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Gastroenterology: Inflammatory Bowel Disease

# Biomarkers predicting the effect of anti-TNF treatment in paediatric and adult inflammatory bowel disease

**Dwight A. Winter**[1] | **Pauline de Bruyne**[2] | **Janneke van der Woude**[3] | **Dimitris Rizopoulos**[4] | **Lissy de Ridder**[1] | **Janneke Samsom**[5] | **Johanna C. Escher**[1]

[1]Department of Paediatric Gastroenterology, Erasmus MC-Sophia Children's Hospital, Rotterdam, Netherlands

[2]Department of Pediatrics, Ghent University Hospital, Ghent University, Ghent, Belgium

[3]Department of Gastroenterology, Erasmus MC, Rotterdam, Netherlands

[4]Department of Biostatistics, Erasmus MC, Rotterdam, Netherlands

[5]Laboratory of Paediatrics, Erasmus MC-Sophia Children's Hospital, Rotterdam, Netherlands

**Correspondence**

Johanna C. Escher, Division of Paediatric Gastroenterology, Erasmus University Medical Center-Sophia Children's Hospital, Wytemaweg 80, 3015 CN, Rotterdam, Netherlands.
Email: j.escher@erasmusmc.nl

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## Abstract

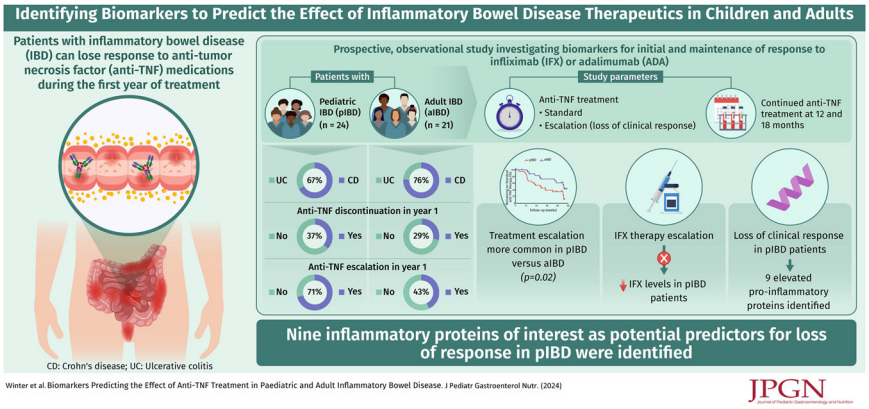
**Objectives:** Paediatric and adult inflammatory bowel disease (pIBD, aIBD) patients may lose response to anti-tumour necrosis factor (TNF) treatment within the first year. Adult-extrapolated weight-based dosing is incorrect in children, due to age-related pharmacokinetic differences. We investigated biomarkers for initial and maintenance of response to infliximab (IFX) or adalimumab (ADA), comparing pIBD and aIBD patients.

**Methods:** In this prospective, observational study, pIBD ($n = 24$) and aIBD ($n = 21$) patients were included when initiating anti-TNF. Escalation from standard dosing and continued anti-TNF at 12 and 18 months were assessed. Biomarkers included clinical laboratory parameters, faecal calprotectin (FCP) and IFX trough levels (TLs). Plasma proteomics was performed in pIBD.

**Results:** During our study, treatment escalation (in clinical loss of response) occurred more common in pIBD versus aIBD ($p = 0.02$). We established that IFX therapy escalation in pIBD patients was not due to low infliximab levels. We identified 9 pro-inflammatory proteins that were elevated in patients losing response.

**Conclusion:** Anti-TNF exposure-response relationship may be different in pIBD versus aIBD. No biomarkers for maintained response were identified, but 9 inflammatory proteins were of interest as potential predictors for loss of response in pIBD.

**Abbreviations:** ADA, adalimumab; aIBD, adult inflammatory bowel disease; ASA, mesalamine-based 5-ASA agents; ASCA, anti-*Saccharomyces cerevisiae* antibody; ATAs, antibodies-to-adalimumab; ATIs, antibodies-to-infliximab; CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; ESR, erythrocyte sedimentation rate; IBD, inflammatory bowel disease; IFX, infliximab; MTX, methotrexate; p-ANCA, perinuclear anti-neutrophil cytoplasmic antibody; PCDAI, Paediatric Crohn's Disease Activity Index; pIBD, paediatric inflammatory bowel disease; PK, pharmacokinetics; PUCAI, Paediatric Ulcerative Colitis Activity Index; TDM, therapeutic drug monitoring; TL, trough level; TNF-α, tumour necrosis factor-alpha; TPM, thiopurine metabolites, that is, azathioprine, 6-mercaptopurine or 6-thioguanine; UC, ulcerative colitis.




**KEYWORDS**

IBD, infliximab, pharmacokinetics, pharmacodynamics

## 1 | INTRODUCTION

The management of inflammatory bowel disease (IBD) changed drastically since the introduction of antitumour necrosis factor agents. Tumour necrosis factor alpha (TNF-α) is a key inflammatory cytokine involved in various inflammatory pathways and has a pivotal role in IBD pathogenesis as it is detected in tissues of the gastro-intestinal (GI) tract of patients with inflammatory bowel disease (IBD). Monoclonal antibodies directed against TNF-α such as infliximab (IFX) and adalimumab (ADA) are now common treatment in adult and paediatric IBD (aIBD and pIBD, respectively). These anti-TNF monoclonal antibodies can induce complete clinical remission within weeks, often accompanied by mucosal healing in the GI tract of both Crohn's disease (CD) and ulcerative colitis (UC). Although anti-TNF monoclonal antibodies are a potent treatment for induction and maintenance of remission in aIBD and pIBD, both primary nonresponse and loss of response can occur.[1,2]

Both the administered dose of a monoclonal antibody as well as its clearance determine serum anti-TNF (trough) levels. In both pIBD and aIBD, monoclonal antibodies can display highly variable and complex pharmacokinetic (PK) behaviour with several factors influencing their clearance, such as body weight and serum albumin.[3] Clearance is also influenced by disease related factors such as disease severity, increased intestinal permeability due to inflammation, or increased proteolytic activity and thus degradation of drug-TNF-α immune complexes in inflamed tissue. Additionally, the presence of antibodies-to-infliximab (ATIs) and the use of concomitant immunomodulators are known to influence clearance of IFX.

In this prospective cohort study, we aimed to evaluate the influence of patient-, disease- and drug related factors on initial and maintenance of response to anti-TNF in both pIBD and aIBD. We have previously reviewed the available data on pharmacokinetics of IFX in paediatric IBD and concluded that current weight-based dosing in children, as extrapolated from adults, is incorrect due to the age-related differences in pharmacokinetic factors mentioned above.[4] In this study we aimed to further explore the exposure-response relation in anti-TNF treatment of children compared to adults. As such, we looked at outcome in both paediatric and adult patients and a possible association with anti-TNF levels as well as baseline and longitudinal characteristics. Furthermore, we aimed to explore the relation between clinical outcome and the extent of inflammation at the start of

**What is Known**

- Paediatric and adult inflammatory bowel disease (pIBD, aIBD) patients may lose response to anti-TNF treatment within the first year, with incidences varying between 4.5% and 40%.
- Literature on infliximab (IFX) treatment in IBD suggest an exposure-response association with higher IFX trough levels leading to better clinical outcome.

**What is New**

- IFX therapy escalation in pIBD patients was not due to low infliximab levels.
- PIBD patients who escalated during induction were more likely to ultimately fail IFX therapy.
- We identified nine inflammatory proteins of interest as potential predictors for loss of response in pIBD.

anti-TNF treatment. Finally, in a selected number of pIBD patients we investigated baseline plasma levels of proteins associated with inflammation using plasma proteomics analysis.

## 2 | METHODS

### 2.1 | Patients and study design

This was a prospective single centre observational cohort study with a follow-up period of 18 months. During a 2-year inclusion period, pIBD and aIBD patients were recruited from the Departments of Paediatric Gastroenterology and Gastroenterology. Inclusion criteria were anti-TNF naïve CD or UC patients failing/intolerant to treatment with immunomodulators or corticosteroids, age above 6 years and written informed consent by patients (and parents in patients aged below 16 years). Patients who initiated anti-TNF immediately after diagnosis or having severe perianal disease as primary indication to anti-TNF treatment were excluded from the trial. This study was approved by the institutional review board of the Erasmus Medical Center (MEC # 2013-021) and was reported online at ClinicalTrials. gov as protocol Record NL-42736.078.13.

The decision to start anti-TNF treatment was made at the discretion of the treating (paediatric) gastroenterologist, adhering to the national and international guidelines. Decisions on treatment escalation were also made by the treating (paediatric) gastroenterologist, based on clinical parameters (including disease activity score as well as objectifiable parameters such as increased serum and faecal inflammatory markers). At initiation, IFX was administered intravenously in the standard weight-based dosing schedule (i.e., 5 mg/kg at weeks 0, 2 and 6 during the induction phase; followed by a maintenance regimen of 5 mg/kg every 8 weeks). ADA was administered subcutaneously in a fixed dose depending on age/weight, starting with a loading dose during the induction phase followed by a maintenance dose every other week from week 4 onwards. In pIBD patients <40 kg: 80 mg, followed by 40 mg every other week from Week 2 onwards; In pIBD patients >40 kg and aIBD patients: 160 mg, followed by 80 mg at Week 2 and 40 mg every other week from Week 4 onwards. In case of inadequate response or when clinical response was lost, anti-TNF therapy could be intensified by increasing the dose, shortening the interval between infusions/injections, or both. This decision was at the discretion of the treating physician, without being informed of the anti-TNF trough level (TL) at the time. After remission induction, treatment response was assessed by the treating (paediatric) gastroenterologist, followed by the choice to continue, or stop anti-TNF treatment.

### 2.2 | Data collection

Patient characteristics were collected prospectively and included age, sex, weight, IBD type (CD and UC), and duration of disease. At the time of each patient visit, clinical disease activity was recorded for paediatric and adult CD (PCDAI, Paediatric Crohn's Disease Activity Index; CDAI, Crohn's Disease Activity Index) and UC (PUCAI, Paediatric Ulcerative Colitis Activity Index; Mayo score), respectively. Also, anti-TNF dose and interval were recorded. The following clinical laboratory parameters were measured in patients receiving ADA, before injections at Week 0, Week 6, Week 8, Week 14, Week 22 and after 1 year of ADA use: haemoglobin, haematocrit, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin. Serum ADA TLs were measured at Week 6 and Week 14, antibodies-to-adalimumab (ATAs) were measured if deemed necessary by the treating (paediatric) gastroenterologist. The following clinical laboratory parameters were measured before each IFX infusion: haemoglobin, haematocrit, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), albumin, serum IFX TLs and ATIs. These measurements were repeated upon first escalation in IFX therapy. This timepoint was defined as time of first escalation (TFE). The commercially available enzyme-linked immunosorbent assay (ELISA) kits from Sanquin Diagnostic Services (Sanquin, Amsterdam, the Netherlands) were used to determine IFX (M2920 kit), ADA (M2910 kit), ATI (M2960 kit) and ATA (M2950 kit) levels. Additionally, pretreatment levels of the following antimicrobial antibodies were assessed: anti-*Saccharomyces cerevisiae* (ASCA) and perinuclear anti-neutrophil cytoplasmic antibody (p-ANCA). As part of clinical care, faecal calprotectin (FCP) was measured at the time of infliximab infusions at baseline, after induction at week 8, during maintenance and at the end of study participation. Potential biomarkers for initial and maintained response to anti-TNF were anti-TNF TLs (at week 6 and TFE), serologic markers (p-ANCA, ASCA), inflammatory markers (CRP, albumin, BSE, thrombocytes, leucocytes, neutrophil number), and FCP.

### 2.3 | Clinical endpoints

Clinical disease activity and response to anti-TNF were evaluated during a total follow-up period of 18 months. Primary endpoint in this study was maintenance of response at 12 months of anti-TNF treatment, defined as ongoing anti-TNF treatment at this timepoint. Primary nonresponse was defined as stopping anti-TNF treatment during the induction phase because of inadequate treatment response. Other timepoint specific outcome parameters in this study were (1) clinical response and remission after induction therapy at week 8 and (2) maintenance of clinical response evaluated at 18 months after start of anti-TNF treatment.

The definition of clinical remission was a PCDAI < 10 points in paediatric CD, a CDAI < 150 points in adult CD, a PUCAI < 10 points in paediatric UC, and a Mayo score ≤2 with no individual subscore > 1 in adult UC. The definition of clinical response was a decrease in PCDAI of >15 points with a total score <30 in paediatric CD, a decrease in CDAI of ≥70 points, >25% reduction in total score in adult CD, a decrease in PUCAI of ≥20 points or a decrease of ≥10 points with a total score <10 in paediatric UC and a decrease in Mayo score ≥ 30% and ≥3 points decrease and decrease in rectal bleeding subscores of ≥ 1 or an absolute rectal bleeding subscore of ≤1 in adult UC.

In addition, the following secondary end points were evaluated: percentage of patients receiving standard dosing of anti-TNF at 12 months and 18 months, development of ATIs or ATAs, need for prednisolone treatment, and need for surgery during the follow up period. TLs of IFX were defined as subtherapeutic if IFX TLs were ≤ 18 μg/mL at week 6[5] or ≤ 5.4 μg/mL at week 14 and during maintenance.[6,7] For ADA, TLs between 5 and 12 μg/mL were deemed therapeutic.[8]

## 2.4 | Plasma proteomics analysis

Peripheral blood samples (from pIBD patients receiving IFX treatment only) were collected for plasma proteomics analyses before the first IFX infusion. The commercially available panel, ProSeek Multiplex Inflammation I 96×96 (Olink Proteomics, Uppsala, Sweden) consisting of 91 preselected proteins, all related to inflammation was used.[9] The concentrations of the proteins in the panel were assessed by performing a proximity extension assay (PEA), where pairs of antibodies with oligonucleotides attached were incubated with patient plasma. Upon ligation to their target protein in plasma oligonucleotides in close proximity produced a template for hybridisation and extension. Pre-amplification was based on universal primers and polymerase chain reaction (PCR). Residual primers were digested before quantification with specific primers on a quantitative real-time PCR chip (Dynamic Array IFC; Fluidigm Biomark) on a Biomark HD Instrument. The analyses were performed at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala. Normalised log2 values corresponding to protein quantities were generated with the Olink Wizard for GenEx (Multid Analyses).

## 2.5 | Population selection for analysis

The total population of IFX and ADA treated patients was used for analysis on clinical response and remission related to anti-TNF treatment, treatment escalation comparison between pIBD and aIBD patients and faecal calprotectin. Personalised logistic regression analysis was performed using data from the total population in relation to clinical response at 8 weeks, at 12 months and at 18 months. Evaluated covariates were age, gender, age at start of anti-TNF, age at diagnosis, disease duration, serum concentrations of p-ANCA, ASCA, CRP, albumin, ESR and peripheral blood concentrations of thrombocytes, leucocytes, neutrophils and FCP. Analysis of clinical outcome related to IFX levels was performed using data from IFX treated patients only. For plasma proteomics analysis the pIBD subgroup on standard infliximab treatment until week 14 was selected.

## 2.6 | Statistical analysis

Normally distributed continuous variables were reported as means and standard deviations and compared with the t-test. Continuous variables not following normal distribution were analysed by the Mann-Whitney U-test and presented as medians and interquartile range (IQR). Univariate analyses were used to determine associations between response to anti-TNF treatment and clinical parameters, serum TLs of IFX/ADA, presence of antibodies to IFX/ADA, and the presence of antibodies to microbial products. In addition, differences between aIBD and pIBD patients in maintenance of treatment response and presence of biomarkers were analysed as follows:

Kaplan–Meier analyses with Mantel-Cox Log-rank tests were performed by dividing the population in pIBD versus aIBD patients. These groups were used to statistically compare the proportions of patients over time regarding the following clinical endpoints (1) escalation of anti-TNF therapy (dose increase above 5 mg/kg and/or maintenance interval shortening to less than 8 weeks) and (2) continued anti-TNF therapy, reflecting maintenance of response at 12 and 18 months. Mixed model analysis was performed to determine possible relationships between covariates and clinical outcome. Personalised logistic regression analysis was used to account for small sample size and the large number of covariates. Both baseline covariates as well as longitudinally measured covariates were considered. For each longitudinal covariate a linear mixed effects model was fitted using time as a fixed effect. Fisher's exact test was used to determine association between clinical outcome and escalation before week 14 and during follow-up in children and adults. Binary logistic regression was used to determine association between clinical outcome and escalation before week 14 and during follow-up in children compared to adults. $p$ values of <0.05 were considered significant.

# 3 | RESULTS

## 3.1 | Patients' characteristics

A total of 45 patients were included (24 pIBD patients and 21 aIBD patients), a large proportion of whom were diagnosed with CD (paediatric CD $n = 16/24$, 67%; adult CD $n = 16/21$, 76%). There was a slight (51%) male predominance (Table 1). Median disease duration at start of anti-TNF therapy was 1.03 years versus 5.97 years in pIBD and aIBD patients, respectively. At the start of anti-TNF treatment, the majority of pIBD were on immunomodulators (21/24; 88% on thiopurine metabolite, no one on MTX) and over half of aIBD patients (10/21 on thiopurine metabolite and 2/21 on MTX; 57%) were on immunomodulators. Total

**TABLE 1** Demographic characteristics.

| | | Paediatric | Adult | Overall |
|---|---|---|---|---|
| **Number of patients** | | 24 | 21 | 45 |
| **Gender (male)** | | 12 (50%) | 11 (52%) | 23 (51%) |
| **Diagnosis CD (%)** | | 16 (67%) | 16 (76%) | 32 (71%) |
| **Disease phenotype according to Paris and Montreal classification** | | **Paris classification** | **Montreal classification** | |
| | Age at diagnosis (CD and UC) | A1a; 2, A1b; 22 | A1; 4, A2; 13, A3; 4 | |
| | Disease location (CD) | L1; 4, L2; 4, L3; 8<br>L4a; 5<br>L4b; - | L1; 3, L2; 4, L3; 8<br>L4; - | |
| | Behaviour (CD) | B1; 15, B2; 1, B3; -<br>p; 3/16 | B1; 3, B2; 5, B3; 1<br>p; 3/12[†] | |
| | Disease Extent (UC) | E1; 1, E2; 3, E3; -<br>E4; 4 | E1; 1, E2; -, E3; 3 | |
| **Age at diagnosis (years - median; IQR)** | (IQR: 25–75) | 13.24 (11.36–14.65) | 25.34 (18.88–36.19) | 15.47 (12.44–23.93) |
| **Age at start anti-TNF (years – median; IQR)** | (IQR: 25–75) | 15.18 (12.68–15.80) | 33.46 (22.29–52.16) | 17.67 (14.9–32.38) |
| **Disease duration at start anti-TNF (years - median; IQR)** | (IQR: 25–75) | 1.03 (0.57–1.97) | 5.97 (1.82–12.62) | 1.7 (0.67–5.89) |

| | pIBD | CD | UC | aIBD | CD | UC | |
|---|---|---|---|---|---|---|---|
| **Patients started on IFX (%)** | 23/24 (96%) | 15/16 (94%) | 8/8 (100%) | 18/21 (86%) | 13/16 (81%) | 5/5 (100%) | 41/45 (91%) |
| **Patients on TPM (%) at start** | 21/24 (88%) | 16/16 (100%) | 5/8 (60%) | 10/21 (48%) | 7/16 (44%) | 3/5 (60%) | 31/45 (69%) |
| **Patients on corticosteroids (%) at start** | 8/24 (33%) | 3/16 (19%) | 5/8 (60%) | 9/21 (43%) | 6/16 (38%) | 3/5 (60%) | 17/45 (38%) |
| **Patients on ASA (%) at start** | 6/24 (25%) | — | 6/8 (75%) | 4/21 (19%) | 2/16 (13%) | 2/5 (40%) | 10/45 (22%) |
| **Patients on MTX (%) at start** | — | — | — | 2/21 (10%) | 2/16 (13%) | — | 2/45 (4%) |

*Note*: Montreal Classification: *Age at Diagnosis* - A1: below 17 years, A2: 17–40 years, A3: Above 40 years. *Location* - L1: terminal ileal ± limited caecal disease, L2: colonic, L3: ileocolonic, L4: Isolated upper disease*. *Behaviour* - B1: nonstricturing, nonpenetrating nonpenetrating, B2: structuring, B3: penetrating, p: perianal disease modifier. Paris Classification: *Age at Diagnosis* - A1a: 0– < 10 years, A1b: 10– < 17 years, A2: 17–40 years, A3: >40 years. *Location* - L1: distal 1/3 ileum ± limited caecal disease, L2: colonic, L3: ileocolonic. L4a: upper disease proximal to Ligament of Treitz*, L4b: upper disease distal to ligament of Treitz and proximal to distal 1/3 ileum*. *Behaviour* - B1: nonstricturing, nonpenetrating nonpenetrating, B2: structuring, B3: penetrating, B2B3: both penetrating and stricturing disease, either at the same or different times, p: perianal disease modifier. Ulcerative Colitis (UC)–Montreal Classification: *Extent* - E1: ulcerative proctitis, E2: left-sided UC (distal to splenic flexure), E3: extensive (proximal to splenic flexure). Paris Classification: *Extent* - E1: ulcerative proctitis, E2: left-sided UC (distal to splenic flexure), E3: Extensive (hepatic flexure distally), E4: Pancolitis (proximal to hepatic flexure).

Abbreviations: aIBD, adult inflammatory bowel disease; ASA, mesalamine-based 5-ASA agents; CD, Crohn's disease; IFX, infliximab; IQR, interquartile range; MTX, methotrexate; pIBD, paediatric inflammatory bowel disease; TNF, tumor necrosis factor; TPM, thiopurine metabolites, that is, azathioprine, 6-mercaptopurine or 6-thioguanine; UC, ulcerative colitis.

*In both the Montreal and Paris Classification systems L4 and L4a/L4b may coexist with L1, L2, L3, respectively.

[†]$n = 4$ missing data.

population demographic characteristics are summarised in Table 1. A total of 41 (91%) patients started anti-TNF treatment with IFX (23 pIBD and 18 aIBD patients) and 4 patients with ADA (1 pIBD patient and 3 aIBD patients). As the number of patients treated with ADA was low, we will not discuss data of patients on adalimumab treatment separately.

## 3.2 | Clinical outcome of anti-TNF treatment in pIBD versus aIBD

Clinical outcome data analysis included all patients on anti-TNF. Based on clinical disease activity scores at Week 8, clinical response was achieved in 37 patients (82%) and clinical remission in 30 patients (67%) (Table 2). Two patients (4.4%; 1 pIBD and 1 aIBD patient) showed primary nonresponse to anti-TNF therapy. Both were UC patients receiving IFX. One adult CD patient was lost-to-follow-up because of nonadherence to ADA therapy. After 12 months, 15 pIBD patients and 15 aIBD patients (63% and 71%, respectively) were still receiving anti-TNF therapy. After 18 months, 14 pIBD patients and 12 aIBD patients (58% and 57%, respectively) were still receiving anti-TNF therapy (see File S1: Supplemental Digital Content 1 Figure, Flow Chart of pIBD and aIBD patients starting anti-TNF treatment within our study). Median IFX dose (during induction and maintenance treatment combined) was 5.02 [4.11–10.78] mg/kg in pIBD patients and 4.57 [3.61–9.23] mg/kg in aIBD patients. Maintenance of response, defined as continued anti-TNF therapy at 12 months was not significantly different between the two age groups (Figure 1A). There was no difference in IFX continuation between pCD and pUC ($p = 0.29$), while aUC patients stopped anti-TNF treatment earlier than aCD patients ($p = 0.035$) (data not shown).

## 3.3 | Treatment escalation in pIBD versus aIBD

Treatment escalation data analysis included all patients on anti-TNF. More than half of all patients underwent therapy escalation ($n = 26/45$ patients, 58%), either by shortening interval, increasing dose or both (Table 2). In our cohort, therapy escalation was more often seen in pIBD compared to aIBD patients (71% vs 43%, respectively; Table 2). Median time of first escalation (TFE) was 18.57 weeks [12.36–31.29] in pIBD patients and 28.00 weeks [18.43–50.15] in aIBD patients ($p = .07$). At 12 months after start of anti-TNF the proportion of patients on standard dose and interval treatment was lower for pIBD patients compared to aIBD patients (33% vs 60%, respectively), and anti-TNF escalation over time differed significantly between pIBD and aIBD patients ($p = 0.02$, Figure 1B). While pUC patients escalated earlier compared to pCD patients ($p = 0.0097$), there was no difference in escalation rate between aCD and aUC patients ($p = 0.48$) (data not shown).

Those pIBD patients who ultimately failed anti-TNF escalated earlier than those who continued anti-TNF, both after 12 months and 18 months of follow-up ($p = 0.03$ and $p = 0.01$, respectively) (Figure 1C, 18-month data not shown). In contrast, aIBD patients who failed anti-TNF within 1 year did not show a significant difference in escalation rate compared to aIBD patients who continued anti-TNF ($p = 0.3535$, Figure 1D).

## 3.4 | FCP

FCP data analysis included all patients on anti-TNF. There was no statistical difference in FCP between pIBD and aIBD patients at baseline, week 8, during maintenance and at the end of study (Table 2). In both pIBD and aIBD patients median FCP declined most between baseline and week 8. Median FCP levels ranged between 268 [66–273]–325 [112–642] µg/g for pIBD patients at the end of study and during maintenance, respectively. In aIBD patients, median FCP levels ranged between and 208 [62–558]–211 [82–472] µg/g at the end of study and during maintenance, respectively. There was no statistical difference in median FCP between patients that continued IFX and those who stopped IFX.

## 3.5 | IFX levels and antibodies to anti-TNF

The complete cohort was considered for analysis of data regarding antibody levels, while data from IFX treated patients was considered for IFX levels. Analysis of clinical outcome related to IFX levels was performed, using either IFX TLs at Week 6 (before the third infusion), at week 14 (before the fourth infusion) or IFX (trough) levels at TFE (see File S2: Supplemental Digital Content 2 Table, table of demographic and clinical characteristics of IFX treated patients). Overall, a majority of pIBD and aIBD patients had adequate IFX TLs > 18 µg/mL at week 6 (73% vs. 71%, respectively) and a small majority >5.4 µg/mL at Week 14 (52% vs. 67%, respectively) (Table 2). aIBD patients that continued standard dosing had significantly higher median IFX TLs at Week 6 compared to therapy escalation (37.3 vs. 16.7 µg/mL, respectively; $p = 0.036$).

At week 6, there was no association between discontinuing IFX treatment and subtherapeutic IFX TLs in pIBD or aIBD patients ($p = 0.72$ vs. $p = 0.82$, respectively) (see File S1: Supplemental Digital Content 3 Figure, with Kaplan-Meier curves for IFX

**TABLE 2** Clinical outcome characteristics, anti-TNF specific outcomes and faecal calprotectin.

| | Paediatric n = 24 | Adult *n* = 21 | Overall *n* = 45 |
|---|---|---|---|
| **Clinical endpoints** | | | |
| Primary nonresponse | 1 (4.2%) | 1 (4.8%) | 2 (4.4%) |
| Remission at Week 8[a] | 15 (63%) | 15 (71%) | 30 (67%) |
| Response at Week 8[b] | 20 (83%) | 17 (81%) | 37 (82%) |
| Continued anti-TNF after 12 months | 15 (63%) | 15 (71%) | 30 (67%) |
| Continued anti-TNF after 18 months | 14 (58%) | 12 (57%) | 26 (58%) |

| **Anti-TNF specific** | | ***p* value** | **Paediatric** | **Adult** | **Overall** |
|---|---|---|---|---|---|
| Overall treatment intensification | | 0.0616 | 17 (71%) | 9 (43%) | 26 (58%) |
| *Dose intensification* | | *0.0079 | 14 (58%) | 4 (19%) | 18 (35%) |
| *Interval shortening* | | 0.2006 | 16 (67%) | 10 (48%) | 26 (51%) |
| **Treatment intensification in relation to discontinued anti-TNF** | | | 8/9 (89%) | 3/6 (50%) | 11/15 (73%) |
| **Immunomodulator combination treatment** | | | 22 (92%) | 19 (90%) | 41 (91%) |
| **Need for systemic prednisone** | | | 7 (29%) | 5 (24%) | 12 (27%) |
| ATI development | | | 4 (17%) | 2 (10%) | 7 (16%) |
| ATI level (IQR) U/mL | | | 150 [40.8–402.5] | 106 [12-200] | |
| ATA development | | | | 1 (5%) | 1 (2%) |
| Median IFX dose [IQR] (*mg/kg*) | | | 5.02 [4.11–10.78] | 4.57 [3.61–9.23] | |
| IFX TL > 18 μg/mL overall | Week 6 | | 16/22 (73%) | 12/17 (71%) | 28/39 (72%) |
| IFX TL > 18 μg/mL[¥] | Week 6 | | 11/16 (69%) | 3/8 (38%) | 14/24 (58%) |
| IFX TL > 18 μg/mL[P] | Week 6 | | 12/15 (80%) | 10/14 (71%) | 22/29 (76%) |
| IFX TL > 5.4 μg/mL overall | Week 14 | | 11/21 (52%) | 12/18 (67%) | 23/39 (59%) |
| IFX TL > 5.4 μg/mL[¥] | Week 14 | | 8/15 (53%) | 5/9 (55%) | 13/24 (54%) |
| IFX TL > 5.4 μg/mL[¥] | TFE | | 9/17 (53%) | 3/8 (38%) | 12/25 (48%) |

| | Paediatric | Adult | ***p* value** |
|---|---|---|---|
| **Median time of first escalation* [IQR]** (weeks) | 18.57 [12.36–31.29] | 28.00 [18.43–50.15] | 0.0709 |

| | | **Standard dosing** | **Therapy Escalation** | ***p* value** | **Standard dosing** | **Therapy Escalation** | ***p* value** |
|---|---|---|---|---|---|---|---|
| **Median IFX TL [IQR]** (μg/mL) | Week 6 | 32.6 [17.9–43.8] | 30.8 [15.2–40.4] | 0.5286 | 37.3 [29.5–41.9] | 16.7 [14.9–35.5] | *0.0360 |
| | Week 14 | 7.6 [1.4–18.0] | 6.8 [2.0–28.2] | 0.8457 | 8.9 [4.5–11.1] | 6.4 [2.1–9.3] | 0.3401 |
| | TFE[¥] | | 6.5 [1.5–10.6] | | | 2.6 [0.2–12.5] | 0.4484 |

| | | **Stop IFX** | **Continued IFX** | ***p* value** | **Stop IFX** | **Continued IFX** | ***p* value** |
|---|---|---|---|---|---|---|---|
| **Median IFX TL [IQR]** (μg/mL) | Week 6[¥] | 33.1 [18.2–40.4] | 24.2 [14.9–41.7] | 0.7923 | 39.9 [15.1–40.9] | 15.2 [9.7–19.0] | 0.1667 |

**TABLE 2** (Continued)

| | | Stop IFX | Continued IFX | *p* value | Stop IFX | Continued IFX | *p* value |
|---|---|---|---|---|---|---|---|
| | Week 14[¥] | 8.8 [2.7–28.2] | 5.5 [1.8–29.8] | 0.9551 | 1.6 [0.2–6.5] | 6.9 [2.6–10.6] | 0.1167 |
| | TFE[¥] | 8.4 [1.6–23.5] | 3.1 [1.5–9.9] | 0.5635 | 0.1 [0.0–0.3] | 8.0 [2.6–18.7] | *0.0357 |
| | ATI development[¥] | 2/8 (25%) | 1/8 (12.5%) | | 2/3 (66%) | 0 (0%) | |

| Faecal calprotectin | | Paediatric | Adult | *p* value |
|---|---|---|---|---|
| **Median time of FCP measurement [IQR]** (weeks) | Week 8 | 8.07 [7.75–9.0] | 8.29 [8.1–9.1] | 0.1643 |
| | Maintenance | 22 [20–22.7] | 22.14 [21.9–22.4] | 0.2304 |
| | End of study | 51.93 [47.3–54.9] | 54.29 [53.9–55.4] | 0.0846 |
| **Median FCP [IQR]** (μg/g) | Baseline | 1240 [703.3–1800] | 1090 [369–1800] | 0.7448 |
| | Week 8 | 225 [98–846] | 260 [136.3–1202] | 0.5436 |
| | Maintenance | 325 [112–642] | 211 [82–472] | 0.4454 |
| | End of study | 268 [66–723] | 208 [62–558] | 0.7556 |

| | | Stop IFX | Continued IFX | *p* value | Stop IFX | Continued IFX | *p* value |
|---|---|---|---|---|---|---|---|
| **Median FCP [IQR]** (μg/g) | Baseline | 1800 [524–1800] | 1240 [749–1800] | 0.9656 | 1800 [70.5–1800] | 1800 [404–1800] | 0.7233 |
| | Week 8 | 139 [87.5–1342] | 390 [102–809] | 0.879 | 1800 [208–1800] | 183.5 [139.5 –727.5] | 0.1376 |
| | Maintenance | 405.5 [153.3–840.5] | 234 [30.5–696.3] | 0.4093 | 240 [64–1604] | 171.5 [84–463.8] | 0.6134 |
| | End of study | 268 [77–1800] | 380 [66–723] | 0.8151 | 402.5 [153.3–1490] | 205 [60–422] | 0.1704 |

Abbreviations: ATA, antibodies-to-adalimumab; ATI, antibodies-to-infliximab; FCP, faecal calprotectine; IFX, infliximab; IQR, interquartile range; TFE, time of first escalation; TL, trough level; TNF, tumour necrosis factor.

[a]Definition of Clinical remission in paediatric CD: PCDAI < 10 points; adult CD: CDAI < 150 points; paediatric UC: PUCAI < 10 points; adult UC: Mayo score ≤ 2 with no individual subscore > 1.

[b]Definition of Clinical response in paediatric CD: decrease in PCDAI of >15 points with a total score <30; adult CD: decrease in CDAI of ≥70 points, >25% reduction in total score; paediatric UC: decrease in PUCAI of ≥20 points or a decrease of ≥10 with a total score <10 points; adult UC: decrease in Mayo score ≥ 30% and ≥3 points decrease and: decrease in rectal bleeding subscores of ≥ 1 or an absolute rectal bleeding subscore of ≤ 1.

*Statistically significant, [¥]Only patients with infliximab treatment intensification included, [P]Peripheral blood leucocyte gene expression subgroup ($n = 29$), on standard IFX treatment until week 14.

continuation related to IFX TLs; A and B). A subtherapeutic IFX TL at week 6 was associated with early escalation of IFX treatment in aIBD, but not pIBD patients ($p = 0.002$ vs. $p = 0.12$, respectively) (see File S1: Supplemental Digital Content 4 Figure, with Kaplan-Meier curves for escalation of IFX during study related to IFX TLs; A and B).

At week 14, a subtherapeutic IFX TL was associated with discontinuing IFX treatment in aIBD patients, but not pIBD patients ($p = 0.03$ vs. $p = 0.86$, respectively) (see File S1: Supplemental Digital Content 3 Figure, with Kaplan-Meier curves for IFX continuation related to IFX TLs; C and D). There was a near-significant association with subtherapeutic IFX TLs at week 14 and early escalation of IFX treatment in aIBD, but not pIBD patients ($p = 0.07$ vs. $p = 0.45$) (see File S1: Supplemental Digital Content 4 Figure, with Kaplan-Meier curves for escalation of IFX during study related to IFX TLs; C and D).

At TFE, the proportion of aIBD patients with IFX TLs >5.4 μg/mL was low compared to pIBD patients (38% vs. 53%, respectively) (Table 2). In aIBD patients that escalated IFX therapy, IFX TL at TFE was significantly higher in patients continuing IFX therapy compared to those who ultimately stopped IFX (8.0 vs. 0.1 μg/mL, respectively; $p = 0.0357$). In pIBD patients that escalated IFX therapy, IFX TLs at week 6, at week 14 and TFE were higher in patients who subsequently stopped anti-TNF compared to patients continuing (Table 2). In children who ultimately stopped IFX due to loss of response, IFX levels at TFE were higher on average and more variable (between 0.0 and 33.29 μg/mL) compared to adults. This variability was mainly due to those pIBD patients who escalated before Week 14 ($n = 4/8$; 50%, IFX levels at TFE between 8.0 and 33.3 μg/mL) (data not shown).

Overall, 4/24 (17%) paediatric and 2/21 (10%) adult patients developed ATIs during follow-up with median ATI levels of 150 [40.8–402.5] and 106 [12–200], respectively

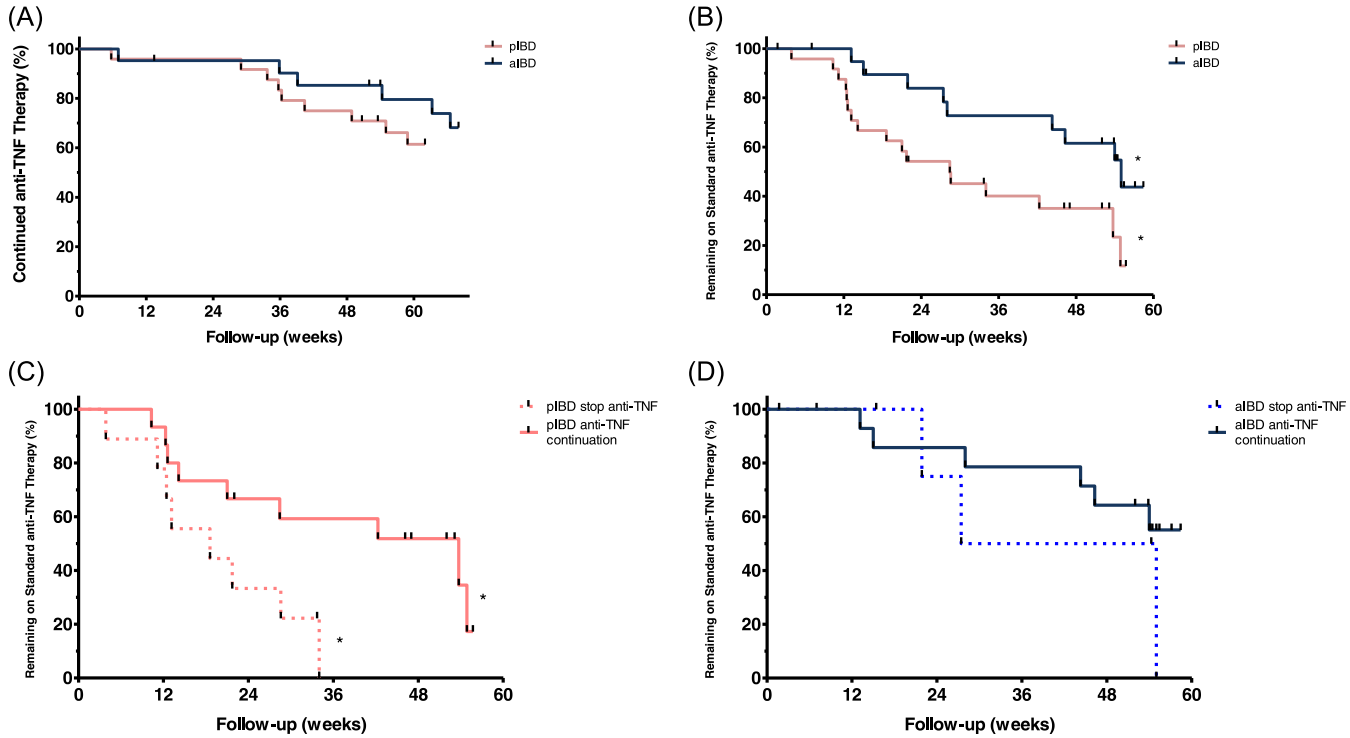



**FIGURE 1** Kaplan-Meier curves depicting differences in anti-TNF continuation and percentage remaining on standard anti-TNF therapy between children and adults (A and B), and differences in percentage remaining on standard anti-TNF therapy between children and adults, based on anti-TNF continuation. Clinical endpoints were stop or continuation of anti-TNF therapy (C and D). (A) Comparison between pIBD and aIBD patients regarding continuation of anti-TNF therapy. Maintenance of response, defined as continued anti-TNF therapy at 12 months was not significantly different between the two age groups. (B) Comparison between pIBD and aIBD patients regarding percentage remaining on standard anti-TNF therapy. Anti-TNF escalation over time differed significantly between pIBD and aIBD patients ($p = 0.02$). (C) Percentage remaining on standard anti-TNF therapy (without dose escalation) in pIBD patients. Paediatric patients who ultimately failed anti-TNF escalated significantly earlier than those who continued anti-TNF after 12 months ($p = 0.03$). (D) Percentage remaining on standard anti-TNF therapy (without dose escalation) in aIBD patients. No significant difference between adult patients who failed anti-TNF within 1 year compared to patients who continued anti-TNF ($p = 0.35$). aIBD, adult IBD; pIBD, paediatric IBD; TNF, tumour necrosis factor.

(Table 2). One adult patient ($n = 1/21$; 5%) developed ATAs during follow-up. The majority of pIBD and aIBD patients with ATIs stopped IFX therapy during follow-up (3/4; 75% vs. 2/2; 100%, respectively). ATIs were present in 2/3 adult and 2/8 paediatric patients who stopped IFX therapy despite escalation (66% vs. 25%, respectively) (data not shown). The largest differences in immunomodulators (IM) between pIBD and aIBD at study start were seen in thiopurine metabolites (TPM; overall 88% vs. 48%, CD; 100% vs. 44%, respectively) and methotrexate (MTX; 10%, only in aIBD patients) (Table 1). The overall proportion of patients on mesalamine-based 5-ASA agents and corticosteroids at start were comparable between pIBD and aIBD, but differed across disease phenotypes. Mesalamine-based 5-ASA agents use was more frequent in paediatric UC patients, while treatment with corticosteroids was more frequent in adult CD patients. There was no difference between pIBD and aIBD patients in concomitant IM treatment during our study either in all anti-TNF treated patients (pIBD; 22/24, 92% vs. aIBD; 19/21, 90%) (Table 2) or IFX treated patients (pIBD; 21/23, 91% vs. aIBD; 17/18, 94%) (see File S2: Supplemental Digital Content 2 Table, table of demographic and clinical characteristics of IFX treated patients).

## 3.6 | Relationship between baseline covariates, biomarkers and longitudinal covariates and outcome

Statistical analysis with personalised logistic regression analysis included all patients on anti-TNF. CRP at baseline showed a trend towards a significant relation to remission at 8 weeks: a doubling of CRP at baseline had an odds ratio of 1.39 (95% confidence interval: 0.94–2.05) for remission. Other baseline covariates and longitudinal covariates were also not significantly associated with outcome; not with primary response nor with response at 12 and 18 months. Evaluated covariates were age, gender, age at start, age at diagnosis, disease duration, serum concentrations of p-ANCA, ASCA, CRP, albumin, ESR and peripheral blood concentrations of thrombocytes, leucocytes, neutrophils and FCP.

IFX serum TLs before third and fourth infusion (at Week 6 and Week 14, respectively) were not predictive for maintenance of response at 12 months and 18 months. Cessation of anti-TNF (due to nonresponse or loss of response) was not significantly different between patients who escalated before Week 14 compared to after Week 14, both in children and adults (Fisher's exact test $p = 1.00$ for both). Also, cessation of anti-TNF was not significantly different between patients who escalated or did not escalate during follow-up both in children and adults (Fisher's exact test $p = 0.352$ and 1.00, respectively). Binary logistic regression analysis showed that cessation of anti-TNF was not statistically different between children and adults based on escalation before week 14 or during follow-up ($p = 0.99$ and 0.502, respectively).

## 3.7 | Patients with loss of response have increased plasma immune protein concentrations at baseline

For plasma proteomic analysis, a subgroup of 15 pIBD patients (five patients who discontinued IFX vs. ten patients that continued IFX) was selected. All patients had plasma samples available from baseline (Week 0) and received standard infliximab therapy until Week 14. Comparability of this subgroup to the overall pIBD population within this study was assessed by comparing the escalation rate (Kaplan-Meier), IFX-TL at Week 6 and IFX (trough) levels at TFE. The subgroup was deemed comparable to the overall pIBD population (data not shown).

At baseline (before start of IFX), concentrations of seven plasma immune proteins were significantly increased in pIBD patients who discontinued IFX (loss of response) versus those who continued IFX treatment (Figure 2A). Concentrations of interleukin-8 (IL-8), hepatocyte growth factor (HGF), eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), monocyte chemotactic protein 3 (MCP-3), oncostatin-M (OSM), transforming growth factor alpha (TGF-alpha) and matrix metalloproteinase-10 (MMP-10) were significantly increased (Figure 2A). Concentrations of two additional proteins, latency-associated peptide-transforming growth factor beta 1 complex (LAP. TGF-beta-1) and interleukin-17A (IL-17A), were increased near-significantly at baseline in pIBD patients who discontinued IFX ($p = 0.055$ for both) (data

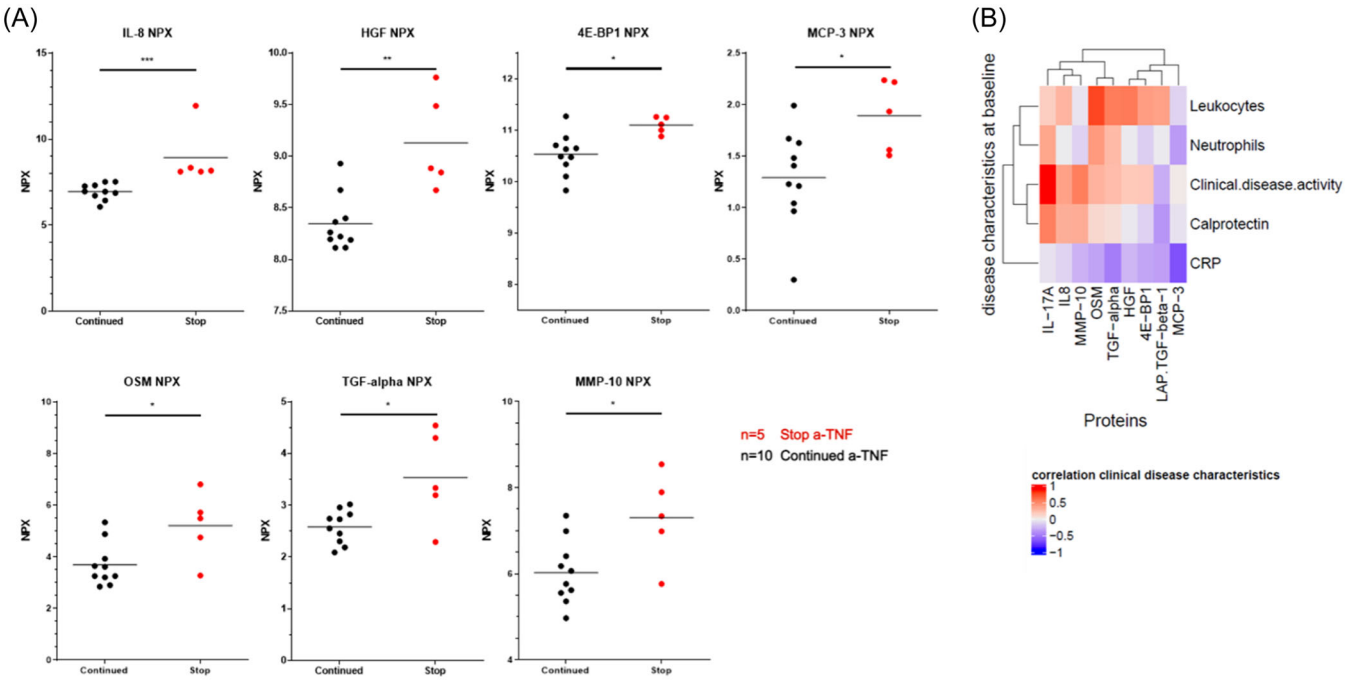



**FIGURE 2** A subgroup of 15 pIBD patients (5 patients who discontinued anti-TNF vs. 10 patients that continued anti-TNF) was selected for plasma proteomic analysis using Olink. All patients had plasma samples available from baseline (Week 0) and received standard infliximab therapy until Week 14. (A) At baseline (before start of anti-TNF), concentrations of seven plasma immune proteins were significantly increased in pIBD patients who discontinued anti-TNF (loss of response) versus those who continued anti-TNF treatment. (B) Heatmap depicting the correlation of baseline clinical disease characteristics with seven significantly increased proteins and two near-significant proteins. *p* values; $^{*}p \le 0.05$, $^{**}p \le 0.01$, $^{***}p \le 0.001$. 4E BP1, eukaryotic translation initiation factor 4E binding protein 1; CRP, C-reactive protein; HGF, hepatocyte growth factor; IL-17A, interleukin-17A; IL-8, interleukin-8; LAP. TGF-beta-1, latency-associated peptide transforming growth factor beta 1 complex; MCP-3, monocyte chemotactic protein 3; MMP 10, matrix metalloproteinase-10; NPX, normalised protein expression; OSM, oncostatin-M; TGF-alpha, transforming growth factor alpha.

not shown). As these data may suggest that patients with subsequent loss of response to IFX treatment have increased immune activation before start of IFX we assessed whether the concentration of the combined nine immune proteins at baseline related to clinical disease activity, FCP, CRP, circulating neutrophil concentrations, and leucocyte concentrations at baseline. Indeed, concentrations of the nine immune proteins positively correlated to several of the clinical disease parameters at baseline (Figure 2B).

## 4 | DISCUSSION

In our study we have shown that continued response to anti-TNF (until 12 and 18 months) was similar in pIBD and aIBD patients, while pIBD patients escalated significantly earlier and more often than aIBD patients. In addition, pIBD patients (but not aIBD patients) who ultimately failed anti-TNF escalated earlier than those who continued anti-TNF, both after 12 months and 18 months follow-up.

Several studies on infliximab treatment in IBD suggest an exposure-response association with higher IFX TLs leading to better clinical outcome.[6,10–16] For this reason, pro-active measurement of anti-TNF TLs or proactive therapeutic drug monitoring (TDM) is currently recommended in both pIBD and aIBD.[12,17–19] In this study, the treating physicians did not have access to TLs at the time of treatment escalation. We expected that early escalation in pIBD patients might be explained by low IFX (trough) levels during induction (Week 6), start of maintenance (Week 14) or at TFE. However, in our study IFX TLs at all timepoints were higher in escalated pIBD patients who subsequently stopped IFX compared to patients continuing. The rate of ATI in this group of pIBD patients was lower compared to aIBD patients who stopped IFX despite escalation (25% vs. 66%, respectively). In adults, it is known that use of concomitant IM treatment and prolonged subtherapeutic IFX TLs may lead to ATI development.[20–24] At study start more paediatric UC patients were on ASA, while more adult CD patients were on corticosteroids or MTX. However, in our cohort there was no difference in concomitant IM treatment during follow-up that could explain the difference in ATI rate. A possible explanation for a lower ATI rate in children with therapy escalation, could be a shorter exposure time to (subtherapeutic) IFX (trough) levels because of earlier escalation compared to adult patients.

Besides clinical remission, the goal of anti-TNF treatment is also to achieve endoscopic remission, with mucosal healing as important treatment outcome parameter. Previous data shows that FCP can be used as a surrogate for mucosal healing.[25–27] In our study, median FCP levels in both pIBD and aIBD patients decreased most after induction with comparable ranges during maintenance and at the end of study. Interestingly, median FCP levels were consistently lower in aIBD patients on continued IFX therapy compared to those stopping IFX. In contrast, results for pIBD patients were less consistent. Possibly the higher escalation rate in pIBD patients might have influenced these results. Furthermore, in our study continuation of anti-TNF treatment was a clinical endpoint and not endoscopic remission. Continuation of anti-TNF does not necessarily equate to endoscopic remission and mucosal healing. Thus, the variability of FCP within our population might have been the result of a combination of complete and partial mucosal healing.

It is unclear whether the differences in escalation rate are the result of pharmacokinetic factors or also pharmacodynamic differences between paediatric and adult patients. Young paediatric patients (<10 years of age) are reported to often have suboptimal TLs during standard induction and at the start of maintenance treatment and thus will benefit from treatment escalation up front.[28] However in our study, where we included only 2 patients below 10 years of age, the early treatment escalation of paediatric compared to adult patients could not be explained by low IFX (trough) levels. In pIBD patients, subtherapeutic IFX TLs were not associated with early escalation or cessation of IFX treatment while in aIBD patients subtherapeutic IFX TLs were associated with early escalation of IFX treatment (Week 6) and cessation of IFX treatment (Week 14). In the current study, we defined therapeutic cut-offs for IFX as TLs ≤ 18 μg/mL at Week 6, ≤5.4 μg/mL at Week 14 and during maintenance.[5–7,22] Higher cut-off TLs have been suggested for Week 6 (30–35 μg/mL) and Week 14 (7 μg/mL).[29] It may be that specifically paediatric patients need these higher TLs to achieve and maintain response to anti-TNF. This would suggest that there is also a difference in exposure-response relationship between paediatric and adult patients. In addition, pharmacodynamic loss of response might have been a cause for more frequent treatment escalation in paediatric patients, and not pharmacokinetic loss of response.

Proteomics might be a helpful tool in distinguishing immune heterogeneity.[30] Immune protein profiles based on multiple parameters seem more promising than single biomarkers and may provide mechanistic insights into IBD pathogenesis. In a large adult cohort ($n = 552$, 328 IBD, 224 non-IBD) a multi-protein model distinguished a subgroup with a high risk of surgery or biologicals after initial disease remission.[31] In a paediatric CD cohort ($n = 265$) distinct immune protein profiles were found for stricturing and penetrating disease at baseline.[32] Recently it was shown that in therapy-naïve paediatric CD patients ($n = 91$) proteomics can be helpful in the prediction of maintenance of

remission in case of IFX top-down treatment. Grouping based on pretreatment profiles of immune proteins modulated by IFX, was directly related to maintenance of remission.[33] These findings suggest a possible role for proteomics in predicting disease progression and informed decision making on IFX treatment on an individual basis. In our study we examined the degree of pretreatment inflammation at immune protein level in pIBD patients who ultimately discontinued anti-TNF compared to patients who continued anti-TNF treatment. In line with the published data, plasma proteomics analysis revealed seven IBD-associated proteins that were significantly increased at baseline in those who discontinued anti-TNF and two proteins with near-significant increase. Biological function of these proteins in relation to IBD and their association with (non-) response to anti-TNF are summarised in (see File S2: Supplemental Digital Content 5 Table, table depicting function of (near-)significant proteins found in our study using proteomics, in relation to Inflammatory Bowel Disease (IBD) and their association with nonresponse to anti-TNF in IBD). Increased immune activation at baseline is associated with loss of response to anti-TNF over time. Indeed, our study showed that most clinical disease characteristics positively correlated with these 9 proteins, except for CRP. CRP correlated with all proteins in a negative manner where a positive correlation at least was expected for IL-17A and IL-8. The negative correlation was strongest with MCP-3. In conclusion, the findings from our peripheral blood proteomics analysis in this subgroup suggest that measuring the expression of these proteins in pIBD patients, associated with loss of response to anti-TNF, could help determine whether to start anti-TNF treatment or to explore alternative therapeutic options. However, our findings need to be validated in a larger cohort with more homogeneity.

One important strength of this study was the longitudinal, observational design of the study. Another strength was the inclusion of both paediatric and aIBD patients, which enabled us to compare the two populations. Blinding the treating physicians for IFX TL data reduced the chance of bias and thus was another strength of this study. The amount of in-depth data collected during this study on clinical outcome as well as samples for analyses on routine laboratory parameters, FCP, IFX (trough) levels and IBD-associated proteins via proteomics was a major strength. A limitation of this study was the low number of patients included. Although common in paediatric studies, the low sample size may have prevented identification of significant findings in analyses such as the mixed-model analysis. The lack of difference between pUC and pCD in continuing anti-TNF could also be due to the low number of patients. Our study was not powered to answer this question clearly, thus our data could be more reflective of pCD rather than pUC in this overall cohort of pIBD. Another limitation might have been the lack of routine TDM at the time of the study. Theoretically, treating paediatric gastroenterologists might have been more defensive than adult gastroenterologists in their treatment approach which could have led to earlier and more frequent escalation of anti-TNF therapy in pIBD patients. In our study pUC patients had anti-TNF escalation more often compared to pCD patients. As escalation was decided by the treating physician, this may have been due to the knowledge (or experience) of the physician that anti-TNF needs to be dosed higher in UC patients with high disease activity. Despite these limitations we were able to report several findings of interest to daily clinical care.

## 5 | CONCLUSION

In conclusion, we have again shown that standard anti-TNF dosing does not seem adequate in pIBD patients. Anti-TNF therapy needed to be escalated in most pIBD patients, but this was not due to low infliximab levels. We also showed that pIBD, but not aIBD patients who escalated during induction were more likely to ultimately fail anti-TNF therapy. Similarly, (dis)continuation of IFX treatment was not associated with low IFX TLs at Week 6, Week 14 or at TFE. FCP results were not consistent for pIBD patients, possibly due to a higher escalation rate and variability in endoscopic remission in this population. Thus, we did not find any clear biomarkers for maintenance of response at 12 months. However, proteomic analysis has shown 9 IBD-associated plasma immune proteins of interest that need to be studied more in-depth as potential predictors of loss of response in paediatric IBD.

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### CONFLICT OF INTEREST STATEMENT

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### DATA AVAILABILITY STATEMENT

Source data available at https://www.ClinicalTrials.gov, record NCT01971970.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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