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Dynamics of Some Routine Immunological Parameters During Anti - TNF Therapy in Patients with Crohn's Disease

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Abstract

Background: Fecal and immunological biomarkers can be used to diagnose and manage patients with Crohn's disease (CD). Anti - tumor necrosis factor (TNF) should be evaluated in addition to biomarkers to determine the response to therapy.
Objectives: The current study aimed at following up fecal calprotectin (FC), perinuclear anti - neutrophil cytoplasmic antibodies (pANCA), anti - Saccharomyces cerevisiae antibodies (ASCA), and anti - nuclear antibodies (ANA) in patients with CD on anti-TNF therapy.
Methods: A total of 57 patients with CD and the mean age of 40 $\pm$ 15 years (ranged: 20 - 75) were monitored after initiation of anti - TNFa treatment. Stool samples were tested for FC (Alegria automated the enzyme - linked immunosorbent assay (ELISA) system), and serum samples for ANCA, ANA (indirect immunofluorescence - IIF), and ASCA (ELISA) in the beginning and after six months on immunosuppressive therapy plus anti - TNFa agents.
Results: It was observed that all patients with CD had significantly decreased FC levels after anti - TNFa therapy (963.97 mg/kg initially vs. 268.42 mg/kg after treatment; P = 0.043). Moreover, in 75% of patients, FC levels dropped below the cutoff value of 50 mg/kg. Positive for ASCA IgA/IgG were 17/24 tested patients, but no differences were observed regarding the application of anti - TNFa therapy. However, the titers of pANCA decreased in four patients after anti - TNFa treatment.
Conclusions: Initial and follow - up measurements of some immunological markers such as FC and pANCA could be of benefit for patients with CD in anti - TNF therapy, whereas others such as ANA and ASCA were not useful to monitor the therapy.

Keywords: TNFa Inhibitors, Immunological Biomarkers, Therapy Monitoring

1. Background

Tumor necrosis factor (TNF) inhibitors are well - accepted treatment options for Inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC), especially in case of steroid and standard immunosuppressive drugs failure. These chronic intestinal disorders are characterized by frequent flare - ups alternating with periods of remission, where the disease - modifying therapy is a desirable option, especially for young adults. Furthermore, anti - TNF therapy is a useful approach for severe refractory or fistulising CD (1). Infliximab, adalimumab, golimumab, certolizumab pegol, etc., induce and maintain clinical remission, reduce the need for surgery and hospitalization, and also improve the quality of life (2). Approximately 70% of patients respond, and up to 30% of patients enter clinical remission by the fourth week after a single dose infusion (1). Thus, anti - TNFa may influence direct and indirect costs associated with these chronic inflammatory debilitating disorders. However, there is a proportion of patients (up to 30%), which do not respond or lose response to this treatment. One of the leading reasons for the secondary loss of therapy is the formation of antibodies against TNFa inhibitors (1). Due to this background for treatment refractoriness, reliable markers are needed to predict the success of treatment.

There are numerous fecal and serological biomarkers that are well - accepted to diagnose IBD. Fecal markers such

as calprotectin (FC) and lactoferrin are extensively studied for their ability to distinguish patients with IBD, assess disease activity, and predict relapse. FC is useful to suggest colonoscopy, assess mucosal healing, monitor therapy, and predict the risk of relapse or postoperative recurrence of CD (3). Among serological markers, autoantibodies (i e, perinuclear anti - neutrophil cytoplasmic antibody (pANCA) and antibodies against microbial antigens (i e, anti - Saccharomyces cerevisiae antibodies (ASCA) are the most commonly studied ones in patients with CD and UC (4). They can be used to diagnose IBD, distinguish CD from UC, and predict the risk of complications in patients with CD (5). ASCA are described as antibodies against oligomannosidic epitopes on the cell wall of the fungi *S. cerevisiae*, and pANCA are directed against different antigens, including cathepsin G, elastase, lactoferrin, and lysozyme (1). Up to 60% of patients with CD may possess these antibodies, whereas only 5% - 15% of them have pANCA (6). Previous studies on Bulgarian patients with CD showed that the prevalence of ASCA varied 14.8% to 50% depending on the test used (ASCA IgA alone, or ASCA IgG + IgA, respectively) (7-9). ASCA and pANCA show high specificity, which makes them useful to differentiate between various phenotypes of IBD, but their low sensitivity makes their diagnostic value questionable (1). It should be noted that pANCA is mainly established as a diagnostic tool for UC and sclerosing cholangitis, particularly in a pediatric practice. However, their clinical significance is essential for contribution to diagnosis, identification of subjects at risk, and classification of clinical phenotypes (6). Nevertheless, the prediction of response to treatment, especially to new therapeutic agents such as anti - TNFa drugs is suggested (10).

2. Objectives

The current study aimed at following up the dynamics of some routinely tested fecal, i e, FC, and serological biomarkers, such as pANCA, ASCA, and antinuclear antibodies (ANA) in patients with CD during anti-TNF therapy.

3. Methods

3.1. Design of the Study

A prospective study was presented in a Bulgarian cohort of patients with CD. The stool and serum samples of the patients were tested before and after six months of anti - TNF therapy with a panel of routine immunological tests: FC, pANCA, ANA, and ASCA. The study protocol was approved by the Ethics Committee of Sofia Medical University, Sofia, Bulgaria.

3.2. Patients

A total of 57 consecutive patients with CD (31 males and 26 females) and the mean age of 40 $\pm$ 15 years (ranged: 20 - 75) were included in the study after initiation of anti - TNFa treatment. The majority of patients (62.8%) were less than 44 years old. The patients were recruited from the clients of the Clinic of Gastroenterology at University Hospital of St. Ivan Rilski, Sofia. The diagnosis of CD was based on the standard criteria of European Crohn's and Colitis Organization (ECCO) consensus for CD (2010), including a set of anamnestic, clinical, laboratory, and instrumental studies (11). The exclusion criteria for patients were as follows, but not limited to previous treatment with anti - TNFa agents, acute diarrhea or proved infectious diarrhea, melena, and other systemic severe or psychiatric diseases.

The subjects with CD were newly diagnosed or known patients, without prior therapy or on 5 - aminosalicylates $\pm$ immunosuppressants. The study included only the patients with the first anti - TNFa treatment. The anti - TNFa agents used in the study were as follows: golimumab (26 patients), adalimumab (21), and infliximab (10) on individual doses and regimens.

All patients were informed about the purpose of the experiment, and a written confirmed consent was obtained from all participants.

3.3. Immunological Methods

3.3.1. Stool Samples

All participants were provided with a tube equipped with a spatula to collect fecal material. Every sample was processed according to the extraction protocol applied to the kit immediately after transportation to the laboratory and tested on automated quantitative enzyme immunoassay test (Calprotectin, Alegria, Orgentec Diagnostika, Germany).

3.3.2. Serum Samples

Serum samples of 24 patients with CD were tested for the presence of pANCA by indirect immunofluorescence (IIF) (NOVA Lite® ANCA, Inova Diagnostics, USA) as well as ANA by IIF (anti - nuclear antibodies HEp - 2 (ANA - HEp - 2), Biosystems, Spain), and ASCA (ASCA IgG, ASCA IgA, Alegria, Orgentec Diagnostika, Germany).

All immunological methods were conducted in the Laboratory of Clinical Immunology, University Hospital "St. Ivan Rilski", Sofia, strictly following the instructions of the manufacturer.

3.4. Statistical Methods

Statistical analysis of the raw data was performed with SPSS version 19 (IBM 2009). $P < 0.05$ was considered the level of significance.

4. Results

It was observed that FC levels during anti-TNF therapy significantly decreased in all of the patients with CD. The initial mean ± standard deviation (SD) level of 963.97 ± 59.07 mg/kg decreased to 268.42 ± 145.25 mg/kg (P = 0.043) (Figure 1).

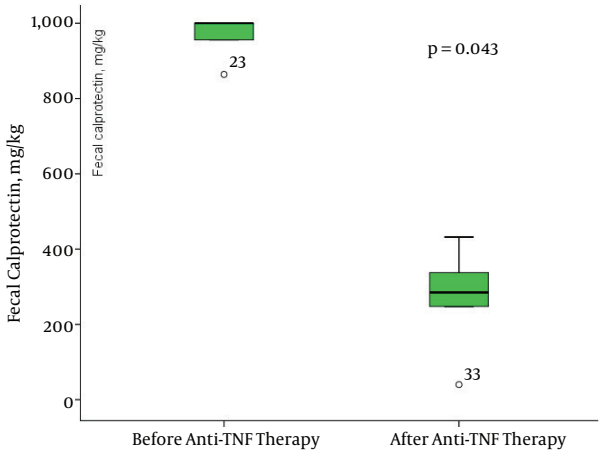

Figure 1. Fecal Calprotectin Level in Patients with Crohn's Disease Prior and After Six Months of Anti - TNF Therapy

Moreover, in 75% of the patients with CD, the FC level dropped below the cutoff value of the test (50 mg/kg).

Positive for ASCA IgA/IgG were 17/24 tested patients: ASCA IgG positive were 10 patients, ASCA IgA - seven patients, positive for both ASCA IgG and IgA - seven patients, negative for both - seven patients (Figure 2). All ASCA IgA positive patients were also positive for ASCA IgG.

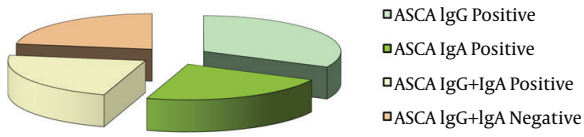

Figure 2. ASCA IgG/IgA in patients with Crohn's disease before anti - TNF therapy

No differences in ASCA were observed before and after application of anti - TNF therapy. No correlations were observed between FC and ASCA.

Five out of 24 patients had pANCA (two patients with 1:20 titer, one - 1:40, and two - 1:80) at baseline. The titers of pANCA decreased in 4/5 positive patients as follows: pANCA titer dropped from 1:80 to 1:20 in two patients, and two other patients with 1:20 titer showed negative results after implementation of anti - TNF therapy. One patient had no change in pANCA titer, which remained 1:40 (Figure 3).

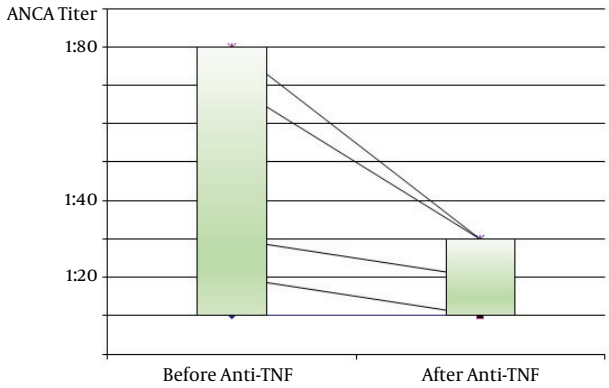

Figure 3. The Dynamics of Panca Titter in Patients with CD at Baseline and After Anti - TNFa Treatment

ANA positive was two out of 24 tested patients with CD (1:80). The titters remained the same after the anti - TNF therapy.

5. Discussion

The current study aimed at evaluating the dynamics of some routine immunological testing such as FC, pANCA, ASCA, and ANA, during anti - TNF therapy of patients with CD. Both fecal and serological markers were tested for their association with responses to specific treatments (6, 12).

5.1. Fecal Calprotectin and Anti - TNF Therapy

FC utility in disease activity and response to treatment, including the anti - TNFa treatment, was described in an extensive review of Smith et al. (13). The FC levels of the current study patients with CD significantly decreased after six months on anti - TNF therapy. The hopeful prospect of the current study was that the FC level dropped below the cutoff value of the test in 75% of the patients.

Sipponen et al., also observed normalized levels of FC and lactoferrin in patients treated with anti - TNFa agents, along with documented mucosal healing. In a small number of patients, there was lack of mucosal healing and the levels of FC and lactoferrin simultaneously increased (14).

Previous studies showed that the levels of fecal markers did not decrease in the non - responders to anti - TNF therapy (15). Other studies also supported the significance of FC to monitor anti - TNFa treatment, along with the prediction of remission or subclinical recurrence of mucosal inflammation when their levels increased (16-19). All these findings suggested the useful role of FC as a non - invasive marker of mucosal response to anti - TNFα treatment and secondary loss of response. However, the results of the

study by Laharie et al., conflicts with the results of these studies (20). They could not demonstrate a relationship between FC and clinical relapse in patients with CD after initiation of infliximab. This non - coincidence may be due to the subjective nature of clinical indices such as Crohn's disease activity index (12).

5.2. ASCA and/or ANCA and Anti - TNFa Therapy

It is observed that high ASCA levels, along with the ileal CD, and FC above 250 mg/kg could be considered as risk factors of relapse in patients with CD after discontinuing anti - TNF therapy (21). Indeed, ASCA serology was stable in time, regardless of treatment modality, and might be a prognostic tool at any time in the disease course (4).

However, the published data regarding ASCA and ANCA in patients with CD on anti - TNF therapy are often conflicting. A prospective clinical study of patients with CD on anti - TNFa drugs did not find a significant relationship between ASCA and ANCA and response to therapy (1). Other studies, however, demonstrated a lower response rate among pANCA positive, but ASCA - negative patients with CD on infliximab (1, 5). However, a further study did not confirm this observation (22). Nevertheless, the combination of pANCA+/ASCA- deserves further investigation for its value to predict nonresponse in patients with refractory luminal CD (1).

The current study observed no response to therapy, but the dynamics of markers after anti - TNF therapy. ASCA levels did not alter during the treatment. In contrast, the titters of pANCA decreased in 4/5 of the positive patients with CD. The obtained results were surprising since the large proportion of pANCA positive patients were influenced towards reducing the titters of the autoantibodies. These findings suggested the possible useful role of pANCA during anti - TNFa treatment. However, to confirm the utility, it is necessary to increase the samples size of the positive ANCA CD patients.

5.3. ANA and Anti - TNF Therapy

ANA testing during anti - TNF therapy is a delicate topic. On one hand, some patients with CD may possess ANA as an autoimmune feature along with their gastrointestinal disease such as the current study patients. On the other hand, patients exposed to anti - TNFa drugs induced apoptosis of immune cells and consequently formed autoantibodies such as ANA and anti - dsDNA (23).

Furthermore, these autoantibodies may be associated with loss of response to therapy. A study of infliximab showed the induction of ANAs in 63.8% of patients with rheumatoid arthritis and 49.1% of patients with CD, anti - dsDNA antibodies in 13% of the former patients, and 21.5% of the latter patients, respectively (24). In the current study, during the six month period, no patients with newly formatted ANA were observed. However, it should be noted that drug - induced lupus erythematosus also represents a possible complication of anti - TNFa treatment for CD, which may lead to a severe diagnostic dilemma (25, 26).

In conclusion, serologic markers during anti - TNFa treatment are not sufficiently predictive of response to therapy when used alone. They should be preferably included in a predictive model with clinical and other predictive factors (12).

5.4. Conclusions

A significant reduction of FC was observed after anti - TNF therapy. The majority of tested patients with CD were ASCA positive. However, the titters of ASCA remained stable over time. However, the titers of pANCA decreased during anti - TNFa therapy.

All in all, the current study results showed that initial and follow - up measurements of some immunological markers such as FC and pANCA could be beneficial for patients with CD on anti - TNF therapy; whereas others such as ANA and ASCA are not useful to monitor the therapy.

However, a case - by - case decision making should be considered along with the assessment of clinical and histological improvement of patients.

Footnote

Conflict of interests: Authors declared no conflict of interest.

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