Practical Use of Infliximab Concentration Monitoring in Pediatric Crohn Disease

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See ''Time for Personalized Biologic Therapy: One Size Does Not Fit All'' by Griffiths on page 666.

ABSTRACT

Objectives: 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 population with Crohn disease (CD).

Methods: Single-center retrospective study of patients with CD receiving IFX that had TDM from December 2009 to September 2013. We defined subtherapeutic trough as a drug level below the detection limit of the Prometheus enzyme-linked immunoabsorbant assay and Anser™ reference values (1.4 and 1 μg/mL, respectively) or a mid-interval level <12 μg/mL.

Results: One hundred ninety-one IFX concentration tests were performed on 72 patients with CD with loss of response to therapy as the primary indication (72%). 34% of all TDM were subtherapeutic. After initial TDM, 25 of the 72 patients received regimen intensification with 72% in clinical remission at 6 months. Including all of the TDM that resulted in IFX dose intensification, we found a significant improvement in 6-month remission rates whether intensification followed mid-interval (88% remission) or trough (56% remission) testing ($P = 0.026$). Antibody to infliximab was found in 14 patients with 5 occurring in the first year of therapy. Furthermore, 71% of patients with antibody to infliximab that were switched to an alternative anti-tumor necrosis factor achieved clinical remission at six months. In multivariable regression analysis, we found IFX dose (mg/kg), IFX dosing frequency (weeks), and the erythrocyte sedimentation rate at the previous infusion were significantly associated with the IFX concentration.

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

Key Words: antibodies to infliximab, Crohn disease, inflammatory bowel disease, infliximab concentration

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What Is Known

- Although there is a high primary response rate to infliximab in pediatric Crohn disease, up to 40% of patients 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.

What Is New

- Fifty-two percent of the patients with subtherapeutic infliximab levels or antibodies to infliximab were presently receiving an intensified (≥7.5 mg/kg) infliximab dose.
- Routine use of inflammatory biomarkers (erythrocyte sedimentation rate, albumin) with infliximab infusions can prioritize infliximab concentration monitoring because 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 disease.

The inflammatory bowel diseases (IBD), Crohn disease (CD), and ulcerative colitis (UC) are chronic gastrointestinal conditions with noted increases of proinflammatory cytokines because 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% to 40% of initial responders will lose response with time, whereas 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-TNFα) leading to chronic intestinal

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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 before 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 because 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 immunosorbent assay (ELISA), radioimmunoassay, and the homogeneous mobility shift assay (HMSA) 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. Because of the IFX drug interference, the ELISA, however, 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. With the potential paradigm shift toward more frequent IFX testing in asymptomatic patients and the rising cost of TDM for IFX, we retrospectively reviewed our practice management of TDM in patients with CD 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 used 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.

METHODS

Subjects

We performed a single-center retrospective study of patients with CD receiving recurring IFX infusions that had either the Prometheus ELISA or Anser^{TM} test sent from December 2009 to September 2013. The study was approved by the Institutional Review Board at Cincinnati Children's Hospital Medical Center. We reviewed the chart of every patient with CD who had TDM for an IFX concentration during this time and included those patients meeting criteria for suspicion for an infusion reaction or mid-interval and/or trough level. Data extracted from electronic medical records included subject's age, sex, race, weight, IFX dose (mg/kg), age at diagnosis, duration of CD, length of CD until IFX was started, time on IFX therapy, present medications, and the results of routine laboratory testing before initiating IFX induction therapy, at the IFX infusion before TDM, and at the time TDM was performed. Routine laboratory monitoring included a complete blood count with automated cell differential, liver profile, and albumin with the nonspecific 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 of the 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 to 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 nonresponse 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 and 1 μg/mL, respectively, whereas 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. Because there was no formal protocol for TDM, we divided the IFX testing into mid-interval and 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, whereas we defined trough samples as those collected immediately before 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^{TM}) for all variable infusion regimens. We based our cut-off for a therapeutic mid-interval IFX level on the Baert et al (15) pharmacokinetic study because they found the median mid-interval IFX level in patients with CD receiving every 8-week infusions as 12 μg/mL. ATI status is reported qualitatively (detected, not detected) because we were not able to compare quantitative ATI values given the different laboratory techniques used 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, Vienna, Austria). 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 t test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. The Fisher exact test was used for comparison of categorical data. Receiver operating characteristic 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 used a linear mixed regression analysis for our multivariable model and performed the likelihood ratio test to attain P values. $P < 0.05$ were considered statistically significant.

TABLE 1. Baseline patient demographics

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

6MP = 6-mercaptopurine; IFX = infliximab; IM = immunomodulator; MTX = methotrexate; SD = standard deviation.

RESULTS

During the 4-year period, 191 IFX concentration tests were sent on 72 patients with CD. Most 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 secondary loss of response to IFX (72%), primary nonresponse to IFX (7%, including levels drawn before the fourth infusion), and studying 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 of 72 (25%) patients were receiving concurrent immunemodulators (IM), either 6MP or methotrexate (MTX), at the time of TDM. The median time on IFX before 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 before initial IFX testing.

Infliximab Trough Concentration

Altogether, there were 140 IFX trough concentration tests from 55 patients with CD. We found 24% of TDM were subtherapeutic (undetectable), whereas 38% were <3 μg/mL and 10 ATI events (2 of 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) μg/mL compared with a median trough of 5.2 (2.4–14) μg/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 of 47 (28%) were subtherapeutic, 47% were <3 μg/mL, and 6 of 42 had ATI.

Infliximab Mid-Interval Concentration

Most published data for TDM during IFX therapy has focused on trough concentrations. We, however, found that 51 of 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 5 incidents of ATI. The median (min-max) days on IFX until undetectable mid-interval levels were discovered (including 5 ATI events) were 313 (41–1653) days. In addition, we found 68% of the 41 mid-interval levels obtained from patients receiving IFX every 8 weeks were subtherapeutic (<12 μg/mL) with 18 of 28 occurring during the first year of therapy.

Antibody to Infliximab

The true incidence of ATI in our pediatric population with CD 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 60% of our entire CD population with CD receiving 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 of 72 (19%) of the patients with CD had ATI events (there was a total of 15 events as 1 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 with 33% of all HMSA samples. We, however, found a 20% rate of ATI detection with ELISA testing

TABLE 2. Drug monitoring results by testing indication

Testing indication	Subtherapeutic	Therapeutic	Antibody to infliximab
Primary nonresponse	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 <12 μg/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)‡,*
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 5 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. Disease activity at the time of drug monitoring was compared with the disease activity at 6 months following the change in therapy by the Fisher exact test. 6MP = 6-mercaptopurine; A = active; IFX = infliximab; MTX = methotrexate; PGA = physician global assessment; Q = quiescent.

* <0.05.

† <0.01.

‡ One patient with antibody to infliximab was lost to follow-up.

after excluding tests with a detectable IFX concentration because ATI discovery with this technique is limited as noted above ($P = 0.34$ compared with HMSA testing). Two of the 15 ATI-positive samples were associated with a detectable IFX concentration (1.2 and 1.3 μg/mL, respectively, both HMSA samples), whereas 5 of 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 of 14 patients with ATI were receiving concurrent IM at the time of TDM. Overall, TDM led to 12 of 14 patients with ATI receiving an alternative anti-TNF immediately after ATI detection with the remaining 2 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 of 60 in this group were receiving an intensified IFX regimen before TDM, 10 of 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 8 patients with ATI. Evaluating all 137 drug monitoring tests this group had performed, we found 28 of 101 trough and 9 of 36 mid-interval levels were undetectable with 12 ATI events (11 patients, 2 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 of 60 patients with the majority receiving an intensified (n = 19) IFX regimen, 6 were switched to an alternative biologic, 3 had IM added, and 1 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 6 months. In comparison, 74% (23/31) were in clinical remission at 6 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, whereas only 37% were PGA-active 6 months following regimen intensification ($P < 0.001$). The 6-month clinical remission rate in patients who had IFX intensification following their ''initial'' TDM was 72% (18/25) compared with 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 6 months) compared with patients who received intensification following trough TDM (53% PGA-quiescent at 6 months, $P = 0.026$). Overall, all therapy changes (IFX dose intensification, addition of 6MP 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 with 29% PGA-quiescent before TDM, $P < 0.001$).

Routine Laboratory Testing and TDM

We were interested in whether TDM could be prioritized by assessing the relation 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). In addition, we found subtherapeutic IFX trough concentrations were associated with elevations in ESR (Fig. 1). We also found that an ESR value ≥15 mL/h was significantly associated with an undetectable trough with a sensitivity of 78%, specificity of 70%, 43% positive predictive value, and a 90% negative predictive value (area under curve 0.70, 95% confidence interval 0.59–0.81, $P < 0.01$).

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/h	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, g/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 at the time of TDM or at the previous infusion before TDM. Drug levels were obtained either mid-interval or as a trough. Alb = albumin; ATI = antibody to infliximab; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; IFX = infliximab; PGA = physician global assessment.

*$P < 0.05$.

†$P < 0.01$.

‡$P < 0.001$.

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/hour before starting IFX compared with a mean (SD) ESR of 28 (19) mm/h in patients with a detectable trough or mid-interval level during this time ($P = 0.20$). We found the hematocrit before starting IFX was significantly lower in this group because patients with undetectable IFX concentrations had a mean hematocrit of 33% (SD 6.6) compared with a mean hematocrit of 37% (SD 3) in patients who were found to have a detectable level ($P = 0.015$) during the first year. Preinduction CRP, platelet count, or albumin were not 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 patients with CD. 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 before 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 before 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 before TDM and were included in this analysis. The model found that IFX trough concentration could be calculated with the following equation: 25.1 (β_0) + 0.5 (β_1) × IFX dose (mg/kg)−2.6 (β_2) × frequency (weeks)−0.15 (β_3) × ESR (mm/h). 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 before TDM significantly influenced the IFX trough concentration ($x^2(1) = 217.5$, $P < 0.001$) compared with a model that only included IFX dosing frequency and IFX dose.

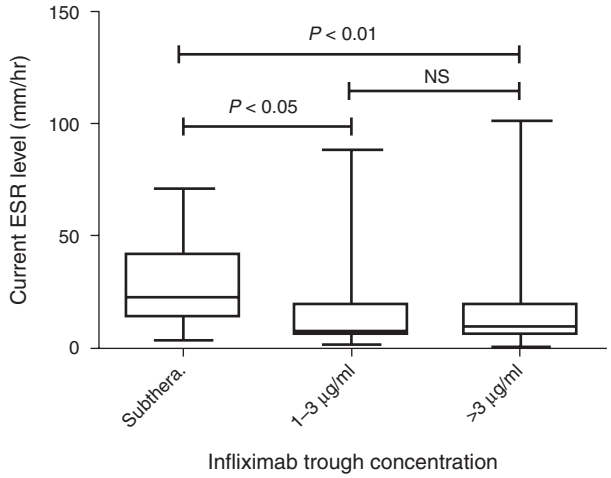

FIGURE 1. Subtherapeutic infliximab trough concentrations are significantly associated with ESR elevations. ESR was obtained at the same time as infliximab drug monitoring. The data were evaluated by Kruskal-Wallis with Dunn posttest analysis. ESR = erythrocyte sedimentation rate; NS = nonsignificant; Subthera = subtherapeutic subtherapeutic trough.

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 (13,31,32). We performed this large retrospective review to investigate the number of occurrences of subtherapeutic IFX levels in those patients with CD 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% to 23% in patients with IBD receiving routine maintenance infusions and as high as 61% in adult patients with IBD receiving episodic treatment (13,33). In addition, studies have found a decreased incidence of ATI when anti-TNF therapy is combined with an IM (31,34,35). Despite the promising results reported by the SONIC trial in which combination IFX-6MP therapy was associated with increased rates of mucosal healing compared with IFX monotherapy (44% vs 30%) in CD (36), combination therapy (6MP-IFX) is less commonly used in the pediatric population with IBD because 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 (37); we reserve combination 6MP-IFX therapy for severe, refractory disease. In addition, an analysis of combination MTX-IFX was found to be no more effective (equal rates of treatment failures) than IFX monotherapy in patients with CD (38). Although the primary outcome measurement was similar, Feagan et al (38) did find rates of ATI were significantly lower in the group that received MTX-IFX compared with IFX monotherapy. In our study, we found 14 of 72 patients with CD had ATI (ELISA and HMSA) following TDM. The true incidence of ATI in our pediatric population with IBD, however, remains unknown because 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 patients with CD at our center had TDM. It is worth noting there was no difference in the rate of ATI detection (33% by HMSA) between the 2 testing methods (20% by ELISA) when we controlled for samples with undetectable concentrations. As our experience grows

with more sensitive TDM methods, our center, however, has been compelled to systematically address ATI in the setting of detectable IFX levels.

It is worth noting that ATI was found in 14 patients, yet only 2 were receiving combination IFX-IM therapy. Overall, combination therapy (IFX-IM) was used in 18 of 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 (34) found that in 83 patients with IBD with ATI, the ATI resolved in two-thirds of patients with a noted clinical response after a median of 4 (range 3–5) infusions. As the sensitivity of the assays change, our reflexive response to abandon the present anti-TNF agent in the setting of ATI has also undergone significant modifications. We largely focus our clinical decisions to ATI on disease activity at the time of TDM, the IFX level in relation to timing (mid-interval vs trough), and the intensity of ATI. For example, a patient with asymptomatic CD 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 (33) previously found that empiric intensification of IFX therapy (without IFX concentration or ATI testing) led to clinical improvement in 69% of the 76 patients with IBD with the majority (89%) achieving clinical remission by 6 months following drug intensification. 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, because clinical decisions were blinded to the results of the assay in the Pariente et al (33) study, they found that the 60% of the ATI-positive patients responded to empiric intensification by 4 to 8 weeks, and this response persisted up to 6 months. This phenomenon of overwhelming anti-TNF antibodies to drug has been documented in patients with rheumatoid arthritis receiving adalimumab. Although patients with rheumatoid arthritis with antibodies to adalimumab had higher nonresponse rates, 30% of patients with drug antibodies no longer had antibodies after dose intensification (39,40).

Various studies have attempted to develop the ideal IFX trough cut point that maximizes anti-TNF response. Levesque et al (30) found that IFX trough concentrations <3 μg/mL were significantly associated with active disease (>70 point increase in the mean Crohn's Disease Activity Index score between infusions) and a higher probability of a CRP >5 mg/L, whereas Marits et al (41) found that patients with a trough of ≥4.1 μg/mL were more likely to experience clinical remission. In a pediatric population with IBD, Singh et al (24) found that a week 14 IFX level (first maintenance dose) of ≥5 μg/mL had a positive predictive value of 83%, negative predictive value of 53%, and an area under curve of 0.68 for persistent remission. They also found that subjects with IBD with persistent remission had a significantly higher median week 14 IFX level (4.7 vs 2.6 μg/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 population with CD 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 postinduction 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 (42) found that predictors of durable sustained response to maintenance IFX included a week 14 trough >3.5 μg/mL and a >60% decrease in CRP in those with an elevated baseline CRP (>8 mg/L). 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) (43). They found no significant association between ESR or CRP and IFX levels during IFX induction (43). In contrast, Singh et al (24) found that week 14 body mass index and CRP improved their predictive analysis of persistent remission compared with week 14 IFX level alone. 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 before 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 patients with CD 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 before 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 of TDM was the clinicians' concern for secondary loss of response (higher probability of subtherapeutic levels) that limits uncovering the true incidence of ATI and subtherapeutic IFX levels in our pediatric cohort with CD because there was not a specific protocol for TDM. Secondly, the majority of those having TDM were performed with the ELISA method that 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 patients with CD who had TDM, we found subtherapeutic IFX concentrations and ATI were relatively common in our pediatric cohort with CD. We found that symptomatic patients with CD 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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REFERENCES

1. Abraham C, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009;361:2066–78.
2. Stange EF, Travis SP, Vermeire S, et al. European evidence-based consensus on the diagnosis and management of ulcerative colitis: definitions and diagnosis. *J Crohns Colitis* 2008;2:1–23.
3. Van Assche G, Dignass A, Reinisch W, et al. The second European evidence-based consensus on the diagnosis and management of Crohn's disease: special situations. *J Crohns Colitis* 2010;4:63–101.
4. Targan SR, Hanauer SB, van Deventer SJ, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med* 1997;337:1029–35.
5. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005;353:2462–76.
6. van Dullemen HM, van Deventer SJ, Hommes DW, et al. Treatment of Crohn's disease with anti-tumor necrosis factor chimeric monoclonal antibody (cA2). *Gastroenterology* 1995;109:129–35.
7. Hyams J, Damaraju L, Blank M, et al. Induction and maintenance therapy with infliximab for children with moderate to severe ulcerative colitis. *Clin Gastroenterol Hepatol* 2012;10:391–9e1.
8. Hyams J, Crandall W, Kugathasan S, et al. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. *Gastroenterology* 2007;132:863–73quiz 1165–1166.
9. Schnitzler F, Fidder H, Ferrante M, et al. Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. *Gut* 2009;58:492–500.
10. Chaparro M, Guerra I, Munoz-Linares P, et al. Systematic review: antibodies and anti-TNF-alpha levels in inflammatory bowel disease. *Aliment Pharmacol Ther* 2012;35:971–86.
11. Gisbert JP, Panes J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. *Am J Gastroenterol* 2009;104:760–7.
12. Hanauer SB, Feagan BG, Lichtenstein GR, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002;359:1541–9.
13. Afif W, Loftus EV Jr, Faubion WA, et al. Clinical utility of measuring infliximab and human anti-chimeric antibody concentrations in patients with inflammatory bowel disease. *Am J Gastroenterol* 2010;105:1133–9.
14. Allez M, Karmiris K, Louis E, et al. Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: definitions, frequency and pharmacological aspects. *J Crohns Colitis* 2010;4:355–66.
15. Baert F, Noman M, Vermeire S, et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med* 2003;348:601–8.
16. Cassinotti A, Travis S. Incidence and clinical significance of immunogenicity to infliximab in Crohn's disease: a critical systematic review. *Inflamm Bowel Dis* 2009;15:1264–75.
17. Ainsworth MA, Bendtzen K, Brynskov J. Tumor necrosis factor-alpha binding capacity and anti-infliximab antibodies measured by fluid-phase radioimmunoassays as predictors of clinical efficacy of infliximab in Crohn's disease. *Am J Gastroenterol* 2008;103:944–8.
18. Billioud V, Sandborn WJ, Peyrin-Biroulet L. Loss of response and need for adalimumab dose intensification in Crohn's disease: a systematic review. *Am J Gastroenterol* 2011;106:674–84.
19. Maser EA, Villela R, Silverberg MS, et al. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. *Clin Gastroenterol Hepatol* 2006;4:1248–54.
20. Seow CH, Newman A, Irwin SP, et al. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. *Gut* 2010;59:49–54.
21. Miheller P, Kiss LS, Lorinczy K, et al. Anti-TNF trough levels and detection of antibodies to anti-TNF in inflammatory bowel disease: are they ready for everyday clinical use? *Expert Opin Biol Ther* 2012;12:179–92.
22. Farrell RJ, Alsahli M, Jeen YT, et al. Intravenous hydrocortisone premedication reduces antibodies to infliximab in Crohn's disease: a randomized controlled trial. *Gastroenterology* 2003;124:917–24.
23. Dubinsky MC, Lamothe S, Yang HY, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology* 2000;118:705–13.
24. Singh N, Rosenthal CJ, Melmed GY, et al. Early infliximab trough levels are associated with persistent remission in pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2014;20:1708–13.
25. Eser A, Primas C, Hauenstein S, et al. Comparison of early measurement of infliximab and antibodies-to-infliximab serum levels with standard trough analysis. *Gastroenterology* 2013;144:S779–879.
26. Wolf DC, Lockton S, Hauenstein S, et al. A multi-center observational study in community gastroenterology practices evaluating the clinical usage of testing for serum levels of infliximab and antibodies to infliximab. *Gastroenterology* 2013;144:S423–523.
27. Wang SL, Ohrmund L, Hauenstein S, et al. Development and validation of a homogeneous mobility shift assay for the measurement of infliximab and antibodies-to-infliximab levels in patient serum. *J Immunol Methods* 2012;382:177–88.
28. Steenholdt C, Ainsworth MA, Tovey M, et al. Comparison of techniques for monitoring infliximab and antibodies against infliximab in Crohn's disease. *Ther Drug Monit* 2013;35:530–8.
29. Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011;17:1314–21.
30. Levesque BG, Greenberg GR, Zou G, et al. A prospective cohort study to determine the relationship between serum infliximab concentration and efficacy in patients with luminal Crohn's disease. *Aliment Pharmacol Ther* 2014;39:1126–35.
31. Baert F, De Vos M, Louis E, et al. Immunogenicity of infliximab: how to handle the problem? *Acta Gastroenterol Belg* 2007;70:163–70.
32. Nanda KS, Cheifetz AS, Moss AC. Impact of antibodies to infliximab on clinical outcomes and serum infliximab levels in patients with inflammatory bowel disease (IBD): a meta-analysis. *Am J Gastroenterol* 2013;108:40–7quiz 48.
33. Pariente B, de Chambrun GP, Krzysiek R, et al. Trough levels and antibodies to infliximab may not predict response to intensification of infliximab therapy in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012;18:1199–206.
34. Steenholdt C, Al-khalaf M, Brynskov J, et al. Clinical implications of variations in anti-infliximab antibody levels in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012;18:2209–17.
35. Ungar B, Chowers Y, Yavzori M, et al. The temporal evolution of antidrug antibodies in patients with inflammatory bowel disease treated with infliximab. *Gut* 2014;63:1258–64.
36. Colombel JF, Sandborn WJ, Reinisch W, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010;362:1383–95.
37. Kotlyar DS, Osterman MT, Diamond RH, et al. A systematic review of factors that contribute to hepatosplenic T-cell lymphoma in patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2011;9:36–41e1.
38. Feagan BG, McDonald JW, Panaccione R, et al. Methotrexate in combination with infliximab is no more effective than infliximab alone in patients with Crohn's disease. *Gastroenterology* 2014;146:681–8e1.
39. Bartelds GM, Krieckaert CLM, Nurmohamed MT, et al. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. *JAMA* 2011;305:1460–8.

40. Bartelds GM, Wijbrandts CA, Nurmohamed MT, et al. Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. *Ann Rheum Dis* 2007;66:921–6.
41. Marits P, Landucci L, Sundin U, et al. Trough s-infliximab and antibodies towards infliximab in a cohort of 79 IBD patients with maintenance infliximab treatment. *J Crohns Colitis* 2014;8:881–9.
42. Cornillie F, Hanauer SB, Diamond RH, et al. Postinduction serum infliximab trough level and decrease of C-reactive protein level are associated with durable sustained response to infliximab: a retrospective analysis of the ACCENT I trial. *Gut* 2014;63:1721–7.
43. Hamalainen A, Sipponen T, Kolho KL. Serum infliximab concentrations in pediatric inflammatory bowel disease. *Scand J Gastroenterol* 2013;48:35–41.

Thomas Phaer on Infant Nourishment and Wet-Nurses

''You are what you eat''

Thomas Phaer (1510-1560), called by some the ''Father of English Pediatrics,'' in 1546 published, in English, a text entitled *The Regiment of Lyfe* in which he commended that a good life begin with maternal nursing or the choosing a good wet-nurse. A passage is reproduced below as written followed by a more liberal rendition with modern spellings.

. . .many wise philosophers. . .affirmeth that yf
lambes be nourished with ye milke of goates
they shall have course wolle like the heare of
goates, and yf kiddes in luke maner sucke upon
shepe ye heare of them shall be soft like wolle . . ..
Wherfore as it is agreeing to nature so is it also
necessarie & comly for the own mother to nource
the own child. Whiche yf it maye be done it shal
be most commendable and holsome, yf not, ye
must be well advised in taking of a nource, not
vil complexion and of worse maners, but such as
chearefull, so that she may accustome the infant
unto myrth, no drunkard, vycyous nor sluttyshe,
for suche corrupteth the nature of the chylde.
But an honest woman (such as had a man chyld
last afore) is best, not within two monethes after her
delyveraunce no approaching nere unto her time againe.[1]

1. Many wise philosophers. . .affirm that if lambs are nourished by goatmilk their wool will be coarse like the hair of goats and if kids nurse upon sheep the hair shall be soft like wool. . ..as this is agreeable in nature, so to it is necessary and comely for a mother to nurse her own child. This is most commendable and wholesome, [but if it is not possible] you must be well advised in choosing a wet-nurse, not of vile complexion and bad manners, but cheerful so that she may accustom the child to mirth, [she should not be] drunkard, vicious or slut-like, for this corrupts the nature of the child. The wet-nurse should be an honest woman who gave birth to a boy within the past two months and who is not pregnant again.

—Submitted by Angel Rafael Colón