Original paper

Antidrug antibody formation during tumor necrosis factor α inhibitor treatment of severe psoriatic patients in the real-life practice

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Adv Dermatol Allergol 2019; XXXVI (5): 589–594
DOI: https://doi.org/10.5114/ada.2019.89507

Abstract

Introduction: Antidrug antibody (ADA) production may be the reason behind secondary inefficacy of anti-TNF-α therapy in psoriasis.

Aim: To investigate the production of ADA, serum tumor necrosis factor α (TNF-α) and drug levels as predictors of clinical response in real-life circumstances.

Material and methods: Serum drug concentrations (TNFi), the presence of ADAs and serum TNF-α levels were measured in 158 patients by the ELISA method. Clinical response was evaluated by calculating PASI. Their correlation has been statistically analysed.

Results: In adalimumab and infliximab treated patients, ADA formation was observed in 18.4% and 33%, respectively, and the serum TNFi concentration was significantly higher in the ADA negative groups. In contrast there was no ADA formation detected among etanercept treated patients. The serum TNFi concentration was significantly lower among non-responders (n = 33). The serum TNF-α level was also measured and the correlation with the concentration of the serum TNFi level was analysed. Having evaluated the results of all patients together, the serum TNFi and TNF-α concentrations showed a significant negative correlation. However, when groups were analysed separately, in case of adalimumab, a significant negative correlation was detected between serum TNFi and TNF-α concentrations. With respect to infliximab, there was no significant correlation, and an inverse correlation was found in the etanercept group. The TNF-α levels and ADA positivity were significantly higher in non-responders.

Conclusions: This study revealed the major role of ADAs against TNFi in case of secondary inefficacy in real-life circumstances. ADA levels show a stronger correlation with PASI failure than serum TNFi or TNF-α levels.

Key words: psoriasis, tumor necrosis factor α inhibitors, antidrug antibodies, clinical efficacy.

Introduction

Tumor necrosis factor α (TNF-α) inhibitor (TNFi) biological agents have been used for years in the treatment of moderate to severe psoriasis with great efficacy. In some cases we have to face primary or secondary inefficacy. Different reasons may be behind this phenomenon, such as the antidrug antibody (ADA) formation. Two different types of ADAs can be distinguished: neutralizing and non-neutralizing antibodies. Neutralizing antibodies may bind to the active cytokine binding place of the agent, they can change the pharmacokinetics and pharmacological dynamical parameters of the drugs, they can also form immunocomplexes with the biologicals and lead to an accelerated clearance. These pathomechanical processes can lead to a decrease in the serum drug level and hence to a consequential treatment failure.

There are three TNFi agents available for treating moderate to severe psoriasis at present: adalimumab, infliximab and etanercept. Antidrug antibodies may significantly alter the clinical efficacy of biological treatments through these mechanisms.

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Received: 26.03.2018, **accepted:** 19.06.2018.

According to previous data such as the review of Hsu *et al*. [1] in which they investigated 25 studies in ADA formation, the authors found a correlation between anti-infliximab antibodies and lower serum infliximab concentrations in three studies and less effective treatment response in five of the investigated studies. They found the prevalence of anti-infliximab antibodies ranging from 5.4% to 43.6% of the patients. Regarding adalimumab, the association between anti-adalimumab antibodies and lower serum concentrations were seen in three of five investigated studies, and in four studies, a decreased treatment efficacy was also demonstrated. The range of appearance of anti-adalimumab antibodies was 6% to 45%. In studies dealing with ADAs against etanercept, six studies measured non-neutralizing anti-etanercept antibodies, and they found no connection with treatment outcomes. The range of appearance of anti-etanercept antibodies was 0–18.3%.

There are some studies on concomitant methotrexate usage and immunogenicity of biologicals. The review article of Farhangian *et al.* [2] pointed that additional methotrexate may lead to better clinical efficacy by reducing the levels of inflammatory cytokines such as TNF-α and IL-12/23 [3]. In the case of rheumatoid arthritis (RA) patients, a 7.5 mg/week dose of methotrexate reduced the prevalence of ADA formation, and this effect seemed to be dose-dependent. This effect was found only in connection with concomitant methotrexate but not with other immunosuppressive drugs such as cyclophosphamide or rapamycin [4]. In addition, certain findings in rheumatology show that the timing of administration of the medicament (the co-administration of methotrexate right after the initiation of biological therapy) may also be important [4, 5].

Krieckaert *et al*. [6] observed 407 RA patients on adalimumab and etanercept treatment in their long-term three years' follow-up study and have found anti-adalimumab antibodies in 26% of adalimumab treated patients. ADA negative adalimumab treated patients had the best clinical outcomes, better than patients on etanercept therapy, while ADA positive adalimumab treated patients had the worst outcome. It has been also demonstrated that the presence of ADA development was more frequent in the case of patients with a longer and more severe disease duration and history, and in patients who did not receive concomitant disease-modifying antirheumatic drugs (DMARDs) such as methotrexate [7].

In the observational study of Chimenti *et al*. [8], 30 patients suffering from psoriatic arthritis and treated with adalimumab were observed. Serum adalimumab concentration showed a significant inverse correlation with serum CRP levels and Disease Activity Score (DAS44)-CRP. They found significantly higher drug concentrations in patients with lower disease activity scores.

Aim

Based on these former studies of large prospective investigations, our aim was to evaluate the correlations of ADA production, serum drug and TNF-α levels simultaneously with clinical outcomes in a large psoriatic population treated in real-life circumstances.

Material and methods

Only those patients were included in this study who had fulfilled the Hungarian national guidelines criteria, which correspond to the European S3 protocol to be entitled to biological therapy.

Informed consent was obtained from all patients in this observational study, which complied with the Helsinki Declaration principles as well as TNF-α inhibitors therapeutic guidelines. The study protocol was approved by the local ethics committee of the Semmelweis University and the Regional and Institutional Committee of Science and Research Ethics.

Patient characteristics

In this observational study, ADA formation, serum drug concentration (TNFi) and serum TNF-α levels were measured and past history was evaluated for 158 patients.

There were 64 (40.5%) patients on adalimumab treatment, 49 (31%) patients on infliximab and 45 (28.5%) patients on etanercept treatment. The applied doses of infliximab, adalimumab and etanercept were the labelled dosing for treating psoriasis and these are the following: 5 mg/kg *i.v.* every 8 weeks, 40 mg *s.c.* every 2 weeks and 50 mg *s.c.* every week, respectively.

Patient groups treated with the different TNFi drugs did not differ significantly in some demographic and clinical aspects: sex, mean age, mean Psoriasis Area and Severity Index (PASI) score at initiation of biological therapy, and mean duration of the current biological therapy (Table 1).

There were 7 patients in the adalimumab group (n = 64), whereas in the infliximab (n = 49) treated group, there was one patient who had been treated with biological therapy before the current therapy, and there were 2 patients among the etanercept treated group (n = 45) who received previous biological therapy.

There were 53 patients, who received concomitant methotrexate therapy among all the 158 patients: 22/49 (44.9%) in the infliximab treated group, while in the adalimumab and the etanercept groups these rates were 21/64 (32.8%) and 10/45 (22.2%), respectively.

Sample collection and method of ADA detection, serum TNFi concentration, serum TNF-α levels

Blood samples were collected from patients on their regular upcoming clinical visit and these were not performed at previously designed and standardized points of

Table 1. Demographic and clinical data

Parameter	Patients (*N* = 158)	Adalimumab (*n* = 64)	Etanercept (*n* = 45)	Infliximab (*n* = 49)
Sex:				
Male	100	37	25	38
Female	58	27	20	11
Age	48	46	47	51
Mean PASI at the time of sample collection	2.54	2.55	2.98	2.11

PASI – Psoriasis Area and Severity Index.

the therapy. Samples were taken prior to the next scheduled administration of TNFi injection/infusion, due to measure the trough drug concentration. During the one-year sample collection time, samples were collected before the change or discontinuation of the therapy in case of non-responder patients. The PASI score was calculated at the time of sample collection.

Whole blood samples were collected in vacutainer tubes without anticoagulant. After centrifugation, serum was obtained and stored at –70°C until batch processed. ADAs of IgG type, as well as levels of TNF-α and TNFi in patients' serum was determined by enzyme-linked immunosorbent assay (ELISA). Calibration curves plotting and calculation of TNF-α, TNFi and ADA concentration was performed by free, online analysis software (www.myassays.com).

Serum trough levels of adalimumab and etanercept were determined by sandwich ELISA, while serum trough levels of infliximab were measured by capture ELISA (Progenika Biopharma SA, Derio, Spain), according to the manufacturer's instructions. Serum drug levels of ≤ 0.024 µg/ml for adalimumab, and ≤ 0.035 µg/ml for infliximab and etanercept were considered negative. For detection of ADAs, a bridging ELISA was used (Progenika Biopharma SA, Derio, Spain), according to the manufacturer's instructions. Serum samples of patients were considered positive if antibody titres were > 10 AU/ml for adalimumab and > 142 AU/ml for etanercept. As for infliximab, serum samples of patients were considered positive if antibody titres were > 5 AU/ml.

Serum TNF-α levels were measured by a highly sensitive quantitative sandwich ELISA (R&d Systems Europe Ltd., Abingdon, UK).

Statistical analysis

Descriptive statistics were performed for serum TNF-α levels and TNFi concentrations. Due to the skewed distribution of the data, we applied non-parametric statistical tests. A Mann Whitney *U* test was used to compare the serum TNF-α levels and serum TNFi concentrations among ADA positive and negative patients. Spearman's correlations were used to test the relationship between serum TNFi concentrations and serum TNF-α levels. A correlation coefficient (r_s) of 0–0.19 was defined as very weak, a coefficient of 0.20–0.39 as weak, a coefficient of 0.40–0.59 as moderate, 0.60–0.79 as strong and 0.80–1 as a very strong correlation. Multiple logistic regressions were performed to explore the predictors of secondary clinical inefficacy. All the statistical tests were two-sided and a *p*-value of < 0.05 was considered statistically significant. Data analysis was carried out using SPSS 22.0 (Armonk, NY: IBM Corp. 2013).

Results

We used the determination of responders in case of patients who achieved PASI 50 from baseline and it turned out that 79% of the patients were responders (*n* = 125). Patients were considered non-responders if they had the 50% PASI reduction but subsequently a loss of efficacy occurred and it turned out that 21% of the patients belonged to this group (*n* = 33). Data are shown in Tables 2 and 3.

ADA formation, serum TNFi concentrations

In case of adalimumab (*n* = 64), 18.4% (*n* = 12) of the patients had ADA expression, the serum TNFi concentration was significantly higher in the ADA negative group ($p = 0.001$).

There were 33% (*n* = 16) of infliximab treated patients (*n* = 49) who had ADA in their sera and the serum drug concentration was significantly higher in the ADA negative group ($p < 0.05$).

We could not identify any ADA in the case of etanercept treated patients (*n* = 45), with 38 (83%) patients who had a measurable drug concentration.

Among non-responder patients (*n* = 33), the serum TNFi concentration was significantly lower than in the case of responders (*n* = 125) ($p = 0.0012$).

Serum TNF-α levels

In the case of adalimumab, we found a moderate inverse correlation between serum TNFi concentration and the serum TNF-α level ($r_s = –0.497$; $p = 0.0001$).

For the total sample (adalimumab, infliximab and etanercept, *n* = 158), a weak negative correlation was observed ($r_s = –0.223$; $p = 0.0049$). Similarly to previous literature data, we also found the following paradoxi-

Table 2. Results: TNF-α levels, TNF inhibitor concentration, ADA positivity, responder/non-responder status in the different TNF-inhibitor drug treated patient groups

Parameter	TNF-α level		TNFi concentration		ADA +	
	Responder	Non-responder	Responder	Non-responder	Responder	Non-responder
Infliximab (*n* = 49)	10.80 (10.62)	13.41 (12.12)	*n* = 33 3.16 (4.5)	*n* = 16 0.83 (1.44)	*n* = 6 (18.2%)	*n* = 10 (62.5%)
	p = 0.8395		***p* = 0.024**		***p* = 0.002**	
Adalimumab (*n* = 64)	2.67 (6.29)	5.40 (7.54)	*n* = 57 5.02 (3.27)	*n* = 7 2.57 (3.12)	*n* = 9 (15.8%)	*n* = 3 (42.9%)
	p = 0.4469		***p* = 0.0488**		*p* = 0.1153	
Etanercept (*n* = 45)	26.80 (17.14)	29.70 (12.63)	*n* = 35 4.25 (3.3)	*n* = 10 4.12 (2)	*n* = 0	*n* = 0
	p = 0.6188		*p* = 0.9038		*p* = N/A	
Adalimumab + infliximab + etanercept (*n* = 158)					*n* = 15 (12%)	*n* = 13 (39.4%)
					***p* = 0.0002**	

TNFi – TNF inhibitor, ADA – antidrug antibody, N/A – not applicable.

Table 3. Results: TNF-α levels and TNF-inhibitor concentration in the different TNF-inhibitor drug treated patient groups

Parameter	TNF-α level		TNFi concentration	
	ADA+	ADA–	ADA+	ADA–
Infliximab (*n* = 49)	18.21 (15.30)	8.46 (6.49)	*n* = 16 0.035 (0.0)	*n* = 33 3.54 (43)
	p = 0.2452		***p* < 0.001**	
Adalimumab (*n* = 64)	3.82 (5.34)	2.77 (6.69)	*n* = 12 2.5 (3.27)	*n* = 52 5.27 (3.14)
	p = 0.2979		***p* = 0.0009**	
Etanercept (*n* = 45)	0	27.45 (16.16)	*n* = 0	*n* = 45 4.23 (3.06)
	p = N/A		*p* = N/A	

TNFi – TNF inhibitor, ADA – antidrug antibody, N/A – not applicable.

cal phenomenon: in the case of etanercept the serum TNF-α level elevated with the serum TNFi concentration, in case of the non-responder group of patients, TNF-α levels were significantly higher in contrast to responder patients ($p = 0.0136$) [9, 10].

ADA positivity was significantly higher among secondary non-responders compared to responder patients. This relation was true for the whole patient group ($p = 0.0002$) and infliximab treated patients ($p = 0.002$).

This was statistically not significant ($p = 0.115$) in the adalimumab treated group but tended to be higher, however the low number of secondary non-responder patients ($n = 7$) is limiting the validity of this result. In case of etanercept, we could not speak about this association because of the lack of ADA+ patients.

There was no significant association between the concomitant use of methotrexate and presence of ADA (data not shown).

Observing the whole patient group, no significant relationship was detected between mean initial PASI score and ADA presence during the therapy. At the time of the sample collection, ADA negative patients had lower PASI scores than ADA positive ones, although this result was not significant ($p = 0.717$). Data are shown in Table 4.

The sample collection was not performed at the previously designed and standardized points of the therapy because of the nature of our study.

However as in a cross-sectional study, at next clinical visits of the patients, we do not have detailed analysis about the connection of ADA presence and the duration of anti-TNF-α therapy. Antibody expression was detected among 58% ($n = 7$) of the adalimumab treated ADA positive patients ($n = 12$) and among 31% ($n = 5$) of the infliximab treated ADA positive patients ($n = 16$) in the first 12 months of therapy. In ADA positive patients the mean duration of therapy was 21 months when we detected the ADAs.

Table 4. PASI scores regarding ADA positivity or negativity

Parameter	Patients (*N* = 158)	ADA negative patients (*n* = 130)	ADA positive patients (*n* = 28)	*P*-value
Mean PASI score (variance)	2.45 (4.15)	2.29 (3.87)	3.20 (5.36)	0.717
Median PASI	0.00	0.00	0.00	

PASI – Psoriasis Area and Severity Index, ADA – antidrug antibody.

In a multivariate logistic model, we found that considering all patients, ADA production was the most important predictor of secondary clinical inefficacy ($p = 0.020$). Having analysed each TNFi treated group separately, a significant impact of ADA was found only in patients treated with infliximab ($p = 0.032$).

Discussion

The aim of our cross-sectional observational study was to analyse the presence of ADA formation, serum TNF-α levels, serum drug-TNFi levels during TNFi therapy and the associations of all these factors with clinical efficacy in a large psoriatic population treated in real-life circumstances.

Previous studies on this topic were performed on smaller patient groups, e.g. one study published by Kui *et al.* [10] observed 77 psoriatic patients. In this study we had the opportunity to examine a relatively wider patient group in real-life clinical situations.

Our findings were consistent with previous literature data [1] regarding ADA prevalence: in case of adalimumab treated patients, 18.4%, in case of infliximab treated patients, 33% and in etanercept treated patients, 0% of them had ADA in their sera. Our study confirmed the literature data stating that ADA positivity is significantly higher in case of the secondary non-responder patient group. ADA positivity is significantly higher in comparison to responders [10–13].

Looking at the serum TNFi drug concentrations we found that in ADA negative patients, significantly higher serum drug concentrations can be measured.

In case of adalimumab, 18.4% of the patients had ADA formation, the serum drug concentration was significantly higher in the ADA negative group.

In the infliximab treated group, 33% of patients had ADA in their sera, the serum drug concentration was significantly higher in the ADA negative group. We could not identify any ADA in the case of etanercept treated patients, among them 38 (83%) patients had measurable drug concentrations. Although we cannot identify any neutralizing antibodies against etanercept, non-neutralizing antibodies can cause an accelerated clearance of the drug and may be behind the secondary inefficacy [14].

The measurable levels of TNF-α may also be a predisposing parameter and may reflect clinical efficacy beside PASI improvement.

In case of adalimumab elevation of serum TNFi concentration resulted significant decrease of serum TNF-α concentration. This observation was also significant analysing all our patients sample (adalimumab, infliximab, etanercept, $n = 158$) together. Similarly to previous literature data, we were also able to confirm that in case of etanercept, there was a concomitant elevation of sera TNF-α concentration and serum TNFi concentration [9, 10]. For this interesting paradoxical phenomenon, there were distinct explanations, such as other molecular mechanisms that can lead to TNF-α elevation through positive feedback during etanercept therapy. Other explanation makes the ELISA method responsible for this result (the ELISA kit can only detect free TNF-α, not TNFi–TNF-α complexes).

In the case of the non-responder group of patients, TNF-α levels were significantly higher in contrast to the responder patients. There was no significant association found between TNF-α serum levels and the presence of ADA, but it tended to be higher in the case of ADA positive patients. Whether there are other inflammatory pathways to be involved in the pathomechanism the serum TNF-α level does not specifically reflect the clinical efficacy of TNFi therapy. Therefore other, more sensitive predisposing parameters should be taken into consideration.

The presence of ADA seemed to be the most relevant and statistically significant ($p = 0.02$) clinical factor in connection with the secondary clinical inefficacy when comparing TNFi concentrations and the serum TNF-α levels.

The heterogeneity of our patients was balanced because of the nature of our study: patient selection and sample collection was performed in a random way, in order of their appearance at our ambulance for the regular upcoming visit and administration of the next TNFi dosage. In contrast to previous studies presenting data only for the first 52 weeks of treatment, one strength of our study is that we were investigating a relatively wide treatment period (average: 92 weeks; 4–304 weeks) with TNFi. On the other hand, this is the reason why an association between the exact time of ADA formation and the duration of TNFi therapy was not analysed.

One limitation of our study was the real-life way of sample collection: prospectively determined occasions at exact visit time points may serve as more representative data. Another limitation can be traced back to the laboratory method we had used.

The ELISA method has the potential of false-positive results because of non-specific bindings. Radioimmunoassay (RIA) could also be used as another method, which is less sensible for drug interference and highly specific as it can detect all subsections of IgG-type of antibodies. Its disadvantage is high complexity and less sensitivity for drug concentration in the blood.

Conclusions

We demonstrated the major role of antidrug antibodies against TNFi drugs in the case of secondary treatment inefficacy between real-life circumstances in a large population of treated psoriatic patients. During TNFi treatment, ADA formation is obviously associated with lower serum TNFi levels and consequential secondary nonresponse. When monitoring the presence of ADAs and serum drug concentration, secondary inefficacy could be predicted leading to a more personalized and efficient therapy in the everyday clinical practice.

Conflict of interest

The authors declare no conflict of interest.

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