Therapeutic Drug Monitoring of TNF Antagonists in Inflammatory Bowel Disease

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Abstract: Although tumor necrosis factor (TNF)-α antagonists play a critical role in the treatment of moderate-to-severe inflammatory bowel disease (IBD), several factors can impact treatment response. The degree of systemic inflammation, serum albumin concentration, disease type, body mass index, gender, concomitant therapy with immunosuppressive agents, and especially development of antidrug antibodies (ADAs) are key determinants of TNF antagonist pharmacokinetics and clinical outcomes. Therefore, measurement of serum drug and antibody concentrations in patients with IBD who are on TNF antagonists has the potential to guide clinical decision-making, optimize treatment, improve outcomes, and reduce healthcare costs. Multiple strategies to prevent ADA formation exist, including multiple clinical algorithms that employ therapeutic drug monitoring to optimize treatment following a secondary loss of therapeutic response. An individualized approach is needed, however, to identify early predictors of ADA development and other confounders of TNF antagonist therapy.

Tumor necrosis factor (TNF)-α antagonists play a critical role in the management of patients with moderate-to-severely active inflammatory bowel disease (IBD).[1-3] Since limited treatment options exist for these patients, optimization of these agents is highly important. However, approximately 40% of patients who initially respond to TNF antagonists eventually lose response.[4] Loss of response is a complex and multifactorial problem because patients fail treatment for many reasons, including inadequate drug exposure,[3,5,6] sensitization,[5,7,8] development of other disease processes,[9] and poor adherence.[9] Given this complicated environment, decision-making based exclusively on assessment of symptoms and conventional diagnostic tests may result in suboptimal outcomes.[10]

In recent years, evidence has accumulated that an inadequate serum drug concentration, due to individual variances in the pharmacokinetics (PK) of TNF antagonists resulting from either sensitization or other factors, is an important cause of treatment failure.[8] The considerable heterogeneity that exists in the PK of infliximab

(Remicade, Janssen), adalimumab (Humira, AbbVie), and certolizumab pegol (Cimzia, UCB) suggests that optimal therapy might ultimately require individualized dosing. Although population-based studies have identified multiple determinants of PK,[8] robust predictive models that would facilitate individualized dosing algorithms are currently lacking. Nevertheless, the recent availability of commercial assays to measure serum drug concentrations and antidrug antibodies (ADAs) has provided gastroenterologists with a new means to optimize the use of TNF antagonists with conventional dose regimens.[9,11-16]

Substantial evidence supports the value of therapeutic drug monitoring (TDM) in both Crohn's disease (CD) and ulcerative colitis (UC).[3,5,7,17-23] Although multiple indications have been proposed for TDM, the most compelling is for the management of patients with secondary loss of response to TNF antagonists.

Heterogeneity in the Pharmacokinetics of Tumor Necrosis Factor Agonists

Although our experience with TNF antagonists in the treatment of IBD spans more than 20 years, the heterogeneity that exists among individuals regarding the PK of these drugs has only recently been recognized. Although the causes of this variability are incompletely understood, several factors are known to be critical. Specifically, the development of ADAs, degree of systemic inflammation as determined by the serum C-reactive protein (CRP) or plasma TNF concentration, serum albumin concentration, disease type, body mass index, gender, and concomitant therapy with immunosuppressive agents have emerged as key determinants of PK.

Antidrug Antibodies

The humoral immune system generates high affinity antibodies against specific microbial proteins. Administration of foreign antigens, such as monoclonal antibodies, as drugs can also lead to sensitization through stimulation of these protective mechanisms.

Antigen recognition and binding to B-cell receptors[24] stimulate cells with the greatest antigen affinity to proliferate[25] and produce specific antibodies that can reduce the efficacy of TNF antagonists[5,7,19,26,27] through either (1) formation of immune complexes that result in accelerated drug clearance and suboptimal serum drug concentrations,[28] (2) neutralization of biologic activity, or (3) immune-mediated adverse reactions, such as serum sickness, that lead to discontinuation of therapy.[29,30] Sensitization has been observed with all biologic drugs, including recombinant insulin, growth hormone, granulocyte macrophage colony-stimulating factor, factor VIII, erythropoietin, interferon,[28] and monoclonal antibodies. Although technologic advances have enabled the creation of chimeric, humanized, and fully human antibodies, the challenge of sensitization remains.[31] Empiric experience has clearly demonstrated that fully human antibodies are capable of inducing ADAs, with negative clinical consequences.[28]

Degree of Systemic Inflammation

TNF antagonists undergo elimination through proteolysis in the reticuloendothelial system (RES).[32,33] Increased inflammatory activity, as measured by serum CRP, is associated with more rapid clearance of drug,[34-36] possibly as a consequence of enhanced proteolytic activity by the tissue macrophages that constitute the RES. Accordingly, patients with a greater inflammatory burden may require higher drug concentrations. Furthermore, a complementary mechanism also may be relevant in patients with a high inflammatory burden. High concentrations of soluble TNF may saturate standard doses of TNF antagonists. This "antigen sink" results in inadequate tissue drug concentrations and poor control of inflammation.

In support of this notion, several studies have demonstrated an inverse correlation between plasma TNF concentrations and clinical efficacy of TNF antagonists in patients with rheumatoid arthritis (RA)[37-39] and IBD. Experienced clinicians also will recognize that high serum concentrations of CRP are uniformly present in hospitalized patients with severe UC. It is noteworthy that the presence of accelerated drug clearance and low drug concentrations is highly prevalent in these patients. TNF is a major driver of interleukin-6 production, which, in turn, upregulates CRP production by the liver. Since TNF has a relatively short half-life (approximately 6 hours), daily measurement of CRP is a potential biomarker to guide infliximab therapy.[40]

Serum Albumin Concentration

Patients with UC or CD with low serum albumin concentrations have both lower trough drug concentrations and lower remission rates following treatment with infliximab.[36,41] Although the exact mechanism for this interaction is unknown, the clearance of monoclonal antibodies and albumin occurs through the same receptor-mediated pathway in the RES.[33] The Brambell receptor (FcRn), which is primarily expressed on vascular endothelial and RES cells,[42] binds albumin and immunoglobulin (Ig) G antibodies[43] and subsequently prolongs their half-lives[44] by preventing their degradation in lysosomes (Figure 1).[32] These receptors become saturated at high concentrations of IgG antibodies or albumin. Although the precise mechanism responsible for the relationship between low albumin concentrations and accelerated drug clearance is unknown, one possibility is the development of enhanced binding of FcRn to albumin in response to hypoalbuminemia, resulting in greater protein catabolism of globulins, including monoclonal antibodies.

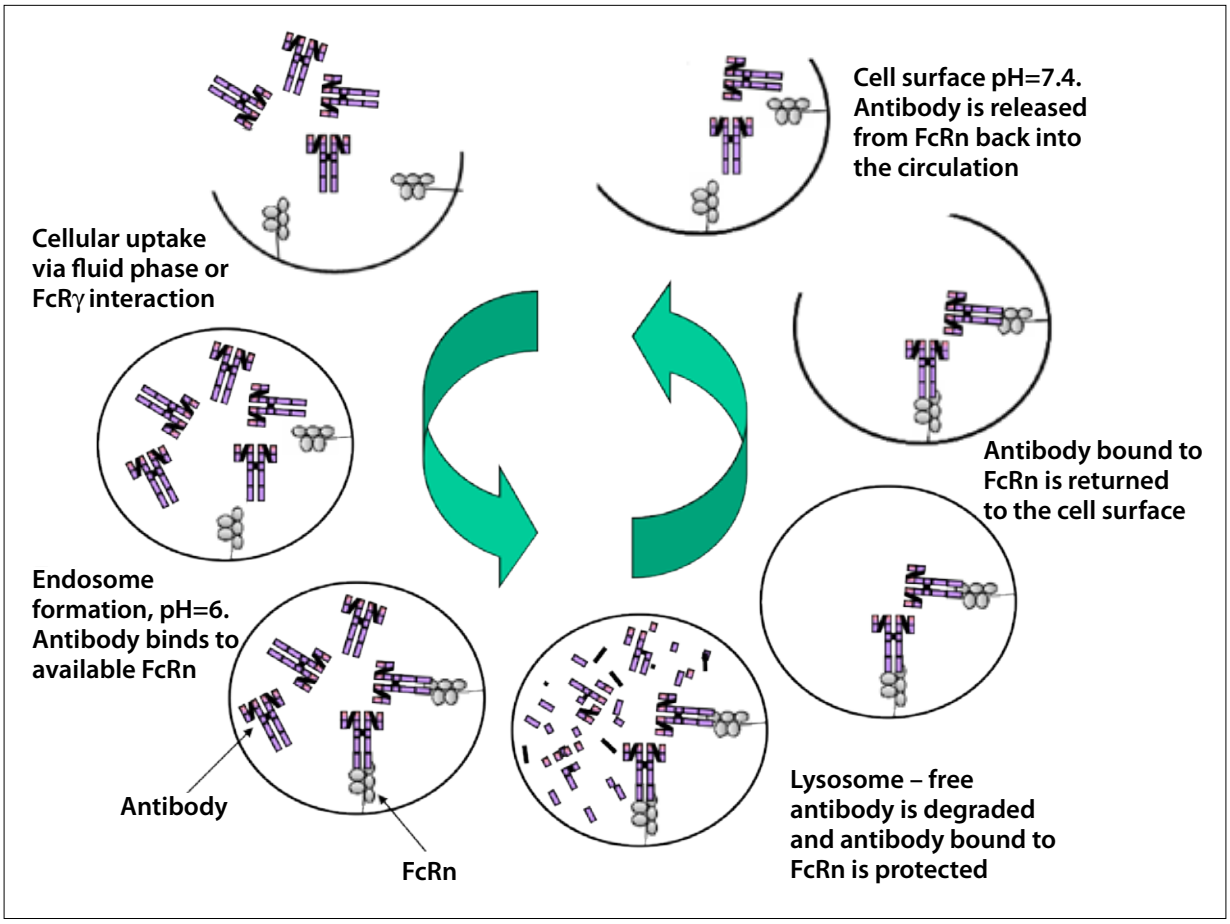

Figure 1. A schematic of the Brambell receptor (FcRn) antibody salvage.

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Disease Type

The proportion of patients with undetectable infliximab concentrations is higher among patients with severely active UC than in those with CD.[5,7] Although this difference has been attributed to the presence of both a higher inflammatory burden and low serum albumin concentration, high stool concentrations of infliximab have recently been observed in these patients,[45] indicating the possibility of a protein-losing colopathy in which monoclonal antibodies and other endogenous antibodies are excreted in the stool. Accordingly, differences in the extent of surface area affected by disease might explain the observed differences in PK between UC and CD.

Body Size

Weight-based dosing of TNF antagonists does not reliably predict patient drug exposure because the relationship between weight and drug clearance is nonlinear.[34,46] These variations in clearance are greatest in patients with low weight and severe inflammation.[34,46] An elevated body mass index has been associated with poor clinical outcomes with infliximab therapy in patients with RA.[47] The production of proinflammatory cytokines by adipose tissue has been proposed as a potential mechanism to explain the higher drug requirements observed in these patients.[48]

Gender

Although gender has been cited as an independent predictor of TNF antagonist clearance,[34,46] this factor is confounded by weight (ie, male gender is associated with both greater drug clearance and greater weight). Further research is required to determine whether these factors are independent predictors of PK.

Concomitant Immunosuppressive Therapy

Concomitant immunosuppressive therapy can reduce clearance of TNF antagonists, both through the prevention of ADAs and likely through direct effects on the RES. (See the section below on prevention of ADAs.)

Table 1. Assays for the Measurement of TNF Antagonist and ADA Levels

	ELISA[46]	HMSA[51]	RIA[52]
Capture Moiety	– Plate-bound to prevent loss during washing steps – Drug used to capture ADAs; TNF-α used for TNF antagonists	– Initial acid-dissociation step separates drug and ADAs in the serum – Dye-labelled capture moiety – Drug used to capture ADAs; TNF-α used for TNF antagonists	– Radioactive capture agents – Drug used to capture ADAs; TNF-α used for TNF antagonists
Process	– Serum with the target molecule is incubated with the plate to facilitate capture-target binding – Washing step removes excess serum – Addition of a color-producing anti–IgG-HRP conjugate	– Initial acid-dissociation step enables detection of both entities – Dye-labelled capture agent is added to the serum – Separation of the free-capture agent and capture-target complex on a size-exclusion HPLC column	– Incubates a known concentration of serum with a radiolabelled capture moiety – Addition of anti-Fc antibody – Centrifugation precipitates target-capture complexes – Capture moiety is separated from the drug/ADA complex with chromatography columns lined with anti-lambda light chains – Columns bind ADAs and other lambda-containing molecules
Quantification of Target	– Estimated by measuring the intensity of color OR – The greatest dilution at which antibodies cannot be detected	– Estimated by measuring the intensity of color	– Quantification of radioactivity determines concentration of drug/ADA
Advantages	– Widely used – Inexpensive – Easily performed	– Allows quantification of ADAs in the presence of drug	– Requires greater technique to perform
Limitations	– Inability to detect ADAs in the presence of drug, as ADAs are bound in ADA/drug complexes that evade detection – Reported as inconclusive	– Less widely available – Less frequently used in clinical trials	– Less widely available – Less frequently used in clinical trials
Special Features	– Only able to quantify free ADA and drug concentrations – Total drug and ADA concentrations cannot be determined because drug/ADA complexes evade detection – Thresholds for positive tests based on detection limit of the assay	– Acid-dissociation step – Estimate of the total serum concentration of target (free and bound in ADA/drug complexes) – Eliminates inconclusive tests	– Infliximab is not bound to columns because it is an IgG antibody with kappa light chains
Comparison[78,86]	– Infliximab detection limit: 0.27 μg/mL – Patients with detectable infliximab/ADA at the time of secondary loss of response: 75%/9%	– Patients with detectable infliximab/ADA at the time of secondary loss of response: 88%/27%	– Infliximab detection limit: 0.07 μg/mL – Patients with detectable infliximab/ADA at the time of secondary loss of response: 88%/34%

ADA, antidrug antibody; ELISA, enzyme-linked immunosorbent assay; HMSA, homogeneous mobility shift assay; HPLC, high-pressure liquid chromatography; HRP, horseradish peroxidase; Ig, immunoglobulin; RIA, radioimmunoassay; TNF, tumor necrosis factor.

Summary

Multiple determinants of PK exist, and they are linked to clinical outcomes through their effects on drug concentrations. Given the heterogeneity of PK observed in patients treated with TNF antagonists, measurement of serum drug and antibody concentrations has the potential to inform clinical decision-making.

Drug and Antidrug Antibody Assays

The most commonly used tests to measure serum drug concentrations and ADAs are plate-based enzyme-linked immunosorbent assays (ELISAs),[19,21,49,50] high-pressure liquid chromatography–based homogeneous mobility shift assays (HMSAs),[51] and fluid-based radioimmunoassays (RIAs).[52,53]

Although these tests have unique operating properties, they are based on 3 common principles: (1) identification of a capture moiety to bind the target molecule, (2) incubation of the capture moiety with a serum sample to facilitate capture-target binding, and (3) quantification of the captured drug or antibody. The properties of these assays are summarized in Table 1. A key distinction between the ELISA-based tests and the other methods is that the former cannot detect ADAs in the presence of drug. Preliminary data suggest that the accuracy of ELISAs decreases below drug or antibody concentrations of 10 μg/mL. In addition, the ability of ELISAs to detect ADAs is compromised with the presence of as little as 1 μg/mL of drug, whereas HMSAs retain the ability to measure antibodies at serum drug titers of 60 μg/mL.[54] Although the pharmacodynamic (PD) consequences of coexistent drugs and ADAs are currently poorly understood, it is possible, and perhaps likely, that this situation may result in higher drug clearance through the formation of immune complexes.

New Assays

Recently, point-of-care assays[55] and tests for the detection of neutralizing antibodies[56] have been developed that facilitate immediate access to TDM. However, these technologies require further validation.

Timing of Sample Collection

Serum samples for measurement of infliximab are generally drawn 4 weeks postinfusion or at trough, meaning that the sample is drawn immediately before the next infusion.[19] However, according to a recent publication on the measurement of drug concentrations immediately after an infusion, the postinfusion concentration of infliximab (Cmax) might be a valuable predictor of clinical outcomes.[57] Continuous responders to infliximab had, on average, a higher Cmax than patients who lost response (149.5 μg/mL vs 126.3 μg/mL; P=.04). This approach has not been replicated and, thus, is not yet recommended for use in clinical practice.

Adalimumab is administered subcutaneously, which results in relatively stable plasma concentrations over the biweekly dosing interval[58] once steady state has been reached. As there is less variation in plasma drug concentrations, drug and ADA sampling is less time-dependent than for intravenously administered products. Although definitive measurement protocols have not been defined for either infliximab or adalimumab, trough sampling has become widely accepted for use in both clinical practice and trials.

Clinical Correlates of Differences in Drug Concentrations

Our understanding of the relationship between TNF antagonist concentrations and clinical efficacy has evolved

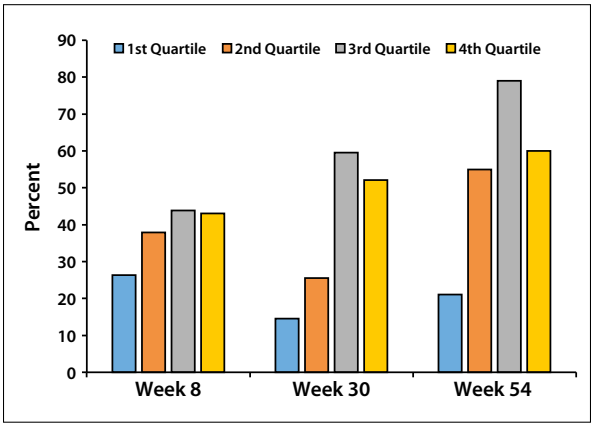

Figure 2. Serum infliximab concentration (μg/mL)/proportion of patients (%).

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over time.[2,3,5,7,19,21,59] In a landmark study in 2003, Baert and colleagues evaluated the efficacy of infliximab in 125 patients with CD[19] who received either single-dose induction therapy (patients with luminal CD) or 3-dose induction therapy (patients with fistulizing CD) and were followed until the disease relapsed, at which time they were re-treated. Those patients with a Week 4 serum infliximab concentration of 12 μg/mL or greater (Prometheus commercial ELISA) had a significantly longer median time to relapse than those with a lower concentration (81.5 days and 68.5 days, respectively; P<.01). A second retrospective cohort study conducted in patients with CD who were treated with infliximab[5] found that remission was maintained continuously in all patients who had detectable trough infliximab concentrations (Prometheus commercial ELISA).

In contrast, remission was only observed for 67% of the observation period in patients with undetectable trough infliximab concentrations (P<.01).[5] Similar results were also observed in SONIC (Study of Biologic and Immunomodulator Naive Patients in Crohn's Disease) and ACCENT I (A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-term Treatment Regimen in Patients With Fistulizing Crohn's Disease).[3,6] In SONIC, which compared combination therapy with azathioprine and infliximab to monotherapy with either agent, patients with inconclusive ADA tests (ie, detectable drug) were more likely to experience corticosteroid-free remission at Week 26 and Week 50 compared with patients with positive ADAs and an undetectable infliximab trough concentration (Week 26: 71.9% [133/185 patients] and 56.3% [9/16 patients]; Week 50: 78.7% [107/136 patients] and 57.1% [8/14 patients], respectively [Janssen research ELISA]). In a post hoc analysis of the ACCENT I results, patients with sustained

Table 2. Serum TNF Antagonist Concentrations That Have Been Reported to Predict Clinical Disease Activity

Study	Number of Patients	Time Point for TDM	Assay	Threshold, µg/mL
Infliximab				
Bortlik et al[66]	84	Trough	ELISA	3.0
Cornillie et al[6]	113 (ACCENT population)	Trough (Week 14)	ELISA	3.5
Arias et al[64]	135	Trough	ELISA	7.19
Lamblin et al[65]	40	Trough	ELISA	1.1
Hibi et al[67]	48	Trough	ELISA	1.0
Levesque (unpublished data, 2013)	327	Trough	ELISA	3.0
Feagan et al[68]	1487 samples from 483 patients	Trough	HMSA	3.0
Adalimumab				
Yarur et al[70]	66 (59 Crohn's disease patients)	Random	HMSA	5.0
Mazor et al[71]	121 patients/161 samples	94 trough samples	Unknown	5.0
Velayos et al[72]	54	Trough	HMSA	5.0

ACCENT, A Crohn's Disease Clinical Trial Evaluating Infliximab in a New Long-term Treatment Regimen in Patients With Fistulizing Crohn's Disease; ELISA, enzyme-linked immunosorbent assay; HMSA, homogeneous mobility shift assay; TDM, therapeutic drug monitoring; TNF, tumor necrosis factor.

response had a higher Week 14 serum infliximab concentration than nonresponders (4.0 µg/mL vs 1.9 µg/mL, respectively; *P*=.03) (Janssen research ELISA).[6]

Similar observations exist for UC. In a retrospective analysis of data from the placebo-controlled ACT (Active Ulcerative Colitis Trial) 1 and ACT 2,[27] which evaluated the efficacy of infliximab in 728 patients with moderate-to-severe UC, a strong correlation was observed between rates of clinical remission, response, and mucosal healing and higher infliximab concentrations when the data were analyzed by trough concentration quartiles (Janssen research ELISA)[23] (Figure 2). In addition, a retrospective cohort study demonstrated lower colectomy rates in patients with UC who had detectable drug at trough[7] in comparison with those without detectable infliximab (Prometheus commercial ELISA). The ELISA-based criterion of any detectable drug was further refined to a trough infliximab concentration of 2 µg/mL using HMSA, which is more sensitive.[60]

In the original ELISA-based study, a subset of hospitalized patients with severe disease had undetectable drug concentrations shortly after the loading doses of infliximab were administered, presumably due to the previously described factors that negatively influence PK in these patients. These patients had a very high risk of colectomy. It is not currently known whether more intensive infliximab dosing regimens can overcome this PK problem, and we speculate that small molecule–based therapies, such as calcineurin or Janus kinase antagonists, may prove to be optimal therapy for a subset of these patients in whom monoclonal antibody therapy is compromised by high drug clearance due to protein-losing colopathy or antigen sink.

Relatively less information is available regarding the relationship between adalimumab drug concentrations and clinical efficacy. Much of the information regarding PK/PD relationships for adalimumab has come from relatively small investigator-initiated studies. In 16 infliximab-naive patients with CD who were in remission following treatment with adalimumab, lower trough drug concentrations (mean drug concentration: 8.1 µg/mL [6.7-9.2], 5.3 µg/mL [5.2-6.1], 3.9 µg/mL [3.2-4.7], and 1.0 µg/mL [0.1-2.6]) were associated with higher Harvey-Bradshaw Index scores (4 [3-4], 6 [5-7], 9 [8-11], and 17 [16-17], respectively) and CRP concentrations (1 [0 to -4], 3 [1.5-6], 10 [3.5-18.7], and 18 [5.9-34.3], respectively; Matriks Biotek adalimumab ELISA).[61]

In a prospective analysis of 21 patients with CD who attained remission with adalimumab, the serum drug concentration was higher in the 16 patients who maintained therapy for 96 weeks (7.8 µg/mL) compared with the 5 patients who failed therapy (3.7 µg/mL; *P*=.0001). Adalimumab concentrations displayed good correlation with clinical disease activity, as measured by the Harvey-Bradshaw Index (r^2=0.6583; *P*<.001), but were not correlated to serum TNF titers (r^2=0.0084; *P*<.692) (Matriks Biotek adalimumab ELISA).[62] Similarly, a retrospective analysis of certolizumab pegol in the treatment of CD suggested that higher drug concentrations at Week 8 were associated with remission at Week 10 (*P*=.03) and Week 54 (*P*=.02).[63]

In summary, observational data consistently support a relationship between higher drug concentrations of both infliximab and adalimumab and better clinical outcomes. Although a causal relationship has not been confirmed by

a randomized controlled trial, target trough concentrations have emerged in the literature for both agents.

Target Serum Drug Concentrations

Defining the optimal trough concentration thresholds for infliximab and adalimumab is an active area of research. The majority of information is based on preliminary data and requires confirmation in definitive large-scale studies. Table 2 summarizes the potential target trough drug concentrations from the existing literature. These results have been generated using different assays, and the results should be interpreted cautiously. Based on Janssen research ELISA assessments of ACCENT I data, a Week 14 trough infliximab concentration of 3.5 μg/mL had a sensitivity, specificity, positive predictive value, and negative predictive value of 0.64, 0.78, 0.56, and 0.83, respectively, for sustained response.[6]

A separate analysis of receiver operating characteristic curves was performed in an attempt to define a discriminating concentration more precisely. The authors of this analysis suggested that an infliximab concentration of 7.19 μg/mL predicted sustained remission with 57% sensitivity and 80% specificity (Leuven research ELISA).[64] However, recent evaluations have suggested lower thresholds. Based on modeling, Lamblin and colleagues reported that an infliximab trough concentration of 1.1 μg/mL would lead to a 50% decrease in CRP, whereas a value of 5.6 μg/mL would result in CRP concentrations below 5 μg/mL (research ELISA).[65]

In another population of patients with CD, a trough infliximab concentration above the ELISA's threshold of 3 μg/mL was associated with sustained remission (Q-INFLIXI ELISA Quantitative Analyses, Matriks Biotek).[66] Hibi and colleagues administered 5 mg/kg of infliximab to a patient with CD at Weeks 0, 2, and 6 followed by maintenance doses every 8 weeks in the responders.[67] In patients with Crohn's Disease Activity Index–defined loss of response, the dosing interval was shortened to 4 weeks. Based on data from the 48 Week-14 responders, a trough infliximab concentration of less than 1 μg/mL was associated with loss of response and elevated CRP concentration (Janssen ELISA).[67]

In a study of 327 patients who received infliximab for maintenance of CD, a trough concentration of less than 3 μg/mL was most predictive of clinical and serologic disease activity (Prometheus commercial HMSA; unpublished data, Barrett G. Levesque 2013). Similarly, Feagan and colleagues analyzed 1487 serum samples from 483 patients with CD who participated in 4 prospective trials of infliximab maintenance therapy.[68] Paired samples obtained over time were used to assess the relationship between infliximab concentrations and CRP. A trough concentration of 3 μg/mL predicted subsequent disease activity (Prometheus commercial HMSA, Leuven research ELISA.)[68] In 52 patients who required dose escalation for secondary loss of response to infliximab, an increase of trough drug concentration by 0.5 μg/mL predicted mucosal healing (positive predictive value, 0.79; negative predictive value, 0.87; Lisa-Tracker Premium ELISA kit, BMD).[69]

Less data are available regarding the relationship between adalimumab concentrations and clinical outcomes. Preliminary data indicate that trough concentrations less than 5 μg/mL were more likely seen in patients with active disease than those in remission (Prometheus commercial HMSA).[70-72]

Although multiple attempts have been made to define drug thresholds that predict clinical disease activity,[6,64-66] these values have varied widely. In clinical trials, the thresholds generally reflect the detection threshold of the assay.[14]

Clinical Correlates of Antidrug Antibodies

The previously described study by Baert and colleagues also provided important information regarding the clinical implications of ADA formation.[19] The presence of ADAs was found to be an independent risk factor for loss of response. Patients with an ADA concentration of less than 8 μg/mL (Prometheus commercial ELISA) had a longer time to relapse than those with higher antibody concentrations (71 days and 35 days, respectively; $P<.001$). Concomitant treatment with immunosuppressive agents was identified as protective against development of ADAs.

In a subsequent retrospective analysis of 53 patients who were given episodic infliximab therapy,[73] higher ADA concentrations were noted in patients with loss of response compared with those who were continuous responders (0.7 μg/mL vs 8.9 μg/mL; $P<.0001$) (Prometheus commercial ELISA). Although a post hoc analysis of the ACCENT I study[22] found no relationship between clinical efficacy and the presence of ADAs, the dose-escalation design of the trial led to a high proportion of patients with inconclusive antibody tests (ie, detectable infliximab in their sample). The Janssen research ELISA used to assess ADAs in this study could not detect ADAs in the presence of infliximab. These factors may have obscured detection of any negative effects of ADAs.

Although CHARM (Crohn's Trial of the Fully Human Antibody Adalimumab for Remission Maintenance) did not assess patients for ADAs, other data sources have provided information regarding the immunogenicity of adalimumab. In CLASSIC (Clinical Assessment of Adalimumab Safety and Efficacy Studied as Induction Therapy in Crohn's Disease) II,[74] a 52-week, open-label, extension study that evaluated adalimumab for maintenance therapy in patients who had responded to 4 weeks of adalimumab

induction therapy, ADAs developed in only 2.6% (7 of 269) of patients (AbbVie research ELISA). However, as in the ACCENT I trial, patients whose disease activity worsened were allowed dose intensification. Again, the ELISA used by the investigators may have underestimated the prevalence of ADAs. Furthermore, selection of a responder population for evaluation also may have underestimated the overall rate of sensitization during induction.

In contrast to these data, a cross-sectional study of 54 patients with IBD treated with adalimumab detected ADAs in 22.2% of trough samples[72] using a Prometheus commercial HMSA. The presence of ADAs was independently associated with an elevated CRP concentration (*P*=.002). Further evidence regarding the immunogenicity of adalimumab has been derived from patients with RA. In a prospective cohort of patients with RA who were treated with adalimumab, ADAs developed in 28% (76 of 272) of patients, detected using a RIA, after 3 years despite concomitant administration of methotrexate in the majority of cases. ADAs and adalimumab were measured by RIAs and ELISAs, respectively. In patients in whom ADAs developed, 67% of ADAs were detectable during the first 28 weeks of therapy.

ADAs were associated with lower trough drug concentrations (median, 5 mg/L intraquartile range [IQR], 3-9 mg/L compared with 12 mg/L IQR, 9-16 mg/L; *P*<.001), decreased rates of sustained remission (4% compared with 34%; *P*<.001), and higher rates of treatment discontinuation (38% vs 14%; hazard ratio, 3.0; 95% CI, 1.6-5.5; *P*<.001).[75]

In the IBD literature, Yarur and colleagues performed TDM in 66 patients with IBD using a RIA and found that one-third of patients with detectable adalimumab concentrations also had ADAs (Prometheus HSMA).[70] The mean adalimumab concentration was lower in patients with ADAs (5.7 µg/mL compared with 12.5 µg/mL; *P*=.001) and in patients with persistent mucosal inflammation (8.5 µg/mL compared with 13.3 µg/mL; *P*=.02).

Collectively, these data suggest that the development of ADAs is associated with poor clinical outcomes.[20,68] Consequently, prevention of ADAs is an important area of interest for clinical investigation.

Prevention of Antidrug Antibodies

A landmark trial by Baert and colleagues[19] led to 3 observations: (1) an ADA concentration of less than 8 µg/mL was associated with a longer time to relapse than higher concentrations (71 days and 35 days, respectively; *P*<.001); (2) an infliximab concentration of 12 µg/mL or greater was associated with longer time to relapse than lower concentrations (81.5 days and 68.5 days, respectively; *P*<.01); and (3) concomitant immunosuppression with azathioprine, in episodically dosed patients, was associated with Week 4 drug concentrations of 12 µg/mL or greater.

In a multivariate analysis within a pivotal study by Farrell and colleagues, scheduled maintenance dosing and use of a concomitant immunosuppressive agent independently protected against the development of ADAs.[73] In a subsequent double-blind, placebo-controlled, single-center, randomized controlled trial, the development of ADAs was lower following administration of preinfusion intravenous hydrocortisone compared with placebo (26% and 42%, respectively; *P*=.06). The median ADA concentrations in these populations were 1.6 µg/mL and 3.4 µg/mL, respectively (*P*=.02; Prometheus commercial ELISA.)[73]

A post hoc analysis of the ACCENT I study suggested that concomitant immunosuppression resulted in lower rates of ADA formation, whereas episodic maintenance therapy was associated with the development of ADAs (8% vs 30%; odds ratio, 0.21; 95% CI, 0.13-0.36; *P*<.0001; Janssen research ELISA).[22]

The relationship between the coadministration of azathioprine and ADA formation was also examined in SONIC (Study of Biologic and Immunomodulator Naive Patients in Crohn's Disease).[3] In this study, concomitant use of azathioprine was associated with lower rates of ADA formation and higher infliximab trough concentrations (3.5 µg/mL vs 1.6 µg/mL; *P*<.001 at Week 30; Janssen research ELISA) compared with monotherapy. Similarly, COMMIT[59] (Combination of Maintenance Methotrexate-Infliximab Trial) evaluated the role of methotrexate for the prevention of ADAs. Patients with active CD who were treated with infliximab and corticosteroid induction therapy were randomly assigned to receive placebo or methotrexate. ADAs were less likely to develop in patients assigned to methotrexate (4% vs 20%; *P*=.01), had a higher median infliximab trough concentration (6.35 mg/mL vs 3.75 mg/mL; *P*=.08), and were more likely to have detectable drug at trough (52% vs 44%; *P*=.84) compared with the placebo group (Prometheus commercial HMSA).

However, it is not currently clear that all ADAs are equally relevant. Specifically, it is possible that some ADAs occur only transiently and at a lesser degree of clinical consequence. Furthermore, a concept has emerged that, in some instances, dose escalation of the TNF antagonist or addition of an immunosuppressive agent may overcome a sensitization response. In support of these theories, a recent study that evaluated 52 patients with CD and ADAs reported persistence of these antibodies in 38 (73%) patients.[76] In the remaining patients, the antibodies regressed spontaneously in 6 (43%) patients and regressed following dose optimization in 8 (57%) patients. It should be noted that even these lower titer and transient ADAs can increase drug clearance, and dose escalation of TNF antagonists to treat this problem comes at a significant economic cost.

Table 3. Categorization of Patients Based on ADA and Drug Concentrations

	ADA-negative	ADA-positive
Infliximab < threshold	Increase dose	Switch within class
Infliximab ≥ threshold	Switch out of class	Switch (high activity) OR monitor (low activity)

ADA, antidrug antibody.

In a subsequent multivariate regression analysis of 2021 serum samples, the presence of ADAs was independently associated with increased disease activity, as measured by an elevated CRP, despite the presence of adequate drug concentrations (Prometheus commercial HMSA).[68] These results suggest that, in the presence of ADAs, active disease may persist despite adequate drug titers.

Extensive experience with vaccines has shown that intermittent exposure to a foreign antigen results in sensitization, whereas continuous exposure is tolerogenic. Thus, maintenance of stable drug trough concentrations might be protective against ADA formation, whereas "dipping" to undetectable concentrations might facilitate their formation. A potential protective effect of adequate drug concentrations was observed in a multicenter cohort study[20] in which a Week 4 infliximab concentration of less than 4 μg/mL had a positive predictive value of 81% for the development of ADAs. In contrast, values greater than 15 μg/mL had an 80% positive predictive value for ADA negativity.

In summary, multiple strategies exist to prevent ADA formation, including scheduled dosing, premedication with hydrocortisone, coadministration of azathioprine[3,22,73] or methotrexate,[59] and, potentially, maintenance of a therapeutic trough drug concentration.

Clinical Algorithms

In a retrospective analysis of the Mayo Clinic's initial experience with TDM,[17] which was based predominantly on assessment of patients with secondary loss of response, 2 distinct patient populations were identified. The first group consisted of patients with subtherapeutic infliximab concentrations, whereas the second group had detectable ADAs. In group 1, dose intensification resulted in higher response rates than switching to a second TNF antagonist (86% vs 33%; $P<.02$). However, for group 2, switching to another TNF antagonist yielded better outcomes than dose escalation (92% vs 17%; $P<.004$). This study provides a rationale for an approach to the management of patients with secondary loss of response to a TNF antagonist. A decision analysis based on this algorithm compared the cost-effectiveness of TDM-based management of patients with secondary loss of response to empiric dose intensification and switching of agents. Compared with empiric dose changes, the testing-based strategy yielded similar rates of remission (66% compared with 63%) and response (26% compared with 28%) but was less expensive ($31,266 compared with $37,266).[10]

TDM-based clinical algorithms have evolved for the management of secondary loss of response. Evaluation of patients in whom symptoms develop after attaining TNF antagonist–mediated remission begins by confirming disease activity and ruling out other disease processes by assessing serum and fecal biomarkers, cross-sectional imaging, and trough TDM sampling.

Once active disease has been confirmed, patients are grouped into 4 categories based on their TDM results (Table 3) to determine the most appropriate management strategy. Those with subtherapeutic drug levels and negative ADAs are managed with dose escalation. For those with therapeutic drug concentrations and negative ADAs in the setting of active disease, an out-of-class therapy is recommended, as the disease may be mediated by non-TNF mechanisms. However, only a limited number of agents are available. As such, adding azathioprine or methotrexate (if not already prescribed), switching immunosuppressive agents, using corticosteroids, or considering surgery are possible options.

Vedolizumab, a selective antagonist of the alpha 4 beta 7 integrin, is now available in the United States for this indication.[77] Those with subtherapeutic drug levels and positive ADAs have conventionally been considered sensitized and are switched to another TNF antagonist. Management of the last group, those with therapeutic drug levels and positive ADAs (who can only be detected with drug-tolerant ADA assays such as HMSAs or RIAs), is controversial. Because the natural history of this population of patients is unknown, experts disagree on management. Because therapeutic drug concentrations are present, some advocate dose escalation despite the presence of drug. Others believe that these patients are sensitized and are unlikely to respond to dose intensification. Given the documented presence of active disease, therapeutic drug concentration, and ADAs, we speculate that these patients may be best managed by either a within-class or out-of-class switch.

Several studies have confirmed the cost-effectiveness of a TDM-based approach. In a randomized controlled study conducted in Denmark, 66 patients with CD treated with infliximab were randomized to empiric treatment intensification/switching or treatment modifications based on the results of TDM. Although response rates were similar between the TDM and empiric therapy groups (53% and 58%; $P=.81$), costs were significantly lower in the TDM arm ($7736 compared with $11,760; $P<.001$.)[78] Finally, in

the TAXIT (Trough Level Adapted Infliximab Treatment) study, 270 patients with therapeutic infliximab concentrations on long-term maintenance therapy were randomized to dosing changes to sustain drug concentrations between 3 μg/mL and 7 μg/mL or dosing changes based on clinical symptoms.[79] Similar to the Steenholdt study,[78] preliminary data from the trial demonstrated no difference between the 2 strategies in 1-year remission rates but lower costs in the TDM group. However, the observed lack of efficacy may have been due to the patient population evaluated because participants were in remission and, thus, could be expected, on average, to have an appropriate concentration of drug. Further trials of TDM are required to determine whether optimization of trough concentration results in greater efficacy. Nevertheless, in patients with supratherapeutic drug concentrations, dose reductions were possible, and dose escalation was avoided in patients with therapeutic drug concentrations and in patients with ADAs, all of which result in a reduction in the cost of care by decreasing the use of expensive biologic therapies. Thus, cost-saving has consistently emerged as a benefit of TDM.[10,78,80]

Despite the benefits of TDM-based management, models that incorporate the large variations in the PK of TNF antagonists to predict outcomes in persons rather than populations have not yet been developed.[8]

Other Applications of Therapeutic Drug Monitoring

Although the most compelling indication for the use of TDM is in the setting of secondary loss of response to a TNF antagonist,[17] a potential role for TDM has been suggested for optimization of induction therapy, assessment of adherence, and evaluation of infusion reactions as well as for use prior to the reintroduction of infliximab after a drug holiday. At present, the role of TDM in clinical decision-making in these situations is not yet well defined.

Retrospective analyses suggest that nonadherence to infliximab maintenance therapy occurs in one-third of patients[81,82] and is associated with higher rates of hospitalization and increased costs. Although infliximab is administered at infusion centers, the subcutaneous TNF antagonists are self-administered. TDM may have a role in monitoring compliance to these subcutaneous agents, but this indication has not been explored in clinical trials.

Although infusion reactions are more prevalent in patients with ADAs,[2,19] their occurrence is not predictive of loss of response[83] because the majority of these adverse events are not immune-mediated. For the most part, infusion reactions are managed by interventions that prevent the release of mediators from mast cells,[83] such as slowing the rate of infusion and premedicating with antihistamines, corticosteroids, and leukotriene antagonists. Measurement of ADAs is primarily useful in patients in whom these interventions have been applied and failed, and in this clinical setting, it could be argued that therapy should just be stopped.

A prolonged interruption of TNF antagonist therapy is associated with the development of ADAs and adverse reactions, such as serum sickness, following reintroduction of therapy.[84] Theoretically, assessment of ADAs might determine which patients are likely to tolerate reintroduction of biologic therapy. However, data supporting this theory are lacking. In a prospective cohort of 22 patients with ADAs who discontinued use of a TNF antagonist, ADAs to infliximab were undetectable in the majority of patients (13/16) by 12 months but ADAs to adalimumab were undetectable in only 2 of 6 patients (P=.04).[85] A separate group of 27 patients were evaluated following reinitiation of TNF antagonists after a drug holiday of greater than 4 months. The presence or absence of ADAs did not reliably predict a severe reaction or nonresponse (odds ratio, 1.5; 95% CI, 0.2-11; P=.7).[85] Furthermore, it should be recognized that ADA titers may decrease over time. Thus, the clinical role of these measurements is limited.

Additional indications for the use of TDM may emerge, such as following induction therapy to ensure that adequate drug concentrations have been obtained. However, the current evidence supports the use of these assessments in patients with secondary loss of response.[17]

Conclusions

The use of TNF antagonists has revolutionized the treatment of IBD, and the use of TDM will further transform clinical management of these patients. Inadequate serum drug concentrations and ADAs are associated with poor clinical outcomes. The use of TDM has the potential to improve clinical outcomes and reduce costs. As limited out-of-class treatment options currently exist, a greater emphasis has been placed on optimization of TNF therapies in the setting of secondary loss of response. As evidence for TDM has evolved, clinical algorithms featuring TDM have developed. Additional data will enable predictive models to emerge that will further enhance care.

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