The New Test for Monitoring Infliximab Therapy: From Laboratory to Clinical Practice

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ABSTRACT: **Background**: Biological agents for anti-tumor necrosis factor-α therapy have revolutionized treatments for autoimmune diseases; however, approximately 20% of rheumatology and 40% of gastroenterology patients do not respond to the therapy, or they show reduced drug efficacy because of anti-drug antibody (ADA) formation.

Objectives: To evaluate laboratory tools for individual monitoring of infliximab therapy and the relationship between ADA and infliximab serum levels, ADA and clinical response, and ADA and autoantibodies.

Methods: Our study comprised patients treated with infliximab and affected by selected rheumatology and gastroenterology diseases. Sera were analyzed for infliximab, total-anti-drug antibodies (Total-ADA), and free-anti-drug antibodies (Free-ADA) serum levels and for the detection of specific autoantibodies.

Results: We analyzed 73 patients. Total-ADA were detected in 26 rheumatology and 21 gastroenterology patients. Serum infliximab levels were significantly lower in Total-ADA positive patients (P = 0.01 for rheumatology group, P = 0.02 for gastroenterology group). A lack of response was observed in 7 rheumatology and 15 gastroenterology samples. Total-ADA serum levels were statistically significantly higher in patients with treatment failure in both groups (P = 0.01 and P = 0.001, respectively). There was no significant association between the presence of Total-ADA and other autoantibodies. Free-ADA were detected in only 27 rheumatology patients. Results showed a significant correlation with clinical outcome (P = 0.006).

Conclusions: The correlation with clinical response suggests that the presence of ADA could interfere with efficacy of therapy. The tests for monitoring therapy may be an important tool to assist clinicians in early detection and prevention of therapy failure.

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KEY WORDS: tumor necrosis factor-alpha (TNF-α), infliximab, anti-drug antibody (ADA), clinical response

Tumor necrosis factor-alpha (TNF-α) is a 17 kDa protein consisting of 157 amino acids. TNF-α is mainly produced by natural killer cells, T lymphocytes, and macrophages and is expressed in some other cells such as fibroblasts, tumor cells, and neurons [1]. Biological TNF-α is a pleiotropic cytokine and has a key role in host defense mechanisms and initiates the response to local injury. However, in excess, the presence of TNF-α can lead to inappropriate inflammation and consequent tissue damage [2]. Unregulated TNF-α can contribute to several pathological situations, including immune-mediated inflammatory diseases such as rheumatoid arthritis (RA), ankylosing spondylitis (SpA), psoriatic arthritis (PA), ulcerative colitis (UC), Crohn's disease (CD), and psoriasis. Both animal and human studies concerning the role of TNF-α in autoimmune disorders led to the development of TNF-α blockage therapy [3].

Infliximab was the first TNF-α inhibitor to be released on the market and it is one of the most commonly prescribed therapies. It is an immunoglobulin G_1 (IgG_1) monoclonal antibody containing a chimeric protein of approximately 25% murine sequences (variable region) and approximately 75% human sequences (constant region) [4]. It presents high specificity, affinity, and avidity for TNF-α, and it is capable of neutralizing the biological activity of TNF by binding to the soluble and transmembrane forms of TNF, preventing it from binding to cellular receptors and inducing the lysis of cells that produce TNF [4]. Infliximab and other biological agents have revolutionized the treatment of chronic inflammatory disease, thereby improving patient outcomes [5].

Although TNF-α antagonists are for the most part well tolerated, their safety and efficacy can be compromised by the development of immune response against anti-TNF-α with the production of anti-drug antibodies (ADA). Neutralizing ADA directly interferes with the ability of TNF-α-inhibitors to block TNF-α signaling through specific TNF-receptors on target cells. ADA may be directed against idiotopes inside or outside the TNF-binding fragments of the anti-TNF-α immunoglobulin construct [6].

The presence of ADA and the development of immunogenicity is associated with primary or secondary failure of anti-TNF-α

therapy and with significant adverse events, such as infection and in-site infusion reaction. The lack of clinical response in patients with ADA may be explained by immune complex formation between TNF-α inhibitors and ADA, thus suppressing drug activity. This situation may be related to an increased drug clearance, which leads to lowering serum drug levels [5].

Due to the clinical and economic implications linked to therapy failure and adverse events, laboratory testing for monitoring TNF-α inhibitor therapy has recently been developed to prevent such complications.

Our study was designed to evaluate laboratory tools for monitoring infliximab therapy in patients with specific autoimmune diseases.

We assessed infliximab serum levels and ADA to study the relationship between ADA and infliximab concentration, ADA and clinical response, and ADA and specific autoantibodies of patients with RA, SpA, PA, UC, and CD.

PATIENTS AND METHODS

STUDY DESIGN

A multi-center study was designed to recruit patients from the gastroenterology department at the University of Parma, Italy and the rheumatology and gastroenterology departments at the University of Modena, Italy. All laboratory tests were performed at the diagnostic laboratory department as OCSAE, Azienda USL, Modena, Italy.

Durning a 12 month period (April 2015–April 2016) two groups of patients were enrolled: patients with rheumatology diseases (RA, SpA, and PA) and patients with gastroenterology disorders (CD and UC). All patients were prescribed infliximab (alone or with immunosuppressors) both naïve and through a follow-up program.

Blood samples were collected prior to individual infliximab administration and serum aliquots were used to test ADA, infliximab levels, and autoantibodies according to each disease.

With the clinicians, we grouped patients as either responders or non-responders to infliximab therapy to study the correlation between the presence of ADA and clinical outcome.

LABORATORY ASSAYS

For infliximab monitoring therapy we detected three parameters: infliximab serum levels, total-anti-infliximab antibodies (Total-ADA), and free-anti-infliximab antibodies (Free-ADA). Total-ADA measure the presence of total anti-drug antibodies (free in the serum and bound with the drug); whereas, Free-ADA detect the ADA free from the drug in the serum.

Anti-infliximab antibodies and infliximab serum levels were measured using an ELISA commercial kit (Immunodiagnostik AG, Benhseim, Germany), following the manufacturer's instructions. The cut-off value of ADA was determined as twice the optical density of the negative control supplied by the manufacturer's instructions. The cut-off value for the presence of anti-infliximab antibodies was set at 10 AU/ml, while the therapeutic range of infliximab was between 3 and 7 μg/ml.

For all patients, ANA, anti-extractable nuclear antigens (ENA), and anti-double strand dsDNA antibodies were detected. In addition, anti-citrullinated protein antibodies (ACPA) were measured in patients with RA, SpA, and PA, while anti-Saccaromyces Cerevisae antibodies (ASCA IgA and ASCA IgG) and anti-neutrophil cytoplasmic antibodies (ANCA) were tested in patients with CD and UC.

ANA and ANCA were detected by indirect immunofluorescence (Hep-2 cells for ANA and ethanol fixed human neutrophil cells for ANCA, respectively), and ENA, ACPA, and ASCA IgA and IgG were detected by immunoassay. dsDNA was tested first by immunoassay and then positivity was confirmed by indirect immunofluorescence (*Crithidia luciliae*).

Statistical analysis was conducted using the Mann–Whitney test to study the association between the presence of anti-infliximab antibodies, infliximab serum levels, and clinical response. The Fisher's exact test was used to evaluate the association between the presence of ADA and other autoantibodies in all diseases.

RESULTS

We enrolled a total of 73 patients (21 females, 52 males) with a mean age of 50.15 years ± 8.44.

We found that 36 rheumatology patients (42 samples, 5 patients were tested several times throughout the study period) were affected with RA, SpA, and PA (6, 24, and 6 patients, respectively) while 37 gastroenterology patients were affected with CD and UC (25 and 12 patients, respectively).

For therapy monitoring, we measured infliximab serum levels and the presence of Total-ADA for all samples.

Total-ADA antibodies were detected in 26 rheumatology samples and 21 gastroenterology samples. Serum infliximab levels were significantly lower in ADA-positive patients with respect to ADA negativity in both rheumatology (mean 2.19 ± 1.95 μg/ml, $P = 0.01$) and gastroenterology samples (mean 5.36 ± 8.78 μg/ml, $P = 0.02$). Data are dispalyed in Figure 1A and Figure 1B.

A lack of response was observed in 7 rheumatology (16.7%) and 15 gastroenterology (40.5%) samples. Total-ADA serum levels were significantly higher in treatment failure patients with respect to patient responders for both groups (mean 37.52 ± 53.23 AU/ml, $P = 0.001$ in RA, SpA, and PA patients; mean 30.30 ± 45.90 AU/ml, $P = 0.01$ in CD and UC patients) as detailed in Figure 2A and Figure 2B.

The association between infliximab serum levels and clinical outcome was not statistically significant ($P = 0.09$ and $P = 0.14$ in the rheumatology and gastroenterology groups, respectively) as shown in Figure 3A and Figure 3B.

Free-ADA was detected in 27 rheumatology patients only. Although no correlation between the presence of Free-ADA and infliximab serum levels was found (mean 2.33 ± 1.99 µg/ml, $P = 0.10$) [Figure 1C], our data show a significant association between the presence of Free-ADA and clinical outcome (mean 37.91 ± 72.40 AU/ml, $P = 0.006$) [Figure 2C].

We studied the association between the presence of Total-ADA and ANA and anti-dsDNA and anti-ENA for all patients. There was no significant association identified between the presence of ADA and other autoantibodies, with the exception of ANCA ($P = 0.01$), as shown in Table 1.

DISCUSSION

Different assays have been developed to measure ADA serum concentrations for laboratory testing. We selected the ELISA assay to detect infliximab serum levels and ADA because this method is simple and quick to perform. It offers the possibility of total automation on laboratory instruments as well as total traceability of results, thus reducing the possibility of errors. However, the limitations of this test, such as the possibility of interference with other drugs and the presence of false-positive results, remain [7].

The results from the current study confirm that there is an inverse correlation between anti-drug antibodies and infliximab levels, as has been identified in other studies [8]. The early detection of ADA could therefore be useful in determining the etiology of low infliximab sub-therapeutic levels, possibly facilitating clinical decision making regarding other therapeutic options for these patients considered non-responders [9]. As with previous findings [6,10], our results also demonstrate that the presence of ADA interferes with clinical outcome. A significant association between the presence of Total-ADA and Free-ADA and treatment failure was confirmed. These data can be applied both for rheumatology and gastroenterology patients [10]. These results could have important implications for the strategy of therapy and the early detection of high ADA levels, thereby preventing a lack of therapeutic response and eventual treatment failure.

In the literature, there is an active discussion concerning the detection of ANA and other autoantibodies during biological therapy treatment, as opposed to disease activity [6]. In the current study, no correlation between ADA and autoantibodies was identified, suggesting that these autoantibodies should be used in the follow-up program only in the presence of specific clinical signs.

Our study design is limited because only five different diseases were tested on a relatively small population size. Patients undergoing infliximab therapy alone or in conjunction with immunosuppressors were tested together, rendering the groups heterogeneous. Further studies should separate these patient groups to include larger populations, stratified according to

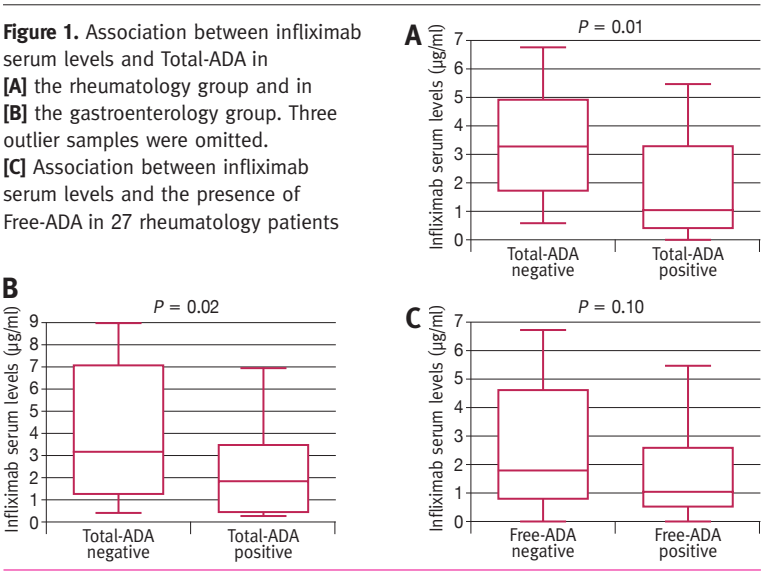

Figure 1. Association between infliximab serum levels and Total-ADA in **[A]** the rheumatology group and in **[B]** the gastroenterology group. Three outlier samples were omitted. **[C]** Association between infliximab serum levels and the presence of Free-ADA in 27 rheumatology patients

ADA = anti-drug antibody

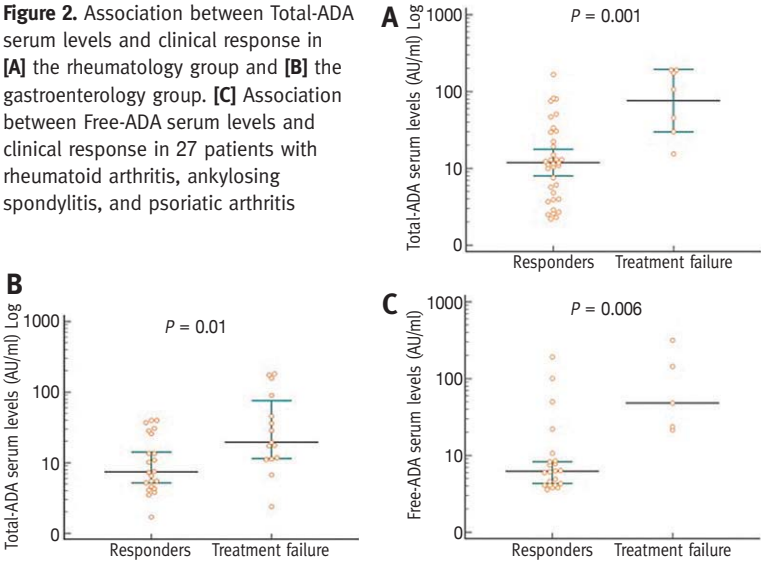

Figure 2. Association between Total-ADA serum levels and clinical response in **[A]** the rheumatology group and **[B]** the gastroenterology group. **[C]** Association between Free-ADA serum levels and clinical response in 27 patients with rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis

ADA = anti-drug antibody

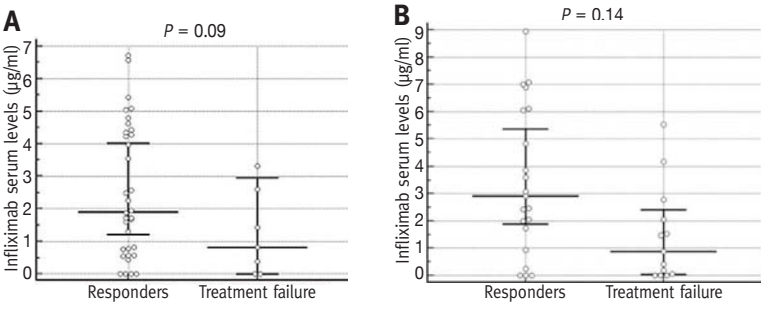

Figure 3. Association between infliximab serum levels and clinical response in **[A]** rheumatology group and **[B]** gastroenterology group

Table 1. Autoantibodies anti-infliximab antibodies in rheumatology and gastroenterology groups of patients

	Autoantibodies	Total autoantibodies (positive)	ADA+	ADA-	*P* value
Rheumatology group	ANA positive	32	22	10	0.14
	ENA positive	0	0	0	–
	dsDNA positive	7	6	1	0.22
	ACPA positive	6	5	1	0.38
Gastroenterology group	ANA positive	14	9	5	0.51
	ENA positive	0	0	0	–
	dsDNA positive	1	1	0	1
	*ANCA positive	8	1	7	0.011
	*ASCA IgA positive	12	7	5	0.72
	*ASCA IgG positive	13	8	5	0.47

*ANCA, ASCA IgA and ASCA IgG were tested only in 28 patients in the gastroenterology group

ANCA = anti-neutrophil cytoplasmic antibodies, ASCA = anti-citrullinated protein antibodies, IgA = immunoglobulin A ,IgG = immunoglobulin G, ENA = anti-extractable nuclear antigens, ANA = anti-nuclear antibodies

the assumption of infliximab alone and infliximab in conjunction with immunosuppressors, to confirm current findings suggesting that immunosuppressors increase patient response to infliximab therapy. We also suggest conducting studies dedicated to the change in therapy response over time, during therapy assumption. Moreover, the correlation between ADA and adverse events is needed.

CONCLUSIONS

In conclusion, our study shows that laboratory tests of ADA and infliximab serum levels for patients with rheumatology and gastroenterology diseases are useful for the monitoring and management of patients assuming anti-infliximab-a therapies. These tests can help clinicians understand the causes for loss of response, treatment failure, or side effects. Data from laboratory tests can assist clinicians in their therapy selection, and the introduction of these tests should be considered for routine biological therapy monitoring of autoimmune diseases.

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Capsule

cGAS drives noncanonical-inflammasome activation in age-related macular degeneration

Geographic atrophy is a blinding form of age-related macular degeneration characterized by retinal pigmented epithelium (RPE) death. the RPE also exhibits DICER1 deficiency, resultant accumulation of endogenous *Alu*-retroelement RNA, and NLRP3-inflammasome activation. How the inflammasome is activated in this untreatable disease is largely unknown. **Kerur** and co-authors demonstrated that RPE degeneration in human-cell-culture and mouse models is driven by a noncanonical-inflammasome pathway that activates caspase-4 (caspase-11 in mice) and caspase-1, and requires cyclic GMP-AMP synthase (cGAS)-dependent interferon-β production and gasdermin D-dependent interleukin-18 secretion. Decreased DICER1 levels or *Alu*-RNA accumulation triggers cytosolic escape of mitochondrial DNA, which engages cGAS. Moreover, caspase-4, gasdermin D, interferon-β, and cGAS levels were elevated in the RPE in human eyes with geographic atrophy. Collectively, these data highlight an unexpected role of cGAS in responding to mobile-element transcripts, reveal cGAS-driven interferon signaling as a conduit for mitochondrial-damage-induced inflammasome activation, expand the immune-sensing repertoire of cGAS and caspase-4 to noninfectious human disease, and identify new potential targets for treatment of a major cause of blindness.

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