Levels of Anti-Double-Strained DNA but not Antinuclear Antibodies are Associated with Treatment Efficacy and Adverse Outcomes in Crohn's Disease Patients Treated with anti-TNFα

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ABSTRACT

Background & Aims: Treatment of Crohn's disease (CD) by infliximab (IFX) has been associated with the induction of antinuclear (ANA) and anti-double strand DNA (dsDNA) autoantibodies and in some studies the formation of dsDNA antibodies was associated with lupus-like syndromes. The aims of this study were to analyse the relationship between the development of ANA and dsDNA antibodies during anti-tumor necrosis factor (TNF)α therapy and the clinical efficacy or adverse outcome in patients with inflammatory bowel disease (IBD).

Methods: Data of 96 CD patients (age at presentation: 25.1 years, folow-up: 5 years, males/females 43/53) treated with anti-TNFα for at least one-year were analyzed. Records of a total of 198 one-year treatment cycles were collected and levels of autoantibodies were determined at induction and after one-year treatment periods.

Results: The majority of CD patients had ileocolonic (67.4%) and complicated disease (B2-B3: 72.6%) with perianal lesions (63.2%). At any time ANA or dsDNA positivity was 28.6% and 18%. Elevated level of ANA at induction or during anti-TNFα therapy was not associated with treatment efficacy or development of adverse outcomes. In contrast, treatment efficacy (dsDNA positivity no/partial response vs. remission: 68.5% vs. 31.5%, P=0.003) was inferior and adverse outcomes were more frequent in patients with dsDNA positivity during the anti-TNFα therapy in both univariate analysis and in logistic regression models (OR efficacy: 4.91, 95%CI: 1.15-20.8; OR adverse outcome: 3.81,95%CI 1.04-13.9).

Conclusions: Our data suggest that development of dsDNA during biological therapy may be associated with suboptimal treatment efficacy and adverse outcomes in CD patients.

Key words: Crohns's disease – antinuclear antibody – double-strand DNA – anti-TNFα therapy – adverse outcome.

INTRODUCTION

Tumor necrosis factor (TNF)α is a pro-inflammatory cytokine that has been shown to play a central role in the pathogenesis of several autoimmune mediated diseases, including rheumatoid arthritis, spondyloarthritis or inflammatory bowel disease (IBD) - Crohn's disease (CD) and ulcerative colitis (UC). The introduction of anti-TNFα therapies over the past decade, along with accumulating evidence from landmark trials and clinical practice, has led to a significant change in patient management and treatment algorithms in these disease groups [1].

However, it has been reported that autoimmune antibodies - antinuclear autoantibody (ANA) and anti double-strand DNA (dsDNA) antibody - may develop during anti-TNFα treatment. In a more recent study from South America, in spondylarthritis patients, the titers of ANA and dsDNA were elevated (7% and 3.5% of patients) after at least one-year anti-TNFα therapy [2]. This was however not a universal finding. A randomized, placebo-controlled trial in adalimumab (ADA) treated patients with rheumatoid arthritis did not find increasing values of ANA positivity [3], whereas infliximab (IFX) treated patients were reported to have a high rate of ANA positivity in rheumatoid arthritis [4].

Similar data were reported in IBD. Infliximab (IFX) treated patients were reported to have a high rate of ANA positivity in CD [5, 6]. In one of the early studies by Garcia-Planella et al, the baseline ANA positivity was 22% of the 36 patients. A further 6/28 patients receiving concomittant immunosuppression became ANA positive after 6 weeks of IFX treatment but none developed dsDNA positivity [7]. In another study from France, induction of ANA and dsDNA autoantibodies was observed in 53% and 35% of IFX treated patients with CD during one-year intermittent therapy [8]. Finally, in an Italian study, 8% of the 63 CD patients were ANA positive before the anti-TNF therapy, while 42% and 17% of patients were ANA and dsDNA positive after 10 weeks IFX treatment [9].

However, concerns about biological drugs remain. Several studies have reported serious infections in patients exposed to biologicals [10] and very recently the risk of serious infections was reported to be independently associated with TNF-antagonists in the TREAT registry [11]. Another side effect is the development of autoantibodies that may be associated rarely with the appearance of systemic lupus erythematosus or lupus like syndromes (LLS). In an early study from Leuven, the cumulative incidence of antinuclear antibodies was 56.8% after 24 months in this cohort of IFX treated CD patients. About 4% of ANA positive patients had developed a LLS, all being ANA negative before IFX treatment and then developed high ANA-titers soon after the first infusion, with antibodies directed against dsDNA and antihistone [5]. In the Italian and French studies drug-induced lupus syndrome was reported in only one and one patients, respectively. In a more recent study from Germany, 44.4% of anti-TNFα treated patients (IFX or ADA, or IFX and ADA consecutively) had ANA positivity, while 15.6% had elevated dsDNA serum levels. The dsDNA antibody levels, but not ANA-titers are associated with clinical symptoms of LLS, while concomitant immunosuppressive therapy may have a protective effect [12]. In contrast, an association between ANA positivity and LLS or clinical efficacy was not reported in patients treated with ADA in randomized clinical trials [13]. However, it is still controversial if the above antibodies (ANA, dsDNA) are associated with the clinical outcome and/or development of complications in the long term.

Therefore the aim of this study was to analyze the relationship between the development ANA and dsDNA antibodies during anti-TNFα therapy and the clinical efficacy or adverse outcome (loss of response, allergy, LLS, induced autoimmune disorders) in patients with IBD.

METHODS

A total of 96 well-characterised, unrelated, consecutive CD patients [males/females: 43 / 53, age at presentation: 25.1 years (SD: 10)] with a complete clinical follow-up were included from three specialised centres providing biological therapy in Hungary.

Diagnosis was based on the Lennard-Jones criteria [14]. The disease phenotype (age at onset, duration, location and behaviour) was determined according to the Montreal Classification [15]. Medical records, including data about the presence of major extraintestinal manifestations, previous frequency of flare-ups (frequent flare-up: > 1 clinical relapse / year [16]) previous surgical procedures (resections or perianal procedures), the presence of familiar IBD, smoking habits and perianal involvement, were determined by a thorough review of the patients medical charts, which had been collected in uniform format. Previous and concomitant medical therapy was meticulously registered.

Infliximab or ADA treatment was initiated in CD patients with luminal (and additional fistulising) disease with a CD activity index (CDAI) > 300 despite adequate previous conventional therapy (including appropriate use of steroids and immunosuppressives for at least 3 months; or failure to respond to, or intolerance to, either steroid or immunosuppressive therapy). The clinical response (partial response) was defined as a ΔCDAI>70 point (and a >50 decrease of the drainage of fistulas in patients with parallel fistulising complications) while clinical remission was defined as a CDAI<150 point (and a complete stop of fistula drainage in patients with parallel fistulising complication). Due to Hungarian health authority regulations, patient follow-up appointments are mandatory at least every 3 months with additional extraordinary visits if needed. These visits include clinical assessment, review of patient diaries, CDAI [17] and perianal disease activity index (PDAI)) determination, laboratory assessment (including C-reactive protein (CRP)), chest x-ray every 6-months, and in fistulising patients, objective assessment of response by MRI, EUA or, rectal ultrasound at 12 months. Consequently, patient selection and follow-up was standardized and uniform in all specialized centers. Of note, all centers were monitored for quality of care and regulation compliance by the Hungarian Insurance Bureau (OEP) in June 2011.

Records of 198 one-year treatment cycles of CD patients were collected and levels of autoantibodies were determined at induction and after the one-year anti-TNFα period. Clinical and laboratory data of CD patients under anti-TNFα therapy were captured prospectively, the data analysis was performed retrospectively. The definition of adverse outcome included secondary loss of response, hyperacute allergic reaction, LLS, severe infectious complications, induced autoimmune disorders leading to the discontinuation of the anti-TNFα therapy. The central coordination and database management was completed at the 1st Department of Internal Medicine, Semmelweis University (by LSK, PLL). The study was approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics.

Detection of autoantibodies

Collected sera were frozen at -80°C until testing. Commercially available kits were used. ANA titers were measured by INOVA QUANTA LiteTM ANA ELISA, an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Sera were diluted at 1:40, 1:80 or 1:160, and the conjugate was a goat antihuman IgG The absorbance was read at 450nm and 620nm. A positivity at 1:40 dilution was interpreted as a positive result. Anti-dsDNA autoantibodies were measured by INOVA QUANTA LiteTM dsDNA ELISA an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. A titer equal to or greater than 200 IU/mL was interpreted as a positive result.

Statistical analysis

Variables were tested for normality using the Shapiro–Wilks W test. The chi-squared test and chi-squared test with Yates correction and logistic regression analysis were used to assess the association between categorical clinical variables and clinical outcome. Variables with P < 0.2 were included in the multivariate testing. A P-value < 0.05 was considered significant. For statistical analysis, SPSS15.0 (SPSS Inc, Chicago, IL, USA) was used.

RESULTS

The clinical data of the patients is summarized in Table I. The majority of CD patients had ileocolonic location (67.4%) and complicated disease behavior (B2-B3: 72.6%) with perianal lesions (63.2%).

Table I. Clinical characteristics of CD patients (*median (IQR))

Charateristic	CD patients n=96
Male/female	43/53
Age at presentation (years)*	25.1 (19-30)
Follow-up (years)*	5 (3-12)
Location	
L1	6%
L2	25%
L3	68%
L4 only	1%
all L4	10%
Behavior	
B1	27%
B2	17%
B3	56%
Perianal disease	63%
Steroid use at induction	61%
Azathioprine use ever/during anti-TNFα therapy	96% / 74%

ANA and dsDNA positivity rates at induction or during anti-TNFα therapy

At the start of the one year cycles, ANA positivity was observed in 12.3% of CD patients. In contrast, no patients were positive at induction for dsDNA. At the start or end of the one-year anti-TNFα therapy cycles, ANA or dsDNA positivity was 28.6% and 18%, respectively. There was an association between dsDNA positivity and female gender (OR: 3.92, 95%CI: 1.25-12.3, p=0.01).

Association between ANA or dsDNA antibodies and clinical outcome

Elevated level of ANA at induction time or during anti-TNFα therapy was not associated with efficacy of therapy or the development of adverse outcomes (data not shown). In contrast, treatment efficacy (between no/partial response and remission group dsDNA positivity: 68.5% vs. 31.5%, P=0.003, OR: 4.81, 95%CI: 1.73-13.3) was inferior (Fig. 1.) and adverse outcomes were more frequent in dsDNA positive cycles (dsDNA positivity in adverse outcomes: 38.9% vs. 14.6%, P=0.01, OR: 3.71, 95%CI: 1.31-10.5, Fig. 2.). The adverse outcomes developed were the following: secondary loss of response (n=12), hyperacute allergic reaction (n=8), severe infectious complications (n=7, i.e. CMV colitis 2, TBC 1, generalized HSV infection 3, severe pneumonia 1), induced autoimmune disorders (n=9, psoriatiform lesions: 5 other skin pustulous lesions: 2, arthritis: 2). No patient developed LLS. The efficacy and adverse event frequency was not different in cycles with IFX (n=132) or ADA (n=66, data not shown).

In a logistic regression analysis, dsDNA positivity (OR: 4.91, 95% CI%: 1.16—20.8, P=0.031) and female gender (OR: 2.42, 95% CI%: 1.08-5.41, P=0.032) but not disease location (OR: 1.78, 95% CI%: 0.91-3.49, P=0.09), disease behaviour (OR: 1.09, 95% CI%: 0.69-1.74, P=0.71), CRP at 3 months (OR: 0.61, 95% CI: 0.24-1.53, p=0.29) or ANA positivity (OR: 0.83, 95% CI%: 0.37-1.86, P=0.65) were associated with the one-year efficacy (no/partial response vs remission) in CD. Similarly, dsDNA positivity (OR: 3.81, 95% CI%: 1.04-13.9, P=0.04), female gender (OR: 4.03, P=0.009) and steroids during the induction phase of the cycle (OR: 0.22, P=0.02), but not disease behavior or location were associated with adverse outcome in CD in a logistic regression model (Table II).

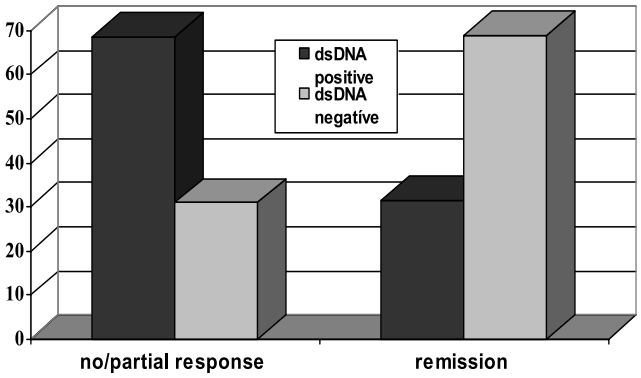

Fig. 1. Association between clinical efficacy and dsDNA positivity (P=0.003, OR: 4.81, 95%CI: 1.73-13)

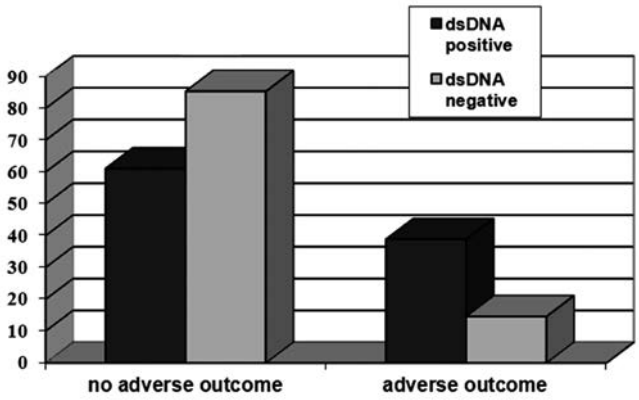

Fig. 2. Association between dsDNA positivity and adverse outcome (P=0.01, OR: 3.71, 95%CI: 1.31-10.5)

Table II. Logistic regression: Association between clinical data and antibody results and adverse outcome in patients with CD

Variable	Coefficient	P value	OR	95%CI
ds-DNA	1.338	0.043	3.81	1.04-13.9
female gender	1.394	0.009	4.03	1.42-11.4
location of CD	0.771	0.170	2.16	0.728-6.51
disease behavior	0.303	0.313	1.35	0.75-2.44
Steroid during anti-TNFα therapy	-1.537	0.002	0.22	0.08-0.57

The coefficient is equivalent to the natural log of the OR; P value: level of significance; OR: odds ratio; 95%CI: 95% confidence interval.

In a sensitivity analysis, the association between the above antibodies and the development of hyperacute allergic reaction or induced autoimmune disorders was analyzed. In a merged analysis allergic reaction/induced autoimmune disorders were significantly more frequent in patients with dsDNA positivity (22.2% vs 6.1%, P=0.04, OR: 4.40, 95%CI: 1.22-15.9).

DISCUSSION

The major finding of the present study was that the development of dsDNA positivity was associated with adverse outcomes and efficacy of long-term anti-TNFα therapy in this large Hungarian CD cohort. DsDNA positivity was also associated with the female gender.

The ANA and dsDNA positivity rates found in the present study were in concordance with the rates reported in previous studies. In one of the largest studies, the IFX treated patients had even higher rates of ANA positivity in CD. In an early study from Leuven [5] in IFX treated CD patients, at the start of the study, before receiving any IFX treatment, 7.2% of patients were ANA positive, but none had titers > 1:40. The cumulative incidence of antinuclear antibodies was 56.8% after 24 months in this cohort of IFX treated CD patients. Of note, almost half of these patients developed ANA after the first infusion, and >75% after fewer than three infusions. ANA positivity persisted beyond one-year of the last infusion and only few patients became seronegative. In this cohort, 43 patients had ANA titers >1:80 and 32.6% of these patients had dsDNA antibody positivity while 39.5% single-stranded DNA positivity. There was a trend for less antibody development in patients receiving immunosupressives [5].

A few years later, in a study from France, 67 CD patients (35 patients in IFX treatment and 32 patients in control group with standard therapy) were observed. ANA positivity was found in 14% and 19% of the patients at the baseline. After one-year of anti-TNFα therapy, the ANA positivity was 53% in the treatment group and this did not change in the control group. In this study, only a titer ≥ 1:160 was interpreted as a positive result. During IFX treatment, concomitant immunosuppressive therapy was given to 88% of patients who became ANA positive during IFX therapy induced ANA, compared to 43% of patients who remained ANA negative (P<0.01). A single patient was dsDNA positive at baseline. After 1 year of therapy, anti-dsDNA autoantibodies were observed exclusively in IFX treated patients. The positivity rate was 35% (12 patients) [8].

In a more recent study by Beigel et al from Germany, 80 out of 180 anti-TNFα treated IBD patients (44.4%) had elevated ANA titers (> 1:240), while 28 patients (15.6%) had dsDNA positivity (serum levels > 9 U/mL). Among the subgroups (IFX, ADA, ADA after IFX, with and without immunosuppression, respectively) there was no statistically significant probability of developing ANA, dsDNA, and LLS [12]. In the landmark CLASSIC II trial by Sandborn et al, 185 CD patients were treated with ADA. Patients had both baseline and week 56 (or last visit) measurements for antinuclear antibodies. Of these, 172 were determined to be ANA negative at baseline, and 33/172 (19%) were ANA positive at their final visits. In addition, all 33 were were positive for dsDNA at their final visits [13].

Conflictive data are available for the clinical utility of autoantibodies. In one of the early studies by Vermeire et al, development of ANA was associated with the female sex (OR: 3.16; P=0.024) and with papulosquamous or butterfly rash (OR: 10.01; P=0.011) but not with disease phenotype, infusion related adverse events or concomitant immunosupressives / steroids in IFX treated CD patients [5]. In contrast, in a study from France, induction of ANA was not associated with any particular clinical pattern such as infections or autoimmune disorders, development of LLS, or reaction to infusion in IFX treated CD patients. This study also showed that IFX treatment did not induce the development of other non–organ-specific autoantibodies [8].

Similarly, in the present study, ANA positivity was not associated with the clinical phenotype, anti-TNFα efficacy or adverse outcomes. In contrast, dsDNA but not ANA positivity was associated with the female gender. In a multivariate analysis, female gender, concomitant steroids at induction and dsDNA positivity were independently associated with adverse outcomes and also inferior clinical efficacy in multivariate analysis. Allergic reaction and induced autoimmune disorders were more frequent in patients with dsDNA positivity (22.2% vs 6.1%, P=0.04, OR: 4.40, 95%CI: 1.22-15.9).

Finally, in the above mentioned study from Germany [12], there was no significant association between even higher ANA titers (> 1:240) and LLS (P=0.26), but there was a significant association between dsDNA antibodies values (> 9 U/mL) and LLS (P=0.02) in a multivariate logistic regression analysis. The frequency of mild LLS with minor clinical symptoms not needing an intervention was only 8.9%, with only 1.1% of the patients requiring to stop the anti-TNFα therapy, thus the clinical relevance of the findings is minor. They identified age as predisposing factor for the development of ANA (P<0.001) and LLS (P=0.002). Immunosuppressive therapy was identified as a protective factor against the development of ANA (P=0.05) and LLS (P=0.04), while there was not association between immunosuppressive therapy and dsDNA values. Furthermore, similar to the results from the present study, there was no association with the type of anti-TNFα therapy (IFX or ADA) and ANA or dsDNA development, or risk of LLS [12]. Interestingly, in a small pilot study by Verma et al, the authors identified 6 CD patients in whom the previous anti-TNFα therapy was switched to certolizumab due to the presence of LLS. Two out of four patients became ANA negative and the LLS symptoms resolved in all patients.

There was also no difference in the clinical activity before and after the switch [18]. Of note however, the development of LLS was a rare event with only 1 or 2 patients reported in most of the previous studies [4, 5, 8, 10]. Similarly, no patients were identified in the present study.

We are aware of the possible limitations of this study. ANA and dsDNA were tested at the beginning and at the end of the one-year cycle only, and thus the dynamics of the antibody development could not be studied. However, according to previous results the antibodies develop quickly after the first few treatment courses [5, 7] so that a second measurement after one-year could detect reliably the presence of the autoantibodies that developed during the anti-TNFα therapy. In addition, drug trough levels and antibody status were not assessed [19], which was reported to be useful for the detection of secondary loss of response in patients receiving long term anti-TNFα therapy.

Additionally, in Hungary, due to regulations of the Hungarian Insurance Bureau (OEP) anti-TNFα therapy was rigorously monitored in this period. Crohn's disease patients with active luminal (and additional fistulising) disease were allowed to be treated with anti-TNFα therapy if CDAI > 300 despite adequate previous conventional therapy (including appropriate use of steroids and immunosuppressives for at least 3 months; or failure to respond to, or intolerance to, either steroid or immunosuppressive therapy). In this study the steroid use at induction (61%) and azathioprine use earlier (96%) or during anti-TNFα (74%) were higher than in other published studies. It may be hypothetized that the relatively lower ANA and dsDNA positivity rate in our cohort may reflect a higher use of immunosuppressant therapy. Finally, the methodology and cut-off values of autoantibody detection were not uniform among the published studies that may account for further differences.

In contrast, the strength of the present study is that we included a cohort of well-characterized CD patients from three high-quality IBD referral centers in Hungary with standardized patient selection and follow-up, according to the regulations of the OEP. Moreover, all centers were monitored for quality of care and compliance with the regulations by the OEP in June 2011. In addition, previous studies have identified a set of clinical, endoscopic and laboratory parameters that were associated with the clinical efficacy of the anti-TNFα therapy (e.g. in one of the earlier studies of our group clinical efficacy and CRP at week 12, need for combined immunosuppression at induction, shorter disease duration and smoking were identified as independent predictors for 12-month clinical outcome) [20] and in the present paper, we performed a multivariate analysis to adjust for the above variables which enabled an unbiased evaluation.

CONCLUSION

ANA and dsDNA antibody positivity rates in the present study were approximatively in the range reported previously. ANA positivity was not associated with the clinical phenotype or other particular clinical patterns such as infections or development of autoimmune disorders, LLS, or efficacy or allergic reaction to anti-TNFα therapy. In contrast, our data suggest that development of dsDNA during biological therapy may be associated with clinically important outcomes. The efficacy of the treatment is inferior and adverse outcomes and/or development of allergic reaction or induced autoimmune disorders were more frequent in patients with dsDNA positivity during anti-TNFα therapy in multiple regression models. In summary, dsDNA positivity during anti-TNFα therapy could identify patients with higher risk for adverse outcome or worse clinical efficacy.

Conflicts of interest: The authors declare that no financial or other conflicts of interest exist in relation to the content of this article.

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