Measurement of Infliximab and Anti-Infliximab Antibody Levels Can Help Distinguish Maintenance Versus Loss of Response

Steenholdt C, Bendtzen K, Brynskov J, Thomsen OØ, Ainsworth MA. Cut-off levels and diagnostic accuracy of infliximab trough levels and anti-infliximab antibodies in Crohn's disease. *Scand J Gastroenterol*. 2011;46:310-318.

Inflammatory bowel disease (IBD), comprised primarily of Crohn's disease (CD) and ulcerative colitis (UC), is estimated to affect approximately 1 million individuals in the United States alone, with approximately 30,000 new cases diagnosed annually.[1] A hallmark of this disease is chronic inflammation of the intestinal mucosa, which results in part from production of the tumor necrosis factor–α (TNF-α) cytokine. Understanding of this pathogenesis has been exploited for treatment purposes, and antibodies directed against TNF-α have proven to be highly effective for the induction and maintenance of remission in both CD and UC. Infliximab (Remicade, Janssen Biotech) was the first anti–TNF-α biologic therapy approved for the treatment of both CD and UC, based on clinical trial data showing robust efficacy in these conditions.[2-6]

Despite its proven efficacy, a subset of patients do not respond to infliximab (and other anti–TNF-α agents).[2] Additionally, some patients achieve an initial response to induction therapy but lose this response over time with maintenance treatment. The reasons for these therapeutic failures remain a matter of debate. One possibility is that loss of response is due to an immunologic mechanism, whereby the patient mounts an immune response to infliximab, thus forming anti-infliximab antibodies. Multiple studies in CD patients have linked the development of anti-infliximab antibodies with loss of treatment response and shorter duration of response.[7-10] Another possibility is that loss of response to infliximab is pharmacologic in nature; under this mechanism, individuals' differing pharmacokinetic or pharmacodynamic profiles may contribute to their inability to maintain a therapeutic serum level of infliximab. Indeed, low serum infliximab concentrations have been linked to a lack of clinical response in both CD and UC.[7,11-14]

Therapeutic failures with infliximab, and the underlying reasons for these failures, pose a significant challenge for clinicians who manage patients with IBD. There are no standard guidelines defining a therapeutic strategy among this patient subset, although treatment algorithms have been proposed.[15,16] The lack of such guidance is primarily due to a paucity of data demonstrating clinically relevant threshold levels of infliximab and/or anti-infliximab antibodies. Further, clinicians do not yet know whether the use of such threshold levels, if identified, would aid in the discrimination of responding versus nonresponding patients. These knowledge gaps led to the design of the recent study by Steenholdt and colleagues, published in the *Scandinavian Journal of Gastroenterology*, which is the first study to establish threshold values for clinically relevant concentrations of circulating serum levels of both infliximab and anti-infliximab antibodies in IBD patients.[17]

Study Description

A total of 106 patients (85 with CD and 21 with UC) were identified over the course of 10 years (January 2001 to June 2010); these patients were all receiving care at a tertiary care center. All patients received infliximab treatment for IBD, as well as concurrent hydrocortisone, acetaminophen, and cetirizine to prevent acute infusion reactions and to limit the development of anti-infliximab antibodies.[8]

IBD patients who received infliximab maintenance therapy (defined as regular infliximab infusions every 4–12 weeks, with the first infusion occurring within 8 weeks following completion of induction therapy) were classified as having 1 of 2 responses: maintenance of response or loss of response. Patients who maintained response had a good clinical response to infliximab induction therapy and

continued this response over the course of maintenance treatment. In contrast, patients with a loss of response initially experienced a good clinical response to infliximab induction therapy but subsequently lost this response during maintenance treatment, resulting in discontinuation of therapy. Classification of infliximab response was based on clinical assessment alone; investigators were blinded to the results of the serum trough level analyses.

Trough levels of infliximab and/or anti-infliximab antibodies were measured; these levels were defined as the serum concentration immediately prior to an infliximab infusion. Infliximab levels were measured using a fluid-phase radioimmunoassay, in which radiolabeled TNF-α (^{125}I-TNF-α) was used as a tracer. When measuring infliximab concentrations, this assay selected for functional infliximab molecules by detecting only those infliximab antibodies that bound the radiolabeled TNF-α tracer. Anti-infliximab antibody concentrations were measured in a similarly designed assay, with radiolabeled infliximab (^{125}I-infliximab) used as a tracer. A receiver operating characteristics (ROC) analysis was performed to identify optimal cutoff levels for each antibody (infliximab and anti-infliximab); the cutoff values that were selected displayed the least difference between sensitivity and specificity.

The majority of patients included in this study had CD (n=85). Of these patients, 69% maintained their response to infliximab, while the remaining 31% were characterized as having lost response to infliximab. The baseline characteristics of the 2 patient groups were well balanced, including patient age, sex, duration of disease, and disease type. Further, there were no significant differences in the total number of infliximab infusions administered to the 2 patient groups.

Infliximab trough levels were significantly increased among CD patients who maintained response to therapy compared to patients who lost response (median infliximab trough level, 2.8 µg/mL vs 0 µg/mL; *P*<.0001). Using data from these patients, a cutoff value of 0.5 µg/mL was defined as clinically relevant for infliximab trough concentrations. Infliximab trough concentrations less than 0.5 µg/mL were associated with a sensitivity of 86% (95% confidence interval [CI], 64–97) and a specificity of 85% (95% CI, 72–94) for identifying patients with a loss of response to infliximab maintenance therapy. In a ROC analysis, the cutoff value of 0.5 µg/mL for infliximab trough level was determined to have significant effectiveness (*P*<.0001), with an 87% accuracy rate. When this cutoff value was considered in a binary manner (<0.5 µg/mL vs ≥0.5 µg/mL), 73% (95% CI, 52–88) of CD patients with low infliximab trough levels showed a loss of response, while 95% (95% CI, 83–99) of CD patients with high trough levels maintained response.

Trough levels of anti-infliximab antibodies were equally revealing. Anti-infliximab antibody trough levels were significantly higher in CD patients who had lost response to infliximab maintenance therapy compared to patients who had maintained response (median anti-infliximab antibody trough level, 35 U/mL vs 0 U/mL; *P*<.0001). Using these data, a cutoff value of 10 U/mL was defined as clinically relevant for anti-infliximab antibody trough concentrations. Notably, this value corresponded to the detection limit of the radioimmunoassay used to measure antibody levels. Anti-infliximab antibody trough levels of 10 U/mL or higher were associated with a sensitivity of 81% (95% CI, 61–93) and a specificity of 90% (95% CI, 79–96) for the identification of CD patients who had lost response to infliximab maintenance therapy. In a ROC analysis, the cutoff value of 10 U/mL for anti-infliximab antibodies was found to have an 87% accuracy rate. This cutoff value was applied to define a binary threshold for anti-infliximab antibody trough levels (<10 U/mL vs ≥10 U/mL); using this binary threshold, 78% (95% CI, 57–91) of CD patients with high anti-infliximab levels had a loss of response to infliximab maintenance therapy, while 91% (95% CI, 80–97) of patients with low anti-infliximab antibody levels maintained their response.

Interestingly, patients who developed detectable (≥10 U/mL) trough levels of anti-infliximab antibodies were nearly 3 times more likely not to have received concomitant immunosuppressive therapy (azathioprine, 6-mercaptopurine, or methotrexate; odds ratio, 2.8; 95% CI, 1.2–6.8; *P*=.02). In addition, patients who had been re-treated with infliximab (defined as a minimum of 2 separate infliximab treatment series occurring ≥6 months apart) were significantly more likely to have developed detectable anti-infliximab antibody trough levels compared to patients who were receiving infliximab for the first time (odds ratio, 3.3; 95% CI, 1.2–9.2; *P*=.03). In contrast, neither concomitant immunosuppressive therapy nor infliximab re-treatment was significantly associated with trough levels of infliximab.

The threshold levels established for both infliximab and anti-infliximab antibodies were evaluated in combination in 69 CD patients (48 patients who had maintained response and 21 patients who had lost response). This combination was shown to be highly accurate (90%) for the association between trough levels and clinical response. The sensitivity and specificity of the combined analysis were 81% (95% CI, 57–94) and 94% (95% CI, 82–98), respectively. Among CD patients with both low infliximab trough levels (<0.5 µg/mL) and high anti-infliximab antibody trough levels (≥10 U/mL), a majority (85%) were classified as having lost response to infliximab maintenance therapy. Similarly, the vast majority (92%) of CD patients with high

infliximab trough levels (≥0.5 µg/mL) and low anti-infliximab antibody trough levels (<10 U/mL) maintained their response to treatment.

Similar determinations of infliximab and anti-infliximab antibody trough levels were made in UC patients, although this group of patients was much smaller (n=21). The association between trough levels and infliximab response in UC patients showed a trend comparable to that observed in CD patients. High infliximab concentrations (median trough level, 3.8 µg/mL) and low anti-infliximab antibody concentrations (median trough level, 0 U/mL) were associated with maintenance of response, while low infliximab concentrations (median trough level, 0 µg/mL) and high anti-infliximab antibody concentrations (median trough level, 85 U/mL) were associated with a loss of response (*P*=.0083 and *P*=.0007 for association of infliximab and anti-infliximab antibody trough levels, respectively). The cutoff values for infliximab and anti-infliximab antibody trough levels were also determined for UC patients: 0.8 µg/mL for infliximab (75% sensitivity [95% CI, 35–97] and 100% specificity [95% CI, 48–100]) and 10 U/mL for anti-infliximab antibodies (80% sensitivity [95% CI, 44–97] and 100% specificity [95% CI, 69–100]).

Clinical Relevance

This study was significant because it defined clinically relevant threshold values for trough concentrations of both infliximab and anti-infliximab antibodies in IBD patients. Separately, these thresholds were shown to each have an 87% accuracy rate for identifying patients who showed maintenance of response versus loss of response to infliximab maintenance therapy. When the 2 threshold values were combined, the accuracy rate increased to 90%. Overall, the majority of patients with high infliximab trough levels and low anti-infliximab antibody trough levels maintained response to infliximab maintenance treatment; in contrast, most patients with low infliximab trough levels and high anti-infliximab antibody trough levels lost response to infliximab during maintenance treatment.

These findings are important because they provide insight into the clinical relevance of infliximab and anti-infliximab serum trough levels during maintenance therapy. While previous studies have also reported these trough levels, their association with response to infliximab therapy has been ambiguous. Some of this confusion may be due to the use of enzyme-linked immunosorbent assays (ELISAs) for the measurement of infliximab and anti-infliximab antibody levels, as use of these assays has met with some controversy in the field. For example, it is unclear whether ELISAs assess the amount of functional (bioactive) infliximab or if they detect only a subset of the total anti-inflix-

imab antibodies formed; also, detection of anti-infliximab antibodies suffers from interference by infliximab present in serum. Additionally, ELISAs are subject to false-positive and false-negative results related to nonspecific binding or epitope masking, among other causes.

To overcome the shortcomings associated with the use of ELISA, Steenholdt and colleagues used a commercially available radioimmunoassay technique to measure infliximab and anti-infliximab antibody trough levels.[17] For measurement of infliximab concentration, the radioimmunoassay selectively detects only functional (bioactive) infliximab by isolating the fraction of infliximab capable of binding TNF-α. When measuring anti-infliximab antibody concentrations, the radioimmunoassay is capable of detecting all immunoglobulin isotypes that bind infliximab. It should be noted that, unlike ELISAs, the methods for radioimmunoassay require more specialized equipment and include the use of a radioactive reagent; thus, the use of radioimmunoassays is limited to laboratories with the required equipment and facilities for handling radioactive materials. Also, because the cutoff values defined in this study were obtained using radioimmunoassays, it may not be possible to extrapolate them to values obtained using ELISA. The authors also acknowledged other important limitations of this study, including the lack of definitive criteria for response to infliximab maintenance therapy (eg, clinical activity index score or endoscopic evaluation, both of which have been previously validated for this purpose).

While cutoff values were established and found to be comparable (although not identical) in both CD and UC patients, limited conclusions can be drawn from this small and retrospective study. Prospective follow-up studies with larger patient sets will potentially help to confirm and validate these results.

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Commentary

Optimizing Infliximab Therapy for Inflammatory Bowel Disease— The Tools Are Getting Sharper

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Infliximab (Remicade, Janssen Biotech) is a chimeric (75% human and 25% murine), monoclonal, immunoglobulin (Ig)G1 antibody that binds to soluble tumor necrosis factor (TNF)-α and prevents the cytokine from triggering the cellular TNF receptor complex and its effects. Infliximab also binds to transmembrane TNF-α and results in apoptosis of TNF-α–producing cells.[1] Up to 40% of Crohn's disease (CD) patients who initially respond to infliximab lose response within the first year.[2] Secondary nonresponse may be due to disease-related factors or drug-related factors, including neutralizing antibodies, altered clearance of the drug, or immunologic escape from TNF-driven inflammation. Recent guidelines from the World Congress of Gastroenterology suggest that a diminished or suboptimal response to infliximab can be managed in 1 of 3 ways: shortening the interval between doses, increasing the dose to 10 mg/kg, or switching to a different anti-TNF agent (in patients who continue to have loss of response after receiving the increased dose).[3]

Failure of infliximab therapy may be due to pharmacokinetic or pharmacodynamic mechanisms or immunogenic mechanisms. Serum albumin may be predictive of infliximab pharmacokinetics.[4] All exogenous proteins have the potential to induce immunogenicity.[5] The formation of anti-infliximab antibodies (ATIs) is associated with a lower serum infliximab level, diminished clinical response, and infusion reactions.[6] In the SONIC study, ATIs were detected at Week 30 in 0.9% of patients receiving combination therapy with azathioprine plus infliximab and 14.6% of patients receiving infliximab monotherapy.[7] Median serum trough levels of infliximab were higher in the combination therapy group than the infliximab monotherapy group.

The most commonly used method for detection of ATIs is a double-antigen enzyme-linked immunosorbent assay (ELISA) that uses specific antibodies for capture and detection.[8] Serum infliximab interferes with ATI measurement in this method. Infliximab is an IgG construct containing κ light chains. An alternative ELISA using an anti–human λ chain antibody for ATI detec-