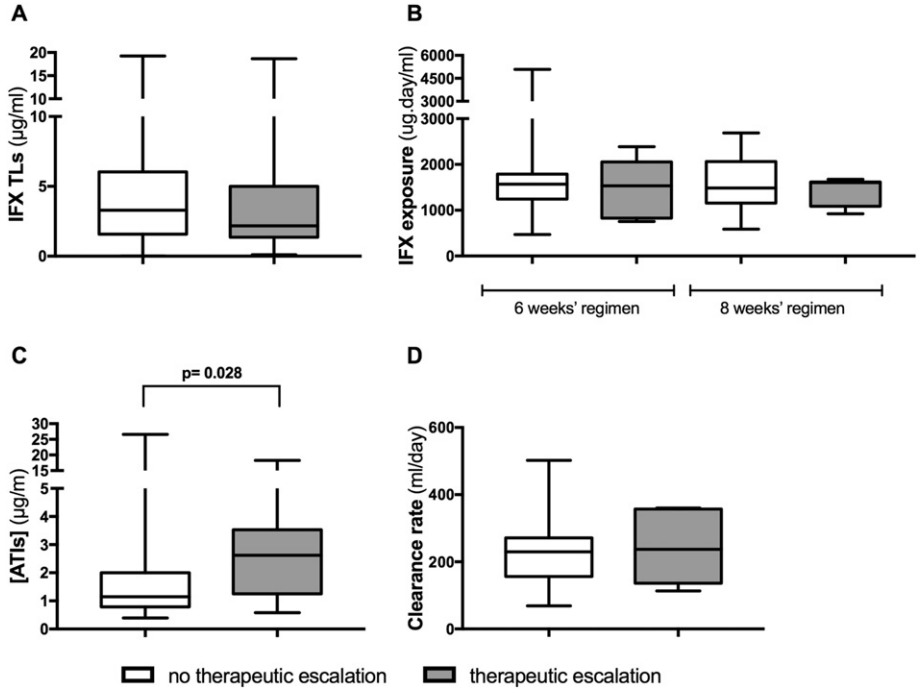

Fig. 2. Relationship between therapeutic escalation episodes and the IFX TLs (A), IFX AUC (B), ATI levels (C) and ATI clearance (D).

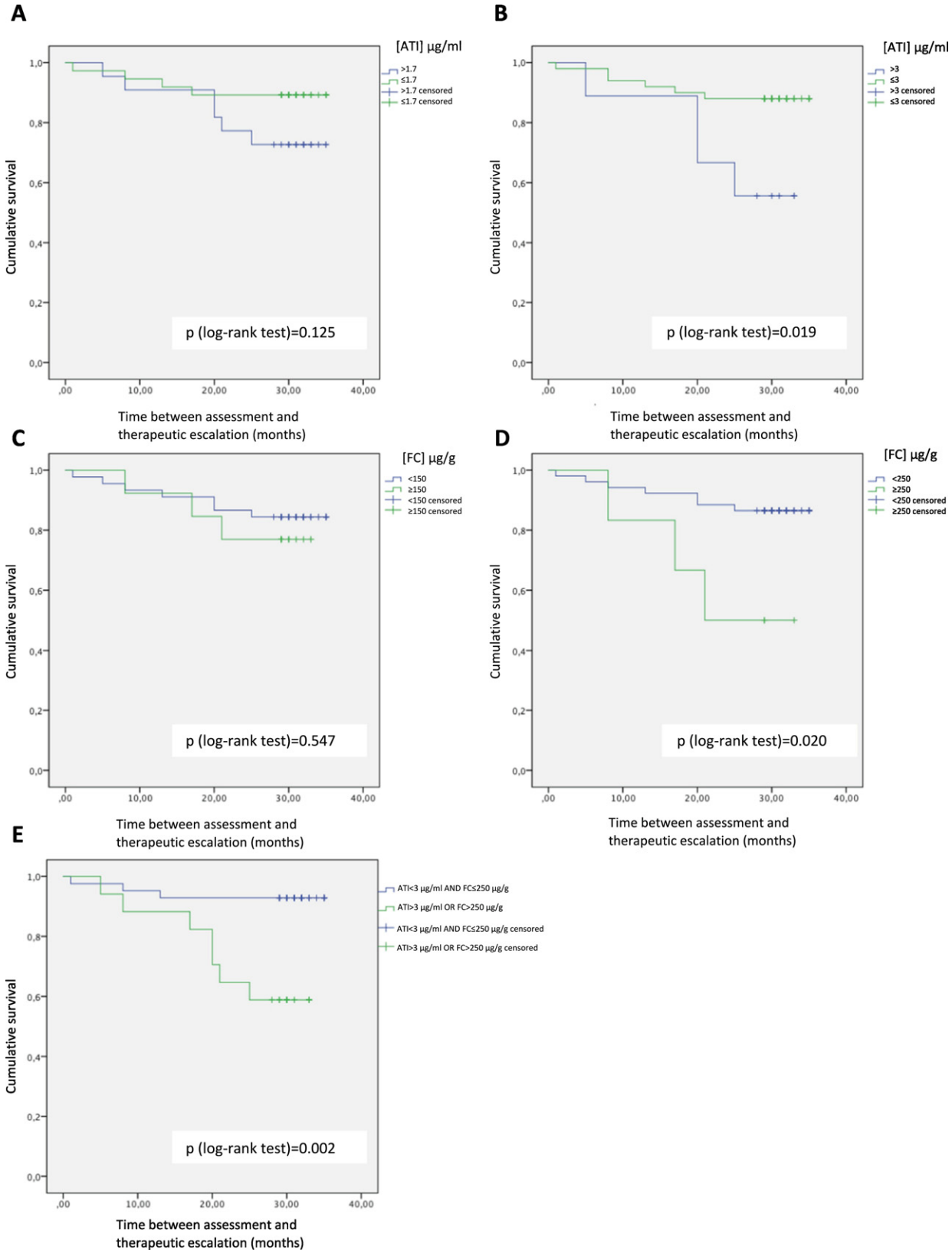

Fig. 3. Kaplan-Meyer survival curves for time to therapeutic escalation of: ATI levels using a cut-off of 1.7 µg/ml (A) or 3 µg/ml (B); FC levels using a cut-off of 150 µg/g (C) or 250 µg/g (D); ATI levels above 3 µg/ml or FC levels above 250 µg/g (E).

These results concur with the data published previously by Edlund et al., who have shown that the presence of ATIs in Crohn's disease (CD) patients, irrespective of their concentration, eventually leads to a drop in IFX levels to values below a critically minimum concentration (Edlund et al., 2016). Moreover, Ungar et al. have shown that ATI development often precedes the onset of a clinical flare (Ungar et al., 2014).

Additionally, a similar analysis including the FC levels has shown that values above 250 µg/g are also significantly associated with the

Table 5
Multi-variate Cox regression to therapeutic escalation.

	p-Value	HR	95% CI	
IFX TLs	0.771	1.021	0.887	1.176
FC (ref: <250 μg/g)	0.018	9.309	1.455	59.561
ATIs (ref: >3 μg/mL)	0.010	0.119	0.024	0.594
Geboes index (ref: <3.1)	0.602	0.648	0.127	3.301
Mayo endoscopic score (ref: 0)	0.851	1.151	0.265	5.008
IFX TLs	0.774	1.020	0.889	1.171
FC (ref: <250 μg/g)	0.019	9.036	1.445	56.511
ATIs (ref: >3 μg/mL)	0.009	0.119	0.024	0.592
Geboes index (ref: <3.1)	0.575	0.619	0.116	3.310
Mayo endoscopic score (ref: ≤1)	0.772	1.318	0.204	8.530

HR- Hazard Ratio; 95% CI-95% confidence interval.

requirement of therapeutic escalation. Such a relationship has been suggested before by Burri et al., who claimed that changes of FC levels between measurements were related to therapeutic escalation (Burri et al., 2015). From a different angle but supporting the same core idea, Papamichael et al. have recently shown that the risk of relapse after IFX de-escalation in CD patients in composite deep remission is relatively low when FC levels are maintained within the normal range (Papamichael et al., 2016). Moreover, the results of a meta-analysis including six different studies suggest that FC is useful to predict relapses in quiescent UC and CD patients (Mao et al., 2012).

The simultaneous analysis of ATI and FC levels shows that the therapeutic escalation is associated to high values of either these variables. Importantly, their impact in the need of a future therapeutic escalation is independent of the disease severity, as is shown by the fact that neither histological score nor endoscopic lesions are significant variables in this context. A combination of a biomarker and ATIs levels to predict disease development has been shown before: in fact, C-reactive proteins levels combined with IFX-TLs and ATI stability were shown to predict LOR in IBD patients (Roblin et al., 2015). Our results, together with the literature, suggest that high levels of ATIs and FC found in otherwise stable UC patients may indicate a future disease flare and its consequent therapeutic escalation. These findings have some important clinical implications: TDM on stable patients is useful if ATI levels are included and should be performed alongside with FC determination: the presence of elevated ATIs of FC levels – even in the absence of clinical symptoms – should alert the physician to act in order to prevent future therapeutic escalations.

This study has several strengths that should be noticed, namely its prospective design with a systematic and multidimensional evaluation of the therapeutic response: endoscopic, histological and clinical data was retrieved, in parallel with the quantification of a biomarker. Nevertheless, there were also a few limitations that should be taken into consideration: the inclusion of a single infusion cycle and the fact that we have not taken into account the amount of IFX lost through the feces.

In short, this study explores the IFX pharmacokinetics and the utility of drug and disease monitoring among UC patients in remission. Our findings show that, in these patients, IFX clearance is mainly related to the presence of ATIs. Moreover, and irrespective of the IFX regimen, IFX TLs, AUC, clearance and ATI concentration are unable to differentiate patients according to their outcome. Conversely, high ATI levels are significantly associated with the long-term need to undergo therapeutic escalation, as are FC levels above 250 μg/g. Therefore, the usefulness of TDM in clinically-stable UC patients relies on the possibility of avoiding future disease progression that can be predicted based on the ATI levels. Moreover, the monitoring of FC should also be carried out in these patients, as this biomarker is also increased in patients that eventually need to undergo a therapeutic escalation.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2017.06.004.

Funding Sources

This work was supported by the Portuguese IBD Group (GEDII – Grupo de Estudo da Doença Inflamatória Intestinal). The funder had no role in study design, data collection, data analysis, interpretation and writing of the report.

Conflict of Interests

FM served as speaker and received honoraria from Merck Sharp & Dohme, Abbvie, Vifor, Falk, Laboratorios Vitoria, Ferring, Hospira and Biogen.

Author Contributions

FM: Study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; study supervision; critical revision of the manuscript for important intellectual content. JA: IFX, anti-IFX antibodies and fecal calprotectin assays; analysis and interpretation of data. JL: histological analysis. CCD: statistical analysis. AF: pharmacokinetic calculations. KG: supervisor of the histological analysis; critical revision of the manuscript for important intellectual content. FC: responsible for the histological analysis; critical revision of the manuscript for important intellectual content. All the other authors: recruitmen of patients and collection of samples.

All authors read and approved the final version of the manuscript.

Acknowledgments

The authors would like to express their acknowledgment to Sandra Dias for her involvement as the GEDII coordinator and all the help during data collection, as well as to Catarina L Santos for scientific writing assistance.

References

Afonso, J., et al., 2016. Detection of anti-infliximab antibodies is impacted by antibody titer, infliximab level and IgG4 antibodies: a systematic comparison of three different assays. Ther. Adv. Gastroenterol. 9 (6):781–794 Available at:. http://www.ncbi.nlm.nih.gov/pubmed/27803733%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5076767.

Anon, Clinical Pharmacology Review of BLA 98-0012, cA2, 6–13.

Ben-Horin, S., et al., 2011. The immunogenic part of infliximab is the F(ab′)2, but measuring antibodies to the intact infliximab molecule is more clinically useful. Gut 60 (1), 41–48.

Benítez, J.M., García-Sánchez, V., 2015. Faecal calprotectin: management in inflammatory bowel disease. World J. Gastrointest. Pathophysiol. 6 (4):203–209 Available at: http://www.ncbi.nlm.nih.gov/pubmed/26600978%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4644884.

Brandse, J.F., et al., 2016. Pharmacokinetic features and presence of antidrug antibodies associate with response to infliximab induction therapy in patients with moderate to severe ulcerative colitis. Clin. Gastroenterol. Hepatol. 14 (2):251–258. http://dx.doi.org/10.1016/j.cgh.2015.10.029.

Burri, E., et al., 2015. Fecal calprotectin and the clinical activity index are both useful to monitor medical treatment in patients with ulcerative colitis. Dig. Dis. Sci. 60 (2), 485–491.

Vande Casteele, N., et al., 2015. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. Gastroenterology 148 (7) 1320–1329.e3.

Cornillie, F., et al., 2014. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. Gut 63 (11):1721–1727 Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4215276&tool=pmcentrez&rendertype=abstract.

Danese, S., Vuitton, L., Peyrin-Biroulet, L., 2015. Biologic agents for IBD: practical insights. Nat. Rev. Gastroenterol. Hepatol. 12 (9):537–545 Available at:. http://www.ncbi.nlm.nih.gov/pubmed/26284562.

Edlund, H., et al., 2016. Magnitude of increased infliximab clearance imposed by anti-infliximab antibodies in Crohn's disease is determined by their concentration. AAPS J.:1–11 Available at: http://link.springer.com/10.1208/s12248-016-9989-8.

Fasanmade, A.A., et al., 2009. Population pharmacokinetic analysis of infliximab in patients with ulcerative colitis. Eur. J. Clin. Pharmacol. 65 (12), 1211–1228.

Geboes, K., et al., 2000. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. Gut 47 (3):404–409 Available at:. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1728046&tool=pmcentrez&rendertype=abstract.

Gecse, K.B., Végh, Z., Lakatos, P.L., 2016. Optimizing biological therapy in Crohn's disease. Expert Rev. Gastroenterol. Hepatol. 10 (October 2015):37–45. http://dx.doi.org/10.1586/17474124.2016.1096198.

Gisbert, J.P., McNicholl, A.G., 2009. Questions and answers on the role of faecal calprotectin as a biological marker in inflammatory bowel disease. Dig. Liver Dis. 41 (1), 56–66.

Kalla, R., et al., 2016. Serum calprotectin – a novel diagnostic and prognostic marker in inflammatory bowel diseases. Am. J. Gastroenterol. 111 (12):1796–1805. http://dx.doi.org/10.1038/ajg.2016.342.

Katz, L., et al., 2012. Doubling the infliximab dose versus halving the infusion intervals in Crohn's disease patients with loss of response. Inflamm. Bowel Dis. 18 (11), 2026–2033.

Mao, R., et al., 2012. Fecal calprotectin in predicting relapse of inflammatory bowel diseases: a meta-analysis of prospective studies. Inflamm. Bowel Dis. 18 (10), 1894–1899.

Moore, C., Corbett, G., Moss, A.C., 2016. Systematic review and meta-analysis: serum infliximab levels during maintenance therapy and outcomes in inflammatory bowel disease. J. Crohns Colitis 10 (5), 619–625.

Mould, D.R., D'Haens, G., Upton, R.N., 2016. Clinical decision support tools: the evolution of a revolution. Clin. Pharmacol. Ther. 99 (4), 405–418.

Papamichael, K., Karatzas, P., Mantzaris, G.J., 2016. De-escalation of infliximab maintenance therapy from 8- to 10-week dosing interval based on faecal calprotectin in patients with crohn's disease. J. Crohn's Colitis 10 (3), 371–372.

Paserchia, L.A., 1999. Clinical Pharmacology Review of BLA 99-0128, Remicade (Supplement).

Paul, S., et al., 2013. Therapeutic drug monitoring of infliximab and mucosal healing in inflammatory bowel disease: a prospective study. Inflamm. Bowel Dis. 19 (12): 2568–2576 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24013361.

Roblin, X., et al., 2015. Combination of C-reactive protein, infliximab trough levels, and stable but not transient antibodies to infliximab are associated with loss of response to infliximab in inflammatory bowel disease. J. Crohn's Colitis 9 (7), 525–531.

Schroeder, K.W., Tremaine, W.J., Ilstrup, D.M., 1987. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. N. Engl. J. Med. 317 (26): 1625–1629. http://dx.doi.org/10.1056/NEJM198712243172603.

Silva-Ferreira, F., et al., 2016. A systematic review on infliximab and adalimumab drug monitoring: levels, clinical outcomes and assays. Infamm. Bowel Dis. 22 (9), 2289–2301.

St Clair, E.W., et al., 2002. The relationship of serum infliximab concentrations to clinical improvement in rheumatoid arthritis: results from ATTRACT, a multicenter, randomized, double-blind, placebo-controlled trial. Arthritis Rheum. 46 (6), 1451–1459.

Strik, A.S., et al., 2016. Optimization of anti-TNF therapy in patients with inflammatory bowel disease. Expert. Rev. Clin. Pharmacol. 2433 (July):1–11 Available at: http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L608510615%5Cnhttp://dx.doi.org/10.1586/17512433.2016.1133288%5Cnhttp://findit.library.jhu.edu/resolve?sid=EMBASE&issn=17512441&id=doi:10.1586%2F17512433.2016.1133288&atitle=Optimiza.

Travis, S.P.L., et al., 2012. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the ulcerative colitis endoscopic index of severity (UCEIS). Gut 61, 535–542.

Ungar, B., et al., 2014. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. Gut 63 (8):1258–1264 Available at: http://www.ncbi.nlm.nih.gov/pubmed/24041539.

Warman, A., Straathof, J.W.A., Derijks, L.J.J., 2015. Therapeutic drug monitoring of infliximab in inflammatory bowel disease patients in a teaching hospital setting: results of a prospective cohort study. Eur. J. Gastroenterol. Hepatol. 27 (3):242–248 Available at: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&CSC=Y&NEWS=N&PAGE=fulltext&D=emed12&AN=2015656340%5Cnhttp://sfx.ucl.ac.uk/sfx_local?sid=OVID:embase&id=pmid:&id=doi:10.1097/MEG.0000000000000279&issn=0954-691X&isbn=&volume=27&issue=3&spage=242&pages=242-248&date=20.

Williet, N., et al., 2016. Pharmacokinetics of infliximab and reduction of treatment for inflammatory bowel diseases. Dig. Dis. Sci. 61 (4), 990–995.