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PRACTICAL USE OF INFLIXIMAB CONCENTRATION MONITORING IN PEDIATRIC CROHN'S DISEASE

Phillip Minar, MD^{1,*}, Shehzad A. Saeed, MD¹, Mahrukh Afreen², Mi-Ok Kim, PhD³, and Lee A. Denson, MD¹

¹Department of Pediatrics, Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

²Dow Medical College, Dow University of Health Sciences, Pakistan

³Division of Biostatistics and Epidemiology, Cincinnati Children's Hospital Medical Center

Abstract

Objective—Therapeutic drug monitoring (TDM) that guides infliximab (IFX) intensification strategies has been shown to improve IFX efficacy. We conducted a review to evaluate the utility of TDM in the assessment and subsequent management of IFX loss of response in our pediatric Crohn's disease (CD) population.

Methods—Single-center retrospective study of CD patients receiving IFX that had TDM from 12/2009–9/2013. We defined subtherapeutic trough as a drug level below the detection limit of the Prometheus® ELISA and ANSER™ reference values (1.4 µg/ml and 1 µg/ml, respectively) or a mid-interval level <12 µg/ml.

Results—191 IFX concentration tests were performed on 72 CD patients with loss of response to therapy as the primary indication (72%). 34% of all TDM were subtherapeutic. Following initial TDM, 25/72 patients received regimen intensification with 72% in clinical remission at six months. Including all TDM that resulted in IFX dose intensification, we found a significant improvement in six month remission rates whether intensification followed mid-interval (88% remission) or trough (56% remission) testing (p=0.026). Antibody to infliximab (ATI) was found in 14 patients with five occurring in the first year of therapy. Further, 71% of patients with ATI that were switched to an alternative anti-TNF achieved clinical remission at six months. In multivariable regression analysis, we found IFX dose (mg/kg), IFX dosing frequency (weeks) and the ESR at the previous infusion were significantly associated with the IFX concentration.

Conclusions—TDM in our pediatric CD population led to informed clinical decisions and improved rates of clinical remission.

*to whom correspondence should be addressed: Phillip Minar, MD, Division of Pediatric Gastroenterology, Hepatology & Nutrition, Cincinnati Children's Hospital Medical Center, MLC 2010, 3333 Burnet Avenue, Cincinnati, OH 45229, Tel: 513-803-4688, Fax: 513-636-5581, phillip.minar@cchmc.org.

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Keywords

infliximab concentration; antibodies to infliximab; inflammatory bowel disease; Crohn's disease

Introduction

The inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC) are chronic gastrointestinal conditions with noted increases of proinflammatory cytokines as a result of a dysregulated immune response to the intestinal microbial flora.(1) The monoclonal antibodies directed against tumor necrosis factor (TNF)- α have been shown to be effective in inducing and maintaining clinical remission in moderate to severe CD and UC.(2–6) Despite early clinical response rates up to 80% to infliximab (IFX), roughly 25–40% of initial responders will lose response over time while a significant subset will require a dose adjustment (intensification) to maintain clinical remission.(7–12) Secondary loss of response to anti-TNF therapy is attributed to increased IFX clearance, differences in individual pharmacogenetics, development of antibodies to IFX (ATI) or alternative inflammatory pathways (non-TNF α) leading to chronic intestinal damage.(13–17) Substantial research efforts have been devoted to investigating loss of response to IFX, particularly to IFX clearance (failure to achieve or maintain adequate therapeutic IFX concentrations) as sustained, detectable IFX concentrations have been associated with higher remission rates, endoscopic healing in CD and UC and lower colectomy rates in UC.(18–21) Further, development of ATI (immunogenicity) is considered to occur in-between infusions following chronically diminished or undetectable serum IFX concentrations.(19, 22)

Similar to therapeutic drug monitoring (TDM) for 6-mercaptopurine (6MP) metabolite concentrations, regular monitoring of IFX serum concentrations is predicted to improve drug efficacy by tailoring dosing regimens to an individual's pharmacokinetics.(13, 23, 24) In clinical practice, IFX TDM has largely been empiric as the clinician bases TDM on the presence of gastrointestinal symptoms or evidence of ongoing mucosal inflammation detected by surrogate biomarkers (serum or fecal) or through repeat endoscopy. Recent research has suggested that more routine TDM for IFX concentration and ATI during disease quiescence or prior to the start of IFX maintenance (following induction) can also improve long-term IFX efficacy.(24–26) In addition, supporters of this approach assert that routine TDM may further improve anti-TNF efficacy as gastrointestinal symptoms may not be clinically evident despite ongoing intestinal inflammation.

Multiple assays have been developed to improve monitoring for adequate circulating IFX levels including the enzyme-linked immunoabsorbant assay (ELISA), radioimmunoassay (RIA) and the homogenous mobility shift assay (HMSA) recently offered by Prometheus® (Prometheus Laboratories Inc., San Diego CA).(15, 27, 28) IFX serum concentrations can be determined quickly and at low cost with the ELISA technique. However, due to IFX drug interference, the ELISA does not detect the presence of ATI if circulating drug is present. Newer technologies have afforded commercial laboratories to offer novel assays that can detect both IFX concentration and ATI in the presence of drug (IFX) using HMSA or the electrochemiluminescence immunoassay (ECLIA). With the potential paradigm shift

towards more frequent IFX testing in asymptomatic patients and the rising cost of TDM for IFX, we retrospectively reviewed our practice management of TDM in CD patients receiving IFX with the hypothesis that independent patient covariates (such as elevated laboratory values and dosing regimens) would be associated with subtherapeutic IFX levels or ATI and could be utilized to prioritize testing. We were also interested in discovering the indications for testing, number of subtherapeutic occurrences, the subsequent clinicians' decision following TDM and whether the regimen adjustments following TDM improved the short-term remission rates.

Materials and Methods

Subjects

We performed a single center retrospective study of CD patients receiving recurring IFX infusions that had either the Prometheus® ELISA or ANSER™ test sent from 12/2009–9/2013. The study was approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center. We reviewed the chart of every CD patient who had TDM for an IFX concentration during this time and included those patients meeting criteria for (a) suspicion for an infusion reaction or (b) mid-interval and/or trough level. Data extracted from electronic medical records included subject's age, gender, race, weight, IFX dose (mg/kg), age at diagnosis, duration of CD, length of CD until IFX was started, time on IFX therapy, current medications, and the results of routine laboratory testing (1) prior to initiating IFX induction therapy, (2) at the IFX infusion prior to TDM and (3) at the time TDM was performed. Routine laboratory monitoring included a complete blood count with automated cell differential, liver profile and albumin with the non-specific inflammatory markers including C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) collected at the discretion of the clinician. CD phenotype was characterized by the Paris Classification.(29)

All IFX concentration and ATI testing was ordered at the discretion of the treating clinician. We recorded the indication for testing, disease activity at time of testing and the rationale for changing the treatment following TDM. Disease activity was defined by the physician global assessment (PGA), either active or quiescent, and was recorded from the most recent clinic visit surrounding when TDM was ordered as well as the clinic visit 6–9 months following IFX intensification. In addition, patients could not be receiving systemic corticosteroids to qualify for PGA-quiescent at 6 months. Secondary loss of response was defined by the primary gastroenterologist as worsening gastrointestinal symptoms following an initial clinical response to IFX induction whereas primary non-response was defined as continued (no improvement) symptoms during IFX induction.

The Prometheus® HMSA testing method replaced the ELISA in 2013. The ELISA and HMSA reference values for detectable IFX concentrations are 1.4 µg/ml and 1 µg/ml respectively while ATI detection is 1.69 µg/ml equivalents with the ELISA (Prometheus® ELISA reports ATI as indeterminate if there is a detectable serum IFX concentration) and 3.1 U/ml for the HMSA. In addition to recording the IFX concentration and ATI status for each subject, we also recorded the timing of the collection in relation to their last infusion. As there was no formal protocol for TDM, we divided the IFX testing into (1) mid-interval

and (2) trough. We defined the result as mid-interval if collected at 28 (+10) days for patients receiving infusions every 8 weeks or at the half-point (+/- 3 days) for those receiving IFX infusions every 4 to 6 weeks while we defined trough samples as those collected immediately prior to the patients next scheduled infusion (whether 4, 6, or 8 weeks). We defined therapeutic IFX concentrations as either a mid-interval IFX concentration >12 µg/ml for patients receiving IFX every 8 weeks (15) or a detectable trough IFX concentration (1.4 µg/ml for the ELISA and 1 µg/ml for ANSER) for all variable infusion regimens. We based our cut-off for a therapeutic mid-interval IFX level on the Baert et al. pharmacokinetic study as they found the median mid-interval IFX level in CD patients receiving every eight week infusions was 12 µg/ml.(15) ATI status is reported qualitatively (detected, not detected) as we were not able to compare quantitative ATI values given the different laboratory techniques utilized for ATI detection in this study.

Statistical Analysis

Statistical analyses were conducted in GraphPad Prism (Version 5 for Windows, GraphPad Software, San Diego CA) and with the statistical software R (Core Team 2012). Continuous variables are presented as mean (standard deviation, SD) or median (25–75% interquartile range) depending on the data distribution. Differences in groups were assessed using Student's *t* test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. The Fisher's exact test was used for comparison of categorical data. Receiver operating characteristic (ROC) curve analysis was used to identify IFX concentration thresholds associated with clinical remission and ESR values. Multiple regression analyses were conducted to test the significant independent variables associated with the trough IFX concentration as a continuous response. To account for multiple IFX observations per patient, we utilized a linear mixed regression analysis for our multivariable model and performed the Likelihood Ratio Test to attain p-values. P values <0.05 were considered statistically significant.

Results

During the four year period, 191 IFX concentration tests were sent on 72 CD patients. The majority of the TDM sent were ELISA (n=170). Baseline patient demographics and IFX dosing characteristics are listed in Table 1. The indication for initiation of IFX included severe disease/growth failure (n=48), internal/perianal penetrating behavior (n=12), steroid dependence (n=7) and following a surgical resection (for stricturing/penetrating behavior) in 5 patients. The indication for TDM included the clinicians concern for (a) secondary loss of response to IFX (72%), (b) primary non-response to IFX (7%, including levels drawn prior to the fourth infusion) and (c) following a reported infusion reaction (3%). The remaining IFX concentration tests (18%) were sent to establish the IFX level during IFX maintenance therapy (92% were PGA-quiescent). The results of TDM by indication and PGA are shown in Table 2. We found that 18/72 (25%) patients were receiving concurrent immunomodulators (IM), either 6MP or methotrexate (MTX), at the time of TDM. The median time on IFX prior to initial TDM for all patients was 7 (3–17) months whereas median time to TDM for secondary loss of response was 12.5 (7–25) months. Thirty (42%)

patients were receiving an intensified (7.5 mg/kg or 6 week intervals) IFX dosing regimen prior to initial IFX testing.

Infliximab Trough Concentration

Altogether, there were 140 IFX trough concentration tests from 55 CD patients. We found 24% of TDM were subtherapeutic (undetectable) while 38% were $<3 \mu\text{g/ml}$ and 10 ATI events (2/10 ATI had detectable IFX concentrations by HMSA testing) were detected. The median (min–max range) time on IFX until subtherapeutic levels were detected was 232 (97–1418) days with 69% of the subtherapeutic levels occurring during the first year of therapy. Although we did not find a difference in the rate of subtherapeutic events by disease phenotype (Paris classification), we found that patients with the penetrating and/or stricturing CD phenotype had a median trough of 3.6 (0–7.8) $\mu\text{g/ml}$ compared to a median trough of 5.2 (2.4–14) $\mu\text{g/ml}$ in patients with the inflammatory CD phenotype ($p<0.05$). In a sub-cohort analysis of those with their initial TDM as an IFX trough, we found 13/47 (28%) were subtherapeutic, 47% were $<3 \mu\text{g/ml}$ and 6/42 had ATI.

Infliximab Mid-interval Concentration

The majority of the published data for TDM during IFX therapy has focused on trough concentrations. However, we found that 51/191 of the IFX tests sent on 37 unique patients were mid-interval samples. We, surprisingly, found 28% of mid-interval TDM were associated with undetectable IFX concentrations and five incidents of ATI. The median (min–max) days on IFX until undetectable mid-interval levels were discovered (including 5 ATI events) was 313 (41–1653) days. Additionally, we found 68% of the 41 mid-interval levels obtained from patients receiving IFX every 8 weeks were subtherapeutic ($<12 \mu\text{g/ml}$) with 18/28 occurring during the first year of therapy.

Antibody to Infliximab

The true incidence of ATI in our pediatric CD population who had TDM performed could not be determined by this study as the majority of the testing was analyzed by the ELISA method. In addition, TDM was clinician driven as only approximately 60% of our entire CD population on IFX had TDM during our review period. Despite the inability of ELISA testing to detect ATI in the presence of a detectable serum IFX concentration (indeterminate ATI), we nonetheless found that 14/72 (19%) of the CD patients had ATI events (there was a total of 15 events as one patient had 2 separate ATI events). When we evaluated the total occurrences of ATI by testing method for all tests, ATI occurred in 5% of all ELISA testing compared to 33% of all HMSA samples. However, we found a 20% rate of ATI detection with ELISA testing after excluding tests with a detectable IFX concentration as ATI discovery with this technique is limited as noted above ($p=0.34$ compared to HMSA testing). Two of the 15 ATI positive samples were associated with a detectable IFX concentration (1.2 and 1.3 $\mu\text{g/ml}$ respectively, both HMSA samples) while 5/15 were discovered in the first year of IFX therapy (median of 483 days and min–max range of 149–1699 to detection). Only 2/14 patients with ATI were receiving concurrent IM at the time of TDM. Overall, TDM led to 12/14 patients with ATI receiving an alternative anti-TNF immediately after ATI detection with the remaining two patients ultimately receiving an alternative anti-TNF following a poor clinical response to IFX dose intensification.

Secondary Loss of Response

The primary indication (72% of all tests, 60 patients) for TDM in our cohort was secondary loss of response. We found 28/60 in this group were receiving an intensified IFX regimen prior to TDM, 10/60 were on combination IM therapy and yet, their initial drug monitoring (including both trough and mid-interval) found 38% were subtherapeutic, 28% had undetectable levels and there were eight patients with ATI. Evaluating all 137 drug monitoring tests this group had performed, we found 28/101 trough and 9/36 mid-interval levels were undetectable with 12 ATI events (11 patients, two on combination IM). The median time on IFX for those patients with an undetectable level (trough or mid-interval) was 12.5 (7–18) months. TDM led to a change in therapy in 29/60 patients with the majority receiving an intensified (n=19) IFX regimen, six were switched to an alternative biologic, three had IM added and one patient had IFX discontinued. In the 29 patients with a change in therapy (of which 20/29 had subtherapeutic levels), 76% were in clinical remission at six months. In comparison, 74% (23/31) were in clinical remission at six months if no change in therapy was made following TDM (only 3/31 had subtherapeutic levels).

Infliximab testing outcomes

Drug monitoring led to a therapeutic change by the clinician in 44% of all TDM tests sent, however, 81% of the subtherapeutic events led to an alteration in therapy. The clinical decisions made following testing and rates of disease activity following these changes are summarized in Table 3. Focusing exclusively on IFX regimen intensification, we found there were 52 events (35 patients) when TDM led to an increase in dose (n=24) or an increase in dosing frequency (n=28). The variable clinic follow up times in this review compelled us to define a 6 month clinical remission as a PGA-quiescent assessment obtained by the treating clinician between >6 months and <9 months from TDM led intensification. For this group, 73% were PGA-active at the time of TDM while only 37% were PGA-active six months following regimen intensification ($p<0.001$). The six month clinical remission rate in patients who had IFX intensification following their *initial* TDM was 72% (18/25) compared to a pre-IFX intensification remission rate of 16% (4/25, $p<0.001$). In terms of TDM timing and type of drug intensification, we found there was a significant higher rate of clinical remission in patients who received IFX intensification following mid-interval TDM (88% were PGA-quiescent at six months) compared to patients who received intensification following trough TDM (53% PGA-quiescent at six months, $p=0.026$). Overall, all therapy changes (IFX dose intensification, addition of 6-MP or corticosteroids and/or following a switch to an alternative biologic) following TDM was associated with a 64% disease remission rate at 6 months (compared to 29% PGA-quiescent prior to TDM, $p<0.001$).

Routine Laboratory Testing & TDM

We were interested in whether TDM could be prioritized by assessing the relationship between IFX concentration and disease activity (PGA) or nonspecific serum markers of inflammation. As expected, undetectable IFX concentration and/or ATI were significantly associated with gastrointestinal symptoms (PGA-active) and elevated serum biomarkers (ESR and albumin) (Table 4). Additionally, we found subtherapeutic IFX trough concentrations were associated with elevations in ESR (Figure 1). We also found that an

ESR value 15 ml/hr. was significantly associated with an undetectable trough with a sensitivity of 78%, specificity of 70%, 43% positive predictive value (PPV) and a 90% negative predictive value (NPV, AUC 0.70, 95% confidence interval 0.59–0.81, $p<0.01$).

Forty-six (64%) patients had their initial TDM within the first year of starting IFX therapy. We found those who had an undetectable IFX concentration (trough or mid-interval) had a trend toward an elevated mean (SD) ESR of 39 (30) mm/hr. prior to starting IFX as compared to a mean (SD) ESR of 28 (19) mm/hr. in patients with a detectable trough or mid-interval level during this time ($p=0.20$). We found the hematocrit prior to starting IFX was significantly lower in this group as patients with undetectable IFX concentrations had a mean hematocrit of 33% [SD 6.6] compared a mean hematocrit of 37% [SD 3] in patients who were found to have a detectable levels ($p=0.015$) during the first year. Pre-induction CRP, platelet count, nor albumin were associated with TDM outcomes in the first year of IFX therapy.

Fixed linear regression models were used to determine the continuous independent variables (dosing regimens and routine laboratory testing) that were significantly associated with IFX trough concentrations in our CD patients. We found that the dosing frequency (weeks, $\beta = -1.8$, $p<0.01$), IFX dose (mg/kg, $\beta = 0.63$, $p=0.05$), and ESR ($\beta = -0.17$, $p<0.001$) obtained at the previous infusion prior to TDM was the most predictive of the IFX trough concentration (continuous data, $R^2 = 0.24$, $p<0.001$).

In order to statistically account for multiple TDM per patient, we subsequently evaluated a multivariable *mixed* regression model based on the IFX dosing regimen and ESR values obtained at the previous IFX infusion prior to TDM. Of the 140 trough samples available to analyze, there were 105 samples from 48 patients that had an ESR sent at the previous IFX infusion prior to TDM and were included in this analysis. The model found that IFX trough concentration could be calculated with the following equation; $25.1 (\beta_0) + 0.5 (\beta_1) \times \text{IFX dose (mg/kg)} - 2.6 (\beta_2) \times \text{frequency (weeks)} - 0.15 (\beta_3) \times \text{ESR (mm/hr.)}$. In order to analyze the statistical significance of this model, we performed the Likelihood Ratio Test on various regression models and found the addition of the ESR obtained from the previous infusion prior to TDM significantly influenced the IFX trough concentration ($\chi^2(1) = 217.5$, $p<0.001$) compared to a model that only included IFX dosing frequency and IFX dose.

Discussion

ATI and subtherapeutic IFX trough concentrations have been the most extensively investigated contributors of loss of response in patients receiving maintenance IFX therapy. Previous investigations have shown that persistent undetectable IFX levels likely permit the development of clinically significant ATI.(13, 32, 33) We performed this large retrospective review to investigate the number of occurrences of subtherapeutic IFX levels in those CD patients who were referred for TDM. Given the increased cost of TDM, we were also interested in discovering distinct clinical factors that may guide TDM to improve the pretest probability of detecting subtherapeutic IFX levels while also investigating whether TDM improved clinical outcomes in our cohort.

The development of ATI has been reported as low as 5–23% in IBD patients receiving routine maintenance infusions and as high as 61% in adult IBD patients receiving episodic treatment.(13, 34) In addition, recent studies have found a decreased incidence of ATI when anti-TNF therapy is combined with an IM.(32, 35, 36) Despite the promising results reported by the SONIC trial in which combination IFX-6MP therapy was associated with increased rates of mucosal healing compared to IFX monotherapy (44% vs. 30%) in CD,(37) combination therapy (6MP-IFX) is less commonly utilized in the pediatric IBD population as there is a heightened awareness of its association with hepatosplenic T cell lymphoma (HSTCL).(3) The association of IBD and HSTCL in young (<35 years of age) males receiving this combination has significantly altered clinical practice at Cincinnati Children's (38); we reserve combination 6MP-IFX therapy for severe, refractory disease. Additionally, recent analysis of combination MTX-IFX was found to be no more effective (equal rates of treatment failures) than IFX monotherapy in CD patients.(39) Although the primary outcome measurement was similar, Feagan et al. did find rates of ATI were significantly lower in the group that received MTX-IFX compared to IFX monotherapy.(39) In our study, we found 14/72 CD patients had ATI (ELISA & HMSA) following TDM. However, the true incidence of ATI in our pediatric IBD population remains unknown as the ELISA testing method (89% of the tests reported on in this review) were limited in only detecting ATI if the IFX concentration was undetectable and not all CD patients at our center had TDM. It is worth noting there was no difference in the rate of ATI detection (33% by HMSA) between the two testing methods (20% by ELISA) when we controlled for samples with undetectable concentrations. However, as our experience grows with more sensitive TDM methods, our center has been compelled to systematically address ATI in the setting of detectable IFX levels.

It is worth noting that ATI was found in fourteen patients yet, only two were receiving combination IFX-IM therapy. Overall, combination therapy (IFX-IM) was used in 18/72 patients receiving maintenance IFX. As the concern for HSTCL has altered our pediatric IBD practice, it is interesting to note more recent findings of transient ATI. Steenholdt et al. found that in 83 IBD patients with ATI, the ATI resolved in 2/3 of patients with a noted clinical response after a median of 4 (range 3–5) infusions.(35) As the sensitivity of the assays change, our reflexive response to abandon the current anti-TNF agent in the setting of ATI has also undergone significant modifications. We largely focus our clinical decisions to ATI on (1) disease activity at time of TDM, (2) the IFX level in relation to timing (mid-interval vs. trough) and (3) the intensity of ATI. For example, an asymptomatic CD patient found to have a subtherapeutic IFX trough with low level ATI during routine TDM would be advised to undergo IFX intensification first with subsequent TDM repeated to monitor ATI response following the dose adjustment.

For the clinician, there is a paucity of data to guide subsequent strategies to optimize therapy with the newer IFX and ATI detection assays. Pariente et al. previously found that empiric intensification of IFX therapy (without IFX concentration or ATI testing) led to clinical improvement in 69% of the 76 IBD patients with the majority (89%) achieving clinical remission by 6 months following drug intensification.(34) With further investigation, they found there was no difference in the mean IFX trough concentration from those who responded (3.3 µg/ml) and those who did not respond (2.3 µg/ml) to empiric IFX

intensification. In addition, since clinical decisions were blinded to the results of the assay in the Pariente et al. study, they found that the 60% of the ATI positive patients responded to empiric intensification by 4–8 weeks and this response persisted up to 6 months.(34) This phenomenon of overwhelming anti-TNF antibodies to drug has been documented in rheumatoid arthritis (RA) patients receiving adalimumab. Although RA patients with antibodies to adalimumab had higher non-response rates, 30% of patients with drug antibodies no longer had antibodies after dose intensification.(40, 41)

Various studies have attempted to develop the ideal IFX trough cut point that maximizes anti-TNF response. Levesque et al. found that IFX trough concentrations $<3 \mu\text{g/ml}$ were significantly associated with active disease (>70 point increase in the mean CDAI score between infusions) and a higher probability of a $\text{CRP} > 5 \text{ mg/L}$ while Marits et al. found that patients with a trough of $4.1 \mu\text{g/ml}$ were more likely to experience clinical remission.(30, 42) More recently in a pediatric IBD population, Singh et al. found that a week 14 IFX level (first maintenance dose) of $5 \mu\text{g/ml}$ had an PPV of 83%, NPV of 53% and an AUC of 0.68 for persistent remission.(24) They also found that IBD subjects with persistent remission had a significantly higher median week 14 IFX level (4.7 vs. $2.6 \mu\text{g/ml}$). (24) Our study design did not allow for an analysis of the ideal IFX trough cut-off given the variability in timing of TDM. Further studies will be needed to fully address the ideal IFX concentration cut off for clinical response in the pediatric CD population in addition to assessing the utility of companion biomarkers of disease activity.(24) In addition, the preferred IFX concentration to maintain clinical remission is likely to change depending on the phase of IFX treatment as higher post-induction IFX levels may be required during the early course of therapy whereas patients in clinical remission and on maintenance therapy may tolerate lower IFX trough concentrations.

In post hoc analysis of the ACCENT 1 trial, Cornillie et al. found that predictors of durable sustained response to maintenance IFX included a week 14 trough $>3.5 \mu\text{g/ml}$ and a $>60\%$ decrease in CRP in those with an elevated baseline CRP ($>8 \text{ mg/L}$). (43) There have been very few pediatric IBD studies focused on anti-TNF response, drug levels and inflammatory markers. In a pediatric cohort of 37 subjects, it was shown that IFX concentration was associated with body weight and the level of intestinal inflammation (by fecal calprotectin). (44) They found no significant association between ESR or CRP and IFX levels during IFX induction.(44) In contrast, Singh et al. found that week 14 body mass index and CRP improved their predictive analysis of persistent remission compared to week 14 IFX level alone.(24) We performed a multivariate linear mixed regression analysis to provide clinicians with further guidance on IFX testing. We found that the IFX dose, IFX dosing frequency and the ESR (at the previous infusion prior to TDM) were significantly associated with the IFX trough concentration at the next infusion. Although our proposed regression model may be an over simplification, consideration of reserving IFX testing to patients with persistent ongoing intestinal inflammation as detected by serum/fecal biomarkers may limit unnecessary testing or uncovering transient ATI that may have otherwise responded to empiric IFX dose intensification.

The strength of performing a retrospective review in this setting is that we were able to assess the impact of the clinician's decision on remission in the subsequent 6 months

following TDM. We had a relatively large, diverse population of CD patients with 191 IFX tests to analyze. We also were able to evaluate the effect of routine laboratory testing (nonspecific biomarkers) can have on guiding future IFX testing and response to therapy. The laboratory tests obtained prior to each infusion allowed us to build a practical regression model that could guide further TDM. In contrast, a weakness of this study is that the primary indication for the majority the TDM was the clinicians concern for secondary loss of response (higher probability of subtherapeutic levels) which limits uncovering the true incidence of ATI and subtherapeutic IFX levels in our pediatric CD cohort as there wasn't a specific protocol for TDM. Secondly, the majority of those having TDM were performed with the ELISA method which does not detect ATI in samples with IFX drug present and this testing is no longer commercially available. Although our sample size was large, our cohort included patients who had multiple levels analyzed. In order to minimize over-estimating our linear regression model based on multiple levels per patient, we performed a mixed regression model. Finally, we did not include a control cohort to determine whether empiric IFX intensification would have led to similar rates of remission as we found with the TDM guided group. Future investigations will be needed to determine the utility of IFX concentration and ATI testing in patients with both active and quiescent disease during the induction and maintenance phases with the new laboratory techniques available as well as the ideal week 14 IFX trough that increases the likelihood of sustained, steroid-free remission.

Although limited to CD patients who had TDM, we found subtherapeutic IFX concentrations and ATI were relatively common in our pediatric CD cohort. We found that symptomatic CD patients who received IFX dose intensification following TDM experienced favorable clinical responses, particularly when dose intensification followed mid-interval TDM. We also found by regression analysis that serum biomarkers (specifically, ESR) can be utilized to prioritize future TDM. There is an urgent need for future TDM studies in pediatric CD to better define 'therapeutic' IFX levels (at multiple time courses of IFX maintenance) and further delineate the utility of serum and fecal biomarkers in guiding TDM.

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What is known

- Although there is a high primary response rate to infliximab in pediatric Crohn's, up to 40% of patient's on maintenance infliximab will require a dose adjustment to sustain a clinical response.
- Therapeutic drug monitoring, although costly and not recommended to be assessed with each infusion, has been shown to be effective in guiding infliximab intensification strategies.

New findings/impact on clinical practice

- Fifty-two percent of patients with subtherapeutic infliximab levels or antibodies to infliximab were currently receiving an intensified (7.5 mg/kg) infliximab dose.
- Routine use of inflammatory biomarkers (ESR, albumin) with infliximab infusions can prioritize infliximab concentration monitoring as we found significant associations with abnormal inflammatory markers and subtherapeutic infliximab concentrations.
- Infliximab dose intensification guided by therapeutic drug monitoring was associated with subsequent, steroid free remission in pediatric Crohn's disease.

Figure 1.

Subtherapeutic infliximab trough concentrations are significantly associated with ESR elevations. ESR was obtained at the same time as infliximab drug monitoring. The data was evaluated by Kruskal-Wallis with Dunn's posttest analysis. Subthera, subtherapeutic trough, NS, non-significant.

Table 1**Baseline patient demographics.**

Female:male	27:45
Age at diagnosis, mean (SD)	12 (4) years
Age at IFX initiation, mean (SD)	13 (4) years
Crohn's location	
Ileal only (L1)	8 (11%)
Colon only (L2)	16 (22%)
Ileocolonic (L3)	48 (67%)
Perianal location (p)	13/72
Crohn's behavior	
Inflammatory (B1)	46 (64%)
Strictureing (B2)	5 (7%)
Penetrating (B3)	14 (19%)
Both penetrating/strictureing	7 (10%)
Age at time of testing, mean (SD)	15 (4) years
Time from diagnosis to IFX initiation, median (range)	10 (4–27) months
Time on IFX prior to first drug monitoring, median (range)	7 (3–17) months
Concurrent IM (6MP:MTX)	18 (8:10)

IFX, infliximab; IM, immunomodulator; 6MP, 6-mercaptopurine; MTX, methotrexate.

Table 2

Drug monitoring results by testing indication.

Testing indication	Subtherapeutic	Therapeutic	Antibody to infliximab
Primary non-response	8	5	0
Secondary loss of response	39	86	12
Immediate infusion reaction	0	1	2
Delayed infusion reaction	0	3	1
Maintenance level	2	32	0
Physician global assessment			
Quiescent	17	82	4
Active	32	45	11

Two patients with antibody to infliximab also had a detectable infliximab concentration. Physician global assessment determined at time of ordering drug concentration testing. Subtherapeutic, trough or mid-interval infliximab concentration was not detectable or mid-interval level for every 8 week infusions <12µg/ml.

Table 3

Clinical decisions following therapeutic drug monitoring.

	Subtherapeutic trough	Trough <3 µg/ml	Therapeutic trough	Subtherapeutic mid-interval	Therapeutic mid-interval	Antibody to infliximab
Total events	24	43	106	25	21	15
Number of IFX regimen altered	20	24	23	17	9	15
PGA-prior to change (A:Q)	(15:5)	(18:6)	(17:6)	(13:4)	(4:5)	(11:4)
PGA-6 month follow up (A:Q)	(9:11)	(10:14)*	(9:14)*	(4:13)**	(4:5)	(4:10) ^Ø *
Increased dose	7	11	8	8	1	2
Shortened dosing interval	10	10	9	6	1	0
Lengthened dosing interval	0	0	0	0	1	0
Discontinued infliximab	1	1	4	1	5	13
(Started alt. biologic)	(1/1)	(1/1)	(4/4)	(1/1)	(4/5)	(13/13)
Added 6MP or MTX	2	2	2	2	1	0

The first five columns do not include patients with antibody to infliximab. Subtherapeutic trough, undetectable infliximab concentration by assay; subtherapeutic mid-interval, undetectable concentration or infliximab level <12 µg/ml for patients receiving every 8 week infusions; PGA, physician global assessment; A, active; Q, quiescent. Disease activity at time of drug monitoring was compared to the disease activity at 6 months following the change in therapy by the Fisher's exact test.

^ØOne patient with antibody to infliximab was lost to follow up.

* <0.05;

** <0.01

Table 4

Global assessment, serum biomarkers and therapeutic drug monitoring.

	Undetectable/ATI	Detectable level
IFX dose (mg/kg)	8 (5–10)	8 (5–10)
PGA active:quiescent	34:14 ***	54:89
ESR with TDM (mm/hr.)	24 (15–44) **	10 (7–24)
CRP with TDM (mg/dL)	0.6 (0.5–3.4)	0.5 (0.5–2)
Alb with TDM (gm/dL)	3.6 (3.2–4.1) **	4.1 (3.6–4.3)
ESR previous infusion	19 (9–40) *	11 (7–23)
CRP previous infusion	0.7 (0.5–2.5) *	0.5 (0.5–0.9)
Alb previous infusion	3.8 (3.4–4.2)	4.1 (3.6–4.3)

Biomarkers were drawn (1) at time of therapeutic drug monitoring (TDM) or (2) at the previous infusion prior to TDM. Drug levels were obtained either mid-interval or as a trough; ATI, antibody to infliximab.

*
p<0.05,

**
p<0.01,

***
p<0.001