Utility of Measuring Serum Concentrations of Anti-TNF Agents and Anti-Drug Antibodies in Inflammatory Bowel Disease

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Abstract: Tumor necrosis factor alpha (TNFα) is a cytokine with a critical role in the pathogenesis of some chronic inflammatory diseases, such as inflammatory bowel diseases. Anti-TNF agents, which neutralize the biological activity of TNFα, are widely used among the different therapeutic options for the treatment of patients with inflammatory bowel diseases. These drugs are very useful in clinical practice, but some patients experience lack and loss of response during the treatment. Drug serum concentration, antibodies against anti-TNF agents, clearance of the drug, formation of immune complexes, a more severe disease and probably other unknown factors can influence the treatment's efficacy. Nowadays, the management of patients with lack or loss of response is empirical. The measurement of drug concentrations and antibodies against anti-TNF agents might be useful for improving the selection of patients that will benefit from the maintenance treatment. In clinical practice, these methods may help us decide which strategy will be used in cases of loss of response: treatment intensification, shortening the infusion interval, increasing the dose, switching to another anti-TNF agent or to a drug with another mechanism of action. The optimal strategy in the future may be comprised of an early detection of loss of response to the treatment by assessing clinical symptoms and finding evidence of activity of the disease on endoscopic or radiological examinations when necessary, as well as a better management of anti-TNF treatment aided by measuring the serum concentration of the drug and antibodies against the drug.

Keywords: Adalimumab, antibodies, infliximab, immunogenicity, through levels, tumor necrosis factor alpha.

INTRODUCTION

Inflammatory bowel diseases (IBD) are chronic diseases that include Crohn´s disease (CD) and ulcerative colitis (UC). Although its etiology is unknown, genetic and environmental factors are important in the pathogenesis of the disease, and the gene–environment interaction determines disease susceptibility and behavior [1]. Dysregulation of intestinal mucosal immunity with an imbalance between pro-inflammatory and anti-inflammatory cytokines of the intestinal mucosa is very important for chronic development and progression [2]. Tumor necrosis factor alpha (TNFα) is a pleiotropic cytokine with a critical role in the pathogenesis of some inflammatory chronic diseases, such as IBD, rheumatoid arthritis and ankylosing spondylitis, as well as degenerative and neoplastic diseases. TNFα is produced mainly by monocytes and macrophages in response to bacterial antigenic stimuli, and in a lower proportion by T and B lymphocytes, mast cells and fibroblasts. The main roles of TNFα are the induction of cytokine and fatty acid derivatives release (IL-1, IL-6, IL-8, leukotrienes, tromboxane A2, prostaglandins), increase the production of polymorphonuclear leukocytes by the bone marrow, stimulation of mononuclear and polymorphonuclear antibacterial activity, expression of adhesion molecules on activated endothelial cells, activation of the complement system and the coagulation cascade, the promotion of changes in vascular permeability and protein catabolism, and stimulation of gluconeogenesis [3]. The TNF receptor exists as one of two isomers, a p55 receptor (TNFR I) and a p75 receptor (TNFR II) [4]. These two isoforms of TNF receptor may be found on the membrane of monocytes and T lymphocytes (mTNFR), or circulating in the serum as a soluble receptor (sTNFR). Binding of TNF with a circulating receptor essentially neutralizes its action and protects patients against shock in the context of infection or inflammation [5]. In IBD patients, TNFα levels are unusually increased in inflamed intestinal tissues, promoting the perpetuation of inflammation [6]. Blockade of TNFα significantly decreases inflammatory activity in IBD patients [7].

ANTI-TNF AGENTS

TNFα plays a key role in the etiopathogeny of IBD, therefore anti-TNF agents that neutralize the biological activity of TNFα have become a widely used treatment option. Currently, infliximab (IFX) and adalimumab (ADA) are the most frequent anti-TNF drugs used. Certolizumab pegol, another anti-TNF drug, has been approved for IBD patients in United States, but not in Europe. IFX and ADA are monoclonal IgG1 antibodies showing high affinity and specificity binding to both serum and trans-membrane TNFα, inhibiting its connection to its receptors [8]. IFX, chimeric monoclonal antibody, composed of human constant regions and murine variable regions, was the first anti-TNF agent used in IBD patients. ADA, approved later, is a 100% human anti-TNF monoclonal antibody. The binding of anti-TNF with trans-membrane TNFα produces different intracellular signals that induce apoptosis, suppression of cytokine production and cell cycle arrest [9] and can cause cell lysis by complement activation or through effector cells [10]. The induction of T-cell apoptosis by IFX and ADA is a key point of the drug's effect in IBD patients. Therefore, etanercept, another anti-TNF agent that lacks this action, is effective in some chronic rheumatic diseases such as rheumatoid arthritis, but not in IBD [11]. Another anti-TNF action is the modulation of myofibroblasts, avoiding tissular damage and aiding in epithelial barrier reparation [12].

The objective of the treatment of IBD is to control the inflammatory response so as to maintain clinical and endoscopic remission. In luminal CD, IFX and ADA are indicated for the induction of remission in steroid-refractory, -dependent or intolerant moderate-to-severe active disease. Both drugs have been approved for the maintenance of remission in patients with CD who have a clinical response to induction therapy, and IFX has been approved for perianal CD [13]. In UC, only IFX is approved for the induction and maintenance of remission [14].

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EFFICACY OF ANTI-TNF TREATMENT

Anti-TNF treatment has been an important advancement in the treatment of patients with IBD over the past decade. These drugs are very useful in clinical practice, but they have some limitations. A significant number of patients do not respond to induction treatment. The ACCENT I study included patients with moderate-severe CD, and only 58% of 573 patients treated with IFX 5mg/kg responded after 2 weeks of induction treatment [15]. In the CLASSIC I study, only 36% of active CD patients had responded to ADA 160/80 mg (first and second doses respectively) after 4 weeks [16]. Nearly two thirds of CD patients initially respond to anti-TNF induction treatment in clinical practice [17]. On the other hand, in patients that initially respond to the treatment, loss of response has been reported in 25-40% of CD patients in maintenance treatment with anti-TNF, causing an adjustment of the dose or a switch to another drug. The annual loss of response rate is about 13% per patient-year of treatment with IFX [17]. Less data has been published about loss of response to the ADA treatment, but figures seem to be similar to those described for IFX [17].

ANTI-TNF TROUGH LEVELS

One of the possible factors associated with the lack and loss of response to anti-TNF treatment is drug serum concentration. In IBD's routine practice, only the levels of cyclosporine or tacrolimus are monitored and only in patients with severe UC. The measurement of trough serum concentrations of anti-TNF agents could be important to decide the best management option for these patients, although its role has not been well established [18]. The optimal treatment would consist in a regime in which the administered dose ensures a level of the drug within the therapeutic range before the administration of the next dose, and that its peak and mid levels that do not cause an increase in toxicity [19]. It is important to consider the pharmacokinetics of anti-TNF drugs as well as intra-individual and inter-individual variations. At present, the doses usually administrated for maintenance therapy are 5mg/kg of intravenous IFX every eight weeks or 40mg of subcutaneous ADA every two weeks. Trough anti-TNF concentrations have been associated with clinical and endoscopic remission [20]. CD patients with detectable IFX trough serum concentrations have presented better remission rates, lower C-reactive protein levels and an improved endoscopic activity [20]. A recent study on UC patients showed that detectable trough serum IFX predicts clinical remission, endoscopic improvement and a lower risk of colectomy [21]. Detectable serum IFX concentration is the strongest factor associated with the prediction of response to anti-TNF therapy, regardless of disease activity, in both CD and UC patients [20-23]. Some factors can decrease drug serum levels: antibodies against anti-TNF agents, clearance of the drug, the formation of immune complexes, a more severe disease and probably other unknown factors [18, 21, 24]. Nowadays, the clinical utility of measuring the concentration of anti-TNF agents remains unclear.

ANTI-TNF ANTIBODIES

The formation of antibodies to IFX (ATI) or ADA (ATA) can affect the treatment's efficacy. ATI are present in 37-61% of patients with episodic treatment [25], and only in 6-16% of cases with maintenance treatment (13, 18). ATI formation could be caused by the chimeric nature of IFX, but similar rates of antibody formation have been reported in patients treated with ADA, even though it is entirely human [26]. This finding could be explained by the fact that any exogenous protein, although human in structure, can induce an immune response [27].

Antibodies against anti-TNF agents have been associated with a decrease in drug serum concentration, a lower remission rate and an increase in acute infusion reactions [26, 28-30]. The presence of ATIs and ATAs are weakly and variably associated with clinical response, but discordant results have been reported [31]. While some studies have reported an association between these antibodies and a loss of response to IFX or ADA [30, 32], other studies have not confirmed it [15, 20]. Sixteen to thirty-nine percent of the patients with scheduled IFX therapy have undetectable serum drug concentrations in the absence of ATI [20, 28, 33]. Most trials only include CD patients, but in the studies that include UC patients, no significant association has been found [21]. The implication of other factors in the loss of response, other than the formation of ATI and ATA, could explain this lack of association: an increased drug clearance or the formation of immune complexes [18]. High antibody levels against the drug, and not merely their presence, could be related to loss of response in some patients. Hence, ATI levels above 8 μg/ml have been significantly associated with a shorter response to IFX (35 vs. 71 days) [25]. Different factors have been associated with a higher probability of ATI or ATA formation: episodic anti-TNF treatment, therapy interruption for over 4-6 months, especially without a previous induction regimen, and no concomitant immunomodulating drug in patients with episodic treatments [28, 32].

Antibodies against anti-TNF agents have been associated with an increased risk of mild-to-moderate infusion reactions. Some immediate and acute reactions are associated with ATA and ATI, but other reactions like influenza-like reactions, arthralgia, rashes, fatigue and myalgia have not been related to the development of antibodies against the drug. A concomitant immunosuppressive treatment could prevent the formation of antibodies, thus reducing the incidence of infusion reactions. This action of the immunosuppressive treatment could be associated with an indirect increase in the serum concentration of anti-TNF agents and hence, with the improvement of the clinical response to the treatment. However, there is no evidence of better clinical or endoscopic remission rate in these patients, although combination therapy could be useful in episodic treatments [21, 25, 34-35]. Co-treatment with immunosuppressors was shown to decrease non response but only in immunosuppressor-naïve patients [36-37]. Because of the higher risk of adverse events such as infections and neoplastic diseases, combination therapy is not widely recommended in routine practice to prevent anti-TNF antibodies formation.

METHODS OF MEASUREMENT

Several methods have been described to detect drugs and antibodies, based mainly on enzyme-linked immunosorbent assay (ELISA) and radioimmunoassays [38-40]. Fluid-phase radioimmunoassays are more complex than solid-phase ELISAs, but they are more sensitive. Other advantages include a lack of interaction with immunoglobulins such as rheumatoid factor, and a detection of functionally monovalent IgG4 antibodies that is not influenced by artifacts induced by the solid-phase adsorption of proteins [41]. Nowadays, there is more experience with the ELISA test, but due to their higher sensitivity, fluid-phase radioimmunoassays could be a better measurement test.

LEVELS OF DRUGS AND ANTIBODIES

Minimum effective serum concentrations of anti-TNF drugs are unknown. At present, a detectable IFX concentration at dosing trough is considered to be a therapeutic concentration [18]. Circulating IFX and ADA may mask the presence of antibodies so that the measurement of ATI or ATA can only be done after the drug has been cleared from the serum, although novel assays could improve current measurement methods with a lower detection limit [42-43].

It has been described that an IFX serum concentration below detectable limits is necessary for the validity of ATI measurement. The binding of anti-TNF-α molecules and the antibodies against them form immune complexes that cannot be detected by ELISA, producing false negative results. When antibody levels are detectable, regardless of the serum levels of the anti-TNF-α agent, the

result is a true positive. However, when antibody levels are negative, it is necessary to know the levels of the anti-TNF-α drugs: if anti-TNF-α serum levels are undetectable, it is a true negative result, but if anti-TNF-α serum levels are detectable, the negative result must be considered inconclusive [21]. This is a handicap for measuring ATI and ATA levels in patients with maintenance treatment. The serum level for considering whether the concentration of antibodies is low or high has not been defined. Some authors have considered ATI levels above 8-10 μg/ml as high levels, but these are arbitrary [25].

TIME OF MEASUREMENT

The best time to measure anti-TNF agents and ATI or ATA levels is just before the next dose of the drug. At this point, the trough concentration is obtained, which has shown to be a good predictor of response to the treatment, and the drug interferes to a lesser extent in the determination of antibodies, thus allowing a better interpretation of the results [28]. Aybay reported that a prominent amount of IFX could be detected in the serum of patients up to the third week of the post-infusion period [4]. After the third week, the concentration decreases in a time-dependent manner. At the sixth post-infusion week, the serum IFX concentration decreased to threshold levels. Other authors have reported that serum IFX levels are maintained above the detection limit for 8 weeks. The measurement method and other factors like clearance of the drug can influence this disparity. The moment in which it is useful to determine the anti-TNF therapy is still unclear. Measurement during the treatment induction with these drugs, and subsequently during maintenance therapy if there is loss of response, are probably the most helpful moments for deciding on the most appropriate approach to follow.

CLINICAL UTILITY OF MEASUREMENT

Nowadays, anti-TNF agents and levels of antibodies against the drugs are not widely measured in routine practice. The optimal patient management based on the results of testing drug levels has not been clearly evaluated, and no prospective studies have been published. Hence, the management of patients with lack or loss of response is empirical. After loss of response, doctors usually choose to shorten the infusion interval (ADA 40 mg every week or IFX every 6 weeks) or to escalate the dose (IFX 10 mg/kg). Applying dose escalation in all patients is not cost-effective and could increase adverse events. The measurement of trough levels of anti-TNF agents and antibodies against the drug could be very useful in these cases (Table **1**). Therefore, if there is loss of response during the maintenance treatment, and if antibodies against the drug are present, an increased dose may not be effective, therefore switching to another anti-TNF agent or a concomitant immunomodulator treatment for decreasing ATI or ATA levels could be a better choice [27, 36]. In the retrospective study performed by Afif *et al*, switching to another anti-TNF-α drug showed higher efficacy than the escalation of the treatment in these patients (92% vs. 17%, $p<0.004$) [27]. This finding suggests that the escalation of therapy in patients who have antibodies against anti-TNF-α drug is less likely to be successful than switching to another anti-TNF-α drug.

In patients losing response, not presenting ATI or ATA, and with undetectable anti-TNF drug serum levels, a dose escalation or shortening the administration interval could be effective [18, 37]. Low trough levels of the drug can result from altered kinetics due to low bioavailability or decreased half-life in the circulation, as for example, due to high consumption in the case of severe disease activity. In such cases, escalation of the treatment has been associated with a greater response, compared to switching to another anti-TNF-α (86% vs. 33%, $p<0.016$) [27]. After escalation, the time up to discontinuation IFX in these patients was similar to the time up to discontinuation in patients with therapeutic levels of the drug [27]. As the remission rate is lower after a prior anti-TNF treatment [44], these patients would theoretically benefit from the administration of an increased amount of the currently-used anti-TNF-α drug.

In patients with detectable levels of anti-TNF, and no antibodies against the drug, if the patient presents clinical symptoms, radiological and endoscopic examinations may be performed in order to confirm that the symptoms are related to the presence of inflammation. If active disease is found in endoscopic or radiological tests, switching to another anti-TNF agent or, to another therapeutic class, could be the management of choice [27].

CONCLUSIONS

Anti-TNF drugs are widely used in the treatment of IBD patients. A significant proportion of patients experiences loss of response during maintenance treatment. Nowadays, the management of patients with loss of response is empirical. The measurement of drug concentrations and probably, the measurement of ATI or ATA, might be useful for improving the selection of patients who will benefit from the maintenance treatment with IFX or ADA, and would help to avoid inappropriate treatments. In clinical practice, these methods may help us to decide which strategy should be followed in case of loss of response: treatment intensification, shortening the infusion interval or increasing the dose, or switching to another anti-TNF agent or to a drug with another mechanism of action. The optimal strategy in the future may be comprised of: an early detection of loss of response by assessing clinical symptoms and finding evidence of activity of the disease on endoscopic or radiological examinations when necessary; as well as a better management of anti-TNF treatment by measuring the serum concentration of the drug and antibodies against the drug. New studies are needed to assess the clinical utility of these measurements, the best

Table 1. Treatment Algorithm in Patients with Clinical Symptoms During Anti-TNF Maintenance Treatment. [1]Endoscopic or Radiological Activity. TNF, Tumor Necrosis Factor; IBD, Inflammatory Bowel Disease; ATI, Antibodies to Infliximab; ATA, Antibodies to Adalimumab

Trough Anti-TNF Agent Concentration		Interpretation	Management
Detectable	Active disease[1]	Anti-TNF drug is not useful	Switch to a non-anti-TNF agent
	Inactive disease[1]	IBD is controlled	Investigate other diseases
Undetectable	ATI/ATA positive	Antibodies could decrease anti-TNF levels	Change to another anti-TNF agent or add an immunomodulator
	ATI/ATA negative	Bioavailability or pharmacokinetic problem	Treatment intensification (increase dose or shorten the interval)

measuring method, and the points during treatment in which these determinations may be more useful, in order to improve results and, therefore, the management of the patient.

ABBREVIATIONS

ADA	=	Adalimumab
ATA	=	Antibodies to adalimumab
ATI	=	Antibodies to infliximimab
CD	=	Crohn´s disease
ELISA	=	Enzyme-linked immunosorbent assay
IBD	=	Inflammatory bowel disease
IFX	=	Infliximab
TNFα	=	Tumor necrosis factor alpha
UC	=	Ulcerative colitis

CONFLICT OF INTEREST

María Chaparro has served as speaker and has received research funding from MSD and Abbott. Fernando Bermejo has served as advisory board member for MSD and has received research funding from Abbott. Javier P. Gisbert has served as speaker, consultant and advisory board member for and has received research funding from MSD and Abbott.

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