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Infliximab Trough Levels Are Associated With Mucosal Healing During Maintenance Treatment With Infliximab in Paediatric Crohn's Disease

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Abstract

Background and Aims: Mucosal healing is an important treatment goal in Crohn's disease. We investigated the association between serum infliximab trough levels and mucosal healing, and the infliximab cut-off levels required for mucosal healing in paediatric patients.

Methods: In this multicentre, retrospective, cross-sectional study, medical records and electronic data of paediatric patients with luminal Crohn's disease, who had received infliximab for ≥1 year, were examined. Ileocolonoscopy was performed on the same day as the infliximab infusion, and serum samples for trough levels were collected immediately before infusion. Mucosal healing was defined as a Simple Endoscopic Score for Crohn's Disease of 0. Univariate, multivariate logistic regression, and receiver operating characteristic curve analyses were performed.

Results: Overall, 105 patients [median age 14.8 years] were included, with mucosal healing observed in 48.6%. Median serum infliximab trough levels were higher in patients with mucosal healing [4.5 μg/mL] than without [3.3 μg/mL, $p = 0.002$]. In the final multivariate model, infliximab trough level ≥4.2 μg/mL [$p = 0.002$] and ≥1-year duration from diagnosis to infliximab treatment [$p = 0.003$] were positively and negatively associated with mucosal healing, respectively. The infliximab trough level for achieving mucosal healing with a specificity of 80% was ≥5 μg/mL.

Conclusions: Associations between serum infliximab trough concentrations and mucosal healing were observed in paediatric patients. Identification of the infliximab trough level that positively associates with mucosal healing in most paediatric patients with Crohn's disease [≥5 μg/mL] may guide treatment decisions to optimise therapeutic response in the era of treat-to-target.

Key Words: Crohn's disease; mucosal healing; infliximab

1. Introduction

Crohn's disease [CD] is a chronic inflammatory bowel disease [IBD] driven by interactions between genetic and environmental factors and the immune system.[1] Approximately 20–25% of CD manifests during childhood or the adolescent period, and the incidence of disease in paediatric populations is continuing to rise.[2] Paediatric CD is typically more severe than adult CD,[3] and young patients with IBD may experience growth impairment due to decreased food intake, malabsorption, higher metabolic demand, and other issues.[2,4] Psychological disorders including depression and anxiety, as well as impaired quality of life, are also more prevalent in paediatric patients than in adults or children without the disease.[2,5]

A number of medical therapies are available to treat paediatric CD including corticosteroids, exclusive enteral nutrition, aminosalicylates, immunomodulators, and anti-tumour necrosis factor [anti-TNF] agents.[6] Mucosal healing [MH] has been identified as a treatment goal that is able to predict sustained corticosteroid-free clinical remission, and is determined by resolution of endoscopically visible ulcers.[7] Achieving MH may modify the natural course of CD and is associated with reduced hospitalisation and surgery rates, as well as higher response and corticosteroid-free remission rates.[8–11]

Several trials in children have shown an improvement in MH with anti-TNF therapy, with enhanced MH leading to improved growth, bone formation, and disease outcome.[6] Infliximab [IFX] is an anti-TNF monoclonal antibody that may be considered for first-line therapy in paediatric CD patients with high risk for poor outcome, or for second-line therapy following failure of, or intolerance to, conventional therapies.[6] However, 13–40% of patients with CD do not respond to anti-TNF induction therapy, and a further 23–46% of patients require dose intensification due to secondary loss of response [LOR] during maintenance treatment with anti-TNF agents.[12] The mechanisms underlying primary non-response and secondary LOR are multifactorial and include factors related to the pharmacokinetics of anti-TNF agents. Such pharmacokinetic-related causes of treatment failure may be overcome with dose intensification to increase drug levels to within the therapeutic range.[12–15]

Higher anti-TNF drug trough levels are generally associated with positive clinical and endoscopic outcomes, including MH, in adult patients with IBD.[16–19] There is, however, a lack of data regarding the association between anti-TNF drug trough levels and MH in paediatric patients with CD. Separate evaluation of the paediatric population is important due to differences in the pharmacokinetics and pharmacodynamics of IFX in children compared with adults.[20] The primary aims of the current retrospective, cross-sectional study were to investigate the association between MH and serum trough levels of IFX in paediatric patients with luminal CD under maintenance IFX treatment, and to identify the cut-off level required for MH. The potential association of other clinical and demographic variables with MH was also examined.

2. Materials and Methods

2.1. Study design and participants

This was a multicentre, retrospective, cross-sectional study conducted between March 2013 and June 2016 in the paediatric departments of three tertiary medical centres in the Republic of Korea; Samsung Medical Center, Samsung Changwon Hospital, and Kyungpook National University Hospital. Eligible patients had paediatric-onset luminal CD and were aged <18 years at both diagnosis and the time of their first treatment with IFX. In addition, eligible patients had received scheduled IFX for ≥1 year, after which time they underwent ileocolonoscopy on the same day as an IFX infusion, with the ileocolonoscopy being performed before the infusion. Patients were also required to have data on serum IFX trough levels, obtained from blood samples taken immediately before the IFX infusion. Patients were excluded if they had a diagnosis of indeterminate-type IBD or had received IFX for the treatment of refractory perianal fistulas. CD was diagnosed according to the revised Porto criteria of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition.[21] Disease phenotype was classified according to the Paris classification.[22] The study was approved by the institutional review board of each centre, and was conducted in accordance with the Declaration of Helsinki.

Baseline demographic and clinical data at diagnosis, including sex, age, disease phenotype, growth indicators, and family history of IBD, were collected from electronic medical records. Data corresponding to the point of ileocolonoscopy examination were collected retrospectively from electronic charts or electronic test results, including: duration from diagnosis to initiation of IFX treatment, IFX treatment duration, number of IFX infusions, dose intensification, concomitant azathioprine [AZA] usage, corticosteroid usage, CD-related gastrointestinal [GI] tract surgery, Pediatric Crohn's Disease Activity Index [PCDAI] score, white blood cell count, haematocrit, platelet count, serum albumin level, erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], 6-thioguanine nucleotide [6-TGN] level, IFX trough level, presence of antibody to IFX [ATI], and Simple Endoscopic Score for Crohn's Disease [SES-CD]. Blood tests, except IFX trough level and ATI, were conducted either the day before or on the same day as ileocolonoscopy and IFX administration. Serum IFX trough levels and ATI were quantified using enzyme-linked immunosorbent assay kits [ELISA] [Matriks Biotek Laboratories, Ankara, Turkey].

Associations between IFX trough levels and MH, as well as other outcomes such as clinical remission and biochemical remission, were investigated. Clinical remission was defined as PCDAI <10, and biochemical remission was defined as a CRP <0.3 mg/dL. MH was defined as an SES-CD of 0. Partial MH was defined as an SES-CD <3.

2.2. Statistical analysis

For statistical comparison between groups, a Student's t-test or Wilcoxon rank sum test was used for continuous variables, and a chi square test or Fisher's exact test was used for categorical variables. Comparative data for continuous variables are reported as median (interquartile range [IQR]) or mean [standard deviation]. Pearson or Spearman correlations were used to investigate the correlation between continuous variables. Univariate and multivariate logistic regression analyses were performed to examine the association between MH and demographic, clinical, and biological variables [including serum IFX trough levels]. Univariate logistic regression analysis was first conducted to investigate the crucial odds ratio [OR] for each factor. A multivariate logistic regression analysis was then conducted using a stepwise selection procedure with a 5% significance level for a covariate to enter or remain in the model. The results were expressed as adjusted ORs with 95% confidence intervals [CIs]. Receiver operating characteristic curve analysis was performed to derive the best cut-off point for continuous variables identified as statistically significant in the multivariate logistic regression analysis. These statistically significant continuous variables were also converted to dichotomous variables and then included in a final multivariate logistic regression model, alongside other dichotomous variables that showed statistical significance in the original multivariate model. Youden's index analysis was used to assess the association of trough IFX cut-off levels with MH. Data were considered statistically significantly different if p was <0.05. All analyses were conducted using SAS software Version 9.4 [SAS Institute, Cary, NC, USA].

3. Results

3.1. Baseline characteristics

This study included 105 patients [74 males, 31 females]. All patients were anti-TNF naïve, as adalimumab was approved for paediatric CD in Korea in May 2015. The median age of study patients at diagnosis was 14.8 years [IQR 13.0–16.0 years]. The median treatment duration of IFX at follow-up ileocolonoscopy was 1.2 years [IQR 1.2–1.4 years]. Dose intensification of IFX had been conducted in 11 patients [10.5%], and 95 patients [90.5%] had been receiving concomitant AZA treatment. Other baseline demographics and clinical characteristics, as well as data collected at the follow-up ileocolonoscopy examination, are summarised in Table 1.

3.2. Association between IFX trough levels and outcomes including MH

Clinical remission was observed in 90.5% [95/105] of patients, and biochemical remission was observed in 82.9% [87/105] of patients. Serum IFX trough levels were significantly higher in patients with clinical remission compared with those without clinical remission [median 4.2 vs 1.2 μg/mL; IQR 3.0–5.4, 1.0–3.2, respectively;

Table 1. Baseline demographics/clinical characteristics and data collected at follow-up ileocolonoscopy.

	n = 105
Demographics/clinical characteristics at baseline [diagnosis]	
Male sex, *n* [%]	74 [70.5]
Median age [IQR], years	14.8 [13.0–16.0]
Lower GI tract involvement, *n* [%]	
L1 [distal 1/3 ileum ± limited caecal disease]	8 [7.6]
L2 [colonic]	6 [5.7]
L3 [ileocolonic]	91 [86.7]
Upper GI tract involvement, *n* [%]	
None	35 [33.3]
L4a [upper disease proximal to ligament of Treitz]	28 [26.7]
L4b [upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum]	15 [14.3]
L4a+b	27 [25.7]
Disease behaviour, *n* [%]	
B1 [non-stricturing, non-penetrating]	84 [80.0]
B2 [stricturing]	18 [17.1]
B3 [penetrating]	3 [2.9]
Perianal modifiers, *n* [%]	67 [63.8]
Linear growth failure, *n* [%]	18 [17.1]
Family history of IBD, *n* [%]	11 [10.5]
Clinical findings and treatment history at follow-up ileocolonoscopy	
Median disease duration [IQR], years	1.5 [1.3–2.5]
Median duration from diagnosis to IFX [IQR], years	0.1 [0.1–0.8]
IFX initiation within 1 year of diagnosis, *n* [%]	80 [76.1]
Median IFX treatment duration before follow-up ileocolonoscopy [IQR], years	1.2 [1.2–1.4]
Median number of IFX infusions before follow-up ileocolonoscopy [IQR]	8.0 [8.0–9.0]
Anti-TNF naïve, *n* [%]	105 [100.0]
Dose intensification during IFX treatment, *n* [%]	11 [10.5]
Concomitant AZA treatment, *n* [%]	95 [90.5]
Corticosteroid usage during IFX treatment, *n* [%]	6 [5.7]
Any lifelong CD-related GI surgery, *n* [%]	6 [5.7]
Median PCDAI score [IQR]	0 [0–5.0]
Median WBC [IQR], /μL	6010 [4390–7450]
Median haematocrit [IQR], %	41.7 [38.4–44.2]
Mean platelet count ± SD, $\times 10^3$/μL	258.7 ± 60.3
Median albumin [IQR], g/dL	4.5 [4.3–4.7]
Median ESR [IQR], mm/h	11 [4–18]
Median CRP [IQR], mg/dL	0.04 [0.03–0.13]
Mean 6-TGN [SD], pmol/8 × 10^8 RBC [*n* = 82]	253.1 ± 101.4
Median IFX trough level [IQR], μg/mL	3.89 [2.58–5.19]
ATI-positive, *n* [%]	8 [7.6%]
Median SES-CD [IQR]	1 [0–4]
Clinical remission,[a] *n* [%]	95 [90.5]
Biochemical remission,[b] *n* [%]	87 [82.9]
Mucosal healing,[c] *n* [%]	51 [48.6]

ATI, antibody to IFX; AZA, azathioprine; CD, Crohn's disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GI, gastrointestinal; IBD, inflammatory bowel disease; IFX, infliximab; IQR, interquartile range; PCDAI, Paediatric Crohn's Disease Activity Index; RBC, red blood cell; SD, standard deviation; SES-CD, Simple Endoscopic Score for Crohn's Disease; 6-TGN, 6-thioguanine nucleotides; TNF, tumour necrosis factor; WBC, white blood cell.

[a]PCDAI <10.

[b]CRP <0.3 mg/dL.

[c]SES-CD = 0.

p <0.001], and in patients with biochemical remission compared with those without biochemical remission [median 4.2 vs 2.3 µg/mL; IQR 3.1–5.4, 1.4–3.9, respectively; p = 0.006] [Figure 1A, B].

Overall, MH—defined as an SES-CD of 0 at follow-up ileocolonoscopy—was observed in 48.6% [51/105] of patients. Median serum IFX trough levels were significantly higher in patients with MH compared with those without MH [median 4.5 vs 3.3 µg/mL; IQR 3.3–6.1, 2.1–4.5, respectively; p = 0.002] [Figure 1C]. Partial MH was observed in 60.0% [63/105] of patients. Median serum IFX trough levels were also significantly higher in patients with partial MH compared with those without partial MH [median 4.5 vs 3.2 µg/mL; IQR 3.4–5.9, 1.7–4.0, respectively; p < 0.001].

In addition, rates of MH were higher in patients with higher serum IFX trough levels [Figure 2A, B]. According to the quartile analysis, MH rates increased with quartiles of IFX trough levels [Figure 2A]. Likewise, 80% of patients achieved MH when IFX trough levels were ≥8 µg/mL, compared with only 20% of patients when these levels were <2 µg/mL [Figure 2B].

3.3. Relationship between IFX trough levels and ATIs, concomitant azathioprine, and 6-TGN levels

ATI positivity was observed in 7.6% [8/105] of patients. Serum IFX trough levels were significantly lower in patients who were ATI positive compared with those who were ATI negative [median 1.0 vs 4.2 µg/mL, respectively; p < 0.001] [Figure 1D].

Regarding concomitant AZA medication, there was no significant difference in IFX trough levels between those who had received concomitant AZA and those who had not (median 4.0 [IQR 2.6–5.3] vs 3.3 µg/mL [IQR 2.6–4.2], respectively; p = 0.259). The ATI positivity rate also did not differ significantly between patients who had or had not received concomitant AZA (7.4% [7/95] vs 10% [1/10]; p = 1.000).

Of the 95 patients who were receiving concomitant AZA, 6-TGN levels were available for 81. These did not differ significantly between those who were ATI-positive and those who were ATI-negative (mean ± standard deviation: 295.6 ± 98.6 vs 249.1 ± 101.4 pmol/8 × 10^8 red blood cells [RBC]; p = 0.249). No significant correlation was observed between IFX trough levels and 6-TGN levels [r = –0.06; p = 0.57]. Further, 6-TGN levels did not significantly differ between patients with and without MH (252.9 ± 91.5 pmol/8 × 10^8 RBC [n = 42] vs 253.3 ± 112.3 pmol/8 × 10^8 RBC [n = 39]; p = 0.987], or between patients with and without biochemical remission [250.7 ± 103.5 pmol/8 × 10^8 RBC [n = 68] vs 265.8 ± 92.5 pmol/8 × 10^8 RBC [n = 13]; p = 0.625), or between patients with and without clinical remission (246.5 ± 99.3 pmol/8 × 10^8 RBC [n = 74] vs 322.8 ± 104.3 pmol/8 × 10^8 RBC [n = 7]; p = 0.057).

3.4. Factors associated with MH

According to univariate analysis, B1 disease behaviour, duration from diagnosis to IFX treatment, CRP level, and IFX trough level were significantly associated with MH [Table 2]. When these factors were included in a multivariate logistic regression analysis with stepwise selection, B1 disease behaviour, duration from diagnosis to IFX treatment, and IFX trough level were significantly associated with MH [OR: 3.524, 95% CI: 1.061–11.701, p = 0.04; OR: 0.529, 95% CI: 0.344–0.813, p = 0.004; OR: 1.479, 95% CI: 1.176–1.860, p <0.001; respectively] [Table 2].

According to receiver operating characteristic curve analysis, the most accurate IFX trough level cut-off associated with MH was 4.2 µg/mL (area under the curve [AUC]: 0.679, 95% CI:

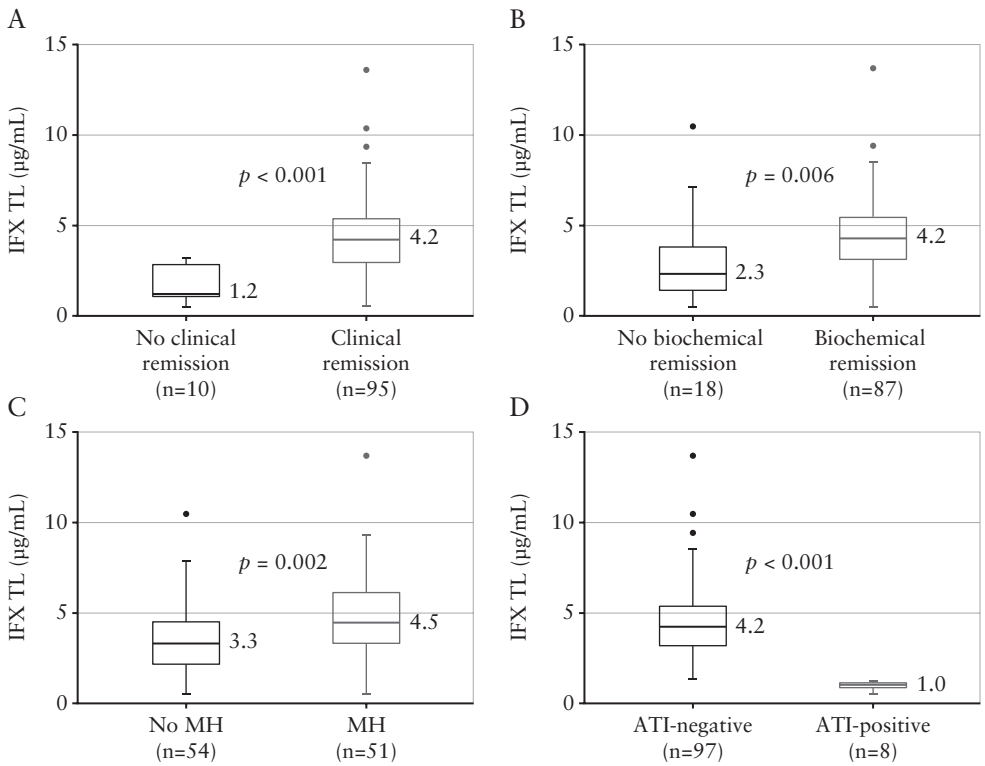

Figure 1. Comparison of infliximab trough levels according to [A] clinical remission, [B] biochemical remission, [C] mucosal healing, and [D] ATI positivity. Filled circles are outliers. ATI, antibody to IFX; IFX, infliximab; MH, mucosal healing; TL, trough level.

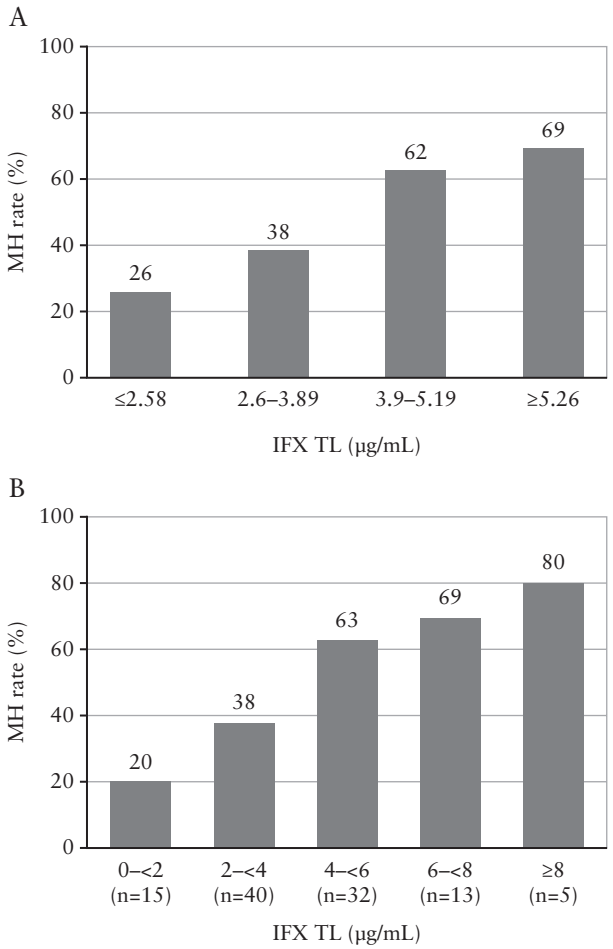

Figure 2. Mucosal healing rates according to infliximab trough level: [A] quartiles of trough levels, [B] 2-µg/mL increments; *n* = 105. IFX, infliximab; MH, mucosal healing;TL, trough level.

0.576–0.782, sensitivity 64.7%, specificity 70.4%, positive predictive value [PPV] 67.3%, negative predictive value [NPV] 67.9%, $p < 0.001$) [Figure 3D]. The most accurate duration from diagnosis to IFX treatment for MH association was 1.0 year [AUC: 0.617, 95% CI: 0.508–0.726, $p = 0.001$].

In the final multivariate model, IFX trough level ≥4.2 µg/mL and ≥1-year duration from diagnosis to IFX treatment remained significantly associated with MH [in a positive and negative manner, respectively] [Table 3].

3.5. Optimal IFX trough levels required for achieving MH and other outcomes

According to receiver operating characteristic curve analysis, the most accurate IFX trough level cut-off associated with clinical remission was 3.26 µg/mL [AUC: 0.902, 95% CI: 0.817–0.987, sensitivity 70.5%, specificity 100%, PPV 100%, NPV 73.3%, $p < 0.001$], and for biochemical remission was 2.52 µg/mL [AUC: 0.708, 95% CI: 0.551–0.865, sensitivity 86.2%, specificity 55.5%, PPV 90.3%, NPV 45.5%, $p < 0.001$] [Figure 3A, B]. For partial MH, the most accurate IFX trough level cut-off was 3.71 µg/mL [AUC: 0.733, 95% CI: 0.633–0.833, sensitivity 69.8%, specificity 71.4%, PPV 78.6%, NPV 61.2%, $p < 0.001$] [Figure 3C].

According to the Youden's index, the IFX trough level cut-offs for achieving biochemical remission, partial MH, and MH with a specificity of ≥80%, were ≥4.9 µg/mL [sensitivity 32.2%, specificity 83.3%, PPV 90.3%, NPV 20.3%], ≥4.5 µg/mL [sensitivity 49.2%, specificity 81.0%, PPV 79.5%, NPV 51.5%], and ≥5.0 µg/mL [sensitivity 37.3%, specificity 80.0%, PPV 63.3%, NPV 57.3%], respectively. Cut-off levels of IFX trough levels required to reach MH are shown in Table 4.

4. Discussion

In this study, we retrospectively evaluated the relationship between endoscopy findings, specifically MH, and various demographic, clinical, and treatment-related factors in paediatric patients with CD who had been receiving IFX therapy for at least 1 year. We found a significant association between serum IFX trough levels and MH, and the most accurate trough level cut-off was calculated to be 4.2 µg/mL. We also calculated that to achieve MH in 80% of paediatric CD patients during IFX maintenance therapy, an IFX trough level of ≥5.0 µg/mL would be required. Our study also revealed a significant association between MH and duration from diagnosis to IFX treatment.

Our findings provide evidence that therapeutic drug monitoring [TDM] of IFX trough levels may prove a useful and simple tool for increasing the rate of MH in paediatric patients. The association between serum IFX trough concentrations and clinical efficacy in adult patients with IBD has been the subject of a number of studies, in which efficacy was assessed in terms of symptoms and/or endoscopic outcomes.[17–19,23–32] *Post-hoc* analysis of the ACCENT I study found that higher post-induction IFX trough levels, specifically ≥3.5 µg/mL, were associated with a durable sustained response to maintenance IFX.[24] *Post-hoc* analysis of data from the SONIC trial identified statistically significant differences in median trough levels in patients who achieved MH compared with those who did not [3.51 vs 1.72 µg/mL; $p = 0.018$], and in patients who achieved a combination of MH and clinical remission compared with those who did not [3.51 vs 1.80 µg/mL; $p = 0.053$].[23] Further analysis of SONIC identified a significant association between trough IFX levels ≥3 µg/mL at Week 30 and MH at Week 26 [OR: 3.34, 95% CI: 1.53–7.28; $p = 0.002$].[28] Recent studies based on real-life clinical practice have also shown the association between IFX trough levels and MH.[18,31] In a recent study by Ungar *et al.*, the authors proposed a therapeutic window for MH in adult IBD patients as IFX trough levels of 6–10 µg/mL, which was defined as the range of drug levels associated with MH rate of 80–90%.[18] Another recent study by Papamichael *et al.* showed that an IFX trough level of ≥9.7 µg/mL was associated with MH in adult patients with CD [OR: 3.6, 95% CI: 1.4–9; $p = 0.006$].[31]

Our study has confirmed that an association between IFX trough levels and MH is present in paediatric patients with CD. However, the cut-off levels in our study are lower than those reported in studies of adult patients. There is evidence that the pharmacokinetic properties of IFX are comparable in paediatric and adult patients with CD and that they are not influenced by age.[33,34] The pharmacokinetics of IFX are highly variable between individuals and are affected by factors such as the presence of ATIs, concomitant use of immunomodulators, serum albumin levels, baseline CRP, body mass index, and sex.[35] Variations in these factors may be responsible for the differences in trough levels observed, but the question remains why MH occurred at a lower trough level in paediatric patients than previously reported in adults. This discrepancy could be explained by differences in disease factors that are related to therapeutic response to IFX treatment, such as disease behaviour, previous

Table 2. Analyses of factors associated with mucosal healing.

	Univariate logistic regression [n = 105]			Multivariate logistic regression with stepwise selection [n = 105]		
	OR	95% CI	p	Adjusted OR	95% CI	p
Sex [male vs female]	0.581	0.249–1.357	0.21			
Age at diagnosis, years	0.941	0.802–1.104	0.457			
Any colonic involvement [yes vs no]	0.541	0.122–2.391	0.418			
Any TI involvement [yes vs no]	0.174	0.020–1.540	0.116			
Upper GI tract involvement [yes vs no]	1.682	0.738–3.831	0.216			
B1 disease behaviour[a] [yes vs no]	3.874	1.299–11.547	**0.015**	3.524	1.061–11.701	**0.04**
Perianal modifiers [yes vs no]	1.078	0.486–2.392	0.853			
Linear growth failure [yes vs no]	0.516	0.505–3.891	1.402			
Duration from diagnosis to IFX treatment, years	0.585	0.386–0.888	**0.012**	0.529	0.344–0.813	**0.004**
Duration of IFX treatment, years	0.504	0.196–1.295	0.155			
Dose intensification during IFX treatment [yes vs no]	0.571	0.157–2.083	0.396			
Concomitant AZA treatment [yes vs no]	2.382	0.581–9.766	0.228			
WBC, /μL	1.000	1.000–1.000	0.306			
Haematocrit, %	0.977	0.890–1.074	0.631			
Platelet count, /μL	0.997	0.990–1.003	0.297			
Albumin, g/dL	3.698	0.917–14.907	0.066			
ESR, mm/hr	0.99	0.968–1.012	0.362			
CRP, mg/dL	0.032	0.003–0.407	**0.008**			
IFX trough level, μg/mL	1.354	1.096–1.673	**0.005**	1.479	1.176–1.860	**<0.001**
ATI [yes vs no]	0.327	0.063–1.699	0.183			

ATI, antibody to IFX; AZA, azathioprine; CI, confidence interval; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GI, gastrointestinal; IFX, infliximab; OR, odds ratio; TI, terminal ileum; WBC, white blood cell.

[a]Non-stricturing, non-penetrating.

anti-TNF use, and disease duration. Compared with the recent study by Papamichael *et al.*,[31] our study included a higher proportion of patients with B1 behaviour [49% vs 80%] and a higher proportion of patients who were anti-TNF naïve [93% vs 100%]; disease duration at investigation was also shorter in our study [median 8 years vs 1.2 years]. As the annual risk for LOR to IFX is approximately 13% per patient-year,[36] it is not surprising that a greater proportion of patients in the study by Papamichael *et al.* required dose intensification compared with our study [43% vs 10.5%]. Interestingly, in the study by Papamichael *et al.,* dose intensification was negatively associated with endoscopic remission. The authors suggest that this was due to a high disease burden and rapid IFX clearance in patients who received increased IFX doses. In our study, a negative trend between dose intensification and MH was also observed, although this was statistically insignificant [OR = 0.571, 95% CI = 0.157–2.083; p = 0.396].

Another important factor that affects response to IFX is the time from diagnosis to initiation of IFX treatment.[10,23] Whereas this variable was not evaluated in the study by Papamichael *et al.,* 76.1% of patients in our study started IFX within 1 year of diagnosis. Moreover, in the current study, the time from diagnosis to IFX treatment was an independent factor significantly associated with MH, with patients initiating treatment ≥1 year after diagnosis less likely to experience MH than those starting treated within 1 year. This finding is consistent with the concept of a 'therapeutic window of opportunity' in early CD, during which treatment is more effective compared with later in the course of the disease.[10,37–39] Thus, despite similar specificity, lower IFX trough cut-off levels for MH may have been observed in our study than in previous adult studies, because our patient population possessed more favourable factors for treatment response to IFX, compared with the adult patients.

Although data on the relationship between trough IFX levels and efficacy are less abundant for the paediatric population, results from relevant studies have been reported, which are comparable to those of the present study.[14,20,40–43] Median IFX trough levels were significantly higher in paediatric patients with IBD [CD or ulcerative colitis] on maintenance therapy who achieved clinical remission [defined by the absence of symptoms in the first year after initiation of IFX therapy] than in patients who did not achieve clinical remission [3.99 μg/mL vs 0.88 μg/mL; p = 0.002].[40] Similarly, in paediatric IBD patients who were in remission or continuing with IFX therapy [median follow-up of 3.9 years after first IFX infusion], the median IFX trough level was 3.7 μg/mL, compared with a trough level of 1.2 μg/mL in those who had failed IFX therapy due to lack of efficacy or to adverse events [p = 0.005].[14] In a separate study, mean trough IFX levels [assessed at several different visits] were significantly higher when patients were in clinical remission than when they had active disease [7.2 μg/mL vs 4.5 μg/mL; p <0.05].[20] In another recent study, IFX trough levels >9.2 μg/mL at Week 2 were capable of predicting clinical remission at Week 14 [AUC: 0.73, sensitivity 71.4%, specificity 81.2%; p = 0.02], and IFX trough levels >2.2 μg/mL at Week 6 were capable of predicting IFX durability beyond 1 year of treatment [AUC: 0.974, sensitivity 88.9%, specificity 100.0%; p <0.0001].[42]

In the current study, we identified an IFX trough level of 5.0 μg/mL as the minimum that should be attained in order that 80% of paediatric CD patients would achieve MH. To our knowledge, no other studies have calculated a cut-off value for IFX trough levels

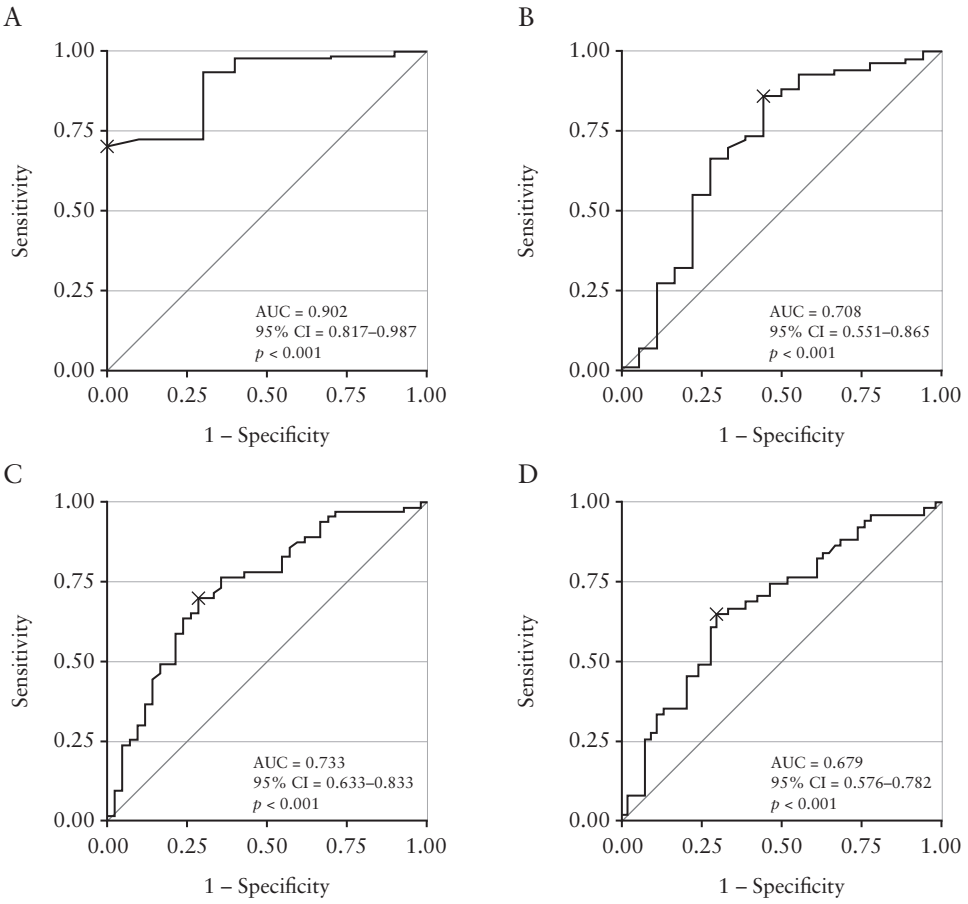

Figure 3. Receiver operating characteristic curve showing the association between infliximab trough level and [A] clinical remission, [B] biochemical remission, [C] partial mucosal healing [SES-CD <3], and [D] mucosal healing [SES-CD = 0]; *n* = 105; AUC, area under curve; CI, confidence interval; SES-CD, Simple Endoscopic Score for Crohn's Disease.

Table 3. Final multivariate model of factors associated with mucosal healing.

	OR	95% CI	*p*
B1 disease behaviour[a]	3.312	0.973–11.273	0.055
Duration from diagnosis to IFX treatment ≥1 year	0.149	1.641–31.571	**0.003**
IFX trough level ≥4.2 µg/mL	3.999	1.608–8.185	**0.002**

n = 105 for all analyses.
CI, confidence interval; IFX, infliximab; OR, odds ratio.
[a]Non-stricturing, non-penetrating.

Table 4. Cut-off levels of IFX trough levels required to reach mucosal healing.

IFX TL cut-off level, µg/mL	Sensitivity, %	Specificity, %	PPV, %	NPV, %
≥3	80	39	55	68
≥3.5	71	57	61	64
≥4.5	49	74	64	61
≥5	37	80	63	57
≥5.3	35	85	69	58
≥6	27	91	74	57
≥8.5	8	98	80	53

n = 105 for all analyses.
IFX, infliximab; NPV, negative predictive value; PPV, positive predictive value; TL, trough level.

in paediatric CD patients based on an endoscopic outcome such as MH. In a previous study, trough IFX level cut-offs of >3, >5, and >7 µg/mL at Week 14 were associated with PPVs of 64%, 76%, and 100%, respectively, in terms of predicting persistent clinical remission at Week 54.[41] Another recent study that used a cut-off level of 3.5 µg/mL, chosen by agreement between study physicians, showed that TDM of IFX trough levels could inform clinical decision making within the paediatric IBD population.[44] Likewise, the use of reactive TDM in paediatric patients with IBD is increasing in clinical practice, to overcome loss of response and to improve outcomes.[45] Our results provide additional evidence that, similar to adults, a cut-off level higher than that needed to achieve clinical remission should be used to reach MH in children receiving IFX, and may further serve as fundamental data for future proactive TDM regimens in children with CD.

Regardless of the effect on MH, combination treatment with AZA during IFX treatment is well-known to be positively associated with IFX trough levels and negatively associated with ATIs.[46,47] Furthermore, a recent study has shown that 6-TGN levels correlate with IFX trough levels in patients with IBD on combination therapy.[48] However, we were unable to observe these findings in our study. One explanation for the discrepancy between the results

of our study and of the former studies[46,47] is the small number of patients who were under IFX monotherapy in our study. An explanation of the discrepancy with the latter study[48] is that AZA dosing had been changed periodically based on clinical status and 6-TGN levels in patients included in our study. This may also explain why 6-TGN levels were relatively higher in patients without clinical remission compared with those with clinical remission, whereas 6-TGN levels between patients with and without MH were comparable. In patients without clinical remission, AZA doses may have been increased if 6-TGN levels were at the lower end of the therapeutic range [235–450 pmol/8 × 10^8 RBC],[49] whereas in those with clinical remission, AZA doses were unlikely to have been changed even if 6-TGN levels were below 235–450 pmol/8 × 10^8 RBC.

The retrospective design of this study is its principal limitation; possible confounding and introduction of bias may affect the results of our study. In addition, limitations due to the cross-sectional nature of the study may not well reflect the dynamically changing pharmacokinetics of patients in real-life clinical practice. Moreover, some patients who developed significant ATIs before follow-up ileocolonoscopy may have stopped IFX treatment, resulting in their eventual exclusion from the study. This may have resulted in a low ATI rate compared with previous studies, as well as comparable rates of ATI and MH between patients receiving combination treatment and those receiving IFX monotherapy. Despite these limitations, the minimal time gap between trough level sampling and colonoscopic examination compared with previous studies is a notable strength of this study. In addition, this is the first study in paediatric CD patients to investigate the association between IFX trough levels and MH.

In conclusion, this study shows that there is an association between IFX trough levels and treatment outcomes in paediatric CD patients. The identification of the IFX trough level required for MH in most paediatric patients with CD [≥5.0 μg/mL] may help to guide dosing decisions, potentially leading to improved future treatment outcomes in the era of treat-to-target.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Author Contributions

BK contributed to the conception and design of the study, acquisition, analysis, and interpretation of data, drafting of the initial manuscript, and critical revision of the manuscript for important intellectual content. SYC contributed to the acquisition, analysis, and interpretation of data, and drafting of the initial manuscript. YOC contributed to the acquisition, analysis, and interpretation of data, and drafting of the initial manuscript. S-YL contributed to the acquisition, analysis, and interpretation of data, and drafting of the initial manuscript. S-YB contributed to statistical analyses and drafting of the initial manuscript. IS contributed to statistical analyses and critical revision of the manuscript for important intellectual content. B-HC contributed to the analysis and interpretation of data and critical revision of the manuscript for important intellectual content. HJL contributed to the acquisition of data and critical revision of the manuscript for important intellectual content. YHC contributed to the conception and design of the study, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content. All authors approved the final version of the manuscript, had full access to all the data in the study, and had final responsibility for the decision to submit for publication.

References

1. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007;**369**:1627–40.
2. Rosen MJ, Dhawan A, Saeed SA. Inflammatory bowel disease in children and adolescents. *JAMA Pediatr* 2015;**169**:1053–60.
3. Pigneur B, Seksik P, Viola S, *et al.* Natural history of Crohn's disease: comparison between childhood- and adult-onset disease. *Inflamm Bowel Dis* 2010;**16**:953–61.
4. Choi J, Kang B, Kim MJ, Sohn I, Lee HJ, Choe YH. Early infliximab yields superior long-term effects on linear growth in pediatric Crohn's disease patients. *Gut Liver* 2018;**12**:255–62.
5. Mikocka-Walus A, Knowles SR, Keefer L, Graff L. Controversies revisited: a systematic review of the comorbidity of depression and anxiety with inflammatory bowel diseases. *Inflamm Bowel Dis* 2016;**22**:752–62.
6. Ruemmele FM, Veres G, Kolho KL, *et al.*; European Crohn's and Colitis Organisation; European Society of Pediatric Gastroenterology, Hepatology and Nutrition. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis* 2014;**8**:1179–207.
7. Neurath MF, Travis SP. Mucosal healing in inflammatory bowel diseases: a systematic review. *Gut* 2012;**61**:1619–35.
8. De Cruz P, Kamm MA, Prideaux L, Allen PB, Moore G. Mucosal healing in Crohn's disease: a systematic review. *Inflamm Bowel Dis* 2013;**19**:429–44.
9. Baert F, Moortgat L, Van Assche G, *et al.*; Belgian Inflammatory Bowel Disease Research Group; North-Holland Gut Club. Mucosal healing predicts sustained clinical remission in patients with early-stage Crohn's disease. *Gastroenterology* 2010;**138**:463–8; quiz e10–1.
10. 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.
11. Peyrin-Biroulet L, Ferrante M, Magro F, *et al.*; Scientific Committee of the European Crohn's and Colitis Organization. Results from the Second Scientific Workshop of the ECCO. I: Impact of mucosal healing on the course of inflammatory bowel disease. *J Crohns Colitis* 2011;**5**:477–83.
12. Ding NS, Hart A, De Cruz P. Systematic review: predicting and optimising response to anti-TNF therapy in Crohn's disease algorithm for practical management. *Aliment Pharmacol Ther* 2016;**43**:30–51.
13. Steenholdt C, Bendtzen K, Brynskov J, Ainsworth MA. Optimizing treatment with TNF inhibitors in inflammatory bowel disease by monitoring drug levels and antidrug antibodies. *Inflamm Bowel Dis* 2016;**22**:1999–2015.
14. 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.
15. Joosse ME, Samsom JN, van der Woude CJ, Escher JC, van Gelder T. The role of therapeutic drug monitoring of anti-tumor necrosis factor alpha agents in children and adolescents with inflammatory bowel disease. *Inflamm Bowel Dis* 2015;**21**:2214–21.
16. Adedokun OJ, Sandborn WJ, Feagan BG, *et al.* Association between serum concentration of infliximab and efficacy in adult patients with ulcerative colitis. *Gastroenterology* 2014;**147**:1296–307.e5.

17. Roblin X, Marotte H, Rinaudo M, *et al.* Association between pharmacokinetics of adalimumab and mucosal healing in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2014;**12**:80–4.e2.
18. 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.
19. Kelly OB, Donnell SO, Stempak JM, Steinhart AH, Silverberg MS. Therapeutic drug monitoring to guide infliximab dose adjustment is associated with better endoscopic outcomes than clinical decision making alone in active inflammatory bowel disease. *Inflamm Bowel Dis* 2017;**23**:1202–9.
20. 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.
21. Levine A, Koletzko S, Turner D, *et al.*; European Society of Pediatric Gastroenterology, Hepatology, and Nutrition. ESPGHAN revised Porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. *J Pediatr Gastroenterol Nutr* 2014;**58**:795–806.
22. 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.
23. Colombel JF, Reinisch W, Mantzaris GJ, *et al.* Randomised clinical trial: deep remission in biologic and immunomodulator naïve patients with Crohn's disease a SONIC post hoc analysis. *Aliment Pharmacol Ther* 2015;**41**:734–46.
24. Cornillie F, Hanauer SB, Diamond RH, *et al.* Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut* 2014;**63**:1721–7.
25. Imaeda H, Bamba S, Takahashi K, *et al.* Relationship between serum infliximab trough levels and endoscopic activities in patients with Crohn's disease under scheduled maintenance treatment. *J Gastroenterol* 2014;**49**:674–82.
26. Levesque BG, Greenberg GR, Zou G, *et al.* A prospective cohort study to determine the relationship between serum infliximab concentration and efficacy in patients with luminal Crohn's disease. *Aliment Pharmacol Ther* 2014;**39**:1126–35.
27. 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.
28. Reinisch W, Colombel JF, Sandborn WJ, *et al.* Factors associated with short- and long-term outcomes of therapy for Crohn's disease. *Clin Gastroenterol Hepatol* 2015;**13**:539–47.e2.
29. Steenholdt C, Bendtzen K, Brynskov J, Thomsen OØ, Ainsworth MA. Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. *Scand J Gastroenterol* 2011;**46**:310–8.
30. 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.
31. 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;**24**:2266–71.
32. 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.
33. 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.
34. 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.
35. 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; quiz e85–6.
36. Gisbert JP, Panés J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. *Am J Gastroenterol* 2009;**104**:760–7.
37. Kang B, Choe YH. Early biologic treatment in pediatric Crohn's disease: catching the therapeutic window of opportunity in early disease by treat-to-target. *Pediatr Gastroenterol Hepatol Nutr* 2018;**21**:1–11.
38. Danese S, Fiorino G, Fernandes C, Peyrin-Biroulet L. Catching the therapeutic window of opportunity in early Crohn's disease. *Curr Drug Targets* 2014;**15**:1056–63.
39. Pariente B, Cosnes J, Danese S, *et al.* Development of the Crohn's disease digestive damage score, the Lémann score. *Inflamm Bowel Dis* 2011;**17**:1415–22.
40. 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.
41. 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.
42. Ungar B, Glidai Y, Yavzori M, *et al.* Association between infliximab drug and antibody levels and therapy outcome in pediatric inflammatory bowel diseases. *J Pediatr Gastroenterol Nutr* 2018;**67**:507–12.
43. 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.
44. 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.
45. Carman N, Mack DR, Benchimol EI. Therapeutic drug monitoring in pediatric inflammatory bowel disease. *Curr Gastroenterol Rep* 2018;**20**:18.
46. Colombel JF, Sandborn WJ, Reinisch W, *et al.*; SONIC Study Group. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010;**362**:1383–95.
47. Van Assche G, Magdelaine-Beuzelin C, D'Haens G, *et al.* Withdrawal of immunosuppression in Crohn's disease treated with scheduled infliximab maintenance: a randomized trial. *Gastroenterology* 2008;**134**:1861–8.
48. Yarur AJ, Kubiliun MJ, Czul F, *et al.* Concentrations of 6-thioguanine nucleotide correlate with trough levels of infliximab in patients with inflammatory bowel disease on combination therapy. *Clin Gastroenterol Hepatol* 2015;**13**:1118–24.e3.
49. Dervieux T, Meyer G, Barham R, *et al.* Liquid chromatography-tandem mass spectrometry analysis of erythrocyte thiopurine nucleotides and effect of thiopurine methyltransferase gene variants on these metabolites in patients receiving azathioprine/6-mercaptopurine therapy. *Clin Chem* 2005;**51**:2074–84.