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Higher Infliximab Trough Levels Are Associated With Better Outcome in Paediatric Patients With Inflammatory Bowel Disease

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Abstract

Background: The role of therapeutic drug monitoring for infliximab [IFX] therapy in children with inflammatory bowel disease [IBD] is poorly investigated. We determined if IFX exposure correlates with long-term remission in children.

Methods: In this retrospective study, all children with Crohn's disease [CD] and ulcerative colitis [UC], receiving maintenance IFX at our centre, were included. Serum trough levels and cumulative drug exposure were correlated with clinical, biological, and endoscopic remission. All children received proactive drug monitoring and dose adaptation aiming to target a therapeutic window of 3–7 µg/mL. All data are presented as median [interquartile range].

Results: A total of 686 serum levels during IFX maintenance in 52 paediatric patients [33 CD and 19 UC] were included (median 9 [4–18] per patient). With a median of 17 [8–36] months under IFX therapy, 39/52 [75%] patients were in clinical remission and 29/40 [73%] patients were in endoscopic remission. Median IFX trough levels were significantly higher when children achieved clinical remission (5.4 [3.8–8.0] µg/mL versus 4.2 [2.6–6.7] µg/mL), biological remission (5.2 [3.7–7.7] µg/mL versus 4.2 [2.6–6.5] µg/mL), combined clinical and biological remission (5.7 [4.0–8.2] µg/mL versus 4.4 [2.7–6.8] µg/mL) and endoscopic remission (6.5 [4.2–9.5] µg/mL versus 3.2 [2.3–5.6] µg/mL) compared with not meeting these criteria [all $p \leq 0.001$].

Conclusions: In this large paediatric cohort, children with clinical and/or endoscopic remission had significantly higher IFX exposure during maintenance therapy. We showed excellent outcome data using serial and systematic measurements of drug levels. This could provide a rationale for the use of proactive drug monitoring in children in order to improve long-term outcomes.

Key Words: Inflammatory bowel disease; infliximab; therapeutic drug monitoring

1. Introduction

The management of paediatric inflammatory bowel disease [IBD] has changed drastically since the introduction of anti-tumour necrosis factor [TNF]-alpha agents.[1–3] Anti-TNF treatment has shown to induce mucosal healing and improve long-term outcome in paediatric IBD patients.[4] Nevertheless, approximately one-third of patients who initially respond to anti-TNF therapy will lose response over time and may require treatment optimisation.[5,6] A possible mechanism of loss of response [LOR] is immunogenicity with development of anti-drug antibodies.[7–9] LOR might be seen more frequently in children than in adults, because of different pharmacokinetics.[8,10] Since therapeutic options in children with IBD are still limited, optimisation of treatment by preventing LOR and immunogenicity is therefore of utmost importance in order to improve outcome.

Therapeutic drug monitoring [TDM] has been proposed as one of the ways to improve outcome.[11] Introducing TDM may also provide a potential benefit in avoiding side effects while maintaining effective drug levels and preserving normal development and growth, which is especially important in children. However, the role of TDM during infliximab [IFX] maintenance therapy in paediatric IBD patients is still poorly studied. Available literature suggests that low IFX trough levels correlate with poor clinical outcome.[12–19] However, inconsistencies in the definition of response between studies makes it difficult to cross-examine and to make firm conclusions, especially with regard to specific concentration thresholds for optimal efficacy. Literature in paediatric IBD furthermore suffers from low sample size. Therefore, treating a complex disease such as paediatric IBD, requires more standardised definition of drug efficacy endpoints enabling cross-comparison of results among different studies. In addition, serial measurement of IFX trough levels will greatly improve the robustness of the findings compared with cross-sectional sampling.

Therefore, the primary aim of this study was to determine whether IFX trough levels during maintenance therapy correlate with clinical, biological, and/or endoscopic remission. Second, we wanted to identify factors that correlate with drug levels and outcome. Finally, we searched if a cut-off value during maintenance can be proposed which accurately reflects mucosal healing.

2. Materials and Methods

2.1. Patients and study design

A retrospective study was conducted in all children with Crohn's disease [CD] and ulcerative colitis [UC] who received maintenance IFX therapy initiated for active IBD at our tertiary referral centre since March, 2015. The last date of follow-up was January, 2018 or alternatively the date when the patient was transferred to the adult gastroenterology unit. Because children are transferred before the age of 18, only data during childhood and adolescence were collected. All patients were assessed clinically by the same two paediatric gastroenterologists by means of the PCDAI [Paediatric Crohn's Disease Activity Index][20] and the PUCAI [Paediatric Ulcerative Colitis Activity Index].[21] Primary non-responders to IFX were excluded from the analysis. They were defined as patients with a decrease in PCDAI < 12.5[20] and a decrease in PUCAI < 20 points,[21] and this in association with therapeutic IFX trough levels at the beginning of maintenance therapy [> 3 µg/mL measured as trough at IFX dose 4 or 5, depending on the first available IFX trough level[22,23]]. Enrolled patients were treated with IFX infusion of 5 mg/kg at Week 0, Week 2, and Week 6, but IFX induction regimens could be intensified at the discretion of the treating physician, based on disease severity. All children thereafter received standard proactive drug monitoring during maintenance treatment, even when the patient was asymptomatic. Normally, before each IFX infusion, the drug concentration was measured and dose adaptation was made aiming to target a therapeutic window of 3–7 µg/mL [conforming with adult studies[24]]. This study was approved by the Ethical Committee of our university hospital [Approval No: S59870, April 10, 2017].

2.2. Data collection

Patient characteristics were assessed retrospectively from the medical records and included age, sex, IBD type [CD and UC], Paris classification for disease burden at diagnosis, comorbidity data at last follow-up, and concomitant treatment at the start of the IFX therapy and during the follow-up period [mesalazine, steroids, thiopurines, or methotrexate]. At the time of each patient visit, disease activity was determined and mentioned in the medical records using the PCDAI for CD and the PUCAI for UC. IFX doses and intervals were recorded along with patient biometrics [body weight and height] and age. The anthropometric measures (body weight, height, and body mass index [BMI]) were expressed by standard references using age- and sex-specific references from the Belgium, Flanders 2004 growth charts.[25]

The following laboratory tests were measured before the IFX infusion: haemoglobin, platelets, C-reactive protein [CRP], erythrocyte sedimentation rate [ESR], albumin, and serum IFX trough levels. IFX trough levels were determined by Ridascreen IFX monitoring enzyme-linked immunosorbent assay [ELISA] [R-Biopharm, Darmstadt], whereas anti-IFX antibodies [ATI] were determined by the drug-sensitive anti-IFX bridging assay developed in house.[26] Endoscopy was performed in case of disease flare or to evaluate mucosal healing, typically 6 to 12 months after starting IFX therapy.

2.3. Definitions outcome

Clinical remission was defined as a PCDAI/PUCAI less than 10.[27,28] Biological remission was defined as CRP ≤ 5 mg/L in combination with an ESR ≤ 20 mm/h, in patients with elevated inflammatory markers at the start of IFX therapy only.[14] Patients were considered to be in combined clinical and biological remission if both criteria [clinical and biological remission] were met. Clinical and biological outcomes were evaluated at every visit to the infusion unit. Endoscopic remission was defined as absence of ulcerations.[29] For CD patients, endoscopic remission was evaluated by both gastroscopy and ileocolonoscopy, whereas for UC patients, it could be either sigmoidoscopy or colonoscopy. If these strict criteria were not met, the patients were considered to show lack of response.

2.4. Statistical analysis

Continuous variables are presented as medians with interquartile ranges [IQR]. Categorical variables are presented as frequencies and percentages. All data below and above the limit of quantification were substituted with the value of the lower and upper limit of quantification, i.e. 0.3 and 12.0 µg/mL for IFX trough levels. For the univariable analysis of unpaired continuous variables, an independent two-group Mann-Whitney U test was used. To quantify correlation, Spearman's rank correlation coefficient [r_s] was calculated. For the univariable analysis of discrete variables, Fisher's exact test or chi-square test was used where appropriate. A Kaplan–Meier curve was used to predict the proportion of patients who were free of treatment failure over time. Receiver operating characteristic [ROC] analysis was used to define the optimal cut-off value for the different IFX efficacy endpoints. A Kruskal-Wallis test was used to test differences in IFX trough levels between more than two groups. A linear trend was tested with the Cochran-Armitage trend test for the IFX efficacy

endpoints [proportions of patients who met the criteria of remission at latest follow-up visit were grouped into quartiles according to the overall median IFX trough level per patient]. A binary logistic regression analysis was performed to assess associations between the predefined outcomes and the studied variables. All significant variables obtained in univariable analyses [$p < 0.05$] were integrated into the model for multivariable analysis, followed by a backward Wald method. Except for variables with high collinearity [variance inflation factor > 5], only one of these variables was retained in the model. Thresholds points from the ROC curve were calculated using the Youden's J statistic and the 'closest top-left' method. Results of multivariable analysis were shown as odds ratios and 95% confidence intervals [CIs]; *p*-values were calculated two-tailed and the threshold for significance was set at 0.05. IBM SPSS 25.0 software [SPSS Inc., Chicago, IL, USA] was used to perform all statistical analyses.

3. Results

3.1. Patients' characteristics

A total of 52 children (33 [63.5%] CD and 19 [36.5%] UC) were included, with a slight [55.8%] female predominance [Table 1]. The indications for initiation of IFX included flare under immunomodulators [*n* = 31, 59.6%], perianal penetrating behaviour [*n* = 2, 3.8%], steroid dependence [*n* = 17, 32.7%], and postoperative prevention of recurrence in two patients [3.8%].

Since all children received proactive drug monitoring, changes in IFX doses and interval [based on the IFX trough level of the previous visit] were made to reach the therapeutic window. This resulted in a median maintenance dosing interval of 5 [4.0–6.0] weeks; 67.8% of patients received 5 mg/kg IFX dosing and 32.2% received dose intensification up to 15 mg/kg. To simplify recording, all IFX doses were presented as per 8-week interval. Thus, if the administered IFX dose of the patient was for example 7.5 mg/kg with an interval of 6 weeks, the standardised dose would be 10 mg/kg/8 weeks. The overall median standardised IFX dose was 8 [6.7–13.3] mg/kg/8 weeks.

3.2. Infliximab trough concentration data

In total, 686 samples were collected from these 52 patients during IFX maintenance treatment. Available IFX trough levels ranged from 1 to 48, with a median of 9.0 [4.0–18.0] IFX trough levels per patient. The overall median IFX trough level during maintenance was 5.0 [3.2–7.3] µg/mL. Based on the therapeutic window of 3–7 µg/mL, 29.0 % of all IFX trough levels were supratherapeutic [> 7.0 µg/mL], 50.9% were therapeutic [3–7 µg/mL], and 20.1 % were subtherapeutic [< 3.0 µg/mL], including 0.6% with undetectable IFX trough levels [< 0.3 µg/mL]. Out of the four samples with undetectable serum levels [derived from four different patients], only one patient [25.0%] was positive for ATI [51 ng/mL] and therefore stopped IFX therapy. In the other three patients, ATIs were negative and in these, the IFX trough level could be optimised by treatment intensification. In addition, more than half of these subtherapeutic IFX trough levels [60.5%] were obtained during the first three measurements performed in the framework of proactive TDM during maintenance.

3.3. Outcome

3.3.1. Relationship between IFX trough level and clinical and/or biological remission

Clinical remission was reported in 459 out of the 686 visits [66.9%]. IFX trough levels correlated significantly, although weakly, with disease activity based on PCDAI [r_s = -0.228, $p < 0.0001$] for CD and PUCAI [r_s = -0.200, $p = 0.003$] for UC patients. Median IFX trough levels during maintenance were significantly higher in children who were in clinical remission at a particular time point (5.4 [3.8–8.0] µg/mL) compared with visits at which patients showed lack of response (4.2 [2.6–6.7] µg/mL, $p < 0.0001$).

Biological remission was only evaluated in patients with elevated inflammatory markers at start of IFX therapy and/or diagnosis [*n* = 47, or 90.4%]. Biological remission was reported in 404 out of the 641 visits [63.0%]. Median IFX trough levels during maintenance were significantly higher in children who were in biological remission at a particular time point (5.2 [3.7–7.7] µg/mL) compared with visits at which patients showed lack of response (4.2 [2.6–6.5] µg/mL, $p < 0.0001$).

We then looked at combined clinical and biological remission, which was only reported in in 336 of the 686 visits [49.0%] due to the strict definition of combined clinical and biological remission, which combines low disease activity index and normalisation of

Table 1. Patient characteristics.

Number of patients, *n*	52
Sex, male, *n* [%]	23 [44]
Crohn's disease, *n* [%]	33 [63]
Paris classification for CD at diagnosis[63]	
Age at diagnosis, *n* [%]: A1a, A1b	12 [36], 21 [64]
Disease location, *n* [%]: L1, L2, L3	7 [21], 6 [18], 20 [61]
Upper GI involvement, *n* [%]: L4a, L4b	19 [58], 2 [6]
Disease behaviour, *n* [%]: B1, B2, B3	26 [79], 6 [18], 1 [3]
Perianal disease modifier, *n* [%]	6 [18]
Growth, *n* [%]: G0, G1	26 [79], 7 [21]
Paris classification for UC[53]	
Disease extent, *n* [%]: E1, E2, E3, E4	1 [5], 5 [26], 3 [16], 10 [53]
Disease severity, *n* [%]: S0, S1	13 [68], 6 [32]
Age at diagnosis, year, median [IQR]	12.2 [9.5–14.4]
Age at start of IFX, year, median [IQR]	13.0 [11.1–15.0]
Disease duration before starting IFX, months, median [IQR]	4.5 [2.0–8.8]
Follow-up time under IFX, months, median [IQR]	17 [8–36]
Concomitant immunosuppression at start of maintenance, *n* [%]	40 [75]
Comorbidity, *n* [%]: none, arthritis, psoriasis, atopy, PSC	28 [54], 6 [12], 3 [6], 17 [33], 1 [2]

CD, Crohn's disease; GI, gastrointestinal tract; IFX, infliximab, IQR, interquartile range; PSC, primary sclerosing cholangitis; UC, ulcerative colitis.

CRP and ESR. Median IFX trough levels during maintenance were significantly higher in children who were in combined clinical and biological remission at a particular time point (5.7 [4.0–8.2] µg/mL) compared with visits at which patients showed lack of response (4.4 [2.7–6.8] µg/mL, $p < 0.0001$].

3.3.2. Relationship between IFX trough level and endoscopic remission

Endoscopic data were available at 87 time points in 40 different patients after a median of 12.6 [12.6–22.4] months. In 54.0 % of the visits, the patients were in endoscopic remission at the time of evaluation [$n = 47$]. IFX trough levels used for comparison were measured at the time of endoscopy if the patient received an IFX infusion on the same day. In all other cases, IFX trough levels were measured at the time of the nearest IFX infusion. Median IFX trough levels during maintenance were significantly higher in children who were in endoscopic remission (6.5 [4.2–9.5] µg/mL) compared with those not in remission (3.2 [2.3–5.6] µg/mL, $p = 0.001$; see Figure 1).

In order to empower these results, the exposure to IFX per patient was calculated by means of the median of all IFX trough levels of this patient before the endoscopy. These median IFX trough levels were also significantly higher in children who were in endoscopic remission (6.5 [4.2–9.5] µg/mL) compared with non-responders (4.3 [2.6–5.8] µg/mL, $p = 0.001$). The same results were found when analysing per diagnosis [CD versus UC] or per group when comparing children who achieved endoscopic remission with those who did not: 6.5 [3.8–9.6] µg/mL versus 3.7 [2.7–4.9] µg/mL [$p = 0.012$] in CD patients and 6.2 [4.5–9.3] µg/mL versus 4.8 [2.5–7.1] µg/mL [$p = 0.037$] in UC patients.

The ROC analysis was used to determine the IFX trough level threshold that best discriminated endoscopic remission [Figure 2]. Area under the ROC curve for IFX trough level was 0.703 [95% confidence interval: 0.59–0.82]. An optimal cut-off level for IFX trough concentration was defined as ≥ 5.4 µg/mL [based on Youden's J statistics] with a sensitivity of 66.0% and specificity of 75.0%, resulting in a positive predictive value of 75.6% and a negative predictive value of 65.2%. The ROC analysis for the other investigated outcomes can also be reviewed in Figure 2; however, these are less discriminative.

3.3.3. Outcome at latest follow-up visit

With a median follow-up of 17 [8–36] months under IFX therapy, 39 out of 52 [75.0%] patients were in clinical and 29 out of 40 [72.5%] patients who underwent endoscopy were in endoscopic remission at latest follow-up visit. Importantly, none of the included patients were taking steroids at the latest visit. Only four patients [7.7%] stopped IFX before January, 2018 [or alternatively the date when the patient was transferred to the adult gastroenterology unit]. This was due to secondary LOR in one patient 8 months after start of IFX, to the presence of ATIs in another patient after 2.5 years, and adverse events in two patients [severe skin lesions and a delayed infusion reaction, despite no measurable ATIs]. IFX withdrawal due to LOR or adverse events are represented by a KaplanMeier curve in Figure 3.

The quartile analysis of the median IFX trough levels per patient showed that patients who were in remission at latest follow-up visit were in the higher quartiles of IFX trough levels [Figure 4]. The overall exposure to IFX [derived from the median of all IFX trough levels of each individual patient before the latest endoscopy] was significantly higher in patients who were in endoscopic remission [$p = 0.001$].

No significant difference was observed in disease severity at start of IFX, based on clinical scores or CRP value, between patients in clinical and endoscopic remission versus non-responders at latest follow-up. The median dose of IFX [presented as a standardised IFX doses with a 8-week intervals] that was administered over the total follow-up period was not significantly different between patients in clinical remission at latest follow-up visit (8.0 [6.2–10.0] mg/kg/8 weeks) compared with non-responders (8.0 [6.7–14.7] mg/kg/8 weeks, $p = 0.143$). However, patients in endoscopic remission at latest follow-up visit received even less IFX drugs over time (6.7 [5.7–8.0] mg/kg/8 weeks) compared with non-responders (10.0 [8.0–16.0] mg/kg/8 weeks, $p = 0.006$).

3.4. Correlation between covariates, IFX trough levels, and outcome

The potential covariates that could influence the IFX trough levels were only evaluated at the first available IFX trough level per patient in relation to the different covariates at that specific time

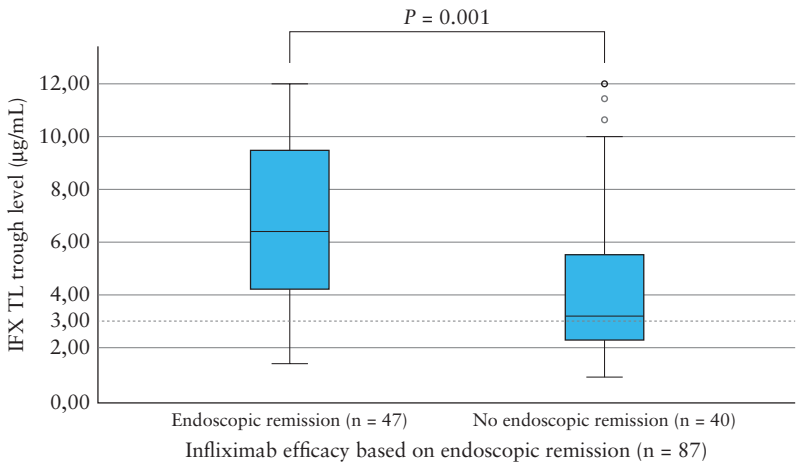

Figure 1. Box-plot presentation of the relationship between infliximab [IFX] trough level [TL] and endoscopic outcome. Endoscopic data were available at 87 time points in 40 different patients where in 47 visits the patients were in endoscopic remission.

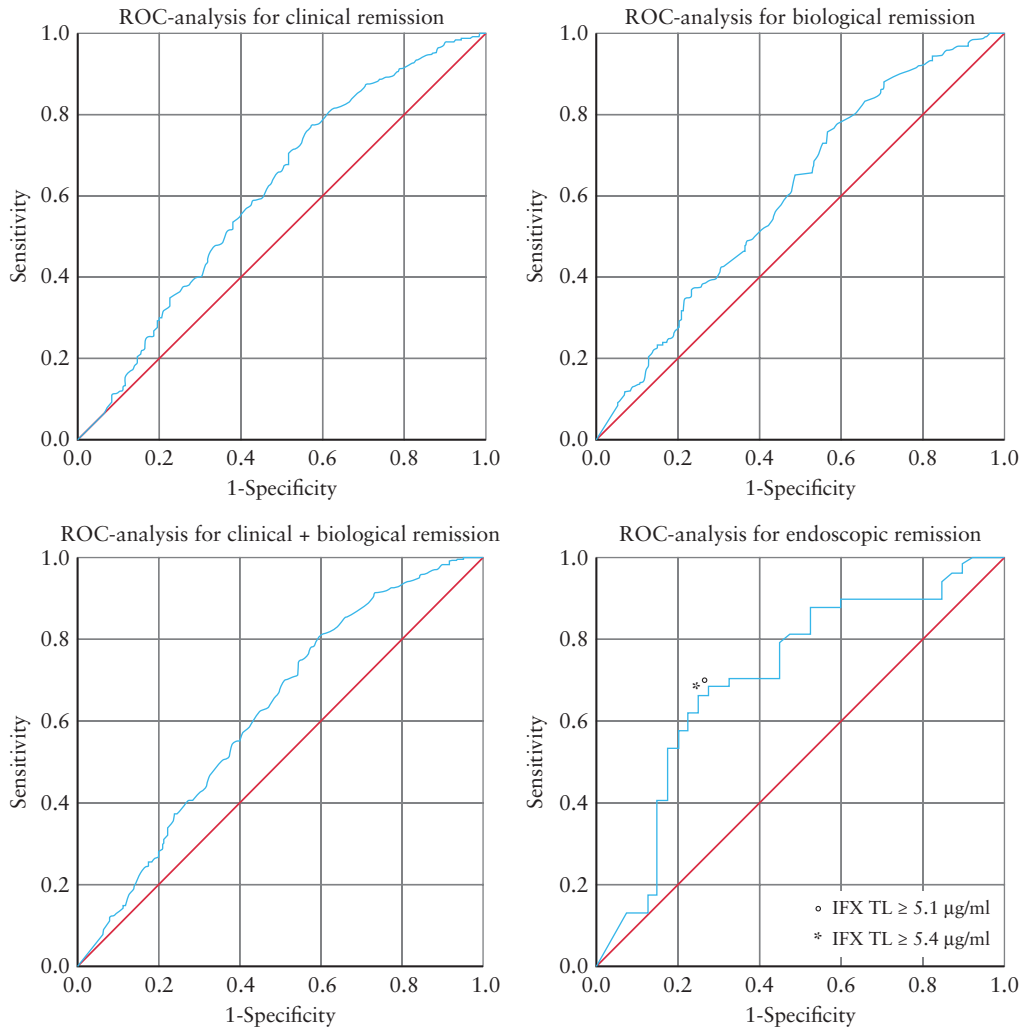

Figure 2. The receiver operating characteristic [ROC] curve was used to determine the infliximab [IFX] trough level [TL] threshold that best discriminated between different outcomes at any time point. Thresholds were calculated using the Youden's J statistic and the 'closest top-left' method. A [upper left]: the area under the ROC curve [AUROC] to achieve clinical remission was 0.610 (95% confidence interval [CI]: 0.563–0.656) with a *p*-value of < 0.001. An optimal cut-off level for IFX trough concentration was defined as ≥ 3.7 or 4.0 µg/mL, based on both methods respectively. B [upper right]: the AUROC to achieve biological remission was 0.621 [95% CI: 0.579–0.662] with a *p*-value of < 0.0001. An optimal cut-off level for IFX trough concentration was defined as ≥ 3.8 or 4.0 µg/mL, based on both methods respectively. C [lower left]: the AUROC to achieve combined biological and clinical remission was 0.604 [95% CI: 0.558–0.650] with a *p*-value of < 0.0001. An optimal cut-off level for IFX trough concentration was defined as ≥ 3.7 or 4.2 µg/mL, based on both methods respectively. D [lower right]: the AUROC for IFX trough level was 0.703 [95% CI: 0.59–0.82] with a *p*-value of 0.001. An optimal cut-off level for IFX trough concentration was defined as ≥ 5.4 or 5.1 µg/mL, based on both methods respectively. Both thresholds have an acceptable sensitivity [66.0% and 68.1%, respectively] and specificity [75.0% and 72.5%, respectively], resulting in a positive predictive value of 75.6% or 74.4% and a negative predictive value of 65.2% or 65.9%, respectively.

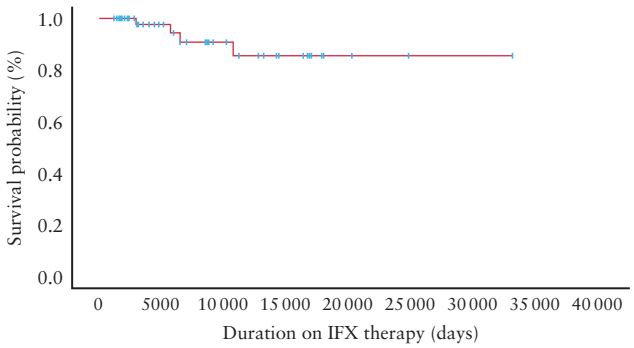

Figure 3. Kaplan–Meier curve of the proportion of paediatric inflammatory bowel disease [IBD] patients with infliximab [IFX] discontinuation due to secondary loss of response or a serious adverse event.

point. Therefore, there was no influence of proactive therapeutic drug monitoring and multiple samples per patient.

No difference was observed in IFX trough levels between CD and UC patients [p = 0.985] or between male and female patients [p = 0.226]. However, IFX trough levels correlated significantly with weight [r_s = 0.291, p = 0.036], height [r_s = 0.280, p = 0.047], and body surface area [BSA] [r_s = 0.305, p = 0.030], but not with body mass index [BMI] [r_s = 0.233, p = 0.100]. In a subgroup of patients with a weight below 34 kg, the IFX trough levels were significantly lower in comparison with the higher weight class [p = 0.043; see Supplementary Figure 1, available as Supplementary data at *ECCO-JCC* online]. After correcting for age and sex, the IFX trough levels did not correlate any longer with weight, height, or BMI [p = 0.157, p = 0.137, and p = 0.327, respectively]. Younger patients did not have significantly lower IFX trough levels [r_s = 0.244, p = 0.082].

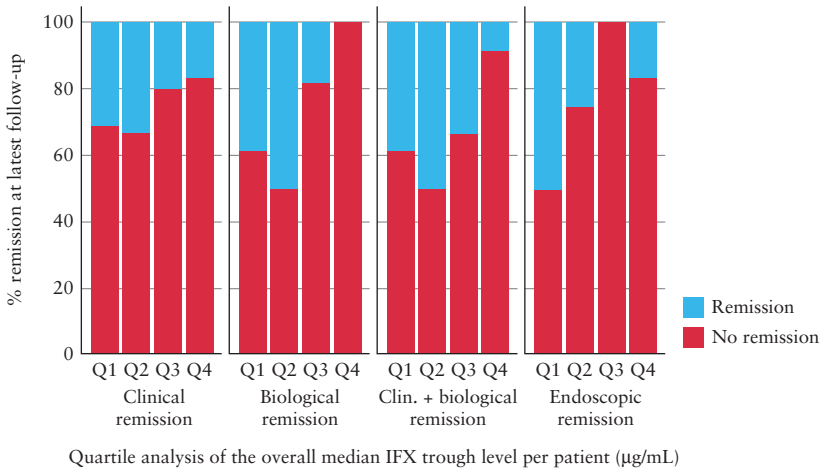

Figure 4. Quartile analysis of the infliximab [IFX] efficacy endpoints divided according to four quartiles of the median IFX trough levels per patient [Q1–Q4]. The overall exposure to IFX per patient was calculated by means of the median of all IFX trough levels of this patient before the latest follow-up visit [for clinical, biological, and combined clinical and biological remission] or the latest endoscopy [for endoscopic remission]. IFX efficacy endpoints were based on latest follow-up visit or latest endoscopy per patient. IFX trough levels [µg/mL] quartiles were as follows: Q1 ≤ 4.4 µg/mL; 4.4 µg/mL < Q2 ≤ 5.2 µg/mL; 5.2 µg/mL < Q3 ≤ 6.7 µg/mL; Q4 > 6.7 µg/mL. The *p*-values derived from the Cochran-Armitage trend test analysis for the different IFX efficacy endpoints were 0.027, 0.003, 0.0001, and 0.001, respectively, for clinical, biological, combined clinical + biological, and endoscopic remission.

Moreover, IFX trough levels correlated significantly with biomarkers of inflammation as measured by CRP [r_s = -0.505, p = 0.001], ESR [r_s = -0.460, p = 0.001], but not with albumin [r_s = 0.260, p = 0.066]. In addition, IFX trough levels correlated significantly with haemoglobin [r_s = 0.323, p = 0.020], but not with platelets [r_s = -0.226, p = 0.107].

Finally, all these covariates were also associated with outcome. Younger patients, those with a lower body weight, height, and BSA at the time of evaluation, had a significantly lower likelihood of being in clinical, biological, and endoscopic remission [Supplementary Table 1, available as Supplementary data at *ECCO-JCC* online]. Similarly, patients with a lower albumin and haemoglobin and higher platelets at time of evaluation had a significantly lower likelihood of being in clinical, biological, and endoscopic remission [Supplementary Table 1].

Binary logistic regression identified IFX trough level (odds ratio = 1.062 [95% CI: 1.003–1.125], p = 0.038 and odds ratio = 1.212 [95% CI: 1.033–1.424], p = 0.019) and albumin (odds ratio = 1.080 [95% CI: 1.017–1.147], p = 0.013 and odds ratio = 1.418 [95% CI: 1.192–1.688], p < 0.0001) as independent predictors for clinical remission and endoscopic remission, respectively. In addition, CRP was an independent predictor for clinical remission only (odds ratio = 0.951 [95% CI: 0.915–0.988], p = 0.010). Results of the binary regression analysis for all investigated outcomes are shown in Table 2.

3.5. Relationship between IFX TLs and immunomodulators

At start of maintenance therapy, 75.5% [n = 40] of children were on concomitant immunosuppressants [thiopurine or methotrexate]. At latest follow-up, only 15 children [28.8 %] were still taking concomitant immunosuppressants. Reasons for withdrawal of these medications included drug intolerance in eight patients [pancreatitis, leukopenia, gastrointestinal intolerance, etc.] or reaching mucosal healing [n = 20]. During the follow-up period, only three patients needed to [re]start immunosuppressants due to flare. No differences were found between median IFX trough levels in patients who were on mono- versus combo-therapy at start of maintenance therapy (4.9 [3.7–7.1] µg/mL versus 5.1 [3.2–7.6] µg/mL, p = 0.994) or versus combo-therapy at the time of the visits (5.0 [3.4–7.2] µg/mL versus 5.8 [3.0–8.4] µg/mL, p = 0.829). Furthermore, no significant difference was found in outcome at a particular time point based on clinical [p = 0.166, n = 167/262 versus n = 292/424] or endoscopic [p = 0.566, n = 16/32 versus n = 24/55] remission in patients with or without immunosuppressants at that time point. However, a significant benefit was seen for combination therapy when considering biological [p = 0.035, n = 140/242 versus n = 264/399] and combined clinical and biological [p = 0.006, n = 111/262 versus n = 225/424] remission.

4. Discussion

In this study, we examined drug exposure to IFX in children with IBD and its correlation with hard outcomes as steroid-free clinical, biological, and endoscopic remission. We demonstrate a strong exposure-response effect in children receiving maintenance IFX. To our knowledge, this study reports the largest cohort of serial IFX trough levels measured in a paediatric IBD population and demonstrates for the first time a clear association between IFX trough levels and mucosal healing.

In adults, the relationship between IFX trough levels and clinical response is well established.[30–35] However, in children, only limited studies on this topic have been published.[12–19] Most of these studies suffer from low sample size and inconsistency with regard to the definition of remission. The novelty of this study is that we comprehensively looked at all different [prospectively collected] IFX efficacy outcomes together, using well-defined definitions of remission. This is in contrast to previous published studies where most of the time only clinical remission was evaluated and, in a minority, biological remission. Hoekman *et al.* reported a significant correlation between IFX trough levels and biological response [CRP and faecal calprotectin], although they could not show the same results for clinical response.[36] Ohem *et al.* only described the correlation of IFX trough levels and biological remission [based on CRP, ESR, and faecal calprotectin], but did not investigate clinical remission.[14] In the study of Hämäläinen *et al.*, there was an association with higher levels of

Table 2. Results of the binary logistic regression analyses for all investigated outcomes.

Covariates	Clinical remission	Biological remission	Clinical + biological remission	Endoscopic remission
IFX trough level [µg/mL]	OR: 1.062 [1.003–1.125] $p = 0.038$	OR: 1.076 [1.012–1.144] $p = 0.019$	OR: 1.104 [1.044–1.167] $p < 0.0001$	OR: 1.212 [1.033–1.424] $p = 0.019$
Albumin [g/L]	OR: 1.080 [1.017–1.147] $p = 0.013$	OR: 1.230 [1.156–1.308] $p < 0.0001$	OR: 1.169 [1.104–1.237] $p < 0.0001$	OR: 1.418 [1.192–1.688] $p < 0.0001$
CRP [mg/L]	OR: 0.951 [0.915–0.988] $p = 0.010$	NA	NA	NS
Haemoglobin [g/dL]	NS	OR: 1.229 [1.006–1.501] $p = 0.044$	OR: 1.209 [1.022–1.431] $p = 0.027$	NS

All relevant data are presented as odds ratios [95% confidence interval] and *p*-value.

IFX, infliximab; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; OR, odds ratio; NA: not applicable [since CRP and ESR are embedded in the definition of both biological and combined clinical + biological remission]; NS, not significant.

intestinal inflammation [as measured with faecal calprotectin] and low IFX levels during induction, but not during maintenance treatment with IFX.[37]

Our results show that median IFX trough levels during maintenance are significantly higher in children with clinical [5.4 µg/mL versus 4.1 µg/mL], biological [5.2 µg/mL versus 4.2 µg/mL], combined clinical and biological [5.7 µg/mL versus 4.2 µg/mL], and endoscopic remission [6.2 µg/mL versus 3.2 µg/mL] at any particular time point. The correlation between IFX trough levels and endoscopic remission was only reported in the randomised, controlled, phase 3 trial by Adedokun *et al*.[12] In this study of 45 children with UC, higher serum IFX levels during induction at Week 8 [≥ 41.1 mg/mL] were associated with greater proportions of patients achieving mucosal healing [92.9%] than children with lower serum concentrations [< 18.1 mg/mL, 53.9%]. IFX trough levels during maintenance therapy [at Week 30] did not correlate with endoscopic remission, an observation which was explained by the authors due to the small sample size [21 patients]. Therefore, our study convincingly demonstrates for the first time in paediatric IBD patients that IFX trough levels correlate significantly with endoscopic remission during maintenance therapy. In the era where mucosal healing is the new gold standard, this is an important observation.

Only limited data are available in the paediatric literature to determine specific IFX cut-off trough levels for predicting remission, where different endpoints will, however, also result in altered therapeutic windows.[13,14] Singh *et al*. showed that IFX trough levels at Week 14, with respectively 3, 4, and 5 µg/mL cut-off levels, had positive predictive values of 64%, 75%, and 83% and negative predictive values of 46%, 52%, and 53% for predicting a sustained response.[13] ROC analysis in the study of Ohem *et al*. revealed that IFX trough levels with, respectively, 1.1, 2.1, and 3.5 µg/mL cut-off, were associated with remission [defined as CRP ≤ 5 mg/L, ESR ≤ 20 mm/h, or faecal calprotectin ≤ 100 µg/g] in a CD population.[14] Our study suggests that even higher cut-off values [≥ 5.4 µg/ml] are necessary to reach mucosal healing. These results are more in line with previously published adult literature.[38–40] Prospective studies are now needed to assess the ideal IFX trough level cut-off for clinical and/or endoscopic remission.

With a median follow-up of 1.4 years under IFX therapy, 39 out of 52 [75.0%] patients were in clinical and 29 out of 40 [72.5%] patients in endoscopic remission at latest follow up visit. Long-term efficacy of IFX beyond 1 year is poorly investigated in paediatric IBD.[41,42] The most important data for clinical remission arise from the randomised, controlled, phase 3 trial[1,3,12] and two real-world cohort studies from Scotland[43] and Italy.[44] These studies reported a clinical remission rate at 1 year of IFX therapy of only 3856%. Some caution should be taken in comparing these results with our study. First, these studies are older and IBD management has evolved, including the use of TDM in case of LOR, which could alter the final outcome.[24,45] Data on mucosal healing are even scarcer in paediatric IBD,[46–48] where in 22–36% endoscopic remission was found.[46,47] Only Olbjørn *et al*. reported a higher mucosal healing rate of 65% in a small cohort of only 17 patients.[48]

The excellent outcome in our study at latest follow-up could possibly be explained by the use of routine proactive drug management in our centre. We strongly believe that proactive TDM [at least in the first year] can contribute to improved long-term outcome, especially since remitters or non-responders cannot be distinguished based on disease severity at start of IFX treatment. In addition, patients with mucosal healing at latest endoscopy had a higher overall exposure to IFX in comparison with non-responders. Therefore we believe that our previous therapeutic window [3–7 µg/mL] should be reset to higher values [≥ 5.4 µg/ml] in order to reach mucosal healing, where proactive TDM can help us to achieve this goal. Although there is evidence that TDM-based treat-to-target strategies can result in a better outcome,[24,49–51] the prospective study TAILORIX[52] could not show benefit of proactive TDM over dose intensification based on symptoms alone. It will be even more important to investigate the role of proactive TDM in children, in whom fluctuations in pharmacokinetic variables are more pronounced compared with adults, due to physiological differences such as volume of distribution and the immaturity of enzyme systems and clearance mechanisms. However, the potential advantages and disadvantages [e.g. possible over-treatment of patients in deep remission] of proactive TDM need to be balanced in each patient individually.[53,54]

Finally, we strongly feel that the short disease duration before starting IFX could certainly have contributed to the excellent outcome, since it has been shown that early treatment with anti-TNF is superior to early immunosuppression.[55,56]

As a final point, we showed associations between IFX trough levels and biomarkers of inflammation and also, but less pronounced, with haemoglobin and biometrics of patients (weight, height, and BSA [all $p < 0.05$]), where patients with a weight below 34 kg were especially at risk for lower IFX trough levels in comparison with patients in higher weight categories. This can probably be explained by the non-linear association between body weight and volume of distribution in the peripheral compartment.[57] This predicts a possible under-dosage of IFX in patients with lower weight. This is the first study reporting the correlation of IFX trough levels with biometrics of the patient beyond the body weight.[12,37,57–60]

Immunogenicity rates were very low and only one child [1.9%] had detectable ATI. Although these were measured with a

drug-sensitive ELISA method, a possible explanation for this low number could be the use of proactive drug monitoring thereby preventing undetectable IFX trough levels and immunogenicity. The high rate of patients on combination therapy with immunosuppressants at start of maintenance therapy could also contribute to this low number.[61,62]

The strength of this study was the availability of > 600 serial IFX drug levels in a very well-phenotyped cohort for all relevant outcomes. To our knowledge, our sample size is the largest among all paediatric studies conducted on this topic. We were able to evaluate the effect of co-medication, biometric, and laboratory data on the IFX trough levels. Since this study was performed as a single centre's experience, there was also a uniform management plan across all patients. One of the limitations of the study was that the impact of drug intensification on IFX trough levels was not investigated, as a result of proactive drug monitoring and continuous dose adaptation aiming to target a therapeutic window. Second, due to the retrospective design of the study, endoscopy was not standardly performed in each patient and at the same time points. Finally, this was not a randomised study to answer whether IFX intensification based on clinical grounds would have led to similar outcome results in comparison with the TDM-guided group.

In conclusion, in this paediatric IBD cohort treated with IFX maintenance, children with clinical and endoscopic remission had significantly higher IFX trough levels. Our data support the value of proactive drug management in children to improve long-term outcome. This now needs to be investigated in prospective studies to show the real benefit of proactive TDM over dose intensification based on symptoms alone.

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Conflict of Interest

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Author Contributions

KvH: literature search, data collection, statistical analysis, data interpretation, and manuscript writing; ED: data interpretation, and manuscript writing; IH, GVA, and MF: manuscript critical revision; AG, SV: study concept and design, data interpretation, and manuscript writing.

Supplementary Data

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

References

1. Hyams J, Crandall W, Kugathasan S, *et al*. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. *Gastroenterology* 2007;**132**:863–73.
2. Hyams JS, Griffiths A, Markowitz J, *et al*. Safety and efficacy of adalimumab for moderate to severe Crohn's disease in children. *Gastroenterology* 2012;**143**:365–74.e2.
3. Hyams J, Damaraju L, Blank M, *et al*. Induction andtenance therapy with infliximab for children with moderate to severe ulcera maintive colitis. *Clin Gastroenterol Hepatol* 2012;**10**:391–9.
4. Nobile S, Gionchetti P, Rizzello F, Calabrese C, Campieri M. Mucosal healing in pediatric Crohn's disease after anti-TNF therapy: a long-term experience at a single center. *Eur J Gastroenterol Hepatol* 2014;**26**:458–65.
5. Cameron FL, Wilson ML, Basheer N, *et al*. Anti-TNF therapy for paediatric IBD: the Scottish national experience. *Arch Dis Child* 2015;**100**:399–405.
6. Topf-Olivestone C, Turner D. How effective is the use of long-term anti-TNF for paediatric IBD? Clues from real-life surveillance cohorts. *Arch Dis Child* 2015;**100**:391–2.
7. Brandse JF, Mathôt RA, van der Kleij D, *et al*. Pharmacokinetic features and presence of antidrug antibodies associate with response to infliximab induction therapy in patients with moderate to severe ulcerative colitis. *Clin Gastroenterol Hepatol* 2016;**14**:251–8.e1–2.
8. Dotan I, Ron Y, Yanai H, *et al*. Patient factors that increase infliximab clearance and shorten half-life in inflammatory bowel disease: a population pharmacokinetic study. *Inflamm Bowel Dis* 2014;**20**:2247–59.
9. Stein R, Lee D, Leonard MB, *et al*. Serum infliximab, antidrug antibodies, and tumor necrosis factor predict sustained response in pediatric Crohn's disease. *Inflamm Bowel Dis* 2016;**22**:1370–7.
10. Momper JD, Wagner JA. Therapeutic drug monitoring as a component of personalized medicine; applications in pediatric drug development. *Clin Pharmacol Ther* 2012;**35**:971–86.
11. van Hoeve K, Hoffman I, Vermeire S. Therapeutic drug monitoring of anti-TNF therapy in children with inflammatory bowel disease. *Expert Opin Drug Saf* 2018;**17**:185–96.
12. Adedokun OJ, Xu Z, Padgett L, *et al*. Pharmacokinetics of infliximab in children with moderate-to-severe ulcerative colitis: results from a randomized, multicenter, open-label, phase 3 study. *Inflamm Bowel Dis* 2013;**19**:2753–62.
13. Singh N, Rosenthal CJ, Melmed GY, *et al*. Early infliximab trough levels are associated with persistent remission in pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2014;**20**:1708–13.
14. Ohem J, Hradsky O, Zarubova K, *et al*. Evaluation of infliximab therapy in children with Crohn's disease using trough levels predictors. *Dig Dis* 2018;**36**:40–8.
15. Choi SY, Kang B, Lee JH, Choe YH. Clinical use of measuring trough levels and antibodies against infliximab in patients with pediatric inflammatory bowel disease. *Gut Liver* 2017;**11**:55–61.
16. Rolandsdotter H, Marits P, Sundin U, *et al*. Serum-infliximab trough levels in 45 children with inflammatory bowel disease on maintenance treatment. *Int J Mol Sci* 2017;**18**:575.
17. Merras-Salmio L, Kolho KL. Clinical use of infliximab trough levels and antibodies to infliximab in pediatric patients with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2017;**64**:272–8.
18. Deora V, Kozak J, El-Kalla M, Huynh HQ, El-Matary W. Therapeutic drug monitoring was helpful in guiding the decision-making process for children receiving infliximab for inflammatory bowel disease. *Acta Paediatr* 2017;**106**:1863–7.
19. Minar P, Saeed SA, Afreen M, Kim MO, Denson LA. Practical use of infliximab concentration monitoring in pediatric Crohn disease. *J Pediatr Gastroenterol Nutr* 2016;**62**:715–22.
20. Turner D, Griffiths AM, Walters TD, *et al*. Appraisal of the pediatric Crohn's disease activity index on four prospectively collected datasets: recommended cutoff values and clinimetric properties. *J Pediatr Gastroenterol Nutr* 2003;**36**:83–9.
21. Turner D, Hyams J, Markowitz J, *et al*.; Pediatric IBD Collaborative Research Group. Appraisal of the pediatric ulcerative colitis activity index [PUCAI]. *Inflamm Bowel Dis* 2009;**15**:1218–23.

22. Gils A. Combining therapeutic drug monitoring with biosimilars, a strategy to improve the efficacy of biologicals for treating inflammatory bowel diseases at an affordable cost. *Dig Dis* 2017;**35**:61–8.
23. Papamichael K, Gils A, Rutgeerts P, *et al.* Role for therapeutic drug monitoring during induction therapy with TNF antagonists in IBD: evolution in the definition and management of primary nonresponse. *Inflamm Bowel Dis* 2015;**21**:182–97.
24. Vande Casteele N, Ferrante M, Van Assche G, *et al.* Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology* 2015;**148**:1320–9.e3.
25. Laboratorium voor Antropogenetica. Vrije Universiteit Brussel. *Growth Charts, Flanders, 2004*. Update July 2006. http://www.vub.ac.be/groeicurven/english.html Accessed January 27, 2018.
26. Van Stappen T, Billiet T, Vande Casteele N, *et al.* An optimized anti-infliximab bridging enzyme-linked immunosorbent assay for harmonization of anti-infliximab antibody titers in patients with inflammatory bowel diseases. *Inflamm Bowel Dis* 2015;**21**:2172–7.
27. Hyams JS, Ferry GD, Mandel FS, *et al.* Development and validation of a pediatric Crohn's disease activity index. *J Pediatr Gastroenterol Nutr* 1991;**12**:439–47.
28. Turner D, Otley AR, Mack D, *et al.* Development, validation, and evaluation of a pediatric ulcerative colitis activity index: a prospective multicenter study. *Gastroenterology* 2007;**133**:423–32.
29. Schnitzler F, Fidder H, Ferrante M, *et al.* Mucosal healing predicts long-term outcome of maintenance therapy with infliximab in Crohn's disease. *Inflamm Bowel Dis* 2009;**15**:1295–301.
30. Baert F, Noman M, Vermeire S, *et al.* Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003;**348**:601–8.
31. Maser EA, Villela R, Silverberg MS, Greenberg GR. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clin Gastroenterol Hepatol* 2006;**4**:1248–54.
32. Bortlik M, Duricova D, Malickova K, *et al.* Infliximab trough levels may predict sustained response to infliximab in patients with Crohn's disease. *J Crohns Colitis* 2013;**7**:736–43.
33. Seow CH, Newman A, Irwin SP, Steinhart AH, Silverberg MS, Greenberg GR. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. *Gut* 2010;**59**:49–54.
34. Reinisch W, Sandborn WJ, Rutgeerts P, *et al.* Long-term infliximab maintenance therapy for ulcerative colitis: the ACT-1 and -2 extension studies. *Inflamm Bowel Dis* 2012;**18**:201–11.
35. Barnes EL, Allegretti JR. Are anti-tumor necrosis factor trough levels predictive of mucosal healing in patients with inflammatory bowel disease?: a systematic review and meta-analysis. *J Clin Gastroenterol* 2016;**50**:733–41.
36. Hoekman DR, Brandse JF, de Meij TG, *et al.* The association of infliximab trough levels with disease activity in pediatric inflammatory bowel disease. *Scand J Gastroenterol* 2015;**50**:1110–7.
37. Hämäläinen A, Sipponen T, Kolho KL. Serum infliximab concentrations in pediatric inflammatory bowel disease. *Scand J Gastroenterol* 2013;**48**:35–41.
38. Ungar B, Levy I, Yavne Y, *et al.* Optimizing anti-TNF-α therapy: serum levels of infliximab and adalimumab are associated with mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2016;**14**:550–7.e2.
39. Papamichael K, Rakowsky S, Rivera C, Cheifetz AS, Osterman MT. Infliximab trough concentrations during maintenance therapy are associated with endoscopic and histologic healing in ulcerative colitis. *Aliment Pharmacol Ther* 2018;**47**:478–84.
40. Papamichael K, Rakowsky S, Rivera C, Cheifetz AS, Osterman MT. Association between serum infliximab trough concentrations during maintenance therapy and biochemical, endoscopic, and histologic remission in Crohn's disease. *Inflamm Bowel Dis* 2018, April 28. doi:10.1093/ibd/izy132. [Epub ahead of print].
41. Oussalah A, Danese S, Peyrin-Biroulet L. Efficacy of TNF antagonists beyond one year in adult and pediatric inflammatory bowel diseases: a systematic review. *Curr Drug Targets* 2010;**11**:156–75.
42. Corica D, Romano C. Biological therapy in pediatric inflammatory bowel disease: a systematic review. *J Clin Gastroenterol* 2017;**51**:100–10.
43. Cameron FL, Wilson ML, Basheer N, *et al.* Anti-TNF therapy for paediatric IBD: the Scottish national experience. *Arch Dis Child* 2015;**100**:399–405.
44. Nuti F, Viola F, Civitelli F, *et al.* Biological therapy in a pediatric Crohn disease population at a referral center. *J Pediatr Gastroenterol Nutr* 2014;**58**:582–7.
45. Ordás I, Feagan BG, Sandborn WJ. Therapeutic drug monitoring of tumor necrosis factor antagonists in inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2012;**10**:1079–87.
46. Nobile S, Gionchetti P, Rizzello F, Calabrese C, Campieri M. Mucosal healing in pediatric Crohn's disease after anti-TNF therapy: a long-term experience at a single center. *Eur J Gastroenterol Hepatol* 2014;**26**:458–65.
47. Nuti F, Civitelli F, Bloise S, *et al.* Prospective evaluation of the achievement of mucosal healing with anti-TNF-α therapy in a paediatric Crohn's disease cohort. *J Crohns Colitis* 2016;**10**:5–12.
48. Olbjørn C, Nakstad B, Småstuen MC, Thiis-Evensen E, Vatn MH, Perminow G. Early anti-TNF treatment in pediatric Crohn's disease. Predictors of clinical outcome in a population-based cohort of newly diagnosed patients. *Scand J Gastroenterol* 2014;**49**:1425–31.
49. Papamichael K, Chachu KA, Vajravelu RK, *et al.* Improved long-term outcomes of patients with inflammatory bowel disease receiving proactive compared with reactive monitoring of serum concentrations of infliximab. *Clin Gastroenterol Hepatol* 2017;**15**:1580–8.e3.
50. Papamichael K, Vajravelu RK, Vaughn BP, Osterman MT, Cheifetz AS. Proactive infliximab monitoring following reactive testing is associated with better clinical outcomes than reactive testing alone in patients with inflammatory bowel disease. *J Crohns Colitis* 2018;**12**:804–10.
51. Vaughn BP, Martinez-Vazquez M, Patwardhan VR, Moss AC, Sandborn WJ, Cheifetz AS. Proactive therapeutic concentration monitoring of infliximab may improve outcomes for patients with inflammatory bowel disease: results from a pilot observational study. *Inflamm Bowel Dis* 2014;**20**:1996–2003.
52. D'Haens G, Vermeire S, Lambrecht G, *et al.*; GETAID. Increasing infliximab dose based on symptoms, biomarkers, and serum drug concentrations does not increase clinical, endoscopic, and corticosteroid-free remission in patients with active luminal Crohn's disease. *Gastroenterology* 2018;**154**:1343–51.e1.
53. Kang B, Choi SY, Choi YO, *et al.* Subtherapeutic infliximab trough levels and complete mucosal healing are associated with sustained clinical remission after infliximab cessation in paediatric-onset Crohn's disease patients treated with combined immunosuppressive therapy. *J Crohns Colitis* 2018;**12**:644–52.
54. Papamichael K, Vande Casteele N, Gils A, *et al.* Long-term outcome of patients with Crohn's disease who discontinued infliximab therapy upon clinical remission. *Clin Gastroenterol Hepatol* 2015;**13**:1103–10.
55. Walters TD, Kim MO, Denson LA, *et al.*; PRO-KIIDS Research Group. Increased effectiveness of early therapy with anti-tumor necrosis factor-α vs an immunomodulator in children with Crohn's disease. *Gastroenterology* 2014;**146**:383–91.
56. Kang B, Choi SY, Kim HS, Kim K, Lee YM, Choe YH. Mucosal healing in paediatric patients with moderate-to-severe luminal Crohn's disease under combined immunosuppression: escalation versus early treatment. *J Crohns Colitis* 2016;**10**:1279–86.
57. Fasanmade AA, Adedokun OJ, Blank M, Zhou H, Davis HM. Pharmacokinetic properties of infliximab in children and adults with Crohn's disease: a retrospective analysis of data from 2 phase III clinical trials. *Clin Ther* 2011;**33**:946–64.
58. Dotan I, Ron Y, Yanai H, *et al.* Patient factors that increase infliximab clearance and shorten half-life in inflammatory bowel disease: a population pharmacokinetic study. *Inflamm Bowel Dis* 2014;**20**:2247–59.
59. Brandse JF, Mould D, Smeekes O, *et al.* A real-life population pharmacokinetic study reveals factors associated with clearance and

immunogenicity of infliximab in inflammatory bowel disease. *Inflamm Bowel Dis* 2017;**23**:650–60.
60. Merras-Salmio L, Kolho KL. Clinical use of infliximab trough levels and antibodies to infliximab in pediatric patients with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 2017;**64**:272–8.
61. Grossi V, Lerer T, Griffiths A, *et al.* Concomitant use of immunomodulators affects the durability of infliximab therapy in children with Crohn's disease. *Clin Gastroenterol Hepatol* 2015;**13**:1748–56.
62. Kansen HM, van Rheenen PF, Houwen RHJ, *et al.*; Kids with Crohn's, Colitis [KiCC] Working Group for Collaborative Paediatric IBD Research in the Netherlands. Less anti-infliximab antibody formation in paediatric Crohn patients on concomitant immunomodulators. *J Pediatr Gastroenterol Nutr* 2017;**65**:425–9.
63. Levine A, Griffiths A, Markowitz J, *et al.* Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011;**17**:1314–21.