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Infliximab Concentrations During Induction Are Predictive for Endoscopic Remission in Pediatric Patients With Inflammatory Bowel Disease Under Combination Therapy

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Key words: children, inflammatory bowel disease, infliximab, outcome, therapeutic drug monitoring

Abbreviations:

ATI:	antibodies to infliximab
AUC:	area under the curve
AUROC:	areas under the ROC curve
CD:	Crohn's disease
CI:	confidence interval
CRP:	C-reactive protein
ESR:	erythrocyte sedimentation rate
GALNT10:	polypeptide N-acetylgalactosaminyl transferase 10
HSD11B1:	hydroxysteroid dehydrogenase 11B1
IBD:	inflammatory bowel disease
IFX:	infliximab
IL:	interleukin
IQR:	interquartile range
MASP:	mannan-binding lectin serine protease
OR:	odds ratio

PCDAI:	Pediatric Crohn's Disease Activity Index
PROK1:	prokineticin 1
PUCAI:	Pediatric Ulcerative Colitis Activity Index
ROC:	Receiver operating characteristics
SES-CD:	Simple Endoscopic Score for Crohn's Disease
SH2D1A:	SH2 domain-containing protein 1A
TDM:	therapeutic drug monitoring
TNF:	tumor necrosis factor
UC:	ulcerative colitis

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Objectives: To study infliximab (IFX) pharmacokinetics in children with inflammatory bowel disease (IBD) during induction therapy to predict outcome and explore if other covariates influenced outcome.

Study design: All children with IBD starting IFX therapy (5 mg/kg at weeks 0-2-6 and 12) for active luminal disease from May 2017 till May 2019 were included and followed prospectively. Patients were sampled at multiple timepoints during induction (trough concentrations (TC) and peak concentration at weeks 0-2-6-12, and intermediate concentration at weeks 1-4). IFX concentrations and cumulative drug exposure were correlated with outcome at six months. Endoscopic remission was defined as Simple Endoscopic Score for Crohn's Disease <3 or Mayo endoscopic sub-score $=0$, and deep remission as endoscopic with clinical remission (PUCAI/PCDAI <10).

Results: 252 serum induction concentrations were obtained from 32 patients (81% on concomitant thiopurines). Children in endoscopic remission (all in deep remission) at six months had significantly higher drug concentrations from week 4 onwards. ROC curve analysis identified IFX TC at week 12 $\geq 5.0 \mu\text{g/mL}$ and AUC week 0-12 $\geq 4056.0 \mu\text{g}\cdot\text{day/mL}$ as minimal target to achieve endoscopic remission at six months (AUROC: 0.796 [95%CI: 0.62-0.97] and AUROC: 0.778 [95%CI: 0.61-0.94], respectively). In addition, our findings suggest that proteomic analysis may help to understand IFX response.

Conclusion: Higher IFX exposure during induction therapy in pediatric IBD patients is associated with significantly better endoscopic and deep remission rates at six months. Drug concentrations differentiate remitters from non-remitters from Week 4 post-induction onwards.

Despite growing insights into the pathogenesis of inflammatory bowel disease (IBD) and recent therapeutic developments, IBD remains a life-long and incurable disease [1-2]. Fortunately, biological agents targeting tumor necrosis factor (TNF)-alpha have improved the treatment of pediatric IBD [1-2]. Currently, anti-TNF is the only class of biologics approved by FDA (Food and Drug Administration) and EMA (European Medicines Agency) for the treatment of pediatric IBD. The future place of anti-TNF agents will depend on the results of novel targeted agents in the expanding therapeutic armamentarium [3].

Several adult studies have shown that patients who fail their first biologic, will have reduced outcomes after initiation of their second or third biologic [4-5]. In addition, achieving deep remission as early as possible in the disease course is crucial for a favorable long-term outcome [6]. Therefore, guidelines recommend optimizing efficacy of the first anti-TNF antibody before switching [7]. Therapeutic drug monitoring (TDM) can help to achieve this goal by tailoring treatment to an individual patient's needs and to differentiate the cause of lack of response [8]. Ideally, these TDM-based interventions should be done as early as possible, including during induction to further improve long-term outcome. However, the ideal timing for sampling and target concentration needed to achieve endoscopic remission are still unknown in children with IBD. Finally, it is still uncertain whether trough, currently being used in TDM-based algorithms, rather than intermediate or peak concentrations, will have a better predictive value for optimizing anti-TNF therapy or whether other pharmacokinetic values, such as area under the concentration-time curve (AUC), would be more useful.

In this prospective study, we evaluated the impact of infliximab (IFX) pharmacokinetics during induction on long-term outcome with the goal to optimize dosing to a target concentration, to further maximize endoscopic remission. The primary aim was to evaluate the association between IFX serum concentrations and cumulative drug exposure throughout induction and outcome measured at six months. For maximal translation into clinical setting, we also studied whether

intermediate or peak concentrations could be of value in predicting outcome. The secondary aim was to define clinical, biological, endoscopic and proteomics predictors of (non)-response to IFX and their correlation to drug concentrations. The ability to identify reliable markers of response prior to treatment initiation may allow more targeted therapy selection and efficient achievement of remission.

Methods

This prospective cohort study was conducted at the University of Leuven (Belgium). Approval was granted by the Ethics Committee of our university (No: S59870, April 10, 2017). All patients and both their parents provided written informed consent before inclusion.

Children aged <18 years with Crohn's disease (CD) or ulcerative colitis (UC) starting IFX therapy for active luminal disease from May 2017 till May 2019 were enrolled. Only anti-TNF naïve patients were included and followed up prospectively till six months after initiation of IFX therapy. IFX was started as per accepted criteria (after treatment failure or intolerance to first-line therapy with 5-aminosalicylates, immunomodulators, steroids, and/or, in the case of CD, exclusive enteral nutrition) in addition to proven endoscopic active disease at baseline (Simple Endoscopic Score for Crohn's Disease (SES-CD) >3 in case of CD [9-10] or a Mayo endoscopic sub-score of ≥ 2 in case of UC [11-12]). Exclusion criteria were the presence of an ileostomy or colostomy, acute disease needing surgery, previous anti-TNF exposure, absence of endoscopic activity or patient's/parental refusal to participate. Patients were treated with standard intravenous IFX 5 mg/kg (2h infusion) at Weeks 0, 2, and 6 followed by a maintenance therapy starting at Week 12 instead of 14 in all patients. The Week 12 timepoint was installed in our pediatric IBD unit after we and others previously showed that most pediatric patients experience low IFX trough concentrations at Week 14 with the standard induction schedule [13-15]. IFX dose and/or interval adjustments were only possible during maintenance therapy after 12 weeks

based on therapeutic drug monitoring aiming to target a therapeutic window between 5-9 $\mu\text{g/mL}$ [16].

Patients received concomitant azathioprine throughout the study unless they had a homozygous or compound heterozygous thiopurine methyltransferase mutation, or intolerance to thiopurines.

Data collection

Baseline patients' characteristics included age, sex, IBD type (in accordance with the revised Porto criteria [17]), co-morbidity, and Paris classification (at diagnosis and at IFX initiation [18]).

Patients were followed-up during induction: visits were scheduled at each IFX infusion at Weeks 0, 2, 6, and 12 and at intermediate visits at Weeks 1 and 4. During maintenance therapy, patient's visits were in accordance with each IFX infusion. Clinical disease activity was assessed and recorded at each visit by the same pediatric gastroenterologist using the PCDAI (Pediatric Crohn's Disease Activity Index) for CD [19] and the PUCAI (Pediatric Ulcerative Colitis Activity Index) for UC [20]. IFX doses and intervals were documented along with patients' age and biometrics (body weight, height, body mass index, and body surface area). If applicable these patients' biometrics were also expressed by standard references using age- and sex-specific references from the Belgium, Flanders 2004 growth charts [21]. At each visit, the following standard laboratory tests were measured: hemoglobin, hematocrit, white blood cells and differentiation, platelet count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, iron panel, and serum IFX concentration.

Serum IFX concentrations were quantified as trough concentrations (at Weeks 2, 6 and 12), as peak concentration (2 hours after cessation of IFX infusion at Weeks 0, 2, and 6), and as intermediate concentration (at Weeks 1 and 4). All samples were prospectively collected, but only measured after the last patient completed the study. As a consequence, no TDM-based

treatment adjustments were made during induction. The samples were centrifuged, and serum was divided into 300 μL aliquots and stored at -20°C . IFX concentrations were measured by Ridascreen IFX monitoring enzyme-linked immunosorbent assay (R-Biopharm) with a lower limit of quantification of 0.3 $\mu\text{g}/\text{ml}$. To account for intra-assay variability, all samples were measured twice independently, and the average was reported. Antibodies to infliximab (ATI) were determined by an in house developed drug-sensitive anti-IFX bridging assay [22].

In addition, prior to the start of IFX therapy and at week 12, serum samples were analyzed by Proximity Extension Assay technology (OLINK, Olink Proteomics AB). Data are presented in Normalized Protein eXpression (NPX) values, which is an arbitrary unit on a log2-scale. Therefore, NPX values can only be used for relative quantification (adjusted for inter-and intra-run variation) and a high value will correspond to higher protein expression. Assay validation data are available on the manufacturer's website (www.olink.com). We measured seven panels each consisting of 96 proteins (Information, Immuno-oncology, Immune Response, Organ Damage, Development, Cell Regulation, Metabolism). Proteins with missing data frequency of $>25\%$ were excluded from further analyses.

Endoscopic re-evaluation was performed six months after IFX initiation to evaluate mucosal healing. For patients with CD, endoscopic remission was assessed by ileocolonoscopy, whereas for patients with UC it could be either sigmoidoscopy or colonoscopy.

Definitions of outcomes

The primary endpoint was assessed at six months for all different outcomes. Clinical remission was defined as PCDAI or PUCAI <10 [23-24]. Biological remission was defined as CRP ≤ 5 mg/L in combination with ESR ≤ 20 mm/h, in patients with elevated inflammatory markers at IFX initiation [16]. Endoscopic remission was defined as SES-CD <3 [9-10] or Mayo endoscopic subscore of 0 [11-12]. Patients were considered in deep remission if both clinical and endoscopic

remission were met. Patients were identified as non-remitter if these criteria were not met or if therapy was stopped due to loss of response before the primary endpoint was achieved.

Statistical analyses

Statistical analyses were performed with the IBM SPSS 26.0 software (SPSS Inc., Chicago, IL, USA) and R (version 3.6.3) using “caret”, “glmnet”, and “corr” packages. Categorical variables are displayed as frequencies with percentages and continuous variables as medians with interquartile ranges [IQR]. Mann-Whitney U test was used for univariable analysis of unpaired continuous variables. Discrete, unpaired variables were compared by the Fisher exact test or Chi-square test, as appropriate. The Spearman rank correlation coefficient (r_s) was used to analyze correlation. AUC for IFX concentrations versus time was calculated by using the linear trapezoidal rule. For cut-off point analysis, a receiver operating characteristics (ROC) curve analysis was performed based on the Youden J statistic and the “closest top-left” method. To evaluate the predictive performance of the different IFX concentrations and drug exposure, the highest area under the ROC curve (AUROC) were defined. To assess associations between predefined outcomes and different covariates, a binary logistic regression analysis was performed using a backward Wald method. Covariates reaching statistical significance in the univariable analysis ($p < 0.2$) were selected for the multivariable models. Except for variables with high collinearity (Variance Inflation Factor > 5), only one of the variables was retained. These results are reported as odds ratios (OR) and 95% confidence intervals [95%CI]. A 2-sided p -value ≤ 0.05 denoted statistical significance. To evaluate the predictive value of serum biomarkers, a false discovery rate correction for multiple testing was applied with a cut-off of 0.05. To generate a prognostic model based on these biomarkers, the dataset was randomly split into a test (40%) and a training (60%) dataset. Multiple penalized logistic regression models were built and optimized using alpha between 0.6 and 1, and 5-fold cross-validation. The performance of each model was evaluated by means of AUROC.

Results

Patients' characteristics

Forty-two pediatric IBD patients started IFX therapy between May 2017 and May 2019. Three of them were excluded as they already received at least one IFX induction dose elsewhere and two others had been exposed to anti-TNF before. Thirty-two patients were eligible for the study as an additional five patients refused to participate in the study (**Figure 1; available at www.jpeds.com**). CD was diagnosed in 20 patients (62.5%) and 12 patients (37.5%) had UC. Median age at start of IFX therapy was 13.8 years [11.3-14.9]. Patient characteristics are displayed in **Table 1**.

Serum infliximab concentration

A total of 252 IFX induction concentrations were obtained from these 32 patients. IFX concentrations were collected from each patient at the indicated timepoints, except three serum samples were missing at Week 1 and one at Week 4. Because there were no significant differences in median IFX concentrations and cumulative drug exposure at different timepoints based on IBD subtype (CD or UC), data from patients with CD and UC were pooled. Only one patient had undetectable IFX concentrations ($<0.3 \mu\text{g/mL}$) at Weeks 6 and 12, but had no measurable ATI.

Impact of IFX concentrations at different time points on different types of remission

Endoscopic remission was achieved in 18/32 patients (56.3%) at six months (all in deep remission). One patient discontinued IFX treatment before six months due to persistent disease despite adequate IFX concentrations, and was reported as treatment failure.

Children who were in endoscopic remission at six months had significantly higher median IFX concentrations already at Week 4 ($38.8 \mu\text{g/mL}$ [24.3-46.0] versus $23.5 \mu\text{g/mL}$ [10.5-36.6], $p=0.017$), at Week 6 at trough ($19.9 \mu\text{g/mL}$ [10.1-26.3] versus $11.1 \mu\text{g/mL}$ [3.7-19.9],

$p=0.031$), and at Week 12 at trough ($9.6 \mu\text{g/mL}$ [5.5-11.9] versus $3.5 \mu\text{g/mL}$ [2.7-7.2], $p=0.004$; **Figure 2**). At Weeks 1 and 2, although numerically higher, IFX concentrations were not significantly different. Peak IFX concentrations could not differentiate remitters from non-remitters at six months. In addition, the overall drug exposure during induction was significantly higher in children who were in endoscopic remission at six months (AUC week 0-12: $4574.7 \mu\text{g}\cdot\text{day/mL}$ [3783.0-5160.8] versus $3722.9 \mu\text{g}\cdot\text{day/mL}$ [3102.2-3991.9], $p=0.008$, **Figure 3** [available at www.jpeds.com]). Only the drug exposure between 3th and 4th IFX infusion could differentiate remitters from non-remitters (AUC week 6-12: $2397.0 \mu\text{g}\cdot\text{day/mL}$ [2053.9-2663.9] versus $1765.7 \mu\text{g}\cdot\text{day/mL}$ [1700.6-2104.5], $p=0.002$) and not the IFX exposure during the first six weeks.

The predictive value of these IFX trough concentrations at Week 12 were reviewed for the different outcomes. Clinical remission was achieved in 24/32 patients (75.0%) at six months and was associated with significantly higher median Week 12 IFX concentration ($8.6 \mu\text{g/mL}$ [5.1-12.0]) versus ($4.3 \mu\text{g/mL}$ [3.1-5.4], $p=0.033$; **Figure 4**). At six months, a total of 19/23 patients (82.6%) were in biological remission and 16/23 patients (69.6%) were in combined clinical and biological remission, but this was not associated with higher median Week 12 IFX concentration ($6.7 \mu\text{g/mL}$ [4.0-12.0] and $6.8 \mu\text{g/mL}$ [4.3-12.0]) versus ($4.3 \mu\text{g/mL}$ [1.2-7.2] and $4.8 \mu\text{g/mL}$ [3.0-7.8], $p=0.250$ and $p=0.135$, respectively; **Figure 4**). However, early and persistent normalization of baseline biomarkers (time between IFX initiation and moment of sustained CRP and ESR normalization) correlated well with these IFX concentrations ($r_s = -0.522$; $p=0.011$).

Use of ROC curves to select cut offs for IFX concentrations

ROC curve analysis was performed to determine the threshold of IFX concentrations and drug exposure that best predicted endoscopic at six months (**Figure 5**; available at www.jpeds.com). Specific characteristics of the optimal cut-off values are represented in **Table II**. Univariable regression analysis identified these cut-off values of IFX trough concentration at Week 12 (≥ 5.0

$\mu\text{g/mL}$), AUC till week 12 ($\geq 4056.0 \mu\text{g}\cdot\text{day/mL}$), and AUC week 6-12 ($\geq 1959.4 \mu\text{g}\cdot\text{day/mL}$) as strong predictors for endoscopic remission (OR:11.0 [95%CI: 1.8-66.4], $p=0.009$, OR:30.6 [95%CI: 3.1-303.4], $p=0.003$, and OR:16.7 [95%CI: 2.8-99.7], $P = .002$, respectively).

Correlation between Week 12 IFX concentrations and potential covariates

On univariable analysis, the different covariates, collected at Week 0 (start of IFX treatment) and at Week 12, were considered as potential factors to influence IFX trough concentrations at first maintenance infusion.

At Week 0, albumin ($r_s = 0.350$; $p=0.050$), height ($r_s = 0.350$; $p=0.050$), hemoglobin ($r_s = 0.479$; $p=0.006$), and PCDAI ($r_s = -0.509$; $p=0.022$) were significantly correlated with Week 12 IFX concentrations. In addition, there was a significant association between these IFX trough concentrations and weight, but only in patients with a body weight $<30 \text{ kg}$ ($r_s = 0.786$; $p=0.036$). The only independent predictor at Week 0 to achieve a Week 12 IFX concentration $>5 \mu\text{g/mL}$ was hemoglobin with an OR of 1.2 (95%CI: 1.0-3.6; $p=0.044$), using a binary logistic regression analysis (first analysis with all separate covariates except for the PCDAI score because this combines all different subcategories, and second analysis with all separate covariates in addition to the clinical disease activity scores expressed as z-score after logarithmic transformation led to the same results).

At Week 12, height ($r_s = 0.362$; $p=0.042$) and biomarkers of inflammation (CRP ($r_s = -0.395$; $p=0.025$), ESR ($r_s = -0.732$; $p < 0.0001$), albumin ($r_s = 0.455$; $p=0.009$), white blood cells ($r_s = -0.385$; $p=0.030$) and neutrophils ($r_s = -0.393$; $p=0.026$)) were significantly associated with these Week 12 IFX concentrations.

No difference in median IFX concentrations at different timepoints were seen in patients on mono- versus combo-therapy with immunomodulators (e.g. at Week 12: 7.3 $\mu\text{g/mL}$ [3.5-11.9] versus 6.4 $\mu\text{g/mL}$ [4.4-10.9], $p=0.889$).

Covariates that modulated the relationship between IFX levels and remission

Different demographic and blood chemistry covariates at start of IFX therapy were considered as potential factors to influence endoscopic outcomes at six months.

Only in patients with CD, a higher disease severity at IFX initiation, as witnessed by higher PCDAI score (31.3 [27.5-40.6] versus 25.0 [20.0-28.1], $p=0.041$) and SES-CD score (18.5 [12.8-25.5] versus 8.5 [6.0-16.5], $p=0.009$), was associated with a worse endoscopic outcome (**Figure 6**; available at www.jpeds.com). This was not the case for patients with UC, neither for the PUCAI score (50.0 [38.8-53.8] versus 47.5 [26.3-68.8], $p=0.683$) as the Mayo endoscopic sub-score (2.5 [2.0-3.0] versus 2.0 [2.0-2.8], $p=0.570$; **Figure 7** [available at www.jpeds.com]). No significant difference in endoscopic outcome at six months was found based on biomarkers of inflammation, patients' biometrics or age at IFX initiation.

On multivariable analysis, clinical disease activity scores (PCDAI and PUCAI) were translated into validated disease severity classes (mild, moderated or severe [23-24]) and in z-scores (after logarithmic transformation) to pool data from patients with CD and UC. Binary logistic regression identified Week 12 IFX concentration $\geq 5.0 \mu\text{g/mL}$ and AUC week 6-12 $\geq 1959.4 \mu\text{g*day/mL}$ as independent predictors for endoscopic and/or deep remission with an OR of 35.5 (95%CI: 2.0-634.3; $p=0.015$) and 21.2 (95%CI: 1.9-239.6; $p=0.014$), respectively.

Beside the clinical characteristics, also the host proteomics is believed to influence a patients' response to IFX. However, univariate analysis of serum biomarkers did not reveal any differently

expressed proteins at IFX initiation after correction for multiple testing between patients with endoscopic remission versus those with lack of response at six months.

Logistic regression models were trained to distinguish patients who developed endoscopic remission from those who did not by using baseline serum protein levels. A six-marker model using top ranked proteins showed the best potential to discriminate responders from non-responders at six months (AUROC: 0.914). Adding Week 12 IFX concentration did not improve the performance (**Figure 8** and **Table 3**; both available at www.jpeds.com). The specific serum protein profile consists of interleukin 17-C (IL-17C), hydroxysteroid dehydrogenase 11B1 (HSD11B1), mannan-binding lectin serine protease 1 (MASP1), SH2 domain-containing protein 1A (SH2D1A), prokineticin 1 (PROK1) and polypeptide N-acetylgalactosaminyl transferase 10 (GALNT10). These proteins did not interact with each other based on protein-protein interaction networks. No significant changes were seen in the level of each protein at baseline with Week 12 (**Table 3**).

Discussion

In this prospective study of children with IBD initiating IFX, we showed that an adequate IFX exposure is needed during induction and leads to better clinical and endoscopic remission rates six months after initiation of IFX therapy.

Children who achieved endoscopic remission at six months had significantly higher IFX concentrations at different timepoints during induction (at Weeks 4, 6, and 12 as start of maintenance therapy). The pharmacokinetics of IFX during induction in children with IBD [13,15,25-28] was previously approached by looking at short-time outcomes at Week 14 [15,28] or less reliable endpoints later on like clinical remission [25-26]. A Week 6 IFX trough concentration $>8.3\mu\text{g/ml}$ increased the chance to induce clinical remission at Week 14 by almost 8.5 times in children with CD [28]. When looking into long-term outcome of IFX, these Week 6

IFX trough concentrations could differentiate clinical remitters from non-responders at Week 54 [25]. Post-induction IFX trough concentrations were also proposed as a good predictor for long-term outcome [13, 25-26]. We previously showed that not only patients with clinical remission, but also with biological or combined clinical and biological remission at Week 52 had significantly higher IFX trough concentrations at the beginning of the maintenance phase [13]. A post-induction IFX trough concentration of at least 4.6 µg/mL was necessary to achieve combined clinical and biological remission at Week 52. The novelty of this study is that we prospectively studied multiple efficacy outcomes: clinical, biological, endoscopic, and deep remission. This is in contrast to previously published studies that almost exclusively looked at clinical remission [25-28].

We sampled intensively and at trough, peak and intermediate timepoints, in order to determine the ideal predictive sampling time and threshold to achieve endoscopic remission. Determination of non-trough concentrations could also be more relevant for translation into clinical practice where patients may not always be seen at trough, and makes treatment adjustments possible before the next IFX administration when the patient is not achieving the expected outcome. We showed that intermediate IFX concentrations ≥ 38.0 µg/mL at Week 4 could already identify patients with endoscopic remission at six months with a high specificity (85.7%). It is noteworthy that the different timepoints, from Week 4 onwards, had the same predictive ability, making it possible to adjust treatment in real-life at different timepoints (intermediate and trough) depending on the clinical situation. Finally, peak IFX concentration could not predict endoscopic outcome at six months.

Although there was no significant difference between the AUROC at different timepoints, the multivariable analysis attracted specific attention to the post-induction IFX trough concentrations as an independent predictor for endoscopic remission at six months. In addition, these post-induction IFX concentrations were significantly higher in patients with clinical, endoscopic, and

deep remission at six months. A post-induction IFX trough concentration of 5.0 µg/mL was confirmed as the minimal target, and maybe even higher levels (≥ 6.4 µg/mL) are necessary to increase the specificity and therefore the success rate of treatment optimization when a patient has an IFX trough concentration below that threshold. Previous studies reported a lower threshold for these post-induction IFX trough concentrations [13,25-26]. However, these thresholds are not generalizable to all different IFX efficacy endpoints, where higher IFX concentrations are necessary to achieve endoscopic remission [29] in comparison to clinical remission [30]. Finally, children who were in endoscopic remission at six months had not only higher post-induction IFX trough concentrations, but also significantly higher drug exposure throughout the whole induction phase (although with the same predictive ability as the Week 12 IFX concentrations). These results are in concordance with a study, showing that children with a fecal calprotectin < 250 µg/g at Week 52 had significantly higher IFX exposure during induction [31]. This suggests that measuring IFX concentration at an earlier stage and adopting pro-active TDM strategy to increase the drug exposure in advance, could result in better long-term success rates. This is also advocated by the recent ECCO/ESPGHAN guidelines, where children at risk for accelerated IFX clearance should undergo TDM during induction [32]. Model-informed precision dosing has been proposed to further assist physicians to achieve optimal exposure as soon as possible which is essential for an optimal outcome. Most pediatric patients have subtherapeutic post-induction IFX trough concentrations when using the current standard regimen [13-15]. This indicates that there is a large opportunity for dose optimization and a strong need for TDM.

Our study showed that in patients with CD, both the post-induction IFX trough concentrations as well as the disease severity at start of IFX therapy (PCDAI and SES-CD score) predicted endoscopic outcome at six months. These results are in concordance with the study of Singh et al, where post-induction IFX trough concentrations at Week 14 together with CRP predicted persistent clinical remission at one year [26]. Patients with a higher disease burden are more

likely to have lower IFX concentrations because the inflamed tissue is thought to be leaky, leading to IFX being shed into the stool [33]. Thus, patients with high baseline inflammatory burden could benefit from an accelerated IFX regimen. Similar results were not found for patients with UC. Possible explanations are the lower number of patients with UC, and the difficulty in capturing the full extent of disease activity by the Mayo endoscopic sub-score.

In addition, identification of variables that could influence the pharmacokinetic profile offers promising concepts for future IBD management. We identified at start of IFX therapy that patient's height and weight (only in patients with a weight < 30 kg) were significantly associated with post-induction IFX trough concentrations. Our data lend support to previous research [13], where patients with lower body weight were more likely to have lower post-induction IFX trough concentrations. The possible under-dosage of IFX in patients with lower weight, is a result of the non-linear association between body weight and volume of distribution in the peripheral compartment [34]. Thus, appropriate weight-based cut-off dosing schemes should be determined and validated in order to account for these specific pediatric needs. In addition, low hemoglobin and albumin levels at IFX initiation (as a degree of intestinal inflammation) were associated with lower post-induction IFX trough concentrations.

In the era of personalized medicine it would be more ideal to predict response to therapy prior to treatment initiation. Thus far, attempts have failed to define a specific predictive biomarker based on genetics and microbiome [35]. Our findings suggest the proteome as a source for stratification of response to IFX therapy in pediatric patients with IBD. The baseline signature of IFX response consisted of six proteins (IL-17C, HSD11B1, MASP-1, SH2D1A, PROK1, and GALNT10). Although, these proteins did not interact with each other based on protein-protein interaction networks, indirect association with efficacy of anti-TNF therapy has been described previous with IL-17, HSD11B1 and MASP [36-42].

First, it has been shown that anti-TNF treatment will impair the TNF- α -induced protein 3 (TNFAIP3)/A20-mediated anti-inflammatory feedback loop in memory CD4⁺ T-cells, which consequently promotes IL-17A expression [36] that in turn increases the TNF- α -induced production of IL-17C production in entero-endocrine and goblet cells [37]. Studies have shown that polymorphisms in the TNFAIP3 gene or region are associated with efficacy of anti-TNF treatment in IBD patients [38-39]. In addition, patients with increased IL-17A expression in CD4⁺ T-cells during treatment seemed to have better clinical response [36]. Secondly, it has been shown that TNF may regulate anti-inflammatory activities through stimulation of intestinal glucocorticoid synthesis in intestinal mucosa [40]. In pediatric IBD patients, expression of HSD11B1 in colonic biopsies was reduced in comparison with non-IBD controls, suggesting that defective local reactivation of cortisone to cortisol could contribute to the pathogenesis of IBD and limit local levels of active immunosuppressive glucocorticoid [41]. Thirdly, in patients with CD who respond to anti-TNF therapy a downregulation of the lectin pathway has been observed with increase in MASP-3 activity and reduction of M-ficolin and MASP-2 levels [42]. Unfortunately, MASP-1 was not investigated in this study.

We can only postulate that these six proteins could play a predictive role in the response to IFX but further studies are warranted to assess the usefulness of these biomarkers in predicting the long-term efficacy of IFX.

However, our study has two important limitations. The study was limited by small numbers. However, data on endoscopic remission in pediatric patients with IBD are lacking because endoscopic re-evaluation is often omitted in pediatric IBD management. Therefore, this study has still an important value. This was a heterogeneous cohort combining both patients with CD and UC. To pool data from both patients with CD and UC, clinical disease activity scores were translated into validated disease severity classes and in z-scores that could have resulted in a loss of information. Finally, the rate of mucosal healing could be underestimated as endoscopic

reevaluation was already done at six months. In many practices, endoscopic re-evaluation is often performed after 9-12 months.

Post-induction IFX trough concentrations and cumulative drug exposure throughout the whole induction phase were both predictive for endoscopic remission at six months. Furthermore, these results suggested the possibility of IFX dose optimization during induction therapy, particularly in patients who are unlikely to maintain efficacy while receiving the standard dose regimen.

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Figure legend

Figure 1 online Flow chart of the study population

Figure 2 Box-plot presentation of the relationship between infliximab (IFX) concentration at different timepoints and endoscopic outcome at six months

Figure 3 online Presentation of the area of the median infliximab (IFX) serum concentrations over time allocated to the endoscopic outcome at month six

Legend: IFX: infliximab; Wk.: week.

Figure 4 Box-plot presentation of the relationship between infliximab (IFX) trough concentration at week 12 and outcome at six months, for clinical, biological, endoscopic and deep remission

Figure 5 online ROC analysis for infliximab (IFX) concentration threshold at different timepoints and cumulative drug exposure threshold that best discriminates endoscopic remission at six months

Legend: AUC: area under the curve; conc.: concentration; IFX: infliximab; TC: trough concentration; wk.: week.

Figure 6 online Presentation of the relationship between endoscopic outcome at six months and disease activity in patients with Crohn's disease

Figure 7 online Presentation of the relationship between endoscopic outcome at six months and disease activity in patients with ulcerative colitis

Figure 8 online ROC analysis for prediction of endoscopic remission at month six of infliximab (IFX) therapy built on baseline serum level of a subset of proteins derived from logistic Lasso regression

Legend: AUC: area under the curve; IFX: infliximab; TC: trough concentration; wk.: week.

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Table 1. Patients' characteristics	Endoscopic remission at month six	No endoscopic remission at month six
Number of patients, n (%)	18 (56)	14 (44)
Sex, male, n (%)	7 (39)	5 (36)
Crohn's disease, n (%)	14 (78)	6 (43)
Paris classification for CD at start of IFX [27]		
Age at diagnosis, n (%): A1a, A1b	5 (36), 9 (64)	1 (17), 5 (83)
Disease location, n (%): L1, L2, L3	7 (50), 3 (21), 4 (29)	0 (0), 3 (50), 3 (50)
Upper GI involvement, n (%): L4a, L4b	8 (57), 0 (0)	2 (33), 0 (0)
Disease behavior, n (%): B1, B2, B3	13 (93), 1 (7), 0 (0)	5 (83), 1 (17), 0 (0)
Perianal disease modifier, n (%)	4 (29)	0 (0)
Growth, n (%): G0, G1	11 (79), 3 (21)	5 (83), 1 (17)
Paris classification for UC at start of IFX [27]		
Disease extent, n (%): E1, E2, E3, E4	0 (0), 3 (75), 0 (0), 1 (25)	0 (0), 3 (38), 0 (0), 5 (63)
Disease severity, n (%): S0, S1	4 (100), 0 (0)	6 (75), 2 (25)
Age at diagnosis, year, median (IQR)	12.7 (9.9-13.9)	11.9 (10.2-14.5)
Age at start of IFX, year, median (IQR)	13.8 (11.4-14.8)	13.3 (10.5-15.5)
Disease activity score at start of IFX therapy:		
PCDAI, median (IQR)*	25.0 (20.0-28.1)	31.3 (27.5-40.6)
SES-CD, median (IQR)*	8.5 (6.0-16.5)	18.5 (12.8-25.5)
PUCAI, median (IQR)	47.5 (26.3-68.8)	50.0 (38.8-53.8)
Mayo endoscopic sub-score, median (IQR)	2.0 (2.0-2.8)	2.5 (2.0-3.0)
Weight at start of IFX, kg, median (IQR)	42.1 (29.6-51.4)	43.9 (30.1-54.1)
Height at start of IFX, cm, median (IQR)	156.5 (142.5-162.4)	153.0 (138.8-164.7)
Concomitant immunosuppression at start of IFX, n (%)	15 (83)	11 (79)
Co-morbidity, n (%): no, atopy, arthritis, psoriasis, coeliac disease, erythema nodosum	13 (72), 5 (28), 0 (0), 0 (0), 0 (0), 0 (0)	10 (71), 1 (7), 2 (14), 1 (7), 1 (7), 1 (7)

Legend: CD: Crohn's disease; GI: gastro-intestinal tract; IFX: infliximab; IQR: interquartile range; n: number; PCDAI: Pediatric Crohn's Disease Activity Index; PUCAI: Pediatric Ulcerative Colitis Activity Index; SES-CD: Simple Endoscopic Score for Crohn's Disease; UC: ulcerative colitis; *: p-value <0.05.

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Table 2 ROC analysis was used to define the optimal cut-off value of the induction serum concentrations and drug exposure that best predicts endoscopic or deep remission

Covariates	IFX conc. at wk. 4		IFX conc. at wk. 6	IFX conc. at wk. 12		AUC wk. 0-12	AUC wk. 6-12
Method	Youden's	CTL	Both	Youden's	CTL	Both	Both
AUROC	0.752	0.752	0.731	0.796	0.796	0.778	0.825
95%CI	0.581-0.923	0.581-0.923	0.555-0.907	0.619-0.973	0.619-0.973	0.613-0.942	0.665-0.984
Cut-off conc.	38.0 µg/mL	30.5 µg/mL	12.7 µg/mL	5.0 µg/mL	6.4 µg/mL	4056.0 µg*day/mL	1959.4 µg*day/mL
Sensitivity	58.8%	70.6%	72.2%	94.4%	72.2%	66.7%	83.3%
Specificity	85.7%	71.4%	61.5%	64.3%	78.6%	84.6%	76.9%
PPV	83.3%	75.0%	72.2%	77.3%	81.3%	85.7%	83.3%
NPV	63.2%	66.7%	61.5%	90.0%	68.8%	64.7%	76.9%

Legend: ROC analysis was used to define the optimal cut-off value of the induction serum concentrations and drug exposure that best predicts endoscopic or deep remission. Thresholds were calculated using the Youden's J statistic and the "closest top-left" (CTL) method. Results were similar when the thresholds were calculated using both methods, except for the serum IFX concentration at week 4 and 12.

AUC: area under the concentration-time curve, AUROC: area under the ROC curve; CI: confidence interval; conc.: concentration; CTL: closest top-left; IFX: infliximab; NPV: negative predictive value; PPV: positive predictive value; wk.: week.

Table 3 online

ASSAY	PANEL	PVALUE	P.ADJ	COEF	COEF
				OLINK	OLINK+TL
CXCL13	IMMUNO-ONCOLOGY (v.3101)	2.22E-06	4.44E-05	-0.266	-0.757
NOV	DEVELOPMENT (v.3511)	1.54E-05	0.000	0.000	-0.253
TNFB	INFLAMMATION (v.3012)	0.103	0.303	-0.447	-0.432
SH2D1A	IMMUNE RESPONSE (v.3202)	0.396	0.539	-1.541	-1.856
GALNT10	ORGAN DAMAGE (v.3301)	0.206	0.360	4.587	5.741
MGMT	IMMUNE RESPONSE (v.3202)	0.952	0.952	-1.124	-1.522
MMP-10	INFLAMMATION (v.3012)	0.174	0.360	0.000	0.011
CD83	IMMUNO-ONCOLOGY (v.3101)	0.174	0.360	-0.008	-0.073
HSD11B1	IMMUNE RESPONSE (v.3202)	0.216	0.360	1.150	1.837
MASP1	IMMUNE RESPONSE (v.3202)	0.051	0.085	-2.147	-2.340
IL-20	INFLAMMATION (v.3012)	0.360	0.449	2.781	2.155
IL-17C	INFLAMMATION (v.3012)	0.217	0.310	0.535	0.326
CCL11	INFLAMMATION (v.3012)	0.077	0.128	2.013	1.853
GAL-9	IMMUNO-ONCOLOGY (v.3101)	0.599	0.665	-0.354	0.000
PROK1	CELL REGULATION (v.3701)	0.548	0.784	0.687	0.466

Title. List of the top ranking proteins and their coefficients, together with results of Mann-Whitney U test to compare the level of each protein at baseline with Week 12 after infliximab therapy

Legend: CCL11: C-C motif chemokine ligand 11 (Eotaxin); CD83: Cluster of Differentiation 83; COEF: coefficient; CXCL13: C-X-C motif chemokine 13; GAL-9: Galectin-9; GALNT10: polypeptide N-acetylgalactosaminyl transferase 10; HSD11B1: hydroxysteroid dehydrogenase 11B1; IL-17C: interleukin 17-C; IL-20: interleukin 20; MASP-1: mannan-binding lectin serine protease 1; MGMT: methylguanine DNA methyltransferase; MMP10: matrix metalloproteinase 10 (stromelysin 2); NOV (nephroblastoma overexpressed); P. ADJ: adjusted p-value; PROK1: prokineticin 1; SH2D1A: SH2 domain-containing protein 1A; TNFB: tumor necrosis factor beta; TL: trough level.

Figure 1 online

Title. Flow chart of study population

Figure 6 online

A. Box-plot presentation of the relationship between endoscopic outcome at six months and disease activity (PCDAI (Paediatric Crohn's Disease Activity Index) and SES-CD (Simple Endoscopic Score for Crohn Disease)) at infliximab initiation in patients with Crohn's disease (CD) (upper panel).

B. ROC analysis for the threshold of PCDAI (Paediatric Crohn's Disease Activity Index) and SES-CD (Simple Endoscopic Score for Crohn Disease) at infliximab (IFX) initiation that best discriminated endoscopic remission at six months (lower panel).

Title. Presentation of the relationship between endoscopic outcome at six months and disease activity in patients with Crohn's disease

Additional information. ROC curve analysis identified a PCDAI score < 28.8 and a SES-CD < 11.0 at IFX initiation as cut-off points to achieve endoscopic remission at six months (area under ROC curve: 0.781 [95%CI: 0.562-1.000] and area under ROC curve: 0.869 [95%CI: 0.712-1.000], respectively). The optimal cut-off level for the PCDAI score and the SES-CD score, respectively, had a sensitivity of 83.3% or 100.0% and a specificity of 78.6% or 60.0% (positive predictive value of 62.5% or 60.0% and negative predictive value of 91.7% or 100.0%).

Figure 7 online

A. Box-plot presentation of the relationship between endoscopic outcome at month six and disease activity (PUCAI (Paediatric Ulcerative Colitis Activity Index) and Mayo endoscopic sub-score)) at infliximab initiation in patients with ulcerative colitis (UC) (upper panel).

B. ROC analysis for the threshold of PUCAI (Paediatric Ulcerative Colitis Activity Index) and Mayo endoscopic sub-score at infliximab (IFX) initiation that best discriminated endoscopic remission at six months (lower panel).

Title. Presentation of the relationship between endoscopic outcome at six months and disease activity in patients with ulcerative colitis

Endoscopic outcome at month 6 based on IFX concentration at different timepoints

IFX exposure over time based on endoscopic outcome at month 6

Outcome at month 6

ROC analysis for endoscopic remission at month 6

ROC analysis for endoscopic remission at month 6

ROC analysis for endoscopic remission at month 6