Reversal of Immunogenicity in Pediatric Inflammatory Bowel Disease Patients Receiving Anti-Tumor Necrosis Factor Medications

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Loss of response to anti-tumor necrosis factor (anti-TNF) agents in the treatment of inflammatory bowel disease (IBD) is a major consideration to maintain sustained response. Reversal of immunogenicity can re-establish response and increase the durability of these agents. Strategies to reverse immunogenicity include dose-intensification and/or the addition of an immunomodulator. However, there is a relative paucity of data on the efficacy of such interventions in pediatric IBD patients. Available reports have not strictly utilized homogenous mobility shift assay, which reports on anti-drug antibodies even in the presence of detectable drug, whereas prior studies have been confounded by the use of drug sensitive assays. We report four pediatric inflammatory bowel disease patients with successful reversal of immunogenicity on an anti-TNF agent using dose intensification and/or addition of an immunomodulator.

Key Words: Child, Biological products, Antibodies

INTRODUCTION

Inflammatory bowel disease (IBD) is an idiopathic chronic inflammatory disease that presents during childhood or adolescence in 25% to 30% of patients [1]. While uncontrolled IBD can have debilitating effects that may necessitate surgical treatment, recent drug development, particularly with anti-tumor necrosis factor (anti-TNF) agents, has decreased the rate of such complications [2].

Anti-TNF agents including infliximab or adalimumab have been shown to be effective in the induction of clinical remission in IBD patients [3,4]. However, loss of response to treatment may occur due to several reasons including individual pharmacogenetics, increased drug clearance, and immunogenicity [5,6]. Strategies to reverse immunogenicity include dose-intensification and/or the addition of an immunomodulator, both of which have been shown to be effective in adult studies [7].
There is limited data on the reversal of immunogenicity in pediatric IBD patients using dose modification and/or the addition of an immunomodulator [8]. Prior studies did not strictly use homogeneous mobility shift assay (HMSA) which report on anti-drug antibodies (ADA) even in the presence of detectable drug levels [9]. We report four pediatric IBD patients with successful reversal of immunogenicity through dose adjustment and/or the addition of immunomodulators.

CASE REPORT

We performed a retrospective analysis of pediatric IBD patients receiving scheduled infliximab or adalimumab therapy who had HMSA testing from 2013 to 2017. All HMSA testing were sent and run at Prometheus Laboratory Inc. (San Diego, CA, USA), where the lower limit of infliximab detection is 0.0074 µg/mL, with a dynamic testing range for antibodies to infliximab in serum of 0.56 to 27 µg/mL, and the lower limit of adalimumab detection is 0.018 µg/mL, with a dynamic testing range for antibodies to adalimumab of 0.063 to 25 µg/mL. The non-radiolabeled, fluid-phase HMSA involves incubating the serum samples with fluorescent-labeled drug. The immune complexes formed by the labeled drug and ADA have increased molecular weight when compared to the free drug. These complexes are separated from the free labeled drug via size-exclusion high-performance liquid chromatography. The ADA load is able to be calculated based on the area under the drug antibody peak [9]. The timing of the test was at the discretion of the treating gastroenterologist. Patients younger than 18 years at diagnosis of IBD who developed ADA, on two consecutive tests, and subsequently demonstrated resolution with any intervention (dose escalation, shortening the interval, or addition of an immunomodulator) were included. This study was approved by the Institutional Review Board at University Hospitals/ Rainbow Babies Children’s Hospital in Cleveland, OH (IRB number 02-17-10).

From 2013 to 2017, 12 patients on biologic medications with positive antibodies were identified. Four of 12 patients met inclusion criteria as described in the methods section (Table 1). Three of 12 patients had positive ADA but were excluded as they were only present on the initial test, suggesting transient ADA. Five of 12 patients who had one positive ADA were also excluded as they were lost to follow up without repeat testing or switched to a different biologic therapy without an attempt to overcome ADA with modification of their current therapy.

Case 1

A 12 year old male presenting with five weeks of abdominal pain was found to have ileocolonic Crohn’s disease (CD) with upper gastrointestinal involvement. The patient was started on standard infliximab dosing with the addition of weekly oral (10 µg per week) methotrexate (MTX) due to his severe and extensive disease. He went into clinical remission and received infusions every 8 weeks with continuation of his once weekly, low dose MTX.

The patient’s course was complicated by disseminated histoplasmosis, which led to the discontinuation of his infliximab and MTX. After treatment for the histoplasmosis, he was restarted on infliximab without an immunomodulator due to recurrence of his CD. The patient had loss of response, characterized by active clinical symptoms, after his 15th infusion, and HMSA testing demonstrated a trough drug level of 11.1 µg/mL and ADA of 5.5 U/mL. His dosage interval was shortened from every seven to every four weeks. At his next titer, trough drug level increased to >34.0 µg/mL and ADA increased to 10.3 U/mL, leading to the clinical decision to add MTX. With this regimen, after his 20th infusion, the patient demonstrated antibody resolution with continued therapeutic levels of IFX (Fig. 1).

Case 2

A 7 year old female who presented with hematochezia and diarrhea was diagnosed with ulcerative colitis (UC), pancolitis distribution. Her initial course was an oral mesalamine, followed by escalation to azathioprine (AZA). At age 17 years, she was admit-
Table 1. Demographics, Disease Location, Details on LOR, and Medication Changes Made for Each Study Patient

| Patient No. | Sex, race, age at diagnosis (y) | BMI at diagnosis (kg/m²) | Disease phenotype, location | Duration of disease prior to anti-TNF agent initiation (y) | Number of infusions until LOR/dose at LOR | Immunomodulator at LOR | PGA at LOR | Change in medical management | Subsequent course |
|-------------|--------------------------------|--------------------------|----------------------------|----------------------------------------------------------|------------------------------------------|------------------------|-----------|-------------------------------|------------------|
| 1           | Male, Caucasian, 12            | 15.51, 9th percentile    | Ileocolonic CD, nonstricturing, non-penetrating UC, pancolitis | 0                                                        | 15 infliximab infusions 5 µg/kg/dose every 7 wk | None                   | Mild      | Shortened interval from 7 to 4 wk; added MTX | Clinical remission |
| 2           | Female, Caucasian, 7           | 13.2, 3rd percentile     | UC, pancolitis              | 9                                                        | 11 infliximab infusions 5 µg/kg/dose every 8 wk | AZA                    | Mild      | Shortened interval to 4 wk; increased dose to 10 µg/kg | Clinical remission and endoscopic healing |
| 3           | Male, Caucasian, 9             | 16.5, 53rd percentile    | Ileocolonic CD, stricturing, non-penetrating, with perianal disease | 7-infliximab 9-adalimumab                                  | 6 adalimumab infusions 40 µg every 2 wk | MTX                    | Mild      | Shortened interval from 2 to 1 wk; increased dose of MTX | Clinical remission |
| 4           | Female, Caucasian, 12         | 14.75, 4th percentile    | CD of small and large intestine | 0                                                        | 5 infliximab infusions 5 µg/kg/dose every 8 wk | AZA                    | Quiescent | Shortened interval from every 8 to 6 wk | Continued to be stable |
tated for her second UC flare while on prednisone and AZA. She was started on standard dosing of infliximab. Her infliximab dose was increased to 7.3 μg/kg/dose due to diffuse colitis found on a subsequent scope. She developed clinical and biochemical loss of response after the 11th infliximab infusion with an undetectable trough drug level and ADA of 4.3 U/mL. Her infliximab dose was increased to 10 μg/kg/dose, drug interval was shortened from every 8 weeks to every 4 weeks, and AZA was continued. After her 14th infusion, ADA resolved and trough drug level increased to 22.9 μg/mL. Additionally, she demonstrated clinical remission with improvement of mucosal inflammation demonstrated with endoscopy (Fig. 1).

Case 3
A 9 year old male diagnosed with CD of the ileum, strictureting phenotype with perianal disease, had an early complication requiring an ileocecostomy. At age 15 years, while taking AZA and mesalamine, he was hospitalized for an acute exacerbation and was started on standard infliximab dosing in addition to oral (12.5 μg once weekly) MTX. The patient was found to have undetectable infliximab trough levels with ADA of 7.7 U/mL after his 6th infusion. He was

**Fig. 1.** Drug serum and anti-drug antibody levels in relation to drug dose intensification and the addition of an immunomodulator. (A) Case 1, (B) Case 2, (C) Case 3, (D) Case 4. IFX: infliximab, ADA: anti-drug antibodies, MTX: methotrexate, ADL: adalimumab.
switched to adalimumab, standard dosing, with oral (22.5 μg once weekly) MTX due to the development of ADA on infliximab. While on adalimumab, the patient demonstrated antibodies with a trough drug level of < 0.6 μg/mL and ADA level of 3.9 U/mL after his 4th injection. The dosing interval was shortened from every 2 weeks to every week, continued MTX. His next titer after his 14th injection demonstrated low drug level of 6.3 μg/mL and ADA of 2.2 U/mL. With this regimen, the patient had achieved clinical remission after his 20th infliximab infusion (Fig. 1).

Case 4
A 12-year-old female presenting with poor weight gain and diarrhea was diagnosed with CD of the small and large intestine. She was induced and started on standard infliximab dosing with AZA due to diffuse inflammation found on endoscopy. The patient initially had ADA of 23.0 U/mL and an undetectable drug trough level after her 5th infusion. Her infliximab interval was decreased from every 8 to every 4 weeks. The subsequent titer after her 7th infusion demonstrated decreased ADA of 9.9 U/mL. She demonstrated eventual resolution of her ADA after her 8th infusion with an infliximab drug trough level of 22.4 μg/mL (Fig. 1).

DISCUSSION
The introduction of anti-TNF agents in the past decade has transformed the management of IBD with longer time spent in remission and reduced need for surgery and/or hospitalizations [2]. However, loss of response, defined as either the need for dose intensification or drug discontinuation, has been reported to be about 13% per patient year of treatment [10]. Various factors have been proposed as potential causes of loss of response [5].

Immunogenicity was studied by Baert et al. [6] in 2003. This prospective study of CD patients reported that ADA of 8.0 μg/mL or greater before an infusion predicted a shorter duration of response when compared to those with lower concentrations of antibodies. The presence of antibodies has been associated with lower drug trough levels, higher risk of infusion reactions, worse clinical outcomes, and increased need to intensify dose or switch to an alternative treatment [6,11]. A systematic review examining the impact of ADA on clinical outcomes and serum infliximab levels demonstrated that ADA are associated with a three times higher risk of clinical loss of response and lower drug trough levels [12].

Recently, several studies have elaborated on the reversal of immunogenicity in adult patients. Vande Casteele et al. [13] first demonstrated immunogenicity reversal for patients on infliximab with either drug interval shortening, dose intensification, or both. Other studies have since demonstrated the efficacy of dose escalation [8]. Addition of an immunomodulator has also been found to be effective in decreasing loss of response to anti-TNF agents, although the exact mechanism remains unclear. Colombel et al. [14] demonstrated in the SONIC (Study of Biologic and Immunomodulator Naïve Patients in Crohn Disease) trial that CD patients had a greater likelihood of achieving corticosteroid-free clinical remission on infliximab and AZA than patients on infliximab monotherapy. Similar clinical improvement with immunomodulators has also been demonstrated in UC patients [15].

Specific ranges for ADA levels have been investigated previously. Vande Casteele et al. [16] reported patients with an ADA level of < 3.13 U/mL, using HMSA testing for infliximab, were more likely to have active disease, defined as a C-reactive protein > 5 μg. In addition, patients with an ADA level more than 9.1 U/mL, at time of loss of response using HMSA testing for infliximab, had a likelihood ratio of 3.6 for an unsuccessful intervention (82% specificity and 65% sensitivity, area under the curve=0.73; p=0.003) [13]. Despite the existing research, the recently published American Gastroenterological Association’s (AGA) guidelines on therapeutic drug monitoring (TDM) state there is not sufficient data to firmly establish high versus low ADA levels and they indicate such interventions are not feasible [17]. Additionally, though higher drug serum levels in the presence of ADA may serve as an indication for
switching to a different biologic therapy, specific recommendations for the maximum level of a drug trough requiring this switch are not available, as the AGA only suggests minimum trough concentrations of $\geq 5 \mu g/mL$ for infliximab and $\geq 7.5 \mu g/mL$ for adalimumab as target drug trough levels [17]. Given some of our patients demonstrated the ability to overcome ADA even in the presence therapeutic drug serum levels, it appears worth considering an intervention to overcome ADA not only for sub therapeutic, but also for therapeutic drug serum trough levels. Pediatric IBD patients will have a longer course of disease given their early age of diagnosis and durability of these biologic agents is a major consideration. This suggests reversal of immunogenicity, even with therapeutic drug serum trough levels, should be considered on a patient-to-patient basis.

More recently, the use of TDM has been proposed to help decrease loss of response and development of ADA. The TAXIT (Trough Level Adapted infliXiMab) trial employed a dose optimization phase prior to randomization of patients to clinically based monitoring versus proactive monitoring. Although clinical outcomes were similar at one year, the rate of complications was lower in the proactive monitoring group [17]. In pediatric patients with CD, a recent retrospective study suggested that infliximab dose intensification guided by TDM was associated with steroid-free remission [8]. Though these studies demonstrated efficacy of TDM, they were limited by the utilization of first generation assays, in which ADA levels cannot be interpreted in the presence of free drug. In addition, transient antibodies have been reported as a complicating factor when considering loss of response [18]. In our case series, all four patients had two consecutive tests confirming the presence of ADA, making this likely transient antibodies but true reversal of immunogenicity.

Measuring drug and ADA levels with each test varies based on whether or not it is a drug sensitive test (first generation testing such as enzyme-linked immunosorbent assay [ELISA]) or drug tolerant (second generation testing such as the HMSA). Drug sensitive assays do not allow for interpretation of ADA levels in the presence of detectable drug levels, whereas drug tolerant assays allow for accurate determination of both drug and ADA levels. HMSA testing is not widely available and can be cost prohibitive, but has been compared with the ELISA in recent publications with favorable results and reliability [9,19]. Specific use of one test over the other has not been well established and is at the discretion of the treating gastroenterologist, based on local resources and availability of each of the tests.

The current case series presents pediatric data on the reversal of immunogenicity for in IBD patients and could aid the clinician by providing a practical approach for increasing the durability of anti-TNF therapy. It is worth noting that in patients 1, 2, and 4 who were on infliximab and experienced clearance of ADA, the drug levels exceeded the current accepted therapeutic range of 3 to 10 $\mu g/mL$. It is unclear what significance these higher drug levels have overall. Our observations suggest that interventions including dose intensification, dose interval shortening, and/or the addition of an immunomodulator in infliximab or adalimumab-treated pediatric IBD patients with immunogenic loss of response may lead to clearance of ADA and restoration of clinical response. To corroborate these findings, larger-scale studies, ideally prospective randomized controlled trials, should examine the impact of both individual interventions and combination therapies on loss of response.

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