European Journal of Pharmaceutical Sciences 150 (2020) 105369

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Biomarkers of disease activity and other factors as predictors of adalimumab pharmacokinetics in inflammatory bowel disease

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ARTICLE INFO

Keywords:
Adalimumab
Inflammatory bowel disease
Pharmacokinetics
Faecal calprotectin
Body mass index
Population pharmacokinetic model

ABSTRACT

Inflammatory bowel disease (IBD) is commonly treated with adalimumab. The main objective of the study was to develop a population pharmacokinetic model of adalimumab in IBD patients evaluating the potential biomarkers of disease activity and other factors and its implications in adalimumab dosing.

A prospective observational study was performed in adult patients diagnosed with Crohn's disease and ulcerative colitis treated with adalimumab and following a proactive therapeutic drug monitoring of serum concentrations. Adalimumab serum concentrations (ASC) were quantified mainly prior the administration using an enzyme-linked immunosorbent assay (ELISA). A population pharmacokinetic model was developed based on 303 ASC data of 104 IBD patients using non-linear mixed effect modelling approach. Sixty-five ASC from 20 additional patients were randomly selected as an external validation group.

A one-compartment model with first order absorption and elimination best describe the ASC time course. Body mass index (BMI), faecal calprotectin (FCP), unexplained decline in ASC and the specific administration pen device exhibited significant influence on apparent clearance (p-value < 0.001).

FCP was the inflammatory activity biomarker showing the most relevant impact on adalimumab exposure, higher than C-reactive protein and albumin, and may be useful for adalimumab dosing adjustment.

The population-based pharmacokinetic model developed adequately characterized adalimumab exposure in IBD patients. The unexplained decline in ASC, FCP, BMI and the specific administration pen device were identified as meaningful variables significantly influencing adalimumab pharmacokinetics.

1. Introduction

Adalimumab is a recombinant fully human immunoglobulin (IgG1) anti-tumour necrosis factor α (TNF-α) that inhibits the binding of TNF-α to its receptors. This drug has dramatically changed the management of chronic inflammatory diseases such as ulcerative colitis (UC), Crohn's disease (CD), rheumatoid arthritis, psoriatic arthritis and plaque psoriasis, among others (FDA, 2019; EMA, 2019).

The relationship between adalimumab serum concentrations (ASC) and clinical outcomes has been highlighted in several studies (Sanchez-Hernandez et al., 2019). In patients diagnosed with inflammatory bowel disease (IBD), trough adalimumab serum concentrations (TASC) at steady state above 5 mg/L (Mitrev et al., 2017) were associated with increased clinical response. Accordingly, a therapeutic range of 5–12 mg/L was proposed for this drug. However, TASC above 8 mg/L were reported to be necessary to reach not only clinical response but also endoscopic remission (Juncadella et al., 2018). Consequently an updated therapeutic range of 8–12 mg/L has been proposed in the clinical practice.

The pharmacokinetics (PK) of adalimumab in CD patients has been commonly characterized using population PK modelling (PopPK) (Berends et al., 2018; Sharma et al., 2015; Ternant et al., 2015; Vande Casteele et al., 2019). However, the potential influence of the main inflammation biomarkers currently taking into account to assess adalimumab treatment response on adalimumab PK has not been evaluated.

Adalimumab exhibits considerable inter- and intra-patient PK variability, which has been associated with treatment failure over time

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https://doi.org/10.1016/j.ejps.2020.105369
Received 21 January 2020; Received in revised form 4 April 2020; Accepted 28 April 2020
Available online 19 May 2020

and/or with the need for dose escalation. Many factors have been identified as sources of this variability, including gender, body weight, hypoalbuminemia, genetics, systemic inflammation and the formation of anti-drug antibodies (ADA) (Vande Casteele and Gils, 2015). Improving knowledge of the PK behaviour of adalimumab together with potential factors related to exposure and therapeutic response may be useful to improve the safety and efficacy of treatment with this drug.

After subcutaneous administration of 40 mg of adalimumab every other week, absorption and distribution were slow and variable, the peak of serum concentrations being reached 5 days after administration (FDA, 2019; EMA, 2019). The elimination half-life of adalimumab was estimated at two weeks. Its bioavailability appeared variable and was estimated to be approximately 64%. This variability in drug disposition could be among the reasons for the high percentage of IBD patients (20–50%) failing to respond satisfactorily to standard adalimumab dosages (González-Fernández et al., 2019; Vande Casteele and Gils, 2015).

The aim of the study was to develop a PopPK model of adalimumab in patients diagnosed with IBD assessing factors with potential clinical relevance.

2. Material and methods

2.1. Study design

This was a prospective observational study performed in adult patients undergoing treatment with adalimumab and following a proactive therapeutic drug monitoring (TDM) program ran between the Gastroenterology Service and the Pharmacokinetics Laboratory of the Hospital Pharmacy Service. The study was conducted at the University Hospital of Salamanca (Spain) between September 2015 and December 2019.

2.2. Subjects and data collection

Serum samples were collected from adult patients diagnosed with moderate or severe CD or UC treated with adalimumab. The inclusion criteria were: Partial Mayo Clinic Score (IMp) > 4 for UC (Lewis et al., 2008) and Harvey-Bradshaw Index Score (HB) > 7 and/or Simplified Endoscopic Activity Score for Crohn´s Disease (SES-CD) > 4 for CD (Daperno et al., 2004; Harvey and Bradshaw, 1980).

The Pharmacy Service provided Humira® solution for subcutaneous injection in two different pre-filled pen devices according to the prescribed dose: 40 mg and 80 mg pen devices. The use of pen devices was explained to patients. The initial dose of adalimumab was selected according to the recommendations provided in the drug data sheet (FDA, 2019; EMA, 2019): 160 mg at week 0 followed by 80 mg at week 2. Afterwards, adalimumab dose adjustment was performed according to TDM. Patient adherence was assessed by checking the medication dispensing records and ratified with a personal care interview with the patient. Non-adherent patients were excluded from the study.

The following information were recorded for each patient: age, gender, height, total body weight (TBW), ideal body weight (IBW), ideal adjusted body weight (IABW), body surface area (BSA), body mass index (BMI), type of disease, extent of the disease, CD behaviour, age at diagnosis, age at start of adalimumab, perianal fistulising disease, concomitant use of immunomodulatory drugs (i.e. thiopurines or methotrexate), extraintestinal manifestations (musculoskeletal, dermatologic, hepatopancreatobiliary or ocular), serum albumin, faecal calprotectin (FCP), C-reactive protein (CRP), type of administration pen device and previous anti-TNF treatments. Disease extent and behaviour were defined according to the Montreal classification (Gomollon et al., 2017). BMI was calculated as: weight (kg)/height^2 (m). IBW was calculated as: 50.0 + 0.9 * (height (cm) – 152) in men and 45.5 + 0.9 * (height (cm) – 152) in women (Chennavasin and Brater, 1982). IABW was calculated as: IBW + 0.4 * (TBW - IBW) (Bauer, 2001). BSA was calculated as: weight (kg)$^{0.425}$ * height (cm)$^{0.725}$ * 0.007184 (Du Bois and Du Bois, 1989). Sampling time and dosing regimen available at the time of the ASC extraction were also recorded.

The selected patients were randomly assigned to either the PopPK development group (80% of patients) or the external validation group (20% of the patients).

2.3. Laboratory tests

Adalimumab serum concentrations and ADA were determined using an enzyme-linked immunosorbent assay (ELISA) developed by Sanquin Laboratories, Amsterdam, the Netherlands. In this technique, serum adalimumab binds to TNF and is detected by an anti-F(ab')2-adalimumab, enzyme horseradish peroxidase labelled antibody. The disadvantages of this assay are: it is a drug sensitive test, since it is not able to reliably detect ADA bound to the drug (when ASC > 0.5 mg/L) and the inability of bridging ELISA to detect antidrug antibodies of IgG4 isotype since these antibodies could be of clinical importance (Aalberse et al., 2009). Therefore, this assay presents a high rate of false negative ADA. Antibodies were measured in patients with ASC < 1 mg/L (Barlow et al., 2016). For these reasons, unexplained decline in ASC (UDASC) was defined as a decrease of at least 33% in ASC during the maintenance phase with no apparent cause (confirmed adherence to treatment, no modification of adalimumab dosage and no significant increase in inflammatory markers or scores (FCP, PCR, albumin, IMp, HB, SES-CD)) and reversible with dose intensification. The lower limit of quantification (LLOQ) of this assay was 0.06 mg/L. ADA were defined as positive when titers were > 12 AU/mL according to laboratory assay. ASC values below the LLOQ were excluded from the formal analysis (Xu et al., 2011).

2.4. Therapeutic drug monitoring and samples

At the beginning of the study, all the patients who were already under chronic treatment with adalimumab were monitored. Patients who started treatment were monitored for the first time at week 4. TDM was repeated after two months in patients for whom dose adjustment had been recommended. Proactive TDM was routinely performed every six months once the TASC were within therapeutic range (8–12 mg/L). For patients with TASC below the therapeutic range, a dose escalation (interval decrease and/or dose increase) was proposed. On the other hand, an interval increase was proposed for patients with supra-therapeutic TASC.

Serum adalimumab concentrations were obtained mainly in the 24 h prior to adalimumab administration. However, in patients with inflammatory signs and symptoms, mostly when low TASC were suspected, additional samples were taken during the drug administration interval in order to determine the optimal therapeutic decision.

2.5. Population pharmacokinetic analysis

Non-linear mixed effects modelling using the first-order conditional estimation method with INTERACTION (FOCEI) was used to develop the PopPK model using NONMEM® version 7.3.0 (Icon Development Solutions, Ellicott City, MD, USA (Beal et al., 2009)). Data visualization and statistical analyses, including evaluation and representation of model and simulation outputs were carried out in R version 3.3.1 (Comprehensive R Network, http://cran.r-project.org).

Adalimumab PK was initially described using a linear one-compartment disposition model. The PopPK model was parameterized in terms of apparent volume of distribution (V/F), apparent clearance (CL/F) and first order absorption rate (Ka). Interindividual variability (IIV) of PK parameters was assumed to follow a log-normal distribution and, consequently, an exponential model was used (Eq. (1)).

$$P_i = P_{pop} \times e^{\eta_i} \quad (1)$$

Where P_i is the PK parameter estimate for the individual i, P_{pop} is the typical value (population median) of the PK parameter and η_i is the inter-individual random effect. η values were assumed to be independently and identically distributed with a mean of 0 and a variance of ω^2: $\eta \sim N(0, \omega^2)$.

Residual unexplained variability (RUV) was modelled using a proportional error model (Eq. (2).).

$$C_{ij} = \hat{C}_{ij} \times (1 + \varepsilon_{1ij}) \quad (2)$$

Where C_{ij} is the jth measured serum concentration in individual i, $\hat{C}_{ij}$ is the model predicted jth value in individual i, and ε_{ij} is the residual random error for measurement j in individual i. ε_{1ij} is the proportional component of the residual random error. ε values were assumed to be independently and identically distributed with a mean of 0 and variance of σ^2: $\varepsilon \sim N(0, \sigma^2)$.

The magnitude of IIV and RUV was expressed approximately as a coefficient of variation (CV,%). Correlation between random parameters and inter-occasion variability (IOV) were graphically explored and evaluated if any trend was observed.

All the previously described variables in Section 2.2. (demographic, clinical, therapeutic, etc.) were considered for the initial covariate analysis. A covariate screening based on physiologically meaningful, visual graphical inspection and stepwise linear regression of the relationships between the IIV of adalimumab PK parameters and the continuous covariates and analysis of variance (ANOVA) for the categorical covariates was performed. Only physiological plausible covariates and sufficiently represented in the studied population that were statistically significant ($p < 0.05$) and had a coefficient of determination $r^2 > 0.10$ with an IIV parameter were considered to be of potential clinical relevance and were further evaluated one by one in the PK model following a stepwise covariate model-building methodology with NONMEM. Additionally, covariates more sensitive to time-varying processes, such as development of immunogenicity, pen device administration or comedications, were also evaluated with NONMEM. For each model, improvement in data fit was assessed using the likelihood ratio test (forward p-value < 0.05; backward p-value < 0.01), the reduction in IIV and RUV, and the precision and bias of PK parameter estimates (Savic and Karlsson, 2009).

2.6. Model evaluation

The PopPK model developed was assessed using goodness-of-fit plots, considering scatterplots of observed versus population predicted concentrations and versus individual predicted concentrations as well as prediction corrected visual predictive check (pcVPC) (Bergstrand et al., 2011; Ette and Williams, 2007; Nguyen et al., 2017). Goodness-of-fit plots were performed for both the development and the validation datasets. The pcVPC was carried out for the development group as internal evaluation. In the pcVPC, the 5th, 50th and 95th percentiles of the observed ASC were presented, as well as the 5th, 50th and 95th percentiles together with the 95% confidence interval (CI) for the corresponding model-based predicted percentiles computed for each bin across time since first dose and replicates. Additionally, for the external validation group, a prediction corrected numerical predictive check (pcNPC) of the ASC obtained at day 7 and day 14 after dose was performed. A total of 1000 replicates of the original dataset were generated for pcVPC and pcNPC analysis.

The accuracy of parameter estimates and robustness of the final PopPK model were assessed using 1000 bootstrap replicates constructed by random sampling from the original dataset. Model parameters were estimated for each bootstrap replicate and the resulting values of the models that converged successfully were used to estimate the median and 95% confidence interval (CI) from the individual replicates.

2.7. Model-based simulations

Deterministic simulations of adalimumab concentration-time profiles were carried out with the final PopPK model to investigate the impact of the factors identified on the expected adalimumab exposure and/or response to adalimumab as well as its potential clinical relevance. A sufficient number of adalimumab administrations was simulated to reach steady state in the different simulated scenarios, based on the variables identified in the final model.

2.8. Ethical considerations

The study was approved by the Biomedical Ethics Committee of the Health Area of Salamanca after evaluating compliance with ethical standards and good clinical practice.

3. Results

3.1. Patient characteristics

A total of 129 patients were included in the study and 389 ASC were determined. Five patients were excluded due to lack of adherence. On the other hand, 21 ASC (5.4%) below LLOQ were discarded with detection of ADA for 19 of them. One-hundred and four patients (303 ASC) were selected for the development of the adalimumab PopPK model and 20 patients were selected for its external validation (65 ASC). No positive ADA were measured in these patients.

The clinical and demographic baseline characteristics of the patients selected for the development of the adalimumab PopPK model in this study, stratified by development and validation group, are shown in Table 1. Table 2 shows the TDM outcomes in both groups.

3.2. Population pharmacokinetic model

Adalimumab PK was best described by a one-compartment model with first order absorption and elimination. Adalimumab absorption rate was fixed at 0.0062 1/h as previously reported by Ternant et al. (2015).

Based on previous adalimumab PK models, corporal size metrics were *a priori* evaluated on the CL/F of the drug. Among the anthropometric parameters evaluated (TBW, IBW, IABW, BMI and BSA), BMI with a power relationship yielded the highest degree of influence on adalimumab elimination and was considered the base model for the following covariates assessment. Interindividual variability of V/F could not be estimated due to the sparse sampling typically carried out in TDM.

In the covariate analysis conducted, the variables UDASC, FCP and administration pen device (40 mg or 80 mg) exhibited significant influence on CL/F (p-value < 0.001) and were incorporated into the final model. The inclusion of these variables in the model reduced IIV on CL/F and RUV by 21% and 10%, respectively. Adalimumab CL/F in the final model is described in Eq. (3).

$$CL/F = 0.0157 \times (BMI/23.7)^{1.11} \times (1 + 1.20 \times UDASC) \times (1 + 0.24 \times PEN) \times (FCP/74)^{0.064} \quad (3)$$

Where adalimumab CL/F, BMI and FCP are apparent clearance, body mass index and faecal calprotectin, expressed in L/h, kg/m^2 and mg/kg, respectively; UDASC and PEN are binary covariates where 0 represents no unexplained decline in TSAC and 40 mg pen device, and 1 represents UDASC and 80 mg pen device, respectively. The pen device seemed to have no effect on patients with BMI < 20 kg/m^2 based on graphical exploration. Diagnosis (CD or UC) did not exhibit significant influence on CL/F (p-value = 0.257).

Table 1
Baseline characteristics of patients selected for the development and validation of the adalimumab PopPK model.

		Development group	Validation group
N		104	20
Gender [male (%)]		58 (55.8)	13 (65.0)
Age at diagnosis [median in years (IQR)]		36 (29–53)	33 (29–46)
Age at start of adalimumab [median in years (IQR)]		43 (32–56)	36 (29–48)
Body weight [median in kg (IQR)]		68 (56–80)	73 (65–82)
Body mass index [median in kg/ m^2 (IQR)]		23.7 (21.1 – 27.1)	23.5 (22.5 – 26.3)
Body surface area [median in m^2 (IQR)]		1.8 (1.6 - 2.0)	1.9 (1.8 - 2.0)
Lean body weight [median in kg (IQR)]		60.0 (51.2 - 67.3)	63.2 (58.7 – 70.7)
IBD type	CD [n (%)]	84 (79.8)	15 (75.0)
	UC [n (%)]	20 (19.2)	5 (25.0)
CD Location	L1 (ileal) [n (%)]	29 (34.5)	8 (53.3)
	L2 (colonic) [n (%)]	13 (15.5)	1 (6.7)
	L3 (ileocolonic) [n (%)]	42 (50.0)	6 (40.0)
	L4 (upper GI disease) [n (%)]	2 (2.4)	0 (0.0)
CD Behaviour	B1 (nonstricturing, nonpenetrating) [n (%)]	40 (47.6)	7 (43.7)
	B2 (stricturing) [n (%)]	27 (32.2)	5 (31.3)
	B3 (penetrating) [n (%)]	17 (20.2)	4 (25.0)
Perianal disease [n (%)]		17 (16.3)	5 (25.0)
UC Extent	E1 (proctitis) [n (%)]	5 (25.0)	1 (20.0)
	E2 (left-side colitis) [n (%)]	3 (15.0)	1 (20.0)
	E3 (pancolitis) [n (%)]	12 (60.0)	3 (60.0)
Extraintestinal manifestations [n (%)]		37 (35.6)	6 (30.0)
Concomitant IMM [n (%)]		47 (45.2)	8 (40.0)
	Thiopurines (azathioprine, 6-MP) [n (%)]	39 (37.5)	7 (35.0)
	Methotrexate [n (%)]	8 (7.7)	1 (5.0)
Previously anti-TNF treatment		34 (32.7)	4 (25.0)
Patients with dose adjustment based on TDM [n (%)]		57 (54.8)	14 (70.0)

CD: Crohn's disease; IBD: inflammatory bowel disease; IMM: immunosuppressants; IQR: interquartile range; UC: ulcerative colitis; TNF: tumour necrosis factor; 6-MP: mercaptopurine; TDM: therapeutic drug monitoring.

Table 2
Therapeutic drug monitoring outcomes of patients included in the development an validation group.

	Development group	Validation group
Serum samples (n)	303	65
Adalimumab serum concentrations [average in mg/L (SD)]	9.8 (4.3)	9.1 (4.8)
Unexplained decline in adalimumab serum concentrations [n (%)]	9 (3.0)	4 (6.2)
Treatment		
Induction (n,%)	48 (15.8)	7 (10.8)
Maintenance (n,%)	255 (84.2)	58 (89.2)
Administration pen device		
40 mg (%)	275 (90.8)	60 (92.3)
80 mg (%)	28 (9.2)	5 (7.7)
Faecal calprotectine [median in mg/kg (IQR)]	74 (17 - 282)	54 (15–217)
C-reactive protein [median in mg/dL (IQR)]	0.15 (0.04 – 0.39)	0.25 (0.10 – 0.62)
Serum albumin [median in g/dL (IQR)]	4.5 (4.3 – 4.7)	4.5 (4.2 – 4.8)

IQR: interquartile range; SD: standard deviation.

3.3. Internal and external validation

Adalimumab PK parameters were estimated with a correct precision (residual standard error ≤ 35% in all cases) and lack of systematic bias (shrinkage ≤ 20% for all parameters). In addition, the magnitudes of the IIV on CL/F and RUV were consistent with previous information (Berends et al., 2018; Vande Casteele et al., 2019). A sensitivity analysis imputing the LLOQ value (0.06 mg/L) to the LLOQ observations and ADA measured in the development group (n = 13) showed the consistency of the final parameter estimates (differences < 20%, data not shown). No significant differences were observed between the median PK parameters obtained from the bootstrap analysis and the final PK parameter estimates (table 3). Moreover, estimated PK parameters were within the 95%CI of parameters obtained in the bootstrap. In addition, only 9 runs out of 1000 were skipped due to near boundary estimates,

Table 3
Adalimumab population pharmacokinetic parameters.

Parameters	Final Model Estimate	RSE (%)	Shrinkage	Bootstrap (n = 1000*) Mean	95% CI
CL/F (L/h)	0.0157	3		0.0158	0.0152 - 0.0166
BMI-CL/F	1.11	16		1.11	0.81 – 1.42
UDASC-CL/F	1.20	28		1.25	0.54 – 1.86
FCP-CL/F	0.0644	24		0.0645	0.0388 – 0.0900
Pen-CL/F	0.239	35		0.253	0.088 – 0.389
V/F (L)	11.2	9		11.3	9.5 – 13.0
Ka (1/h)	0.0062 (fix)			0.0062 (fix)	
IIVCL/F (CV,%)	23.2	9	14	22.7	19.3 – 26.5
RUV (CV,%)	21.7	13	12	21.4	19.2 – 23.9

$CL/F_i = CL/F^*(BMI/23.7)^{BMI\text{-}CL/F} * (1 + UDASC\text{-}CL/F) * (1 + Pen\text{-}CL/F) * (FCP/74)^{FCP\text{-}CL/F}$.

If no UDASC, UDASC-CL/F = 0.

If 40 mg pen device or BMI < 20 kg/m^2, Pen-CL/F = 0.

* 9 runs over were skipped cause of nearly boundary.

BMI: body mass index (mg/m^2); CI: confidence interval; CL/F: apparent clearance; CV: coefficient of variation; FCP: faecal calprotectin; IIVCL/F: interindividual variability on clearance; Ka: absorption rate constant; Pen: adalimumab pen device; RSE: residual standard error; RUV: residual unexplained variability; UDASC: unexplained decline in adalimumab serum concentrations; V/F: apparent volume of distribution.

proving the stability and robustness of the final model.

Goodness-of-fit plots showed adequate descriptive capacity and absence of pronounced bias in both groups, development and external validation (Fig. 1).

Adalimumab serum concentrations and variability were adequately described throughout the assessment of the follow-up treatment in the development population group using the final PopPK model, as shown in Fig. 2. Although concentrations values and shape of the profile are not directly interpretable from the pcVPC, since a correction is applied,

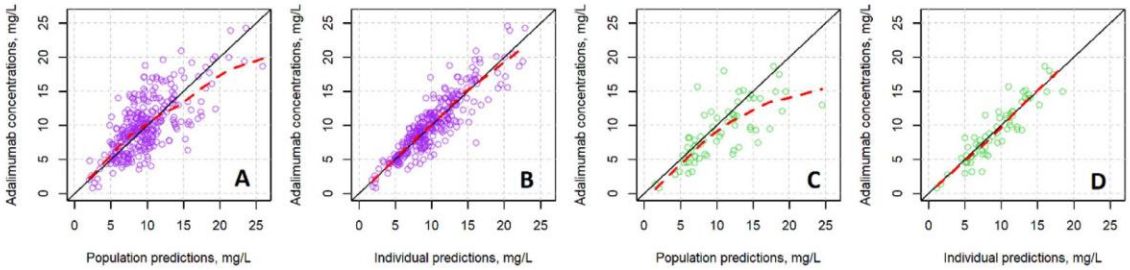

Fig. 1. Goodness-of-fit plot of adalimumab concentrations with the final model for development (A-B) and validation populations (C-D); black solid line, identity line; open circles, adalimumab serum concentrations observed; blue dashed lines, locally weighted scatterplot smoothing (LOWESS).

this methodology is useful an extensively applied for model evaluation with heterogeneous dosage administrations. The pcVPC (Fig. 2) shows the adequate performance and predictions of the adalimumab PopPK model developed. The pcNPC shows a proper prediction performance of the model at day 7 and 14 after adalimumab last dose for the external evaluation group, thus supporting the adequate prediction capacity of the model (supplementary 1).

4. Discussion

Ulcerative Colitis and CD are the most common forms of IBD. These pathologies share many similarities in terms of symptoms, risk factors and treatment, the main difference being the area of the digestive system where inflammation occurs: in UC only the mucosa of the large intestine or the colonic mucosa is affected, whereas in CD, transmural lesions can occur in any part of the digestive tract (Gomollon et al., 2017).

In recent years, adalimumab has contributed to markedly improve the prognosis of IBD (Annese et al., 2016). According to its data sheet (FDA, 2019; EMA, 2019), the dosage of adalimumab is 40 mg every other week via subcutaneous injection, which may be increased to 40 mg every week or 80 mg every other week in cases the recommended dose fails to achieve desired response. PK variability is currently highlighted as a key source of differences (Vande Casteele et al., 2015) in treatment response to adalimumab, supporting that higher drug concentrations are associated with sustained response (Juncadella et al., 2018; Roblin et al., 2014). In addition, the development of immunogenicity associated with this type of drugs is well known and associated with low drug concentrations (Paul et al., 2014). Therefore, improving the understanding of adalimumab PK behaviour is essential to address its therapeutic management and reduce the immunogenicity developed in patients under treatment.

This study aimed at developing a PopPK model of adalimumab in IBD patients based on real-world data obtained from patients under short and long-term treatment. ASC were properly described following a one-compartment open model with first order absorption and elimination. Adalimumab CL/F was demonstrated to be significantly affected by BMI, FCP, the specific drug administration device and the UDASC. No significant differences in adalimumab elimination were found regarding diagnosis (UC or CD), in agreement with previous studies (Ordás et al., 2012; Vande Casteele and Gils, 2015).

The results obtained in this study indicated that a higher CL/F was seen for patients with higher BMI. Therefore, in order to reach target TASC between 8 and 12 mg/L, patients with higher BMI are more likely to require higher doses than those established in the drug data sheet. Several previous studies described TBW as the best anthropometric parameter to describe adalimumab elimination (Sharma et al., 2015; Vande Casteele et al., 2019). By contrast, BMI was the best body size metric affecting adalimumab CL/F in our study population. This difference could be explained by the higher BMI in the development population of the current analysis (23.7 kg/m^2) compared to the BMI of the previous study (Vande Casteele et al., 2019) carried out in adult population (22.6 kg/m^2).

Immunoglobulin drugs administered subcutaneously undergo a high presystemic metabolism (Ordás et al., 2012; Richter et al., 2012 and Wang et al., 2008). Moreover, adipose tissue is a metabolically and immunologically active tissue (Grant and Dixit, 2015). Therefore it can be assumed that patients with increased adipose tissue may experience

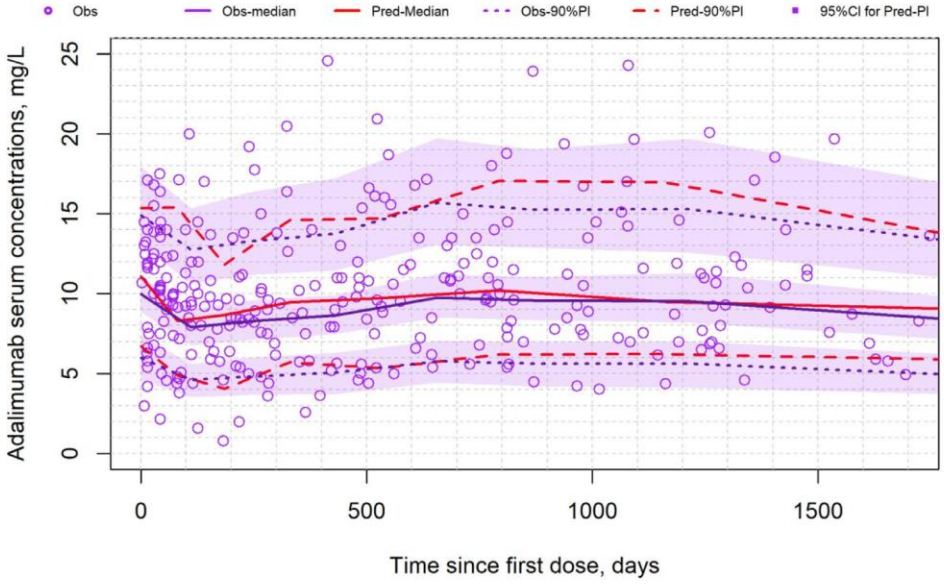

Fig. 2. Prediction-corrected visual predictive check (pcVPC) for the concentration-time after-dose profiles of adalimumab in the development population (internal evaluation). Purple open circles, adalimumab observations (Obs); purple solid line, median of the Obs; red solid line, median of the predicted adalimumab concentrations (Pred); purple dashed lines, 5th and 95th percentile of the Pred (90% prediction interval, PI); red dashed lines, 5th and 95th percentiles of the Obs; purple-shaded area, 95% confidence interval (CI) for the 5th, 50th and 95th percentiles of the Pred.

higher degradation of the drug due to increased of inflammatory mechanisms and consequent catabolism via reticuloendothelial system (Hodkinson, 2017). Therefore, bioavailability could be potentially decreased leading to lower drug concentrations. These findings are supported by previous studies where therapeutic response to adalimumab in IBD patients was lower in patients with higher BMI (Bond et al., 2016; Bultman et al., 2012). Similar results were obtained for patients using adalimumab for the treatment of conditions other than IBD (Højgaard et al., 2016). It should be noted that the effect of BMI on adalimumab PK would be most likely on bioavailability rather than on CL/F. However, bioavailability of adalimumab could not be estimated due to the absence of intravenous drug administration data.

Moreover, the results presented in this work showed a significant influence of the specific administration pen device on adalimumab exposure. Using the 80 mg pen could decrease the ASC reached compared to two administrations with the 40 mg pen device, probably due to a non-linear bioavailability with dose. This could be explained by the previously mentioned metabolism after subcutaneous administration. The deterministic simulations showed the different drug exposures after using 40 mg and 80 mg pen devices of adalimumab when the equivalent intensification dosing of 40 mg weekly and 80 mg each other week were administered accounting for three body size compositions (Fig. 3). According to these results, obese patients (IMC > 30 kg/m^2) could not reach endoscopic remission therapeutic concentrations using the 80 mg pen each other week and dose intensification of 40 mg weekly was proposed for these patients. However, the low percentage of patients treated with the adalimumab 80 mg pen in the presented work (9.2%) justifies the need for additional studies to support these findings.

The influence of the inflammatory burden on the increase of adalimumab CL/F has been previously reported by Vande-Casteele et al. (2015). Among the inflammatory activity biomarkers analysed in our study, FCP and CRP showed a positive influence on adalimumab CL/F, while serum albumin showed a negative influence. Among all these inflammatory variables, FCP had the greatest impact on adalimumab CL/F decreasing its IIV by 16%. One of the novelties regarding previously published adalimumab PopPK models is the assessment of this new biomarker that has been recently introduced in clinical practice. In previous studies, CRP and albumin were the most influential inflammatory markers of adalimumab elimination (Sharma et al., 2015; Vande Casteele et al., 2019). However, the inclusion of FCP in the PK model as a disease activity biomarker seems to provide a better explanation of adalimumab clearance IIV than CRP.

Multiple studies have shown that FCP is a reliable marker of endoscopic activity and therapeutic response as well as a good predictor of relapse and post-operative recurrence in UC (Lin et al., 2014; Mumolo et al., 2018). To this end, the FCP is considered superior to CRP and other faecal biomarkers (Mosli et al., 2015). Thus, FCP is a non-invasive, cost-effective and safe parameter not only helpful in predicting adalimumab exposure but also in related to adalimumab treatment response and mucosal healing in UC.

Recent studies have also shown good correlation between FCP and the endoscopic activity of colonic and ileocolonic CD evaluated by various endoscopic index, such as the Crohn's Disease Endoscopic Index of Severity (CDEIS) and SES-CD (Lin et al., 2014; Lobatón et al., 2013). This correlation is higher than the ones presented by clinical activity index and CRP (Kawashima et al., 2017; Mosli et al., 2015).

On the other hand, there is controversy regarding the influence of the location of CD on the accuracy of the FCP to predict endoscopic lesions. While in some studies the accuracy is similar in different locations (Arai et al., 2016; Jensen et al., 2011), in most cases the correlation between FCP and endoscopic activity is lower in ileal disease than in colic or ileocolic (Lobatón et al., 2013; Stawczyk-Eder et al., 2015). Some authors have questioned the validity of these findings since the exploration of the ileum was performed by ileocolonoscopy and was considered incomplete since visualizing proximal small intestine sections was not possible (Guardiola et al., 2018). Recently, several studies addressing this issue have been conducted through a complete study of the ileum (Arai et al., 2016; Cerillo et al., 2015;

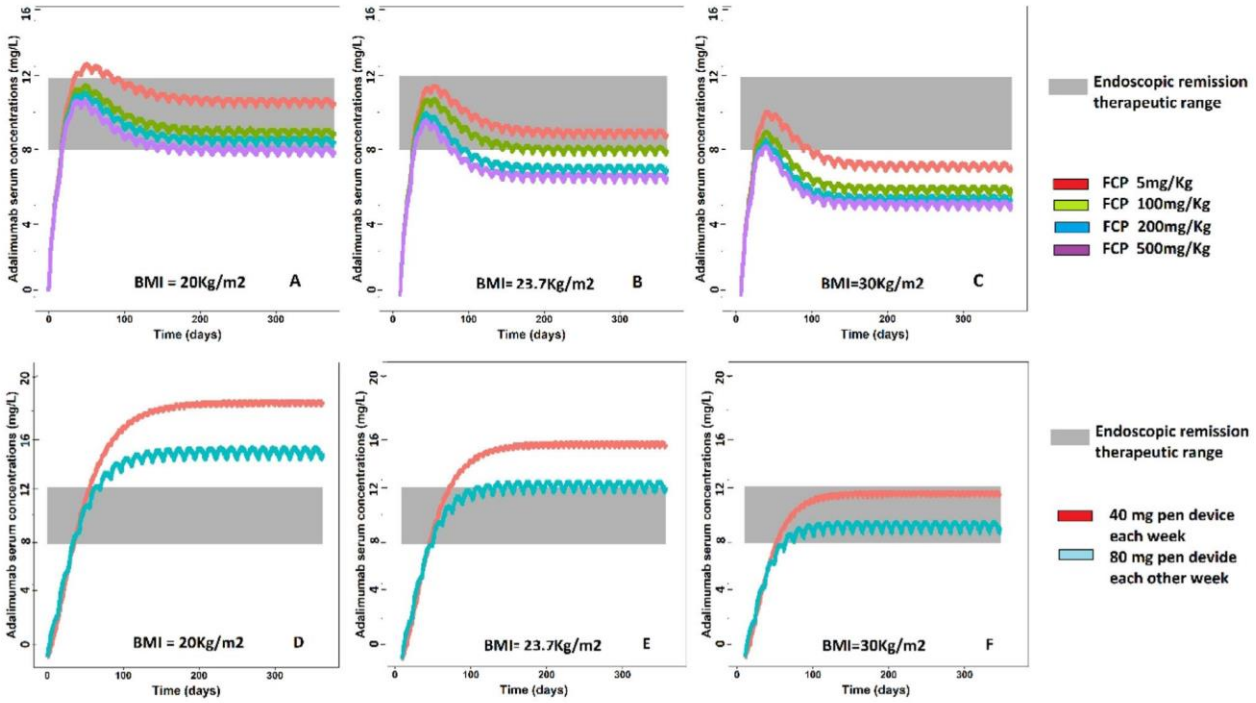

Fig. 3. Adalimumab serum concentrations simulated depending on the faecal calprotectin (FCP) of the patient for body mass index (BMI) of 20 kg/m^2 (A), 23.7 kg/m^2 (median of study population) (B) and 30 kg/m^2(C) when the standard doses of 160 mg (week 0), 80 mg (week 2) and 40 mg each other week is administrated with the 40 mg pen device. On the lower panels adalimumab serum concentrations simulated of equivalent intensification doses of 40 mg weekly and 80 mg each other week for patients with BMI of 20 kg/m^2 (D), 23.7 kg/m^2 (E) and 30 kg/m^2 (F), respectively, with a median FCP of 74 mg/Kg (median of study population) are shown.

Kawashima et al., 2017). All of them suggested that FCP was a reliable marker of ileal endoscopic activity, although less relevant than in colic disease. In the adalimumab PopPK model presented in this research, FCP was the best disease biomarker in patients with ileal CD. Adalimumab PK behaviour in patients with CD located in the small intestine can be characterized by FCP better than CRP or albumin although its impact on the disease activity is not yet well established. The total number of patients (29) with disease exclusively ileal CD was small and did not allow definitive conclusions.

Model-based simulations yielded lower ASC than expected for patients with high FCP. For example, an average patient (BMI = 23.7 kg/m^2) treated with a standard adalimumab dosage would not reach TASC within the therapeutic range for endoscopic remission (TASC > 8 mg/L) with high FCP ≥ 200 mg/kg. These results are consistent with the relationship between TASCs observed at steady state and FCP values in the development group (supplementary). In the raw data, a slight increase in the proportion of patients not reaching the therapeutic range for endoscopic remission (TASCs > 8 mg/L) with increasing FCP values was observed. In addition, obese patients (BMI ≥ 30 kg/m^2) could not reach therapeutic concentrations (TASCs ≤ 8 mg/L) whatever FCP values considered. Therefore, FCP has been identified as a relevant factor to predict adalimumab exposure in absence of the development of immunogenicity and it can be a powerful biomarker for adalimumab dosage individualization in patients with high detectable FCP levels before the onset of clinical symptomatology.

One of the biggest challenges of TDM of anti-TNF and the use of PopPK models is the correct interpretation of the analytical assay used (Gorovits et al., 2018; Sánchez-Hernandez et al., 2019) to measure ADA. Among the available techniques, the most commonly used is ELISA. Most of the commercial ELISA analytical methods to determine ADA are drug-sensitive tests, since they are unable to detect ADA bound to the drug. Thus, ADA can only be measured in the absence of detectable ASC and therefore, the proportion of ADAs and the quantitative effect of them on CL/F could be underestimated. A drug-sensitive test was used in PopPK models developed by Ternant et al. (2015) and Sharma et al. (2015). In addition, Berends et al. (2018) could only measure ADA when ASC was below 5 mg/L. On the other hand, Vande Casteele et al. (2019) developed an in-house ELISA assay applied for accurate quantification of ADA with concentrations up to 25 mg/L. However, different reports (Van Stappen et al., 2018 and Papamichael et al., 2019a) conclude that drug-tolerant assays did not offer clinical benefits over drug-sensitive assays.Taking into account the mentioned limitations of drug-sensitive ELISA, the PopPK presented in this work, can be a very helpful tool to identify the development of immunogenicity prior to the presence of detectable ADA, when a proactive monitoring strategy is applied in the clinical setting and specially since UDASC are mainly related to start of immunogenicity (Bloem et al., 2015). Therefore, model-based assessment of ADA presence could improve the efficacy and persistence of adalimumab treatment (Papamichael et al., 2019b; Sanchez-Hernandez et al., 2020).

One of the strengths of our study is the development of an adalimumab PopPK model with an adequate descriptive and predictive performance including variables that are typically available during the follow-up of IBD patients, supporting its extrapolation to clinical practice. In addition, special emphasis should be placed on the identification of new factors that affect adalimumab exposure such as BMI and FCP, and can be used for optimizing individual drug dosage regimens.

Due to the lack of information in the absorption and distribution phases, the PopPK model developed was focused on characterizeing the elimination of the drug, which can limit the accurate estimation of drug exposure. However, the purpose of this study was not to characterize the drug's whole time-concentration profile, but to establish a model with an adequate description capacity of adalimumab PK and exposure that would be easy to implement in clinical practice as a guiding tool for dosage decision-making. Moreover, adalimumab data reliability was limited by the lack of homogeneity, since the only serum samples collected at protocoled sampling times were coming from patients who started adalimumab treatment after the beginning of the study.

5. Conclusions

In conclusion, the population-based pharmacokinetic model developed adequately characterized adalimumab PK in IBD patients after subcutaneous administration. The UDASC, BMI and FCP have been identified as meaningful variables with significant influence on adalimumab clearance and exposure. The PopPK model presented could be a useful tool both for immunogenicity development detection and for adalimumab dosage individualization, possibly leading to an improvement in adalimumab treatment efficacy and safety. Future prospective studies are required to support the findings obtained.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

JG Sánchez-Hernández and F Muñoz have served as speakers for Abbvie Spain.

Acknowledgments

The authors want to thank the support received from all the staff of our Pharmacokinetics Laboratory, Pharmacy and Gastroenterology Services.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2020.105369.

References

Aalberse, R., Stapel, S., Schuurman, J., et al., 2009. Immunoglobulin G4: an odd antibody. Clin. Exp. Allergy 39, 469–477. https://doi.org/10.1111/j.1365-2222.2009.03207.x.

Annese, V., Duricova, D., Gower-Rousseau, C., et al., 2016. Impact of new treatments on hospitalisation, surgery, infection, and mortality in ibd: a focus paper by the epidemiology committee of ECCO. J. Crohns. Colitis. 10 (2), 216–254. https://doi.org/10.1093/ecco-jcc/jjv190.

Arai, T., Takeuchi, K., Miyamura, M., et al., 2016. Level of fecal calprotectin correlates with severity of small-bowel Crohn's disease, measured by balloon-assisted endoscopy and computed tomography enterography. Clin. Gastroenterol. Hepatol. 15, 56–62. https://doi.org/10.1016/j.cgh.2016.08.015.

Barlow, N.L., Mohammed, P., Berg, J.D, 2016. Serum trough infliximab and anti-infliximab antibodies in a cohort of gastroenterology and rheumatology patients' infliximab therapeutic drug monitoring. Ann. Clin. Biochem. 53 (4), 477–484. https://doi.org/10.1177/0004563215604866.

Bauer, L.A, 2001. Applied Clinical Pharmacokinetics. McGraw Hill, New York, pp. 93–179 Medical Publishing Division.

Beal, S.L., Sheiner, L.B., Boeckmann, A.J., et al., 2009. NONMEM 7.1.0 Users Guides. Icon Development Solutions, Ellicott City 1989–.

Berends, S.E., Strik, A.S., Van Selm, J.C., et al., 2018. Explaining interpatient variability in adalimumab pharmacokinetics in patients with Crohn's disease. Ther. Drug Monit. 40 (2), 202–211. https://doi.org/10.1097/FTD.0000000000000494.

Bergstrand, M., Hooker, A.C., Wallin, J.E., et al., 2011. Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. AAPS J. 13, 143–151. https://doi.org/10.1208/s12248-011-9255-z.

Bloem, K., van Leeuwen, A., Verbeek, G., et al., 2015. Systematic comparison of drug-tolerant assays for anti-drug antibodies in a cohort of adalimumab-treated rheumatoid arthritis patients. J. Immunol. Methods 418, 29–38. https://doi.org/10.1016/j.jim.2015.01.007.

Bond, A., Asher, R., Jackson, R., et al., 2016. Comparative analysis of the influence of clinical factors including BMI on adalimumab and infliximab trough levels. Eur. J. Gastroenterol. Hepatol. 28 (3), 271–276. https://doi.org/10.1097/MEG.0000000000000544.

Bultman, E., de Haar, C., van Liere-Baron, A., et al., 2012. Predictors of dose escalation of adalimumab in a prospective cohort of Crohn's disease patients. Aliment Pharmacol. Ther. 35 (3), 335–341. https://doi.org/10.1111/j.1365-2036.2011.04946.x.

Cerrillo, E., Beltrán, B., Pous, S., et al., 2015. Fecal calprotectin in ileal Crohn's disease: relationship with magnetic resonance enterography and a pathology score. Inflamm. Bowel Dis. 21, 1572–1579. https://doi.org/10.1097/MIB.0000000000000404.

Chennavasin, P., Brater, D.C., 1982. Aminoglycoside dosage adjustment in renal failure: a hand-held calculator program. Eur J Clin Pharmacol 22, 91–94. https://doi.org/10.1007/bf00606431.

Daperno, M., D'Haens, G., Van Assche, G., et al., 2004. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointest Endosc 60, 505–512. https://doi.org/10.1016/s0016-5107(04)01878-4.

Du Bois, D., Du Bois, E.F, 1989. A formula to estimate the approximate surface area if height and weight be known. 1916. Nutrition 5, 303–313.

Ette, E.I., Williams, P.J, 2007. Pharmacometrics: The Science of Quantitative Pharmacology. Wiley-Interscience, New York.

European Medicines Agency (EMA). Humira product information. Avaiable from:https://www.ema.europa.eu/en/medicines/human/EPAR/humira. Accessed September 1, 2019.

Food and Drug Administration (FDA). Adalimumab product approval information. Avaiable from:https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/125057s410lbl.pdf. Accessed September 1, 2019.

Gomollon, F., Dignass, A., Annese, V., et al., 2017. Third European evidence-based consensus on the diagnosis and management of Crohn's Disease 2016: part 1: diagnosis and medical management. J. Crohns. Colitis. 11 (1), 3–25. https://doi.org/10.1093/ecco-jcc/jjw168.

González-Fernández, M.Á., Villamañán, E., Jiménez-Nácher, I., et al., 2019. Persistence of biological agents over an eight-year period in rheumatoid arthritis and spondyloarthritis patients. Farm Hosp. 43 (1), 24–30. https://doi.org/10.7399/fh.11080.

Gorovits, B., Baltrukonis, D.J., Bhattacharya, I., et al., 2018. Immunoassay methods used in clinical studies for the detection of anti-drug antibodies to adalimumab and infliximab. Clin. Exp. Immunol. 192 (3), 348–365. https://doi.org/10.1111/cei.13112.

Guardiola, J., Lobatón, T., Carrillo, E., et al., 2018. Recommendations of the Spanish working group on Crohn's disease and ulcerative colitis (GETECCU) on the utility of the determination of faecal calprotectin in inflammatory bowel disease. Gastroenterol. Hepatol. 41 (8), 514–529. https://doi.org/10.1016/j.gastrohep.2018.05.029.

Grant, R.W., Dixit, V.D., 2015. Adipose tissue as an immunological organ. Obesity (Silver Spring) 23, 508–512. https://doi.org/10.1002/oby.21003.

Harvey, R.F., Bradshaw, J.M., 1980. A simple index of Crohn's-disease activity. Lancet 315 (8167), 514. https://doi.org/10.1016/s0140-6736(80)91372-0.

Hodkinson, J.P., 2017. Considerations for dosing immunoglobulin in obese patients. Clin. Exp. Immunol. 188 (3), 353–362. https://doi.org/10.1111/cei.12955.

Højgaard, P., Glintborg, B., Kristensen, L.E., et al., 2016. The influence of obesity on response to tumour necrosis factor-α inhibitors in psoriatic arthritis: results from the DANBIO and ICEBIO registries. Rheumatology (Oxford) 55 (12), 2191–2199. https://doi.org/10.1093/rheumatology/kew326.

Jensen, M.D., Kjeldsenm, J., Nathan, T, 2011. Fecal calprotectin is equally sensitive in Crohn's disease affecting the small bowel and colon. Scand. J. Gastroenterol. 46, 694–700. https://doi.org/10.3109/00365521.2011.560680.

Juncadella, A., Papamichael, K., Vaughn, B.P., 2018. at alt. Maintenance adalimumab concentrations are associated with biochemical, endoscopic, and histologic remission in inflammatory bowel disease. Dig Dis. Sci. 63 (11), 3067–3073. https://doi.org/10.1007/s10620-018-5202-5.

Kawashima, K., Ishihara, S., Yuki, T., et al., 2017. Fecal Calprotectin More Accurately Predicts Endoscopic Remission of Crohn's Disease than Serological Biomarkers Evaluated Using Balloon-assisted Enteroscopy. Inflamm. Bowel Dis. 23 (11), 2027–2034. https://doi.org/10.1097/MIB.0000000000001202.

Lewis, J.D., Chuai, S., Nessel, L., et al., 2008. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. Inflamm. Bowel Dis. 14 (12), 1660–1666. https://doi.org/10.1002/ibd.20520.

Lin, J.F., Chen, J.M., Zuo, J.H., et al., 2014. Meta-analysis: fecal calprotectin for assessment of inflammatory bowel disease activity. Inflamm. Bowel Dis. 20, 1407–1415. https://doi.org/10.1097/MIB.0000000000000057.

Lobatón, T., López-García, A., Rodríguez-Moranta, F., et al., 2013. A new rapid test for fecal calprotectin predicts endoscopic remission and postoperative recurrence in Crohn's disease. J. Crohns. Colitis. 7, 641–651. https://doi.org/10.1016/j.crohns.2013.05.005.

Mitrev, N., Vande Casteele, N., Seow, C.H., et al., 2017. Review article: consensus statements on therapeutic drug monitoring of anti-tumour necrosis factor therapy in inflammatory bowel diseases. Aliment Pharmacol. Ther. 46 (11–12), 1037–1053. https://doi.org/10.1111/apt.14368.

Mosli, M.H., Zou, G., Garg, S.K., et al., 2015. C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: a systematic review and meta-analysis. Am. J. Gastroenterol. 110, 802–819. https://doi.org/10.1038/ajg.2015.120.

Mumolo, M.G., Bertani, L., Ceccarelli, L., et al., 2018. From bench to bedside: fecal calprotectin in inflammatory bowel diseases clinical setting. World J. Gastroenterol. 24 (33), 3681–3694. https://doi.org/10.3748/wjg.v24.i33.3681.

Nguyen, T.H., Mouksassi, M.S., Holford, N., et al., 2017. Model Evaluation of Continuous Data Pharmacometric Models: metrics and Graphics. CPT Pharmacometr. Syst. Pharmacol. 6 (2), 87–109. https://doi.org/10.1002/psp4.12161.

Ordás, I., Mould, D.R., Feagan, B.G., et al., 2012. Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. Clin. Pharmacol. Ther. 91 (4), 635–646. https://doi.org/10.1038/clpt.2011.328.

Papamichael, K., Cheifetz, A.S., Melmed, G.Y., et al., 2019a. Appropriate therapeutic drug monitoring of biologic agents for patients with inflammatory bowel diseases. Clin. Gastroenterol. Hepatol. 17 (9), 1655–1668. https://doi.org/10.1016/j.cgh.2019.03.037.

Papamichael, K., Juncadella, A., Wong, D., et al., 2019b. Proactive therapeutic drug monitoring of adalimumab is associated with better long-term outcomes compared with standard of care in patients with inflammatory bowel disease. J. Crohns. Colitis. 13 (8), 976–981. https://doi.org/10.1093/ecco-jcc/jjz018.

Paul, S., Moreau, A.C., Del Tedesco, E., et al., 2014. Pharmacokinetics of adalimumab in inflammatory bowel diseases: a systematic review and meta-analysis. Inflamm. Bowel Dis. 20, 1288–1295. https://doi.org/10.1097/MIB.0000000000000037.

Richter, W.F., Bhansali, S.G., Morris, M.E, 2012. Mechanistic determinants of biotherapeutics absorption following SC administration. AAPS J. 14 (3), 559–570. https://doi.org/10.1208/s12248-012-9367-0.

Roblin, X., Marotte, H., Rinaudo, M., et al., 2014. Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. Clin. Gastroenterol. Hepatol. 12 (1), 80–84. https://doi.org/10.1016/j.cgh.2013.07.010.

Sanchez-Hernandez, J.G., Rebollo, N., Munoz, F., et al., 2019. Therapeutic drug monitoring of tumour necrosis factor inhibitors in the management of chronic inflammatory diseases. Ann. Clin. Biochem. 56 (1), 28–41. https://doi.org/10.1177/0004563218782286.

Sánchez-Hernández, J.G., Rebollo, N., Martin-Suarez, A., Calvo, M.V., Muñoz, F, 2020. A 3-year prospective study of a multidisciplinary early proactive therapeutic drug monitoring programme of infliximab treatments in inflammatory bowel disease. Br. J. Clin. Pharmacol. 1–11. https://doi.org/10.1111/bcp.14229.

Savic, R.M., Karlsson, M.O., 2009. Importance of shrinkage in empirical bayes estimates for diagnostics: problems and solutions. AAPS J. 11, 558–569. https://doi.org/10.1208/s12248-009-9133-0.

Sharma, S., Eckert, D., Hyams, J.S., et al., 2015. Pharmacokinetics and exposure efficacy relationship of adalimumab in pediatric patients with moderate to severe Crohn's disease: results from a randomized, multicenter, phase-3 study. Inflamm. Bowel Dis. 21, 783–792. https://doi.org/10.1097/MIB.0000000000000327.

Stawczyk-Eder, K., Eder, P., Lykowska-Szuber, L., et al., 2015. Is faecal calprotectin equally useful in all Crohn's disease locations? A prospective, comparative study. Arch. Med. Sci. 11 (2), 353–361. https://doi.org/10.5114/aoms.2014.43672.

Ternant, D., Karmiris, K., Vermeire, S., et al., 2015. Pharmacokinetics of adalimumab in Crohn's disease. Eur. J. Clin. Pharmacol. 71 (9), 1155–1157. https://doi.org/10.1007/s00228-015-1892-1.

Vande Casteele, N., Gils, A, 2015. Pharmacokinetics of anti-TNF monoclonal antibodies in inflammatory bowel disease: adding value to current practice. J. Clin. Pharmacol. 55 (3), 39–50. https://doi.org/10.1002/jcph.374.

Vande Casteele, N., Baert, F., Bian, S., et al., 2019. Subcutaneous absorption contributes to observed interindividual variability in adalimumab serum concentrations in Crohn's disease: a prospective multicentre study. J. Crohns. Colitis. 13 (10), 1248–1256. https://doi.org/10.1093/ecco-jcc/jjz050.

Van Stappen, T., Vande Casteele, N., Van Assche, G., et al., 2018. Clinical relevance of detecting anti-infliximab antibodies with a drug-tolerant assay: post hoc analysis of the TAXIT trial. Gut 67, 818–826. https://doi.org/10.1136/gutjnl-2016-313071.

Wang, W., Wang, E.Q., Balthasar, J.P, 2008. Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin. Pharmacol. Ther. 84 (5), 548–558. https://doi.org/10.1038/clpt.2008.170.

Xu, S.X., Dunne, A., Kimko, H., et al., 2011. Impact of low percentage of data below the quantification limit on parameter estimates of pharmacokinetic models. J. Pharmacokinet. Pharmacodyn. 38 (4), 423–432. https://doi.org/10.1007/s10928-011-9201-9.