Distinct Cutoff Values of Adalimumab Trough Levels Are Associated With Different Therapeutic Outcomes in Patients With Inflammatory Bowel Disease

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Background and Aims: We aimed to evaluate the relationship of serum adalimumab trough levels (ATL) with disease activity of inflammatory bowel disease (IBD) patients in a large, well-characterized referral center-based cohort.

Methods: We compared serum ATL between those with clinical, biochemical, or endoscopic/radiologic disease activity and those without.

Results: A total of 236 patients with IBD were included. Higher cutoff levels were associated with endoscopic and/or radiologic responses (cutoff value: 5.3 mcg/mL, \( P = 0.003 \)) compared with improvement in C-reactive protein (cutoff value: 4.3 mcg/mL, \( P = 0.031 \)).

Conclusions: Higher cutoff ATL was associated with endoscopic and/or radiologic response.

Key Words: therapeutic drug monitoring, inflammatory bowel disease, adalimumab

INTRODUCTION

Inflammatory bowel diseases (IBD), consisting of Crohn’s disease (CD) and ulcerative colitis (UC), are chronic relapsing inflammatory disorders of the intestinal tract. The pro-inflammatory cytokine, tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)), is known to have a pivotal role in the immunopathogenesis of IBD.\(^1\) Anti-TNF agents are the mainstay of treatment for the induction and maintenance of remission in patients with moderate-to-severe IBD.\(^1-5\) In spite of its efficacy, up to 40% of IBD patients do not respond to induction therapy with anti-TNF agents, and about 20% of initial responders may lose response annually.\(^2-5\) These problems with efficacy and safety of anti-TNF-\( \alpha \) agents may be caused by several factors (including patient-related, disease-related, or drug-related) influencing...
the pharmacokinetics and pharmacodynamics of these drugs. Therapeutic drug monitoring (TDM) may aid clinical decisions during the course of anti-TNF therapy, and monitoring of the drug trough levels and antidrug antibodies has been recommended by some experts.6,7 Several studies showed that serum trough levels of anti-TNF-α agents are correlated with various clinical outcomes of IBD, including clinical activity,8,9 endoscopic improvement,10 and perianal fistula healing.11 Although studies of the utility of infliximab TDM have shown consistent results, adalimumab studies are sparse and have demonstrated relatively discrepant results, partly due to the sample timing and the different pharmacokinetics of agents such as infliximab and adalimumab.12 The absence of a universally accepted therapeutic cutoff level of adalimumab drug levels clearly related to clinical outcomes in IBD makes interpretation of these levels challenging. Adalimumab is usually self-administered at home and the sampling time may be more variable than the scheduled infusion of infliximab.13 Therefore, this study aimed to evaluate the correlation between serum adalimumab trough levels (ATL) and antibodies to adalimumab (AAA) with disease activity of IBD measured by clinical symptoms, C-reactive protein (CRP), and endoscopic and/or radiologic findings, using a large, well-characterized referral center-based cohort.

METHODS
Patients and Setting
We performed a single-center, cross-sectional, retrospective study of all IBD patients treated with adalimumab who had trough levels measured between September 2014 and August 2017. The protocol was approved by the Mayo Clinic Institutional Review Board. Patients with IBD (CD and UC) receiving adalimumab maintenance therapy who attended the IBD Clinic and who had ATL/AAA measured at our institution were eligible for inclusion in this study. The diagnosis of IBD had been established on the basis of standard clinical, endoscopic, radiologic, and/or histologic criteria. Exclusion criteria included patients who only had ATL/AAA ordered at outside hospitals, patients who were diagnosed with indeterminate colitis, and patients who were pregnant (due to variable pharmacokinetics). The medical records of patients in the IBD database were reviewed, and basic demographic data including date of birth, sex, and date of IBD diagnosis were abstracted. Data on previous biological use (including infliximab, certolizumab pegol, golimumab, natalizumab, vedolizumab, and ustekinumab) and previous surgeries were collected. Current use of combination immunomodulator (IMM) therapy (azathioprine, 6-mercaptopurine, or methotrexate) and corticosteroid therapy was noted. Data on dosing and intervals of adalimumab therapy at the time of checking ATL/AAA were gathered.

Adalimumab Assay
Serum ATL/AAA were measured by electrochemiluminescence immunoassay (ECLIA) performed at Esoterix Endocrinology (during the study period, this was a send-out test through Mayo Medical Laboratories). The detection limit for ATL and AAA were 0.6 mcg/mL and 25 ng/mL, respectively. At the time patient management decisions were being made based on these lab results, the AAA detection limit was also used as a positive cutoff and any value above 25 ng/mL was interpreted as positive.

Outcomes
We sought to evaluate the association between ATL/AAA and three different measures of disease activity in patients with IBD: 1) clinical symptoms; 2) CRP; and 3) endoscopic and/or radiologic disease activities. Clinical disease activity was divided into active or quiescent disease at the time of checking ATL/AAA based on the treating physician’s overall assessment. Inflammatory biomarkers, including CRP, and endoscopic and/or radiologic findings near the date of checking ATL/AAA (within 3 months) were also abstracted. Serum CRP concentration of ≤ mg/L was considered as normal.14 No active inflammatory lesions on small bowel cross-sectional images (CT or MR enterography) and colonoscopy in CD and no active lesions on colonoscopy or sigmoidoscopy in UC were considered as endoscopic and/or radiologic response.

Statistical Analysis
Continuous data were reported as means with standard deviations (SD) or medians with interquartile ranges (IQR). Categorical data were reported as counts and proportions. Patients were categorized into groups by disease activities (quiescent vs. active for clinical activity and endoscopic and/or radiologic findings, normal vs. elevated for CRP) and their status of AAA (presence vs. absence). Differences between the groups were compared using the Student t test and the median test as appropriate. The diagnostic power of ATL was investigated using area under the receiver–operating characteristic (ROC) curve analysis to obtain the area under the curve (AUC) and 95% confidence interval. The cutoff value for ATL that identified disease activity was determined by identifying the point closest to the 1.0 angle. A P-value of <0.05 was considered statistically significant. All statistical analyses were carried out using SPSS, version 21 (IBM Corp., Armonk, NY).

RESULTS
Baseline Patient Characteristics
A total of 236 patients (75.4% CD and 24.6% UC) were included in the study. The baseline characteristics of study patients were presented in Table 1. The median age at diagnosis of IBD was 24 years (IQR: 18–34), and 50.8% were male. The median
age at the time of assessing adalimumab level was 34 years (IQR: 25–51). At the time of measurement, adalimumab was administered every other week in 154 (65.3%) and every week in 82 patients (34.7%). The median disease duration of IBD before adalimumab therapy was 4.5 years (IQR: 1–12 years), and the median duration of adalimumab use was 2 years (IQR: 1–4 years) at the time of assessing adalimumab level.

### Association Between Adalimumab Trough Levels and Clinical Disease Activity

At the time of adalimumab measurement, 61 patients (25.8%) had clinically quiescent disease and 175 patients (74.2%) had clinically active disease. The median ATL was 5.1 mcg/mL (IQR: 3.1–9.8) and 4.4 mcg/mL (IQR: 2.2–7.4) in patients in quiescent and active disease, respectively ($P = 0.502$) (Table 2). The mean ATL was 6.8 mcg/mL (SD 5.9) and 5.9 mcg/mL (SD 5.9) in patients in quiescent and active disease, respectively ($P = 0.331$) (Table 2; Fig. 1). The discriminant ability of ATL for clinical disease activity as determined by the area under ROC was 0.557 ($P = 0.183$); thus, a cutoff value could not be obtained (Table 2; Figure 1).

### Association Between Adalimumab Trough Levels and C-reactive Protein

At the time of adalimumab draw, normal CRP was found in 171 patients (72.5%), and 65 patients (27.5%) had elevated CRP (i.e., >8 mg/L). Patients with a normal CRP had a median ATL of 4.9 mcg/mL (IQR: 2.8–8.7) compared with 3.8 mcg/mL (IQR: 1.4–7.3) among those with high CRP ($P = 0.016$) (Table 2). The mean ATL was significantly higher in patients with a normal CRP compared with those with a high CRP (6.7 ± 4.7 vs. 4.7 ± 4.2 mcg/mL; $P = 0.023$) (Table 2; Figure 2). The discriminant ability of ATL for CRP elevation as determined by the area under ROC was 0.591 ($P = 0.031$), and the optimum cutoff ATL value for distinguishing normal vs. elevated CRP was 4.3 mcg/mL (Table 2; Figure 2).

### Association Between Adalimumab Trough Levels and Endoscopic/Radiologic Disease Activity

Endoscopic and/or radiologic response, defined by no endoscopic (n = 53) and/or radiologic disease activity (n = 43), was identified in 53 patients (22.5%) at the time of adalimumab measurement. The remaining 183 patients (77.5%) showed active inflammation in endoscopic (n = 141) and/or radiologic (n = 125) evaluations. Among patients with endoscopic and/or radiologic response, the median ATL was 6.5 mcg/mL (IQR: 3.7–11) compared with 4.1 mcg/mL (IQR: 2.2–7.3) in those who did not achieve endoscopic and/or radiologic response ($P = 0.01$). The mean ATL was also significantly higher in patients with endoscopic and/or radiologic response compared...
with those without endoscopic and/or radiologic response (8.2 ± 7.0 vs. 5.6 ± 5.5 mcg/mL; \( P = 0.016 \)). The diagnostic power of ATL for endoscopic and/or radiologic response as determined by the area under ROC was 0.632 (\( P = 0.003 \)), and the optimum ATL cutoff value for predicting endoscopic and/or radiologic response was 5.3 mcg/mL (Table 2; Figure 3).

**Anti-adalimumab Antibodies**

A total of 95 patients (40%) developed detectable AAA (≥25 ng/mL), and 36 patients with detectable AAA (38%) had undetectable ATL (<0.6 mcg/mL). There were no differences in the proportion of patients developing AAA with various cutoff values (25, 100, and 300 ng/mL) between the groups divided by clinical, biochemical, and endoscopic/radiologic disease activities in this cohort (Table 3). In our cohort, 40 patients had AAA between 25 and 100 ng/mL, 20 had AAA between 100 and 300 ng/mL, and 35 had AAA greater than 301 ng/mL.

**Adalimumab Trough Levels and Clinical Management**

Checking ATL resulted in a change in clinical management in 149 study patients (63.1%), including dose change of adalimumab, which consisted of shortening the administration interval (\( n = 48 \)), lengthening the interval (\( n = 7 \)), or discontinuing adalimumab (\( n = 10 \)); addition of an IMM (\( n = 10 \)), or stopping an IMM (\( n = 2 \)); changing to another biologic (\( n = 60 \); other anti-TNFs 36 [infliximab 19, certolizumab pegol 14, and golimumab 3] and other classes 24 [vedolizumab 22 and ustekinumab 2]); and recommending surgery (\( n = 12 \)).
DISCUSSION

In the present study, we demonstrated that ATL were associated with objective response parameters, including biochemical and endoscopic/radiologic disease activities, but not symptom-based, subjective parameters. A higher cutoff ATL was associated with endoscopic/radiologic response. The use of ATL guided clinical management in 63% of the patients. Our study is in keeping with recent reports in which adalimumab concentrations were correlated with biochemical and endoscopic remission in IBD.15–17 Recently, the Food and Drug Administration (FDA) recommended that targets of treatment in IBD should move away from a composite disease activity index to separate patient-reported outcomes and objective measurements of inflammation, because clinical symptoms alone are not a reliable measure of the underlying inflammation.18,19 Therefore, targeting higher ATL for deep remission, not just for symptom control, may be a reasonable approach for IBD management.

A previous systematic review suggested various cutoff levels of infliximab for predicting drug efficacy, from below 1 mcg/mL to >7 mcg/mL.22 Part of the variation may be due to the differences between assays, such as the enzyme-linked immunosorbent assay (ELISA), homogeneous mobility shift binding assay (HMSA), or ECLIA such as in our study, and the various definitions and timing of outcome evaluations. Although a growing body of evidence supports a therapeutic range of infliximab associated with clinical remission of IBD using ELISA-based assays of between 3 and 7 mcg/mL,23 there has been a relative lack of data regarding therapeutic cutoff

| TABLE 3. Proportion of Patients With AAA by Different Cutoff for AAA |
|--------------------------|--------------------------|--------------------------|
| Symptom Inactive (n = 61) | CRP Normal (n = 171) vs. Elevated (n = 65) | Endoscopic and/or radiologic response Yes (n = 53) vs. No (n = 183) |
| AAA (> 25 ng/mL) (%) | 31% vs. 43% | 39% vs. 45% | 32% vs. 43% |
| P value | 0.092 | 0.4 | 0.168 |
| AAA (> 100 ng/mL) (%) | 16% vs. 26% | 22% vs. 26% | 17% vs. 25% |
| P value | 0.138 | 0.523 | 0.216 |
| AAA (> 300 ng/mL) (%) | 13% vs. 15% | 15% vs. 15% | 11% vs. 16% |
| P value | 0.661 | 0.883 | 0.414 |

Serum CRP concentration of ≤8 mg/L was considered as normal.
levels of adalimumab in IBD patients. In 2016, Zittan et al. analyzed the correlation between ATL and mucosal healing in 60 CD patients receiving adalimumab therapy. They found that higher adalimumab levels were associated with mucosal healing, and they suggested a cutoff level of 8.14 mcg/mL for mucosal healing in CD patients. Yarur and colleagues performed a similar study of 66 IBD patients (59 CD, 7 UC) to analyze the correlation between adalimumab TDM (random adalimumab levels and AAA) and histologic and endoscopic healing. In that study, random adalimumab levels above 7.8 and 7.5 mcg/mL were associated with histologic healing and endoscopic healing, respectively, and the presence of AAA was associated with lower random adalimumab levels. Other studies reported that adalimumab levels above 8.5 and 12 mcg/mL were associated with biochemical and endoscopic remission, respectively. A recent technical review suggested that an ATL cutoff ≥7.5 mcg/mL would be better than a lower target such as ≥5 mcg/mL to gain clinical benefit in IBD patients. In an ATL cutoff ≥7.5 mcg/mL would be better than a lower target such as ≥5 mcg/mL to gain clinical benefit in IBD patients. In the present study, the suggested cutoff values of ATL for biochemical or endoscopic and/or radiologic response in patients with IBD were 4.3 and 5.3 mcg/mL, respectively, somewhat lower than those in previous reports, probably due to diverse methodology and study design. However, we confirmed that higher ATL may be helpful to manage patients with IBD for a treat-to-target approach.

The indications and timing of TDM in patients treated with anti-TNF or other biologics are controversial. There is growing evidence that TDM is cost-effective and associated with favorable outcomes in IBD patients receiving anti-TNF therapy. The use of TDM to guide treatment decisions in patients with loss of response to infliximab was shown to be cost-effective compared with empiric dose escalation. However, two recent randomized controlled trials evaluating the benefit of routine proactive TDM of infliximab over no therapeutic monitoring (TAXIT and TAILORIX) failed to show the efficacy of a proactive approach in IBD management with biologics. So far, the consensus for the indication and timing of TDM in patients with IBD is the “reactive” measurement of trough levels of anti-TNF agents at the time of secondary loss of response rather than the routine “proactive” strategy. One of the major strengths of our study is the large number of patients included in a real-world setting. Also, this study utilizes the measurement of various disease activities including endoscopic and radiologic findings performed by individual chart review and gathers data on a change in the clinical management of IBD after ATL/AAA measurement. Limitations of our study include the retrospective study design, heterogeneity of study populations, lack of standardization of treatment, and timing of ATLs blood draw. Also, we could not evaluate the effect of concomitant IMM on the immunogenicity against adalimumab due to the retrospective design and heterogeneity of study populations, although recent data from randomized controlled trial showed adalimumab in combination with azathioprine-increased ATLs. In contrast to ATL, AAA are typically interpreted qualitatively in the appropriate clinical context. Among the patients of the present study, 40% developed detectable AAA (≥ 25 ng/mL), and 38% of patients with AAA had undetectable ATL (< 0.6 mcg/mL). A significant proportion of patients with AAA had detectable ATL, and AAA in these patients might be “transient” AAA rather than “sustained” AAA. Around 2018, Esotexir changed cutoffs for interpretation of positive results of their test, calling <100 ng/mL as a low antibody titer with no significant impact on free drug level; 101–300 ng/mL as intermediate antibody titer with variable impact on free drug level, and > 301 ng/mL as high antibody titers, with significant impact on free drug level, which prompted us to test the new classifications in our cohort. Moreover, we could not analyze the correlation between various disease activities and ATL according to the disease types due to the small sample size.

In summary, ATL were associated with objective response parameters, including biochemical and endoscopic/radiologic disease improvement, rather than symptom-based, subjective parameters. A higher cutoff value for ATL was associated with endoscopic and/or radiologic response. Also, ATL assessment altered clinical management in the majority of IBD patients. Our findings support the rationale of a reactive TDM approach for clinical assessment in patients with IBD receiving biologies. Also, targeting higher ATL in patients who are not responding at lower ATL may be beneficial to achieve deep remission.

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REFERENCES

1. Danese S. New therapies for inflammatory bowel disease: from the bench to the bedside. Gut. 2012;61:918–932.
2. Hanauer SB, Feagan BG, Lichtenstein GR, et al.; ACCENT I Study Group. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. Lancet. 2002;359:1541–1549.
3. Colombel JF, Sandborn WJ, Rutgeerts P, et al. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. Gut. 2007;56:32–65.
4. Schreiber S, Khalbauten M, Lawrance IC, et al.; PRECISE 2 Study Investigators. Maintenance therapy with certolizumab pegol for Crohn's disease. N Engl J Med. 2007;357:239–250.
5. Singh S, Pardi DS. Update on anti-tumor necrosis factor agents in Crohn disease. Gastroenterol Clin North Am. 2014;43:457–478.
6. Vande Casteele N, Feagan BG, Gilis A, et al. Therapeutic drug monitoring in inflammatory bowel disease: current state and future perspectives. Curr Gastroenterol Rep. 2014;16:378.
7. Vande Casteele N, Herfarth H, Katz J, et al. American gastroenterological association institute technical review on the role of therapeutic drug monitoring in the management of inflammatory bowel diseases. Gastroenterology. 2017;153:835–857.e6.
8. Mauer EA, Villela R, Silverberg MS, Greenberg GR. Association of trough serum infliximab to clinical outcome after scheduled maintenance treatment for Crohn's disease. Clin Gastroenterol Hepatol. 2006;4:1248–1254.
9. Oh EH, Ko DH, Seo H, et al. Clinical correlations of infliximab trough levels and antibodies to infliximab in South Korean patients with Crohn's disease. World J Gastroenterol. 2017;23:1489–1496.
10. Seow CH, Newman A, Irwin SP, et al. Trough serum infliximab: a predictive factor of clinical outcome for infliximab treatment in acute ulcerative colitis. Gut. 2010;59:49–54.
11. Yarur AJ, Kanagala V, Stein DJ, et al. Higher infliximab trough levels are associated with perianal fistula healing in patients with Crohn’s disease. *Aliment Pharmacol Ther.* 2017;45:933–940.

12. Ward MG, Warner B, Unsworth N, et al. Infliximab and adalimumab drug levels in Crohn’s disease: contrasting associations with disease activity and influencing factors. *Aliment Pharmacol Ther.* 2017;46:150–161.

13. Ward MG, Thwaites PA, Beswick L, et al. Intra-patient variability in adalimumab drug levels within and between cycles in Crohn’s disease. *Aliment Pharmacol Ther.* 2017;45:1135–1145.

14. Al-Bawardy B, Ramos GP, Willrich MAV, et al. Vedolizumab drug level correlation with clinical remission, biomarker normalization, and mucosal healing in inflammatory bowel disease. *Inflamm Bowel Dis.* 2019;25:580–586.

15. Juncadella A, Papamichael K, Vaughn BP, Cheifetz AS. Maintenance adalimumab concentrations are associated with biochemical, endoscopic, and histologic remission in inflammatory bowel disease. *Dig Dis Sci.* 2018;63:3067–3073.

16. Plevris N, Lyons M, Jenkinson PW, et al. Higher adalimumab drug levels during maintenance therapy for Crohn’s disease are associated with biologic remission. *Inflamm Bowel Dis.* 2019;25:1036–1043.

17. Watanabe K, Matsumoto T, Hisamatsu T, et al.; DIAMOND Study Group. Clinical and pharmacokinetic factors associated with adalimumab-induced mucosal healing in patients with Crohn’s disease. *Clin Gastroenterol Hepatol.* 2018;16:542–549.e1.

18. Peyrin-Biroulet N, Ferrante M, Van Assche G, et al. Trough concentrations of infliximab guide dosing for patients with inflammatory bowel disease. *Gastroenterology.* 2015;148:1320–1329.e3.

19. Willet N, Sandborn WJ, Peyrin-Biroulet L. Patient-reported outcomes as primary end points in clinical trials of inflammatory bowel disease. *Clin Gastroenterol Hepatol.* 2014;12:1246–56.e6.