

A SPECIAL MEETING REVIEW EDITION

Clinical Research Highlights in IBD: Diagnosis and Anti-Tumor Necrosis Factor Monitoring

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Special Reporting on:

- Serological and Inflammatory IBD Marker Prevalence As Function of Age in a Large Cohort of Patients Presenting IBD-Like Gastrointestinal Symptoms
- Prevalence of Antibodies to Adalimumab (ATA) and Correlation Between ATA and Low Serum Drug Concentration on CRP and Clinical Symptoms in a Prospective Sample of IBD Patients
- Serum Adalimumab Levels and Antibodies Correlate with Endoscopic Intestinal Inflammation and Inflammatory Markers in Patients with Inflammatory Bowel Disease
- Comparison of Early Measurement of Infliximab and Antibodies-to-Infliximab Serum Levels with Standard Trough Analysis
- Trough Levels and Antidrug Antibodies Predict Safety and Success of Restarting Infliximab After a Long Drug Holiday
- A Multi-Center Observational Study in Community Gastroenterology Practices Evaluating the Clinical Usage of Testing for Serum Levels of Infliximab and Antibodies to Infliximab
- Preoperative Serum Biologic Levels Do Not Impact Postoperative Outcomes in Ulcerative Colitis
- Higher Preoperative Serum Biologic Levels Are Associated with Postoperative Complications in Crohn's Disease Patients

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Serological and Inflammatory IBD Marker Prevalence As Function of Age in a Large Cohort of Patients Presenting IBD-Like Gastrointestinal Symptoms

Scott E. Plevy, Steven Lockton, Fred Princen, and Sharat Singh

About 10% of the approximately 1.4 million patients with inflammatory bowel disease (IBD) in the United States are younger than 17 years.¹ Pediatric IBD has traditionally been diagnosed by endoscopy and biopsy; however, less invasive testing methods can be particularly desirable for younger patients. Although the analysis of serologic biomarkers to assist in the diagnosis of IBD has been extensively described,² a comparison of the relative prevalence of markers in adults versus children has not been. A study published in 2009

examined the influence of age at diagnosis on serologic response in children with Crohn's disease (CD).³ The study consisted of 705 children, including 79 patients younger than 8 years and 626 patients aged 8–15 years at diagnosis. A significant difference was observed in the prevalence of anti-*Saccharomyces cerevisiae* antibodies (ASCA) in the 2 age groups (<20% in the younger patients vs nearly 40% in the older patients; $P < .001$). In contrast, anti-CBir1, which binds to flagellin, was more common in the younger patients (66% vs 54%; $P < .05$).

At the 2013 Digestive Disease Week held on May 18–21 in Orlando, Florida, Scott E. Plevy, MD, and colleagues presented results from a study that examined serologic and inflammatory biomarkers across a range of age categories in patients with IBD.⁴ The study was designed to determine whether certain biomarkers of disease vary in prevalence based on age. For example, ASCA bind to glycans, which are known to be weakly immunogenic, particularly in young people. Therefore, a long maturation may be required for an ASCA response to develop in patients

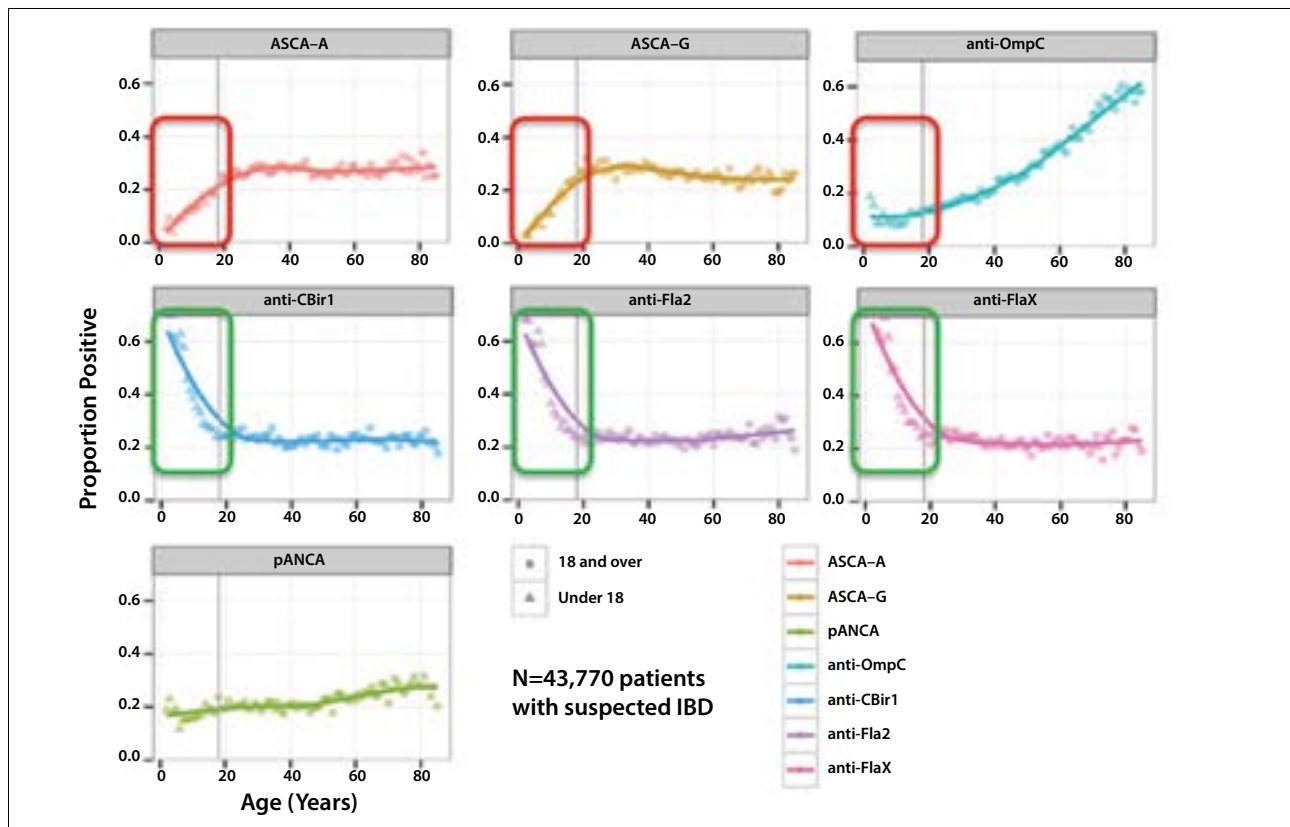


Figure 1. Prevalence of biological markers in inflammatory bowel disease according to age. ASCA, anti-*Saccharomyces cerevisiae* antibodies; OmpC, outer membrane porin C; pANCA, perinuclear antineutrophil cytoplasmic autoantibodies. Data from Plevy SE, et al. Serological and inflammatory IBD marker prevalence as function of age in a large cohort of patients presenting IBD-like gastrointestinal symptoms. Paper presented at Digestive Disease Week; May 18–21, 2013; Orlando, FL. Abstract 1029.

with IBD. In contrast, flagellin proteins are highly immunogenic, enhancing inflammatory proliferation and cytokine production in antigen-specific, CD4-positive T cells; thus antibodies to flagellar proteins might arise earlier in the development of IBD. The study also examined the relationship between clinical phenotypes and biomarker prevalence with the goal of determining whether specific biomarker patterns correlate with more aggressive disease.

The study initially collected data from a large cohort of 43,679 de-identified patients, aged 2–85 years, with a suspected diagnosis of IBD. Biomarker levels registering above the third quartile were considered positive. Patient data were examined for the prevalence of serologic markers (ASCA-A, ASCA-G, antineutrophil cytoplasmic autoantibodies, perinuclear antineutrophil cytoplasmic autoantibodies, and anti-outer membrane porin C [anti-OmpC]), anti-flagellins (anti-CBir1, anti-A4-FLA2, and anti-FlaX), and inflammatory markers (C-reactive protein [CRP], serum amyloid A [SAA], intercellular cell adhesion molecule 1 [ICAM-1], vascular cellular adhesion molecule 1 [VCAM-1], and vascular endothelial growth factor). Marker prevalence was then determined according to patient age: Group 1, 2–7 years (n=932; 3.5%), Group 2, 8–17 years (n=5,907; 16.7%), and Group 3,

18 years or older (n=36,840; 79.8%).

The prevalence rate of ASCA-A was 6.2% in Group 1 and 22.6% in Group 3, thus demonstrating an association between increased prevalence and increased age (Figure 1). Similarly, ASCA-G was present in 3.9% of the youngest patients versus 21.6% of adults. The prevalence rate of anti-OmpC also increased in older patients, from 8.5% in Group 1 to 23.1% in Group 3. In contrast, anti-CBir1 prevalence decreased with patient age, as demonstrated by a prevalence rate of 61.2% in young patients versus 22.9% in patients aged 18 years or older. The latter pattern was also observed for the anti-flagellins anti-A4-FLA2 and anti-FlaX. Antineutrophil cytoplasmic autoantibodies did not vary significantly among the 3 age groups. The prevalence of the inflammatory markers CRP and SAA increased with age, while ICAM-1 and VCAM-1 decreased with age. These findings were further validated by showing that the prevalence of anti-flagellin antibodies decreased in older versus younger patients with CD from 46 centers in North America.

The study results raised the question of whether these markers are stable over time. The cohort included approximately 3,800 patients who had samples drawn at more than 1 time point, with a median time between samples of 13.6 months. Anti-flagellin antibodies

remained stable over time in this cohort. In addition, in a subset of 116 patients, 1 sample was taken when the patient was a child, and a second sample was taken a median of 4 years later, when the patient was an adult. Again, anti-flagellin titers remained stable in these patients over time. Finally, samples were available for a cohort of 33 patients with severe CD. A median 13 years had elapsed between the earliest and latest time points regarding laboratory values. Again, titers of the 3 different anti-flagellin antibodies were relatively stable over time. Furthermore, anti-flagellin antibodies appeared to be more highly associated with aggressive or complicated CD, characterized by the presence of fibrostenosis, need for surgery, and the presence of internal and perianal fistulas.

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Prevalence of Antibodies to Adalimumab (ATA) and Correlation Between ATA and Low Serum Drug Concentration on CRP and Clinical Symptoms in a Prospective Sample of IBD Patients

Fernando S. Velayos, Sarah Sheibani, Steven Lockton, Scott Hauenstein, Sharat Singh, Jonathan P. Terdiman, and Uma Mahadevan

The introduction of biologic therapies to the treatment of inflammatory bowel disease (IBD) has had a major impact on disease course. Adalimumab, a

fully humanized antibody, binds to tumor necrosis factor alpha (TNF- α), which plays a pivotal role in the pathogenesis of IBD.¹ However, antibodies to biologic therapies

develop in many patients with IBD, resulting in a decrease in therapeutic efficacy. Recently, a homogeneous mobility shift assay was developed by Prometheus Laboratories Inc. that

enables the concurrent measurement of antibodies to adalimumab (ATA) as well as adalimumab levels in the same sample. The PROMETHEUS® Anser™ assay can detect low levels of ATA even in the presence of up to 20 µg/mL of adalimumab.²

Little is known about the clinical relevance of adalimumab and ATA levels. To address this need, Fernando S. Velayos, MD, presented results from a clinical study at Digestive Disease Week 2013. The study showed a correlation between ATA, low serum drug concentrations, and clinical symptoms in patients with IBD.³ The independent investigator-initiated, cross-sectional study prospectively recruited 113 patients with IBD who were currently receiving treatment with adalimumab, including 11 patients with ulcerative colitis, from a tertiary care center. Patients were included regardless of the presence or absence of symptoms. The PROMETHEUS® Anser™ mobility shift assay was used to determine the prevalence of detectable ATA or low trough serum drug concentrations.¹ The study then assessed the correlation between these parameters and levels of C-reactive protein (CRP), a marker of inflammation. Variability of trough serum levels also was investigated. Self-reported patient symptoms based on the Harvey-Bradshaw Index (HBI) and the Crohn's Disease Activity Index were collected, and patients were asked to self-report on whether they believed their disease was responding to treatment and was remitting, not responding, or worsening. The study then examined

whether stratified categories of ATA or drug concentrations correlated with the patient's self-described symptoms. Patient symptom reporting, IBD history, adalimumab dosing, weight, and CRP measurement were conducted within 2 weeks of measuring ATA and serum drug concentrations. Trough drug concentration was determined from blood drawn immediately prior to the next dose of adalimumab.

Samples were available for 113 patients, with 5 additional follow-up samples also available, yielding a total of 118 samples. The study population was 59% female and had a median age of 36 years. The majority of patients had long-term Crohn's disease spanning a median 10 years. The majority of patients were receiving adalimumab every 2 weeks for a median of 72 weeks. Approximately half of the patients had received prior anti-TNF-α therapy, and 71% of patients had received prior immunomodulator therapy. Nearly 16% of patients were currently receiving immunomodulator therapy. According to self-reporting, HBI, or Crohn's Disease Activity Index results, the majority of patients were responding to treatment or in remission.

Based on a threshold of any detectable ATA set at 1.7 U/mL or higher, 26 of 118 patient samples had detectable ATA, yielding a prevalence rate of 22%. The median drug concentration was 9.11 µg/mL. Ninety percent of patient samples had detectable adalimumab levels, defined as 1 µg/mL or higher. When a cut-off was defined as a serum level

of 5 µg/mL or higher, the prevalence rate decreased to approximately 66%. Approximately two-thirds of patients with detectable ATA also had detectable drug. In these patients, the median drug level was 4.78 µg/mL. Samples that had ATA levels greater than 1.7 U/mL were more likely to have a serum drug concentration lower than 5 µg/mL.

Multivariate modeling showed that dosing every week increased the odds of development of ATA compared with dosing every other week. Patients with an ATA level of at least 1.7 U/mL were more likely to have elevated levels of CRP, independent of serum drug concentration, supporting an association between increased ATA and increased inflammation. Similarly, levels of adalimumab lower than 5 µg/mL also correlated with increased levels of CRP. Consistent with these findings, the presence of ATA or a serum drug concentration lower than 5 µg/mL was associated with worse self-reported disease symptoms.

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Serum Adalimumab Levels and Antibodies Correlate with Endoscopic Intestinal Inflammation and Inflammatory Markers in Patients with Inflammatory Bowel Disease

Andres J. Yarur, Amar R. Deshpande, Daniel A. Sussman, Scott Hauenstein, Steven Lockton, Jamie S. Barkin, Sharat Singh, and Maria T. Abreu

Despite the fact that adalimumab is a fully human antibody, antibodies to adalimumab (ATA) have been shown to develop in 44% of patients.¹ In a poster presented at Digestive Disease Week 2013, Andres Yarur, MD, and colleagues presented data showing that increasing serum adalimumab and ATA levels correlated with increasing levels of markers of inflammation and endoscopic inflammation.² The cross-sectional study included adult patients with Crohn's disease (CD) or ulcerative colitis receiving adalimumab and collected predictive variables including demographics, disease phenotype, and concurrent use of corticosteroids or immunosuppressive drugs. Levels of adalimumab and ATA were measured with the PROMETHEUS[®] Anser[™] homogeneous mobility shift assay, which can detect levels of ATA as low as 10 U/mL in serum samples containing 20 µg/mL of adalimumab.^{1,3} Primary outcomes were the presence of macroscopic mucosal inflammation (MMI) during endoscopic examination and C-reactive protein (CRP) level.

Of the 66 patients, 59 had CD, 62 (94%) had detectable serum adalimumab, and 18 (27%) had detectable ATA. Elevated CRP levels were best predicted by a minimum serum adalimumab cut point of 5 µg/mL (receiver-

Table 1. Variables Associated With Positive ATA

Variable	Odds Ratio	95% CI
Adalimumab <5 µg/mL	8.6	2.3–31
Macroscopic mucosal inflammation	3.8	1.1–13
Microscopic mucosal inflammation	5.9	1.7–20
Steroid use	3.7	1.1–13
Previous exposure to infliximab	2.6	0.8–8.3
Receiving combination therapy with immunosuppressant	0.7	0.23–2.4

ATA, antibodies to adalimumab; CI, confidence interval.

Data from Yarur AJ, Deshpande AR, Sussman DA, et al. Serum adalimumab levels and antibodies correlate with endoscopic intestinal inflammation and inflammatory markers in patients with inflammatory bowel disease. Poster presented at: Digestive Disease Week; May 18–21, 2013; Orlando, FL. Abstract Tu1147.

operator curve, 0.71). The mean level of serum adalimumab was significantly higher in patients with undetectable versus detectable ATA (12.5 µg/mL vs 5.7 µg/mL; $P=$.001). Mean serum adalimumab levels were significantly higher in patients with mucosal healing versus those with MMI (13.3 µg/mL vs 8.5 µg/mL; $P=$.02). The mean serum CRP level was significantly higher in patients with detectable ATA versus those without (12 mg/dL vs 2.1 mg/dL; $P=$.002). Detectable ATA was associated with several parameters, including a serum adalimumab level of less than 5 µg/mL, MMI, need for corticosteroids, and previous infliximab use (Table 1). Treatment comprising adalimumab plus an immunomod-

ulator yielded a higher mean level of serum adalimumab compared with monotherapy (14 µg/mL vs 9 µg/mL; $P=$.026).

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Comparison of Early Measurement of Infliximab and Antibodies-to-Infliximab Serum Levels with Standard Trough Analysis

Alexander Eser, Christian Primas, Scott Hauenstein, Steven Lockton, Sharat Singh, and Walter Reinisch

Traditional solid-phase assays, such as the enzyme-linked immunosorbent assay and the electrochemiluminescent immunoassay, experience interference in detecting both antibodies to infliximab (ATI) and infliximab when either is present. At Digestive Disease Week 2013, Alexander Eser, MD, presented results from a study demonstrating that the PROMETHEUS[®] Anser[™] homogeneous mobility shift assay can detect ATI at any point in the infusion cycle, regardless of the amount of circulating infliximab.¹ ATI levels at midinfusion were in good agreement with measurements taken at trough levels and could provide important information regarding management decisions. Rates of ATI were lower among patients concomitantly receiving azathioprine, but this difference was not statistically significant.

This study enrolled more than 90 consecutive patients with a documented diagnosis of inflammatory bowel disease (Crohn's disease or ulcerative colitis) who were on maintenance therapy with infliximab. Serum samples were acquired approximately 4 and 8 weeks after drug infusion. Serum levels of infliximab and ATI were determined by the PROMETHEUS[®] Anser[™] homogeneous mobility shift assay.²

The median dose of infliximab was 5.5 mg/kg. Median infliximab concentrations were 23.0 µg/mL at midinfusion and 8.98 µg/mL at trough ($P < .001$). ATI were detectable in 18

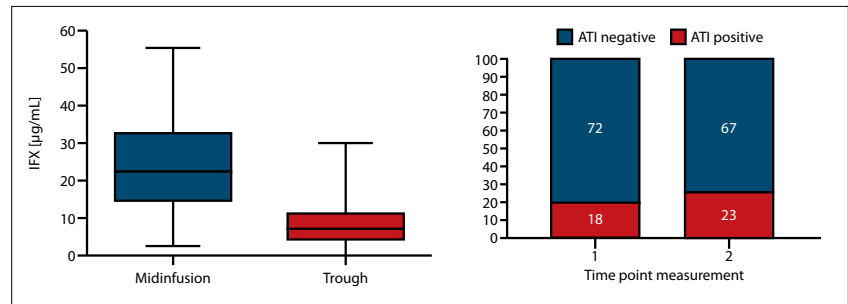


Figure 2. In a study comparing early measurement of infliximab and serum levels of ATI with standard trough analysis, IFX levels were significantly higher at midinfusion versus trough level. ATI, antibodies to infliximab; IFX, infliximab. Adapted from Eser A, et al. Comparison of early measurement of infliximab and antibodies-to-infliximab serum levels with standard trough analysis. Presented at Digestive Disease Week; May 18–21, 2013; Orlando, Florida. Abstract Tu1164.

patients at the midinfusion interval (Week 4) and 23 patients at trough (Figure 2). Sixteen patients were positive for ATI at both time points, with 2 patients positive for ATI only at midinfusion and 5 patients positive for ATI only at trough sampling. One (9%) of 11 patients receiving infliximab plus an immunomodulator was positive for ATI versus 22 (28%) of 79 patients receiving infliximab monotherapy ($P = .277$). ATI most frequently developed in patients with undetectable or very low trough levels. For example, 17 (77%) of 22 patients represented in the first quartile of infliximab trough-level measurements (0–2.31 µg/mL) tested positive for ATI. In contrast, of the 22 patients in the quartile representing the highest infliximab trough-level measurements (14.98–39.59 µg/mL), none tested positive for ATI. Patients with ATI

at Week 4 were likely to test positive for ATI at week 8 (Cohen's κ , 0.80; correlation, Kendall's τ correlation coefficient, 0.651; $P = .001$), confirming agreement for ATI measurement between week 4 and week 8. Dr. Eser concluded that the PROMETHEUS[®] Anser[™] mobility shift assay can detect ATI at any point in the infusion cycle, regardless of the amount of circulating infliximab.

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Trough Levels and Antidrug Antibodies Predict Safety and Success of Restarting Infliximab After a Long Drug Holiday

Filip J. Baert, David Drobne, Vera Ballet, Ann Gils, Niels Vande Castele, Scott Hauenstein, Sharat Singh, Steven Lockton, Paul J. Rutgeerts, and Severine Vermeire

Despite the increased efficacy observed with biologic therapies in patients with inflammatory bowel disease (IBD), loss of response to both infliximab and adalimumab is a significant problem that has been well documented and often leads to treatment discontinuation.^{1,2} However, reintroduction of therapy is often desirable given the chronic nature of IBD and the limited available therapeutic options.² Pharmacokinetic monitoring of drug levels and antidrug antibodies has the potential to predict the response to and safety of reintroduction of infliximab after a long drug holiday. Building on this concept, Filip J. Baert, MD, presented results from a study that identified clinical and biologic predictors for the success and safety of restarting infliximab in a highly immunogenic cohort of patients, including those with prior episodic therapy and patients who restarted infliximab therapy after prior loss of response or infusion reactions.³ The study assessed the rate of infusion reactions and the success rate for the reintroduction of infliximab with a 3-dose induction course after 1 year and at the end of follow-up. Serum samples were collected prospectively during the initial course of infliximab therapy. After the drug holiday, samples were collected upon restarting therapy and prior to the second and third infusions (Figure 3). Serial drug trough and antibodies to infliximab (ATI) levels were determined using the PROMETHEUS® Anser™ homogeneous mobility shift assay enabling the concurrent ATI detection in the presence of the drug.⁴ The study's main outcome was treatment success based on the clinical

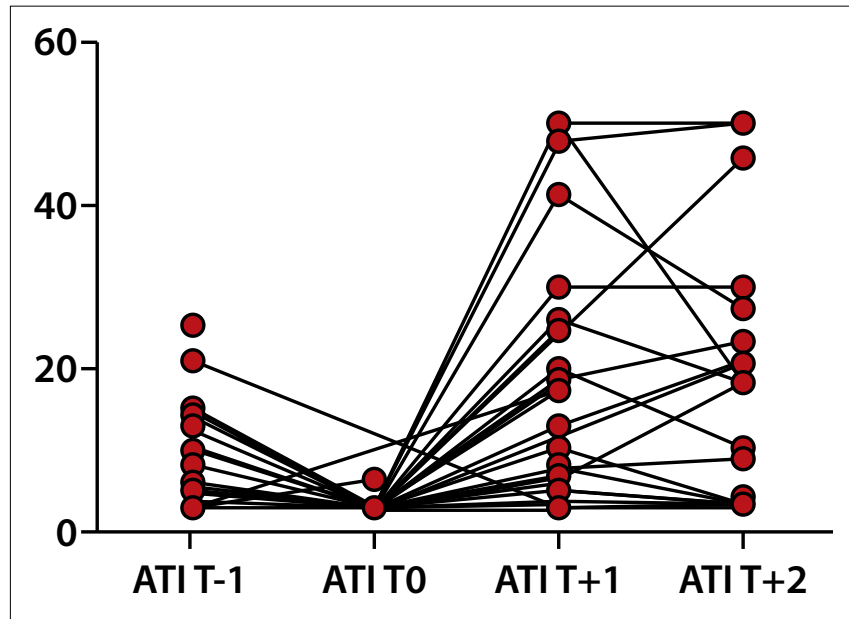


Figure 3. Maturation of ATIs in a study of early trough levels and antidrug antibodies after restarting infliximab following a long drug holiday. Undetectable ATI levels predicted a safe restart. ATI, antibodies to infliximab; T-1, maintenance therapy; T0, restart; T+1, before second infusion; T+2, before third infusion. Adapted from Baert FJ, et al. Trough levels and antidrug antibodies predict safety and success of restarting infliximab after a long drug holiday. Presented at Digestive Disease Week; May 19–22, 2013; Orlando, Florida. Abstract 492.

and biologic responses observed at the described time points. Outcomes were correlated with several clinical variables, including treatment modalities and pharmacokinetic measurements.

The study identified a consecutive cohort of 128 patients with IBD for whom complete clinical data were available. Twenty-three patients had ulcerative colitis, and 105 patients had Crohn's disease. Infliximab was restarted after a median drug holiday duration of 15 months (range, 6–125 months). Reasons for cessation of drug treatment included loss of response and/or infusion reaction in 29 patients and remission or pregnancy in 99

patients. All patients received maintenance therapy during re-treatment. During the initial course of treatment, 70% were concomitantly treated with an immunomodulator. In the second course of treatment, occurring after the drug holiday, 65% received concomitant immunomodulator therapy.

Restarting infliximab treatment was successful in 84% of patients at week 14, 70% of patients at 1 year, and 61% of patients at the end of follow-up (median, >48 months). Multivariate regression analysis showed that 2 clinical factors significantly correlated with short-term response to treatment. Patients who were negative for the pres-

ence of ATI fared better overall than those with ATI (hazard ratio [HR], 0.14; 95% confidence interval [CI], 0.026–0.74; $P=.021$) In addition, concomitant immunomodulator therapy predicted improved short-term response to treatment (HR, 6; 95% CI, 1.3–27; $P=.019$). Infusion reactions occurred in 25 (19.5%) patients, including 10 patients with a delayed reaction. In 16 (12.5%) patients, a severe infusion reaction led to discontinuation of treatment. Long-term response was influenced by the reason for discontinuation (pregnancy and/or remission; HR, 0.37; 95% CI, 0.15–0.92; $P=.033$) and by higher trough levels (HR, 0.34; 95% CI, 0.13–0.85; $P=.021$). Unde-

tectable ATI predicted a safe restart, as shown by the HR for infusion reaction with a detectable ATI of 7.7 (95% CI, 1.88–31.3; $P=.004$). Notably, 15 of the 16 patients who experienced a serious infusion reaction had received intravenous corticosteroid prophylaxis.

A wide range of drug levels was observed at the first time point after restarting infliximab, and long-term responses were correlated with higher drug levels. Concomitant immunomodulator therapy appeared to influence long-term response in patients with low drug levels but not those with high drug levels. During the second course of infliximab treatment, ATI were detected in 40% of patients after

the first infliximab infusion and in 29% of patients after the second infusion.

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A Multi-Center Observational Study in Community Gastroenterology Practices Evaluating the Clinical Usage of Testing for Serum Levels of Infliximab and Antibodies to Infliximab

Douglas C. Wolf, Steven Lockton, Scott Hauenstein, Susan Carroll, Sharat Singh, and Emil Chuang

Despite the very positive impact of targeted antibodies on the treatment of inflammatory bowel disease (IBD), as many as 50% of patients do not respond to infliximab therapy.¹⁻³ For approximately half of patients with non-responsive disease, modification of either the size or the frequency of the dose is required. Several hypotheses have been suggested to account for the lack of response to infliximab, including low drug bioavailability due to individual variation in the rate of drug metabolism; development of antibodies to infliximab (ATI); and involvement of an alternative inflammatory pathway. Monitoring patients for drug levels and the presence of ATI could enable more efficient dose customization for individual patients, rather than reliance on trial-and-error.^{4,5}

Exploring this approach, Douglas C. Wolf, MD, presented results at Digestive Disease Week 2013 from a multicenter, observational study designed to evaluate the impact of measuring serum infliximab levels and the presence of ATI in patients with IBD on physician decision-making regarding infliximab dosing and frequency.⁶ The study was conducted at community gastroenterology practices and enrolled patients with IBD who were receiving infliximab therapy. Serum levels of infliximab and ATI were measured at any time during therapy as decided by the treating physician. Patient blood samples were taken at enrollment (Visit 1) and 6 months later (Visit 2). Disease activity was assessed for patients with ulcerative colitis (UC) using the partial Mayo score and for patients with Crohn's disease (CD) using the Harvey-

Bradshaw Index (HBI). Levels of infliximab and ATI were measured using the PROMETHEUS[®] Anser[™] homogeneous mobility shift assay.⁷

Of the 193 study participants, 128 (66%) had CD, 62 (32%) had UC, and 3 (2%) had nonspecified IBD. Patients had a median age of 38.9 years and a mean disease duration of 10.2 years. Sixty percent of tests for infliximab and ATI level monitoring were ordered for a baseline measurement (ie, for patients who were not experiencing a loss of response to infliximab), and 40% of tests were ordered for patients who were experiencing an inadequate response or loss of response to infliximab, disease flare, autoimmunity, or delayed hypersensitivity reaction. Patients with CD had a mean HBI score of 3.0 (standard deviation, 3.27), and 84% of patients

had a score of 6 or lower. Patients with UC had a mean partial Mayo score of 2.1 (standard deviation, 2.31), and 92% of patients had a score of 5 or lower. Dosing was scheduled at 5 mg/kg every 8 weeks in 48% of patients, at 10 mg/kg every 8 weeks in 17% of patients, and at other dosages and/or frequencies in 35% of patients.

Thirty-six (19%) patients tested positive for ATI at Visit 1, and median levels of infliximab were significantly lower in patients who were positive for ATI compared with patients who were negative for ATI (1 µg/mL vs 15.7 µg/mL; $P < .0001$). Positive testing for ATI was associated with infliximab levels below 3 µg/mL ($P = .0001$). Physician knowledge about the presence of ATI appeared to influence treatment decision-making, as suggested by the observation that different treatments were given to patients who were positive for ATI and those who were negative for ATI. For example, a change to the infliximab regimen, such as a dose increase, modified dosing interval, or discontinuation, was made in 11 (31%) of the 36 patients who tested positive for ATI. In contrast, a change in the infliximab regimen was made in 10 (6%) of 157 patients who

tested negative for ATI ($P < .0001$). A switch to a different biologic agent also was made for a significantly greater percentage of patients who were positive for ATI compared with those who were negative (5 [14%] of 36 patients vs 1 [$< 1\%$] of 157 patients; $P < .0001$). The percentage of patients who received an added medication or for whom more tests or procedures were ordered did not vary significantly between patients with and without ATI.

The median serum concentration of infliximab did not appear to be significantly different in patients who were taking immunosuppressants ($n = 22$) compared with those who were not ($n = 171$; 9.8 µg/mL vs 11 µg/mL, respectively), and the median serum concentration of infliximab did not appear to vary significantly in patients with CD ($n = 128$) compared with those with UC ($n = 62$; 9.85 µg/mL vs 11.45 µg/mL, respectively). A lower median concentration of infliximab was reported for patients with a longer disease duration, ranging from 22.4 µg/mL for patients with 0–2 years' disease duration ($n = 22$) to 10.6 µg/mL for patients with greater than 10 years' disease duration ($n = 78$). Dr. Wolf

concluded that testing for serum levels of infliximab and ATI may help physicians customize infliximab treatment plans for patients with IBD.

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Preoperative Serum Biologic Levels Do Not Impact Postoperative Outcomes in Ulcerative Colitis

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Patients with ulcerative colitis (UC) are frequently treated with biologic agents, such as adalimumab and infliximab. Both of these agents bind to tumor necrosis factor alpha (TNF- α) and increase the risk of infection. Data suggest that the risk of infectious complications and pelvic sepsis increases after surgery in patients receiving biologic therapy for UC.¹ For example, 1 study examined the rate of

postoperative complications in a group of patients treated with infliximab and matched controls.² Patients in the infliximab treatment group underwent 46 two-stage and 39 three-stage restorative proctocolectomies. For patients receiving infliximab therapy, the likelihood of postoperative sepsis was 13.8 times greater (95% confidence interval [CI], 1.82–105; $P = .011$), and the likelihood of a mandatory 3-stage procedure

increased more than 2-fold (hazard ratio [HR], 2.07; 95% CI, 1.18–3.63; $P = .011$). Although patients may be eligible for either a subtotal colectomy (STC) or ileal pouch-anal anastomosis (IPAA), some surgeons advocate performing STC and anastomosis in patients who have received anti-TNF- α therapy to reduce the possibility of infection. However, many studies show no increase in perioperative or postop-

erative infections in patients receiving anti-TNF- α therapy. Some of these studies are limited by their retrospective nature, a small study population, or the inclusion of data from a single center. Additionally, in some studies, many months elapse between the last infliximab treatment and surgical intervention, such that most patients would likely have little or no infliximab in their serum at the time of the intervention, considering that the half-life of infliximab is approximately 9–18 days.

To provide further insights into these issues, Cheryl C. Lau, MD, presented results from a study designed to examine whether higher serum biologic levels pre-surgery increase the risk of postoperative complications in patients with UC.³ The study prospectively collected data on all patients with UC undergoing major abdominal surgery by a single surgeon in a tertiary care center over a 13-year period ending in 2012. The study included patients, identified from a database, who had had serum samples drawn within 7 days prior to surgery. Patients with unclassified inflammatory bowel disease and those undergoing anorectal surgery only were excluded. Data were collected regarding the use of immunomodulators, the type of anti-TNF- α therapy, corticosteroid use, and surgical variables such as whether the surgery was emergency versus elective and whether the index operation was a 2-stage IPAA versus a 3-stage STC. STC or IPAA was performed at the discretion of the operating surgeon. The primary outcome was overall postoperative morbidity, with early postoperative morbidity defined as occurring within 30 days of the operation. Secondary outcomes included all infections, medical and surgical complications (all of which were classified as major or minor), and rate of readmission within 30 days of the operation. Serum levels of anti-TNF- α drugs, including infliximab, adalimumab, and certolizumab, were measured with the homogeneous

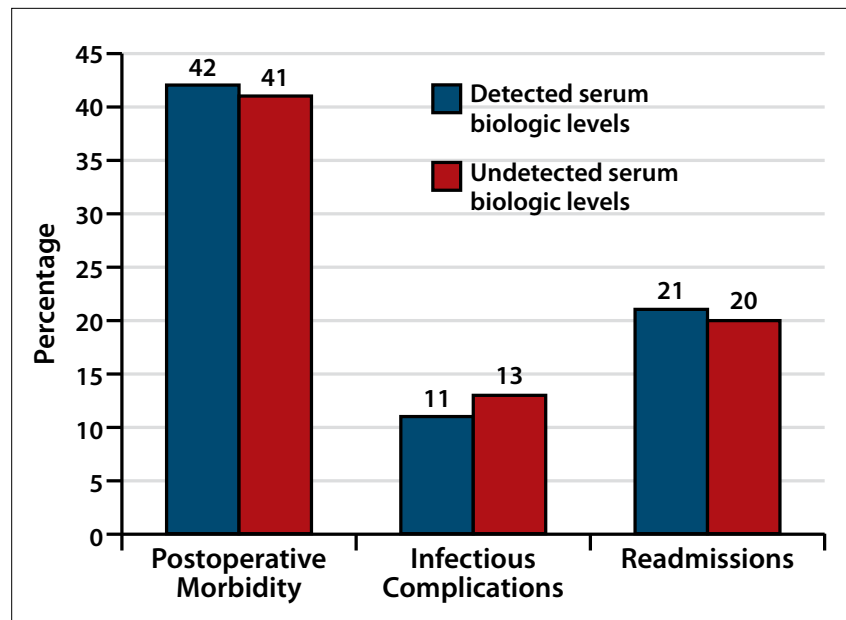


Figure 4. There was no significant difference in serum biologic levels and postoperative outcome in a study of patients receiving treatment for ulcerative colitis. Adapted from Lau CC, et al. Preoperative serum biologic levels do not impact postoperative outcomes in ulcerative colitis. Presented at Digestive Disease Week; May 18–21, 2013; Orlando, Florida. Abstract 1010.

mobility shift assay, with investigators blinded to the clinical outcome.⁴ Anti-TNF- α levels below 0.98 $\mu\text{g}/\text{mL}$ were considered undetectable.

The study cohort included 94 patients with a median age of 35 years (range, 7–76 years). Forty-eight percent were male. Fifty-two (55%) patients underwent IPAA as their index surgery, and 42 (45%) patients underwent STC. Two-thirds of patients were taking preoperative anti-TNF- α agents prior to surgery; however, serum anti-TNF- α levels were detected in only 19 (20%) patients. Patient data were grouped based on the detection or lack of detection of anti-TNF- α levels in patient serum. The 2 groups were similar in age, sex, and history of preoperative immunomodulator use. Among patients with serum biologic levels of anti-TNF- α , low (>0–3 $\mu\text{g}/\text{mL}$), medium (>3–8 $\mu\text{g}/\text{mL}$), and high (>8 $\mu\text{g}/\text{mL}$) measurements were equally represented. The maximum level detected was 66 $\mu\text{g}/\text{mL}$. No difference was observed in the initial use of STC in patients with undetectable versus detectable anti-TNF- α

levels (40% vs 63%; $P=.07$), nor was any difference observed with respect to overall postoperative morbidity (41% vs 42%; $P=.95$), infectious complications (13% vs 11%; $P=.74$), or 30-day readmission rates after surgery (20% vs 21%; $P=.92$).

In the group of patients who underwent STC and anastomy, rates of postoperative morbidity, minor complications, and readmission were slightly higher in patients with detectable serum anti-TNF- α levels, although this difference did not reach statistical significance. In patients who underwent primary index surgery of STC versus IPAA, no significant association was observed between serum anti-TNF- α levels and postoperative outcomes. All patients with detectable serum anti-TNF- α antibody were receiving infliximab, which has a generally accepted clinically relevant threshold of 3 $\mu\text{g}/\text{mL}$. No significant difference in postoperative morbidity or readmission rate was observed for patients above versus below the clinically relevant threshold (Figure 4), although a trend toward increased rates

of these outcomes was noted in patients with higher serum biologic levels.

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Higher Preoperative Serum Biologic Levels Are Associated with Postoperative Complications in Crohn's Disease Patients

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Although biologic therapies have revolutionized the treatment of Crohn's disease (CD), they exert varying clinical responses that may be at least partially accounted for by pharmacokinetic variation among patients. Despite the improved treatment overall with newer therapies, inflammatory bowel disease (IBD) often lasts for many years, and surgical intervention is commonly required for many patients at some point. Analyses of the effect of biologic therapy on surgical outcomes are limited by their retrospective nature, limited patient numbers, and variable timing of infliximab dosing relative to the date of surgical intervention.^{1,2}

To shed light on the controversy regarding the influence of biologic therapies on postsurgical outcomes, Cheryl C. Lau, MD, and coworkers presented results at Digestive Disease Week 2013 from a study that investigated the relationship between preoperative levels of anti-tumor necrosis factor alpha (TNF- α) antibodies and postoperative complications, including infection, in patients with CD undergoing abdominal surgery.³ Data were prospectively collected from patients operated on by a single surgeon, and patients with unclassified IBD were excluded. Serum was collected within 7 days of the operation date. Serum biologic levels were measured by inves-

tigators who were blinded to clinical outcomes using the PROMETHEUS[®] Anser[™] homogeneous mobility shift assay, and a value of at least 0.98 $\mu\text{g/mL}$ or greater was considered detectable.⁴

Most of the 123 patients underwent small bowel right colon resection with anastomosis. The median age of patients was 32 years (range, 12–68 years), and 67% of the patient population was male. Two-thirds of the patients were on anti-TNF therapy prior to the operation, with 40% showing detectable biologic serum levels at the time of the operation. Patient demographics, including preoperative laboratory values, corticosteroid use, and immunomodulator use, as well as the incidence of intra-abdominal abscesses, were similar between the patients with detectable versus undetectable serum levels of a biologic agent.

Within the group of patients with detectable anti-TNF- α antibodies, a wide range of values was observed; patient data were analyzed based on low, medium, or high levels of serum biologic, and a maximum value of 99 $\mu\text{g/mL}$ was reported. The patients with detectable serum biologic showed an increased incidence of postoperative morbidity (32% vs 18%), infectious complications (22% vs 10%), and readmissions after surgery (16% vs 7%)

relative to the group with undetectable levels, but none of these trends reached statistical significance. However, subgroup analysis of patients based on low, medium, or high levels of serum biologic demonstrated a significant increase in infectious complications (26% vs 10%; $P=.03$) and readmission rates (20% vs 7%; $P=.05$) for patients with biologic levels that were greater than 8 $\mu\text{g/mL}$. Overall postoperative morbidity was also higher for this group, but the outcome did not reach statistical significance (34% vs 18%; $P=.06$). Dr. Lau concluded that patients with CD and elevated preoperative levels of serum biologic are at greater risk for postoperative complications.

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Commentary

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There were many interesting presentations at Digestive Disease Week (DDW) 2013 focusing on patients with inflammatory bowel disease (IBD). Several studies evaluated how measurement of drug antibodies and drug concentrations can be incorporated into clinical care. Data were also presented regarding differences in the autoantibody profile patterns of childhood-onset Crohn's disease versus adult-onset Crohn's disease, as identified by serology testing.

Plevy and colleagues presented an interesting analysis of autoantibodies in 43,679 patients with IBD who ranged in age from 2 to 85 years.¹ Autoantibody profiles have been used to create algorithms for the diagnosis of Crohn's disease, and the frequency and level of the antibodies can provide information pertaining to prognosis. This study was the first to examine whether there is an autoantibody profile pattern correlating with childhood-onset Crohn's disease versus adult-onset Crohn's disease. The authors assessed autoantibodies that have been associated with IBD (predominantly Crohn's disease), including anti-*Saccharomyces cerevisiae* antibody (ASCA), antineutrophil cytoplasmic autoantibodies (ANCA), perinuclear antineutrophil cytoplasmic autoantibodies (pANCA), anti-OmpC, and the anti-flagellin antibody anti-CBir1. The study showed that anti-CBir1 and anti-flagellin markers were highly prevalent in younger patients. Adult patients had a higher prevalence of anti-OmpC and ASCA antibodies. The prevalence of ANCA antibodies did not substantially differ among the age groups. These

data confirm the observations made in a 2009 study by Markowitz and colleagues of pediatric patients.² Although these findings are unlikely to impact the operating characteristics of the test panel, they do raise the question of whether exposure to the underlying microbial flora that drives the formation of these antigens differs in childhood and adult Crohn's disease.

Several studies reported on findings made in conjunction with the new PROMETHEUS® Anser™ homogeneous mobility shift assay. Velayos and colleagues determined the prevalence of antibodies to adalimumab and measured concentrations of the drug in 113 patients with IBD (11 with ulcerative colitis and 102 with Crohn's disease).³ They then evaluated the impact of these concentrations on C-reactive protein (CRP) and symptoms. The original clinical trials with adalimumab did not sufficiently test for antibodies, and the reported rates were thought to be underestimated.^{4,6}

Twenty-two percent of samples had detectable antibodies to adalimumab, which is about 10 times higher than the reports from previous clinical trials. Approximately 90% of samples had detectable drug concentrations, as measured by the Prometheus® Anser™ ADA homogeneous mobility shift assay. This assay permits the concurrent measurement of the drug and the antidrug antibody in the same sample without the existing limitations with the enzyme-linked immunosorbent assay (ELISA) or the electrochemiluminescent immunoassay (ECLIA) technologies, information that can be of interest for therapies that are dosed every other week or every week, such as adalimumab. The frequently used ELISA does not permit simultaneous measurement of the drug and the antidrug antibody. The benefit of the new approach is illustrated in the study by Velayos and colleagues.³ Sixty-two percent of the patients who had antibodies to adalimumab also had detectable drug concentrations. This group

of patients was not identifiable with the ELISA used in previous clinical trials. It appears that the presence of antibodies to adalimumab, even if the drug is detectable, somehow interferes with the function of the drug, resulting in higher CRP concentrations and lower rates of clinical response and remission. This same conclusion was reported with infliximab and the associated antibodies at last year's DDW,⁷ and it, therefore, may be a generalizable observation at this point. In the study by Velayos and colleagues, adalimumab concentrations greater than 5 µg/mL were associated with lower CRP concentrations, but interestingly, antibodies to adalimumab were associated with higher CRP concentrations even in the setting of "therapeutic drug concentrations."³ Rates of antibodies to adalimumab were higher than previously reported.^{8,9}

In a similar study by Yarur and colleagues, rates of antibodies to adalimumab were also higher than previously reported.¹⁰ This study included patients with Crohn's disease (n=59) or ulcerative colitis (n=7) who were receiving adalimumab. This study also confirmed that 5 µg/mL was the cut point of adalimumab drug levels that best predicted low CRP concentrations. Higher concentrations of adalimumab were associated with lower concentrations of CRP. Adalimumab concentrations were higher in patients who had undetectable antibodies to adalimumab, for example. Patients without antibodies had drug concentrations of 12.5 µg/mL, and patients with antibodies had concentrations of 5.7 µg/mL.

The study also examined the relationship between mucosal healing and these factors.¹⁰ Patients with higher adalimumab concentrations were more likely to have mucosal healing. The mean drug concentrations were 13 µg/mL in patients who had mucosal healing and 8.5 µg/mL in patients who did not. These findings corroborate the observation in the

study by Velayos and colleagues that antibodies to adalimumab were associated with higher CRP concentrations.³ Patients receiving an immunomodulator or immunosuppressive therapy had higher adalimumab concentrations than patients who were receiving adalimumab as monotherapy.

Overall, in this cross-sectional group of patients with IBD, the presence of higher serum adalimumab concentrations was linked to both mucosal healing and low CRP levels. Higher adalimumab concentrations are more common in patients who are receiving combination therapy. It is now possible in the United States to measure serum levels of adalimumab and antibodies to adalimumab, and there is great interest in how these results can be used.

In a study of 90 patients with either ulcerative colitis or Crohn's disease who were receiving long-term therapy with infliximab, Eser and colleagues used the PROMETHEUS® Anser™ IFX assay to measure antidrug antibodies at the midpoint of an 8-week infusion interval (ie, at 4 weeks) and then again at trough for 8 weeks.¹¹ Most of the patients had a detectable concentration at the midpoint, but a number of the patients had low or undetectable levels of drug at trough. At the midpoint, it was possible to see antibodies of infliximab along with the drug level, which had not been possible with conventional assays. At the trough time point, those patients who had antibodies to infliximab at midpoint typically were unequivocally ATI-positive and had undetectable or very low drug concentrations, which are associated with less efficacy. The PROMETHEUS® Anser™ IFX assay permitted much more flexibility by providing a measurement of drug concentration at times other than trough. It can accelerate treatment decisions without sacrificing accuracy.

A very interesting study by Wolf and colleagues (not shown in this review) examined patients with Crohn's disease who had received adalimumab

therapy for at least 3 months and subsequently lost response.¹² Blood samples were evaluated for drug concentration as well as antibodies, again using the PROMETHEUS® Anser™ ADA assay. Detectable drug was found in 86% of patients. In this study of patients losing response to adalimumab, 47% of patients had antibodies to adalimumab, which is much higher than the approximately 9% that was reported in a previous clinical trial.⁹ Again, adalimumab drug concentrations were inversely associated with antibodies to adalimumab, and patients with lower drug levels had lower efficacy.

This study provided another interesting set of data regarding adalimumab concentrations in patients losing response to therapy. Among patients with detectable adalimumab levels, and consistent with the previous studies in this report, approximately two-thirds had detectable antibodies to adalimumab. Another third of patients, however, had subtherapeutic drug levels and no evidence of antidrug antibodies, meaning that their drug clearance was driven through alternate pathways. Similar findings have been seen previously with infliximab, and it is interesting to see them repeated again with adalimumab. Higher-titer antidrug antibodies are associated with increased clearance, but there is an important group of patients with increased clearance or lower drug concentrations, in whom the altered clearance is not related to antidrug antibodies.

In another study, Wolf and colleagues reported on 174 patients with IBD who were receiving infliximab at several community gastroenterology centers and underwent testing with the PROMETHEUS® Anser™ IFX assay to measure infliximab and antibodies to infliximab.¹³ The study included 115 patients with Crohn's disease, 56 with ulcerative colitis, and 3 with indeterminate or unclassified disease. Approximately 40% of the patients were receiving infliximab at 5 mg/kg

every 8 weeks. Almost all the patients were on monotherapy; fewer than 10% were receiving immunosuppressants. The PROMETHEUS® Anser™ IFX assay had been ordered for a variety of reasons, including loss of response in 38% of patients.

Overall, the median concentration of infliximab was approximately 11 µg/mL. During maintenance therapy, the therapeutic level is thought to be higher than 3 µg/mL. The study found that 18.4% of patients had antibodies to infliximab, which led to changes in therapy. With the PROMETHEUS® Anser™ IFX assay, it is now possible to measure infliximab and the presence of antibodies to infliximab. Physicians were therefore able to nuance the management plan. If a patient had antibodies to infliximab as well as a therapeutic drug concentration and was doing well, the choice could be made to continue treatment and drug monitoring. In contrast, patients who had antibodies to infliximab in the presence of low or undetectable drug concentrations received a different treatment.

Baert and colleagues examined trough levels and antidrug antibodies in 128 patients who had received infliximab, had therapy stopped (ie, had a drug holiday), and then had therapy restarted.¹⁴ The median duration for the drug holiday was 15 months (range, 6–125 months). Infliximab had been stopped for reasons including loss of response, infusion reaction, remission, and pregnancy, so the study population was heterogeneous. Infliximab was restarted successfully in approximately 85% of patients. Serum samples of infliximab and antibodies were measured at several points: during the original course of treatment with infliximab, before the first restart infusion with infliximab (Week 0), and then before the second restart infusion of infliximab (Week 2) and before the third restart infusion of infliximab (Week 6). The goal was to identify the role of drug monitoring in patients who had a drug holiday.

Baert and colleagues found that measuring the drug levels and anti-drug antibodies before the first restart infusion was not particularly helpful.¹⁴ However, the first restart dose has the potential to result in the formation of antidrug antibodies by the memory B cells, which become apparent at the second dose. Rates of response were low among patients who had antibodies prior to their second or third restart dose.

This information has been useful in practice. I do not routinely measure infliximab or antidrug antibodies prior to restarting a patient after a drug holiday, but I do obtain measurements routinely after the drug is restarted. If patients have antidrug antibodies and undetectable drug, therapy will be futile and I will stop it. In the study by Baert and colleagues, approximately 15% of patients met these criteria.

Lau and colleagues presented 2 similar studies on preoperative serum levels on postoperative outcomes in patients with IBD. One study included 94 patients with ulcerative colitis,¹⁵ and the other included 123 Crohn's disease patients.¹⁶ The PROMETHEUS® Anser™ assay, which measures infliximab and adalimumab, was used. In the study of patients with ulcerative colitis, postoperative outcomes did not differ according to preoperative drug levels. In the Crohn's disease study, patients with higher preoperative drug concentrations had higher rates of infectious complications and readmissions after surgery. This observation is potentially confounded by the fact that patients with complications are more likely to dose-escalate and then go to surgery, and, therefore, it is not surprising to

find that patients with higher drug levels had more complications. Thus, there does not appear to be utility in measuring drug levels perioperatively for surgical decision making.

At the 2013 DDW, studies on the measurement of drug antibodies and drug concentrations provided important new data. Overall, these studies add to the cumulative weight of evidence and knowledge that therapeutic drug monitoring of adalimumab and infliximab can play an important role in making clinical decisions regarding continuing, dose-escalating, or stopping these medications.

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