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The association of infliximab trough levels with disease activity in pediatric inflammatory bowel disease

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ABSTRACT

Objective
Low serum trough levels (TLs) of infliximab (IFX) and antibodies to IFX (ATIs) are associated with the loss of therapeutic response in adults with inflammatory bowel disease (IBD) receiving IFX. Until now, pediatric data are scarce. Therefore, we aimed to cross-sectionally investigate the association between ATIs and IFX TLs, and clinical and biochemical disease activity in children receiving IFX for IBD.

Material and Methods
Children aged <18 years receiving IFX maintenance treatment for Crohn’s disease (CD) or ulcerative colitis (UC) at three Dutch hospitals were included. Prior to two consecutive IFX infusions, IFX TLs and ATI levels were measured. Clinical disease activity was determined by Pediatric Crohn’s Disease Activity Index (PCDAI) and Pediatric Ulcerative Colitis Activity Index (PUCAI), for CD and UC, respectively. Biochemical disease activity was assessed by serum C-reactive protein (CRP) and fecal calprotectin (FC). Clinical remission was defined as a PUCAI or PCDAI score of <10. Therapeutic range of IFX was considered 3-7 µg/ml.

Results
Thirty-nine patients were included (31 CD; 16 females). Median age was 15 years. Median IFX TL was 3.5 µg/ml [IQR 2-7]. Subtherapeutic and supratherapeutic TLs were found in 38% and 23% of children, respectively. ATIs were detected in four patients. A correlation was found between IFX TL and CRP \( r_s = -0.51; p < 0.01 \) and FC \( r_s = -0.49; p < 0.01 \). However, when only clinical disease activity was considered, no difference in median TL was found between remission and active disease (resp. 3.5 µg/ml [IQR 2-5] and 2.3 µg/ml [IQR 0.3-4.6]; \( p = 0.2 \)).

Conclusions
IFX TLs are related biochemical markers of disease activity. This could provide a rationale for monitoring TLs in children receiving IFX for IBD.
INTRODUCTION

Infliximab (IFX), a chimeric antibody directed against TNF, is effective in inducing and maintaining remission in adults with Crohn's disease (CD) and ulcerative colitis (UC). Since the introduction of IFX in 1993, the management of inflammatory bowel disease (IBD) has changed drastically, particularly in patients who do not respond to conventional therapy. More recently, IFX has also been shown to be effective in inducing and maintaining remission in children with CD and UC.

Despite convincing evidence of the clinical efficacy of IFX, several challenges exist. A proportion of IBD patients do not respond at all and many more gradually lose response over time. This phenomenon of "secondary loss of response" occurs in probably more than a third of the initial responders. Loss of response is assumed to be related to low serum IFX concentration and to the development of antibodies to IFX (ATIs). Some studies in children with CD report higher loss of response rates than in adults, suggesting that pharmacodynamics and pharmacokinetics in children may differ from that in adults. Results to date, however, have not been unequivocal. To optimize treatment with IFX and achieve long-term IBD control, several studies have recommended therapeutic drug monitoring (TDM), which comprises measurement of serum IFX and ATI levels and adjusting treatment accordingly. High IFX trough levels (TLs) (i.e., serum drug level measured just before the next administration) are associated with a more favorable outcome in adults, as indicated by higher rates of clinical remission and a higher likelihood of mucosal healing. In contrary, the presence of ATIs is associated with a shorter duration of response and loss of response to IFX. Other data suggest that high TLs might lead to side effects, such as psoriasiform eczema and arthralgia.

Based on these findings, algorithms have been developed for the management of adult IBD patients on IFX who develop symptoms of active disease. Although the first data from TDM-guided maintenance treatment with IFX, showed no benefit compared to clinically based adjustment with respect to the primary outcome, data from ongoing trials will determine the potential value of TDM in the treatment of CD (NCT01442025).

Pediatric series investigating the association between disease activity and TLs and ATIs are lacking so far. To our knowledge, two studies on this topic have recently been published. In one study, higher levels of intestinal inflammation (as measured with fecal calprotectin [FC]) were found to be associated with low IFX levels during remission induction with IFX, but not during maintenance treatment with IFX. However, the methodology of this study was not clearly described, e.g., the number of analyzed samples per patient varied to a large extent without further explanation. Also, the association between clinical disease activity and TLs and ATIs was not described. In an analysis of a randomized controlled trial investigating the efficacy and safety of IFX for pediatric UC, higher IFX (non-trough) concentrations 2 weeks after remission induction were associated with greater proportions of patients achieving...
efficacy endpoints. Furthermore, limited data suggested a positive relationship between IFX TL during maintenance treatment and improvement in Pediatric Ulcerative Colitis Activity Index (PUCAI) score.

The aim of our study was therefore to investigate the relationship between TLs, ATIs and clinical and biochemical disease activity in children receiving IFX as maintenance therapy for IBD.

**METHODS**

**Patients**
Between January 2013 and December 2013 all patients aged <18 years receiving scheduled IFX infusions as maintenance treatment for CD, UC or indeterminate colitis, at the department of pediatrics of two academic hospitals and one district hospital in the Netherlands were asked to participate. Prior to each infusion, children’s weight was measured to calculate the exact IFX dose to be administered. No dose rounding to the nearest vial size was performed. All patients had previously responded to an induction regimen with 5 mg/kg IFX at week 0, 2 and 6 followed by infusions every 8 weeks (q8w). There were no exclusion criteria. Approval from the local Medical Ethics Review Committee was obtained.

**Data collection**
Baseline characteristics were abstracted from the medical records, including co-medication at inclusion and during remission induction with IFX. Prior to two consecutive intravenous IFX infusions, the following laboratory tests were performed: hematocrit, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), albumin, IFX TL and ATI. Patients were asked to collect a fecal sample at the time of IFX infusion for the measurement of FC levels. IFX TLs and ATIs were determined by Sanquin Diagnostics Services, the Netherlands, where a home-based ELISA test has been developed and validated. This method does not allow detection of ATIs in the presence of IFX. The lowest level of IFX TL quantification was 0.002 µg/ml.

At both infusion visits, disease activity was determined by a trained physician. For CD, the Pediatric Crohn’s Disease Activity Index (PCDAI) was used. A PCDAI score <10 was defined as clinical remission, 10-30 as mild to moderate disease activity and >30 as severe disease activity. For comparison purposes, the short PCDAI (shPCDAI) was also determined, which does not include laboratory values. For UC, the PUCAI was used. A PUCAI score <10 was defined as clinical remission, 10-34 as mild disease activity, 35-64 as moderate disease activity and >65 as severe disease activity. Participants’ weight and height were recorded at both instances. Although no consensus exists regarding the optimal cutoff of CRP to define remission, we considered a CRP level below 5 mg/l as normal as described previously. TLs were categorized in accordance with a therapeutic range of 3-7 µg/ml.
Statistical analysis
Primary analysis was the relation between IFX TLs and biochemical markers of disease activity. Statistical analysis was performed using IBM SPSS Statistics 21 for Mac. For continuous data with a normal distribution, mean and standard deviation (SD) were reported, and T-tests and Pearson correlations were used. For continuous data with a non-normal distribution, median and interquartile range (IQR) were reported, and Mann-Whitney U tests and Spearman’s Rank correlations were used. For categorical data, Fisher’s exact tests were used. Unless otherwise specified, averages from both measurements were analyzed. In case of missing data at one of the measurements (e.g., when subjects did not provide a stool sample), data from the other measurement were used. When data were missing at both instances, it was omitted from the analysis. Significance was set at \( p < 0.05 \). Analyses were performed with all patients unless specified otherwise (for specified analyses, only patients receiving the standard IFX regimen of 5 mg/kg approximately every 8 weeks [q8w, defined as 49-63 days] were included).

RESULTS

Patients
A total of 39 children were included, of whom 31 (79%) were diagnosed with CD. The median age was 15 years. The median duration of IFX treatment at the time of inclusion was 8.7 months. Seven included children (18%) received previous treatment intensification, and were thus not receiving IFX 5 mg/kg q8w. Patient characteristics at inclusion are shown in Table 1.

Biochemical and clinical data
Laboratory results from both infusions are shown in table 2. Median IFX TL was 3.5 µg/ml. Based on a therapeutic window of 3 to 7 µg/ml, 38% of all TLs were subtherapeutic and 23% supratherapeutic. Detectable ATIs were observed in 4 patients. Two patients had detectable ATI only at one measurement. Based on PCDAI and PUCAI scores, the majority of patients were in clinical remission (72%).

TLs correlated significantly with body height \( (r_s = 0.32; p < 0.05) \), but not with body weight \( (r_s = 0.20; p = 0.23) \) or body mass index \( (r_s = 0.00; p = 1.0) \).
Table 1  Patient characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients</td>
<td>39</td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>15.0 (12.9-16.3)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>16 (41%)</td>
</tr>
<tr>
<td>Length (cm), median (IQR)</td>
<td>166 (156-175)</td>
</tr>
<tr>
<td>Weight (kg), median (IQR)</td>
<td>53.8 (47-62)</td>
</tr>
</tbody>
</table>

**Diagnosis and disease characteristics:**

- Crohn's disease, n (%): 31 (79%)
  - Location, n (% of CD patients):
    - Esophageal | 1 (3%)
    - Gastroduodenal | 11 (35%)
    - Ileal | 18 (58%)
    - Colonic | 29 (94%)
    - Perianal | 8 (26%)
  - Phenotype, n (% of CD patients):
    - Inflammatory | 29 (94%)
    - Obstructive | 1 (3%)
    - Penetrating | 1 (3%)
- Ulcerative colitis, n (%): 8 (21%)
  - Extent, n (% of UC patients):
    - E1 Proctitis | 0
    - E2 Left-sided | 1 (13%)
    - E3 Pancolitis | 7 (88%)

Age at diagnosis (years), median (IQR) | 12.9 (10.8-14.0)
Age at first IFX infusion (years), median (IQR) | 15.0 (12.9-16.3)
Disease duration at 1st infusion (years), median (IQR) | 2.16 (1.37-3.13)
Duration IFX treatment (months), median (IQR) | 8.7 (5.1-18.3)
IFX infusion interval (days), median (IQR) | 55.5 (51-56)
Immunomodulation during IFX initiation, n (%):
  - Purine-analogue | 27 (69%)
  - Methotrexate | 4 (10%)
Current immunomodulation, n (%):
  - Purine-analogue | 17 (44%)
  - Methotrexate | 1 (3%)

IQR = Interquartile range, CD = Crohn's disease, UC = ulcerative colitis, IFX = infliximab
When analyzing only the 32 patients receiving IFX 5 mg/kg q8w, the average IFX TL was related to biochemical markers of disease activity. A significant negative correlation of IFX TL with average CRP ($r_s = -0.51; p < 0.01$) and FC ($r_s = -0.49; p < 0.01$) was found. No significant difference was found between median TL of patients in clinical remission (3.5 µg/ml; IQR 2.0-5.0) and patients with active disease (2.3 µg/ml; IQR 0.3-4.6) ($p = 0.20$). Furthermore, no difference in average TL was found between patients in clinical remission or active disease based on PCDAI or PUCAI when analyzed per diagnosis (CD: $p = 0.28$; UC: $p = 0.67$). No correlation was found between TL and PCDAI ($r_s = -0.19; p = 0.35$) or PUCAI ($r_s = -0.03; p = 0.95$) score.

When analyzing all 39 patients (regardless of IFX dose and interval), no correlation was found between IFX TL and CRP, neither when analyzed as a group ($r_s = -0.15; p = 0.36$), nor when analyzed per diagnosis (CD: $r_s = -0.23; p = 0.21$; UC: $r_s = -0.11; p = 0.80$). A significant moderate negative correlation was found between IFX TL and FC ($r_s = -0.42; p = 0.01$). No

### Table 2  Biochemical and clinical data.

<table>
<thead>
<tr>
<th></th>
<th>CD+UC</th>
<th>CD</th>
<th>UC</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trough level (TL) median µg/ml (IQR)</td>
<td>3.5 (2-7)</td>
<td>3 (1.5-5.0)</td>
<td>7.8 (3.9-15.4)</td>
<td>0.01</td>
</tr>
<tr>
<td>- Undetectable, n (%)</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>- &lt;3, n (%)</td>
<td>15 (38%)</td>
<td>14 (45%)</td>
<td>1 (13%)</td>
<td>*</td>
</tr>
<tr>
<td>- 3-7, n (%)</td>
<td>15 (38%)</td>
<td>12 (39%)</td>
<td>3 (38%)</td>
<td>*</td>
</tr>
<tr>
<td>- &gt;7, n (%)</td>
<td>9 (23%)</td>
<td>5 (16%)</td>
<td>4 (50%)</td>
<td>*</td>
</tr>
<tr>
<td>Detectable ATI, n (%)</td>
<td>4 (10%)</td>
<td>4 (13%)</td>
<td>0</td>
<td>0.56</td>
</tr>
<tr>
<td>Hb, median mmol/l (IQR)</td>
<td>8.1 (7.3-8.4)</td>
<td>8.1 (7.1-8.5)</td>
<td>7.9 (7.3-8.4)</td>
<td>0.96</td>
</tr>
<tr>
<td>CRP, median mg/l (IQR)</td>
<td>2.3 (0.9-3.2)</td>
<td>1.9 (0.7-5.3)</td>
<td>2.4 (1.0-3.0)</td>
<td>0.75</td>
</tr>
<tr>
<td>ESR, median mm/h (IQR)</td>
<td>8.0 (5.0-20.0)</td>
<td>8.0 (5.0-20.0)</td>
<td>7.0 (3.9-21.8)</td>
<td>0.83</td>
</tr>
<tr>
<td>FC, median µg/g (IQR)</td>
<td>366.5 (116-1097)</td>
<td>337 (136-1111)</td>
<td>439 (59-953)</td>
<td>0.95</td>
</tr>
<tr>
<td>Total PCDAI, median (IQR)</td>
<td>5 (0-15)</td>
<td>23 (74%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- remission (&lt;10), n (%)</td>
<td>23 (74%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- mild to moderate (10-30), n (%)</td>
<td>8 (26%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- severe (&gt;30), n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total PUCAI, median (IQR)</td>
<td>5 (0.6-16.9)</td>
<td>5 (63%)</td>
<td>3 (38%)</td>
<td></td>
</tr>
<tr>
<td>- remission (&lt;10), n (%)</td>
<td>5 (63%)</td>
<td>3 (38%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- mild to moderate (10-64), n (%)</td>
<td>3 (38%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- severe (&gt;65), n (%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* TL categories: $p = 0.01$

Data are averages of two measurements. P-values indicate differences between CD and UC patients. ATI = antibodies to infliximab, CRP = C-reactive protein, ESR = erythrocyte sediment rate, FC = fecal calprotectin, PCDAI = Pediatric Ulcerative Colitis Activity Index, PCDAI = Pediatric Crohn’s Disease Activity Index, IQR = Interquartile range

**Relation between TLs and disease activity**

When analyzing only the 32 patients receiving IFX 5 mg/kg q8w, the average IFX TL was related to biochemical markers of disease activity. A significant negative correlation of IFX TL with average CRP ($r_s = -0.51; p < 0.01$) and FC ($r_s = -0.49; p < 0.01$) was found. No significant difference was found between median TL of patients in clinical remission (3.5 µg/ml; IQR 2.0-5.0) and patients with active disease (2.3 µg/ml; IQR 0.3-4.6) ($p = 0.20$). Furthermore, no difference in average TL was found between patients in clinical remission or active disease based on PCDAI or PUCAI when analyzed per diagnosis (CD: $p = 0.28$; UC: $p = 0.67$). No correlation was found between TL and PCDAI ($r_s = -0.19; p = 0.35$) or PUCAI ($r_s = -0.03; p = 0.95$) score.

When analyzing all 39 patients (regardless of IFX dose and interval), no correlation was found between IFX TL and CRP, neither when analyzed as a group ($r_s = -0.15; p = 0.36$), nor when analyzed per diagnosis (CD: $r_s = -0.23; p = 0.21$; UC: $r_s = -0.11; p = 0.80$). A significant moderate negative correlation was found between IFX TL and FC ($r_s = -0.42; p = 0.01$). No
difference in TL was found between patients in clinical remission or active disease based on PCDAI or PUCAI ($p = 0.30$). No correlation was found between TL and PCDAI ($r_s = -0.13; p = 0.50$) or PUCAI ($r_s = 0.27; p = 0.53$) score.

Three patients in clinical remission at inclusion developed symptoms of active disease (according to PCDAI/PUCAI score) prior to the second infusion. This was associated with a marked decrease in TL in two patients (98% and 75%, respectively). Because of signs of active disease, the third patient received an early second infusion only 20 days following the first infusion, which might have masked a potential drop in TL. One patient went from mild/moderate disease activity to severe disease activity between the two infusions, which was associated with a decrease in TL from 0.2 µg/ml to below the limit of detection.

Change in TL between the 2 infusions was associated with change in CRP level ($r_s = -0.46; p = 0.04$). However, no correlation was found between change in TL and change in PCDAI score ($p = 0.97$), change in PUCAI score ($p = 0.67$), or change in FC level ($p = 0.82$).

When measuring clinical disease activity using the shPCDAI instead of the PCDAI, the clinical status of one patient changed from remission to active disease. This did not have an impact on the correlation between TL and clinical disease activity.

Clinical and biochemical data of patients receiving the standard IFX regimen, divided according to a therapeutic window of 3-7 µg/ml is provided in Table 3.

<table>
<thead>
<tr>
<th>IFX TL</th>
<th>CRP, median mg/l</th>
<th>FC, median µg/g</th>
<th>Proportion clinical remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3 µg/ml</td>
<td>2.4</td>
<td>478</td>
<td>69%</td>
</tr>
<tr>
<td>3-7 µg/ml</td>
<td>1.85</td>
<td>457</td>
<td>60%</td>
</tr>
<tr>
<td>&gt;7 µg/ml</td>
<td>1.13</td>
<td>51</td>
<td>75%</td>
</tr>
</tbody>
</table>

Data are averages of both measurements. IFX = infliximab, TL = trough level, CRP = C-reactive protein, FC = fecal calprotectin

### Table 3  Biochemical and clinical data based on a therapeutic window of IFX TL

#### Relation between TLs and albumin
When analyzing only the 32 patients receiving IFX 5 mg/kg q8w, a moderate positive correlation was found between average IFX TL and average albumin level ($r_s = 0.57; p < 0.01$).

#### Immunomodulators
No difference was found between patients who did or did not receive immunomodulators (purine antagonists or methotrexate) during remission induction with IFX in CRP ($p = 0.93$), FC ($p = 0.32$), TL ($p = 0.38$), or clinical status ($p = 0.65$). Furthermore, no difference was found between patients with or without current concomitant immunomodulator use in FC ($p =$
0.15) or TL (p = 0.63). A trend was observed for clinical status (p = 0.07) and CRP (p = 0.08), suggesting more active disease in patients receiving concomitant immunomodulators.

Antibodies to IFX
Four patients had ATIs detected at least at one time point (14, 22, 28 and 485 AU, respectively), which was associated with highly elevated FC levels (>1000 µg/g) and an elevated CRP (>5 mg/l) in three patients. Despite ATIs, two of these patients were in clinical remission. Only one of these four received immunomodulators during IFX induction treatment.

Relation between clinical and biochemical disease activity
CRP levels did not differ significantly between active disease or clinical remission (p = 0.14) in all children, or when analyzed per diagnosis (CD: p = 0.30; CU: p = 0.39). However, when categorizing CRP as either elevated (>5.0 mg/l) or normal, an elevated CRP was found significantly more frequently in patients with active disease (6/11 patients) compared to patients in remission (3/28 patients) (p < 0.01). No correlation was found between CRP levels and PCDAI (r_s = 0.10; p = 0.60) score or PUCAI score (r_s = 0.17; p = 0.69).

FC was significantly higher in patients with active disease (median: 858; IQR: 421-1800 mg/g) than in patients in clinical remission (median: 156; IQR: 56-484 mg/g) (p < 0.01). FC was correlated to PCDAI score (r = 0.52; p < 0.01), and a trend towards a significant correlation to PUCAI score was observed (r_s = 0.63; p = 0.10).

DISCUSSION
This study has shown that low TLs of IFX are associated with elevated biochemical markers of disease activity in children receiving IFX maintenance treatment for CD or UC. However, no relation was found between IFX TL and clinical disease activity, as determined by PCDAI or PUCAI.

A correlation between IFX TL and biochemical markers of disease activity has been described in adults receiving scheduled maintenance treatment with IFX for CD and UC. For the first time, our study confirms this finding in children. In contrast to previous findings in adults, no correlation between IFX TL and clinical disease activity was found in our study. This could be due to several aspects. Firstly, in our practice, children receiving IFX maintenance treatment who develop signs of active disease are likely to receive dose intensification, either by an increase in IFX dose, a decrease in administration interval, or a combination of both. Consequently, 7 of the 39 included children were not receiving the standard dose of IFX 5 mg/kg q8w. This might have masked the association between TL and clinical disease activity, since dose intensification in patients with active disease could potentially result in higher TLs despite more rapid clearance. Furthermore, the majority of the included children were in
clinical remission. When patients receiving IFX maintenance therapy have persisting severely active disease despite dose intensification, IFX is likely to be discontinued. Also, the absence of an association of clinical disease activity and TLs might be related to the weak correlation between clinical disease activity and endoscopic appearance, which can be considered the gold standard for assessing disease activity in IBD. Ideally, the relation between IFX TL and endoscopic appearance should be studied in pediatric IBD, as previously performed in adults, where patients with mucosal healing had significantly higher TLs than patients without healing. However, endoscopy is not routinely performed during the follow-up of pediatric IBD. Therefore, surrogate biochemical and clinical markers are used to monitor disease activity, although the latter has been shown to correlate only weakly with endoscopic inflammation.

For the determination of clinical disease activity in CD, the PCDAI was used. Since the PCDAI includes hematocrit, ESR and albumin, it is not a purely clinical score. However, when using the shPCDAI, (in which biochemical markers, height velocity and perirectal examination are not included), only one patient’s disease activity was reclassified.

It remains to be resolved when and for which patients it is safe to stop IFX. Currently, many children continue to receive IFX for a prolonged period despite the absence of signs of inflammation. This could have affected the strength of the correlation between IFX TLs and biochemical markers of inflammation, since some of the included children might not need ongoing IFX therapy to remain in remission.

Based on a therapeutic range of 3-7 µg/ml, approximately half of all included children had TLs which can be considered subtherapeutic. Since many children with TLs below 3 µg/ml were in clinical remission, a potential explanation might be that children have a different therapeutic range. However, it could also be that the standard IFX dose of 5 mg/kg q8w is insufficient for a significant proportion of children to get therapeutic TLs, and that children might benefit from a higher dose. This could be related to the fact that children often present with a more severe IBD phenotype than adults. Furthermore, differences in body composition between children and adults may result in different IFX dosage requirements.

Future studies are required focusing on the pharmacokinetics and pharmacodynamics of IFX in children with IBD.

It has been shown previously that low albumin levels are associated with increased clearance of IFX in children and adults. In our study, IFX TL correlated relatively strongly with albumin levels ($r_s = 0.57$). This might be related to low albumin levels reflecting active inflammation, since it is a negative acute-phase protein. Also, the Brambell receptor (FcRn) mechanism (which is responsible for the salvage and recirculation of both albumin and immunoglobulin) could play a role. Serum albumin reflects the efficacy of FcRn salvage recycling (by non-competitive binding with IgG). Furthermore, protein loss in patients with active IBD can potentially result in the fecal loss of IFX, resulting in low albumin and IFX levels. In a recent study, it was suggested that low albumin levels may identify children who would benefit from a higher IFX dose.
It has been shown that IFX is more effective in combination with an immunomodulator\textsuperscript{71}. Therefore, most children currently receive immunomodulators during IFX induction. Because of the risk of severe side effects (e.g., hepatosplenic T-cell lymphoma), immunomodulators are frequently stopped after successful remission induction with IFX. In our study, we did not find a relation between IFX TLs or ATIs and concomitant immunomodulator use or immunomodulator use during initiation of IFX. Although not significant, we even observed a trend towards more disease activity in patients receiving concomitant immunomodulators. We assume this is the result of a selection bias, since immunomodulators are unlikely to be discontinued in patients who continue to have symptoms of active disease.

ATIs were found in four patients (10%). This is likely to be an underestimation, since our assay is unable to detect ATI in the presence of detectable IFX levels, which is a limitation of present study. A large variability in ATI detection rate has been reported between different assays\textsuperscript{369}. The impact of ATIs in the presence of detectable TL remains to be elucidated.

As expected, FC levels were significantly higher in patients with clinically active disease. Furthermore, an elevated CRP was found more frequently in patients with active disease. No direct correlation was found between CRP and PUCAI or PCDAI score. However, a significant positive correlation was found between FC and PCDAI score, and a trend towards a positive correlation between FC and PUCAI score. Thus, FC appears to be more directly related to clinical disease activity than CRP.

The majority of children in clinical remission had FC levels $>100 \mu g/g$, and some even had FC levels $>1000 \mu g/g$. It has been shown that FC correlates significantly with endoscopic disease activity in IBD\textsuperscript{370} and is useful to predict relapse in quiescent IBD patients\textsuperscript{371}. However, it still remains to be determined whether monitoring endoscopic appearance and/or biochemical markers in daily practice and adjusting treatment accordingly improves disease control and prevents progression.

Strengths of our study are the unselected inclusion of all children receiving IFX maintenance treatment for IBD in one secondary hospital and two tertiary hospitals, which resulted in the inclusion of both children with and without active disease. Furthermore, the systematic data collection provides insight in the direct correlation between drug levels and disease activity. A limitation is the short follow-up. We did not investigate the relation between IFX TL and long-term outcome. Furthermore, due to the preponderance of CD patients in our study, only a limited number of children with UC were included. Also, there was a marked heterogeneity between the included patients i.a. in terms of age, age at diagnosis and body weight.

In our current clinical practice, children’s TLs are not routinely measured, but only in case of suspected loss of response. This is based on retrospective analyses in adults, showing that TDM of IFX is associated with a favorable outcome in patients who lose response\textsuperscript{349}. Prospective evaluation in adults is ongoing to determine whether TDM can also prevent the loss of response to IFX in CD (NCT01442025).

In summary, we have shown that IFX TL are related to biochemical markers of inflammation
in children with IBD. This could provide a rationale for the use of TDM in children. It might be beneficial to measure TLs in children receiving IFX maintenance therapy, and adjust treatment accordingly. However, no relation between IFX TL and clinical disease activity was found. Since a large proportion of children will eventually lose therapeutic response to IFX, future prospective studies are required to determine the value of TDM in pediatric IBD.