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BRIEF ARTICLE

Utility of serum TNF-α, infliximab trough level, and antibody titers in inflammatory bowel disease

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Abstract

AIM: To assess tumor necrosis factor-α (TNF-α), infliximab (IFX) concentrations, and antibodies against IFX molecules in patients with inflammatory bowel disease (IBD) who develop loss of response, side effects, or allergic reaction during anti TNF-α therapy.

METHODS: Blood samples of 36 patients with response loss, side effects, or hypersensitivity to IFX therapy (Group Ⅰ) and 31 patients in complete clinical remission (Group Ⅱ) selected as a control group were collected to measure trough serum TNF-α level, IFX, and anti-IFX antibody (ATI) concentration. We examined the correlation between loss of response, the development of side effects or hypersensitivity, and serum TNF-α, IFX trough levels, and ATI concentrations.

RESULTS: The serum TNF-α level was shown to be correlated with the presence of ATI; ATI positivity was significantly correlated with low trough levels of IFX. ATIs were detected in 25% of IBD patients with loss of response, side effects, or hypersensitivity, however no association was revealed between these patients and antibody positivity or lower serum IFX levels. Previous use of IFX correlated with the development of ATI, although concomitant immunosuppression did not have any impact on them.

CONCLUSION: On the basis of the present study, we suggest that the simultaneous measurement of serum TNF-α level, serum anti TNF-α concentration, and antibodies against anti TNF-α may further help to optimize the therapy in critical situations.

Key words: Tumor necrosis factor-α; Infliximab; Antibody; Inflammatory bowel disease

Core tip: The clinical utility of measuring serum tumor necrosis factor-α (TNF-α), infliximab, and anti-infliximab levels in the therapeutic decisions of patients with inflammatory bowel disease is still an outstanding question. In this study we assessed TNF-α, infliximab concentrations, and antibodies against infliximab molecules in patients with inflammatory bowel disease who developed loss of response, side effects, or allergic reaction during anti TNF-α therapy. Our results showed that the simultaneous measurement of serum TNF-α level, serum anti TNF-α concentration, and antibodies against anti TNF-α may further help to optimize therapy in critical situations.

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INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are the two types of inflammatory bowel disease (IBD) characterized by alternating periods of relapse and remission. The introduction of anti-tumor necrosis factor (TNF)-α therapy caused a dramatic change in the management of IBD. Approximately 40% of patients that initially responded to anti-TNF-α therapy will subsequently lose that response, thus requiring dose intensification or drug change[1]. The presence of antibodies against anti-TNF-α agents and low drug serum concentrations have been implicated as predisposing factors for therapeutic failure. Dose intensification as a possible method for managing therapeutic failure is only viable in cases of low anti-TNF-α drug trough levels, while switching to another drug may be useful if antibodies develop against the biological agents[2]. Immunogenicity (the formation of antibodies to the biological agents) is the major cause of loss of response and adverse reactions. Scheduled maintenance therapy, concomitant immunomodulators therapy, and pretreatment with high-dose corticosteroids may help to reduce immunogenicity[3].

Although anti-drug antibodies were initially considered to play a role in shorter response duration, recent studies revealed that detectable infliximab (IFX) trough levels, irrespective of anti-drug antibody status, may predict to clinical response and endoscopic improvement[4,5]. The role of TNF-α measurement, together with antibody and drug serum concentration in therapeutic decisions, has not previously been investigated in everyday practice; furthermore, pharmacokinetic monitoring of IFX to control disease activity and optimize the treatment of IBD is not standardized in the daily routine. The aim of this study was to assess TNF-α, IFX concentrations, and antibodies against IFX molecules in patients with IBD who developed loss of response, side effects, or allergic reaction during anti TNF-α therapy.

MATERIALS AND METHODS

Study population

Sixty-seven patients with CD and UC treated in our center with IFX between 2011 and 2012 were enrolled in this prospective observational study and categorized into two groups. Blood samples of 36 patients with response loss, side effects, or hypersensitivity to IFX therapy (Group I) and 31 patients in complete clinical remission (Group Ⅱ) selected as a control group were collected to measure trough serum TNF-α level, IFX, and anti-IFX antibody (ATI) concentration. The study was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee of the University of Szeged. The 3 infusion induction phase was followed by maintenance therapy in every patient. Data on patient demographics, clinical characteristics, concomitant corticosteroid and azathioprine therapies, need of surgery, C-reactive protein level, erythrocyte sedimentation rate (ESR), hematocrit, leukocyte and serum iron levels, and details on biological therapy were prospectively registered. Disease activity was measured by using the Crohn's disease activity index (CDAI) and partial Mayo score. The patients' demographic and clinical data are summarized in Table 1.

Table 1 Demographic and clinical data of patients participating in the study

Therapy	IBD patients with loss of response, side effects, hypersensitivity (n = 6)	Control IBD patients (n = 1)
Mean age at diagnosis, yr	34.9 (17-67)	36.4 (17-66)
Mean disease duration at biological therapy, yr	7.1 (1-20)	7.7 (1-21)
CD/UC	19/17	17/14
Male/female	14/22	14/17
Previous biological therapy	22	15
Concomitant steroid therapy	5	3
Concomitant thiopurine therapy	18	16
Previous surgery	16	7
Active disease	25	0

CD: Crohn's disease; UC: Ulcerative colitis; IBD: Inflammatory bowel disease.

We examined the correlation between loss of response, side effects, or hypersensitivity and serum TNF-α, IFX trough levels, and ATI concentrations.

Measurement of serum TNF-α, IFX trough levels, and ATI

Enzyme-linked immunosorbent assay (ELISA) was applied to determine the serum levels of TNF-α, infliximab trough levels, and ATI. Blood samples were obtained prior to application of IFX infusion. Q-INFLIXI ELISA, Q-ATI ELISA, and Q-TNF-α ELISA kits were obtained from Matriks Biotek, Ankara, Turkey.

Statistical analysis

Continuous data were analyzed using medians with an interquartile range (IQR). All categorical data were compared between groups of patients using the Pearson χ^2 statistic. Mann-Whitney U and Fisher's exact tests were used for comparison of infliximab trough levels and ATIs in a subgroup of patients. Relation between laboratory parameters, IFX trough levels, and ATI was analyzed by Mann-Whitney U test. A P value less than 0.05 was considered to be significant.

Table 2 Serum infliximab and antibody levels in cases of antibody positivity

Patients	Serum IFX level (μg/mL)	ATI level (μg/mL)
1	2.75	3194.90
2	2.68	258.55
3	2.67	1056.25
4	2.66	3055.04
5	2.93	3712.82
6	2.26	3343.07
7	2.66	129.54
8	2.49	4540.33
9	12.40	58.92
10	2.66	3679.21
11	2.65	536.57
12	1.90	555.53
13	1.71	810.87
14	4.67	46.34

IFX: Infliximab; ATI: Antibody.

RESULTS

The median CDAI in groups I and Ⅱ were 138 (IQR 68-186) and 50 (IQR 34-70), respectively; the partial Mayo score in the two groups were 5 (IQR 3-6) and 1 (IQR 0-1), respectively. The median serum TNF-α levels were 10.5 (IQR 3.2-18-9) and 6.3 (IQR 1.5-15.7) pg/mL in groups I and Ⅱ, respectively. The median IFX trough level was 3.1 (IQR 2.6-5.04) and 3.5 (IQR 2.6-4.7) μg/mL in the two groups, respectively. Fourteen patients were found to have ATI positivity with a median of 933 μg/mL (IQR 328-3306). ROC analysis revealed that the cut off value of serum IFX for detecting ATI was 3.01 μg/mL. The serum TNF-α level was significantly higher in the presence of ATI (24.23 pg/mL *vs* 6.28 pg/mL, $P = 0.005$). ATI positivity correlated significantly with low trough levels of IFX (2.66 μg/mL *vs* 3.86 μg/mL, $P = 0.015$). However, no difference was detected in serum IFX and antibody levels between the two groups (2.67 μg/mL *vs* 2.66 μg/mL, $P = 0.821$). Serum IFX and ATI levels in patients with ATI positivity are summarized in Table 2. Two of the IBD patients with antibodies against anti TNF-α developed side effects, 5 patients lost response, and an allergic reaction occurred in 3 patients. 37 patients were previously treated with biologicals, with development of ATI being more frequent those patients ($P = 0.048$). Dose intensification was required in 9 patients. No association was found between dose intensification and the development of ATI. Concomitant immunosuppression had no impact on IFX trough levels or on the development of ATI formation. Increased ESR and C-reactive protein correlated significantly with lower serum IFX level ($P = 0.04$ and $P = 0.002$). The serum TNF-α level was higher in patients not treated concomitantly with steroids ($P = 0.038$).

DISCUSSION

In this prospective observational study, serum TNF-α level was shown to be correlated with the presence of ATI, and ATI positivity correlated significantly with low trough levels of IFX. ATIs were detected in 25% of IBD patients with loss of response, side effects, or hypersensitivity, however no association was revealed between these patients and antibody positivity or lower serum IFX levels. Previous use of IFX correlated with the development of ATI, although concomitant immunosuppression did not have any impact on them.

The prevention and management of therapeutic failure with IFX is a significant challenge for clinicians in the field of IBD. One of the major reasons for loss of response is the development of ATI, which is frequently caused by immunogenicity[6]. Immunogenicity induced by IFX can be determined by measuring antibodies, concentrations of TNF-α, and IFX levels[7]. Use of concomitant immunomodulators and maintenance *vs* episodic IFX therapy has previously been shown to decrease the incidence of ATI[8,9]. Baert *et al*[4] revealed that ATIs reduce serum IFX level, as well as increase the risk of infusion reactions and loss of response. The role of ATI in loss of response to IFX and the lower efficacy of IFX re-treatment have also been confirmed by a study by Farrell *et al*[5]. In this study, both increased TNF-α and decreased IFX levels correlated with the presence of ATI, although neither ATI nor serum IFX influenced the outcome of the therapy. A recent meta-analysis also concluded that the presence of ATIs is associated with a significantly higher risk of loss of clinical response to IFX and lower serum IFX levels in patients with IBD[10]. Although these statements and consequences are logical, the results of clinical practice are conflicting.

In a recently published systematic review, Chaparro *et al*[2] assessed the relationship between the efficacy of TNF-α blockers and their serum levels and the clinical utility of testing for antibodies against TNF-α. A close relationship was revealed between trough levels of anti-TNF-α and maintenance of response. Maser *et al*[11] did not find any difference in the duration of clinical response in patients with detectable IFX serum levels with or without ATI. A higher serum IFX level was proved to predict a longer duration of response and clinical remission by some studies both in CD and UC[9-12]. In contrast, a Japanese study shown that the median trough levels of IFX did not differ significantly in patients who maintained and lost response, suggesting a faster clearance in cases of loss of response[13]. Because of these controversial data, the usefulness of monitoring the trough levels and ATI concentrations in therapeutic decisions may be questionable. Our results do not confirm the clinical utility of trough level and antibody measurement in the differentiation of "problematic" patients with loss of response or adverse reactions *vs* those who respond appropriately to the biological therapy.

In a recently published study by Bortlik *et al*[14], the median trough levels of IFX were significantly higher and antibody titers significantly lower in patients with concomitant thiopurines. In our study, previous biological therapy had a more significant effect on the outcome of IFX therapy than the concomitant use of

thiopurines. According to a study by Afif *et al*[15], dose escalation was associated with a high clinical response in patients with subtherapeutic IFX levels and negative ATI, and better clinical outcome was achieved in ATI positive patients switching to another anti TNF-α. On the basis of previous studies, concomitant corticosteroid therapy is suggested to decrease the effect of TNF-α blocker[16,17], which was confirmed by our results regarding the higher TNF-α level in patients receiving steroids. In conclusion, we found significant association between serum TNF-α level and the presence of ATI, as well as between ATI positivity and low trough levels of IFX. However, antibody positivity and lower serum IFX levels did not correlate with loss of response, side-effects, and hypersensitivity. Previous use of IFX correlated with the development of ATI. When previous studies determined only ATI positivity or negativity, detectable IFX serum concentration suggested many were false-negative results. This factor was decreased by the quantification of ATI titers in our study. On the basis of the present work, we suggest that further prospective studies are needed to determine whether the simultaneous measurement of serum TNF-α level, serum anti TNF-α concentration, and antibodies against anti TNF-α may help to optimize therapy in critical situations.

COMMENTS

Background

The prevention and the management of therapeutic failure with infliximab (IFX) is a significant challenge for clinicians in the field of inflammatory bowel disease (IBD). One of the major reasons for loss of response is the development of anti-IFX antibody (ATI). Due to controversial data, the usefulness of monitoring IFX trough levels and ATI concentrations in therapeutic decisions may be questionable.

Research frontiers

Previous studies have suggested that ATIs reduce the serum IFX level, and therefore increase the risk of infusion reactions and loss of response. The role of ATI in the lower efficacy of IFX retreatment has also been previously confirmed.

Innovations and breakthroughs

Simultaneous measurement of serum tumor necrosis factor-α (TNF-α) level, serum anti TNF-α concentration, and antibodies against anti TNF-α may help to optimize therapy in critical situations of IBD. However, no previous studies have been performed in this topic.

Applications

Significant association was revealed between serum TNF-α level and the presence of ATI, as well as between ATI positivity and low trough levels of IFX. However, antibody positivity and lower serum IFX levels did not correlate with loss of response, side-effects, and hypersensitivity.

Peer review

This is an interesting study with the important message that simultaneous measurement of serum TNF-α level, serum anti TNF-α concentration, and antibodies against anti TNF-α may help to optimize therapy in critical situations.

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