



Drug-neutralizing Antibodies against TNF- α blockers as Biomarkers of Therapy Effect Evaluation

Krasimir Kraev¹, Mariela Geneva-Popova¹, Velichka Popova¹, Stanislava Popova¹, Ana Maneva², Anastas Batalov¹, Teodora Stankova², Ginka Delcheva², Katya Stefanova²

¹ Department of Propaedeutics of Internal Diseases, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria

² Department of Biochemistry, Faculty of Pharmacy, Medical University of Plovdiv, Plovdiv, Bulgaria

Corresponding author: Krasimir Kraev, Department of Propaedeutics of Internal Diseases, Faculty of Medicine, Medical University of Plovdiv, 15A Vassil Aprilov Blvd., 4002 Plovdiv, Bulgaria; E-mail: kkraev@hotmail.com

Received: 24 Aug 2019 ♦ **Accepted:** 2 Dec 2019 ♦ **Published:** 30 June 2020

Citation: Kraev K, Geneva-Popova M, Popova V, Popova S, Maneva A, Batalov A, Stankova T, Delcheva G, Stefanova K. Drug-neutralizing antibodies against TNF- α blockers as biomarkers of therapy effect evaluation. *Folia Med (Plovdiv)* 2020;62(2):282-9. doi: 10.3897/folmed.62.e39402.

Abstract

Introduction: TNF- α blocker therapy is part of the treatment with biologics used in the management of inflammatory joint diseases. In recent years, drug-induced neutralizing antibodies have been shown to have a negative effect on the course of the disease process.

Aim: To investigate drug-induced neutralizing antibodies against TNF- α blocking drugs used in patients with inflammatory joint diseases and their effect on the clinical course of the disease.

Materials and methods: The study included 121 (56.8%) patients with rheumatoid arthritis, 50 (23.5%) patients with ankylosing spondylitis, 42 (19.7%) patients with psoriatic arthritis, and 31 sex and age-matched healthy controls. The patients were monitored at 0, 6, 12, and 24 months after initiation of TNF- α blocker treatment. The demographic data, vital signs and the parameters of inflammatory activity (C-reactive protein, erythrocyte sedimentation rate, and disease activity indices) were analyzed in all patients. Drug-induced anti-TNF- α blockers antibodies (adalimumab and etanercept) were analyzed using ELISA. Statistical analysis was performed with SPSS v. 24.

Results: Drug-induced neutralizing antibodies against adalimumab were obtained in 11.57% of patients at 6 month, in 17.64% at 12 month, and in 24.8% at 24 month. Drug-induced neutralizing antibodies to etanercept were not demonstrated in patients followed up at 6 months, at 7.77% at 12 months, and at 9.63% at 24 months. Between the presence of neutralizing antibodies to blockers of TNF- α and indices available for disease activity, there is a strong positive correlation and Pearson Correlation = 0.701, $p=0.001$. Patients with poor clinical response and available antibodies against adalimumab at 12 months were 82.36% and patients treated with etanercept 71.42%. The difference between the two groups was non-significant ($U = 0.527$, $p > 0.05$). Patients with poor clinical response and available anti-adalimumab antibodies at 24 month were 75%, and in patients treated with etanercept – 87.50%, the difference between the two groups not being able to reach significance ($U = 0.623$, $p > 0.05$).

Conclusion: Drug-induced neutralizing antibodies against TNF- α blockers (adalimumab and etanercept) have a negative effect on the course of inflammatory joint disease and can be used as reliable biomarker to assess the effect of the treatment with these drugs.

Keywords

biomarkers, inflammatory joint disease, TNF- α blockers

INTRODUCTION

Biologics are drugs that use an immunomodulatory approach to control disease, which usually results in rapid and lasting remission. The first biological agents that influence the disease pathogenetically are tumor necrosis factor- α (TNF- α) blockers.¹⁻⁴

The experience gained from the use of biologic medicines has shown that some patients do not have sufficient effect from their treatment and do not achieve remission, others have initial good response, but subsequently “wash away” the effects and worsen the condition.⁴⁻⁶

In recent years, particular attention has been paid to the formation of neutralizing antibodies in the patient's body during treatment with biologics, since, according to some authors, their infusion leads to a decrease or no effect of the applied therapy.^{2,5,7}

Despite numerous studies on this topic and the widespread recognition of the presence of drug-induced neutralizing antibodies against TNF- α blocking drugs, there is still no clear opinion what their impact on the course of the disease is.⁸⁻¹²

Radstake et al.¹³ analyzed patients with active rheumatoid arthritis (RA) treated with a TNF- α blocker (adalimumab) and found that 16 (47%) of the patients had a good clinical response according to EULAR (European League Against Rheumatism) criteria at 6 months of initiation of treatment, evaluated by the DAS-28 activity index, 8 (24%) had a moderate clinical response, and 10 (29%) had a poor clinical response. According to the authors, the formation of antibodies against the used drug strongly correlates with the clinical response of the patients.

Van Kuijk et al.¹⁴ and Lloyd et al.¹⁵ assume that the available anti-adalimumab antibodies in patients with psoriatic arthritis are leading to higher DAS-28 (Disease Activity Score-28) and higher values of the scales of functional capacity impairment.

Vincent et al.¹⁶ analyzed why one-third of patients with inflammatory joint diseases receiving TNF- α inhibitors did not respond to treatment or lost their initial effect. Probably the explanation for this process is the immunogenicity of the medications themselves. According to the authors, in chronic inflammatory diseases correlations have been found between the formation of neutralizing anti-drug antibodies to TNF- α blockers, low serum drug levels and failure or loss of clinical response assessed by disease activity indices.¹⁶

Kleinert et al.¹⁷ evaluated the effect of treatment with anti-TNF- α receptor antagonist on clinical remission and/or minimal disease activity in patients with RA. The authors conclude that during the treatment with the drug, one quarter of patients achieve clinical remission and almost half of them have achieved minimal disease activity. Using the RA activity assessment scales, the authors demonstrated that changes in patients' condition were apparent at 3 months from initiation of treatment and maintained over the 12-month follow-up period.¹⁷

Benucci et al.¹¹ analyzed patients treated with various TNF- α blocking agents. After 6 months of treatment neutralizing adalimumab antibodies had 6% of patients with RA, and 13% of patients had neutralizing antibodies against etanercept. According to the authors¹¹ the existence of neutralizing anti-drug antibodies against TNF- α blockers influenced the activity of rheumatic diseases. Similar results are obtained in the treatment of patients with psoriatic arthritis receiving TNF- α -blocker therapy.¹⁸

Evaluating the importance of drug-induced neutralizing antibodies to various TNF- α blockers in the treatment of patients with RA, Moots et al.¹⁹ have demonstrated that in patients without anti-drug antibodies clinical activity is lower.

Paramarta and Baeten²⁰ evaluated the clinical relevance of neutralizing antibodies against adalimumab in patients with peripheral spondylitis. According to the results obtained, there is no clear relationship between serum levels and drug-induced neutralizing antibodies against adalimumab with the clinical response of patients.²⁰

Most researchers agree that the presence of drug-induced neutralizing antibodies against blockers of TNF- α have also negative effect on the clinical course, but there are authors that disagree on that point.²¹⁻²⁴

All authors agree that the biologics therapy used to treat inflammatory joint diseases has revolutionized their course and prognosis. This obliges the researchers to look for reasons for the lack of effectiveness in their use in some patients.

The purpose of the study was to investigate drug-induced neutralizing antibodies against TNF- α blocker drugs in patients with inflammatory joint diseases and assess their effect on the clinical course of the disease.

MATERIALS AND METHODS

Patients

We studied 121 (56.8%) patients with rheumatoid arthritis (RA), 50 (23.5%) patients with ankylosing spondylitis (AS), 42 (19.7%) patients with psoriatic arthritis (PsA) and 31 age and sex-matched healthy subjects. All patients were diagnosed as having RA, AS, PsA according to EULAR criteria.^{14,25,26} Patients were monitored at 0, 6, 12 and 24 months after initiation of TNF- α blockers (adalimumab and etanercept). Demographic and physical data as well as ESR, CRP, and disease activity indices were analyzed in all patients. The patients included in the present study had moderate ($3.2 < \text{DAS } 28 \leq 5.1$) and high ($\text{DAS } 28 > 5.1$) disease activity. The study was approved by the Ethics Committee of the Medical University of Plovdiv. All participants gave informed consent before being recruited for the study

Laboratory methods

The following parameters were determined: for inflam-

matory activity – erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), for autoimmune disorders – RF (Rheuma Factor), anti – CCP (cyclic citrullinated peptide) antibodies, for disease activity – DAS-28 for patients with RA, BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) for patients with AS, DAPSA (Disease Activity in Psoriatic Arthritis) for patients with PsA. Laboratory tests were performed using standard laboratory methods. Determination of drug-induced neutralizing antibodies against etanercept (Enbrel) and adalimumab (Humira) was performed by ELISA method using Immundiagnostik-TNF- α -blocker-ADA kits. When tested for neutralizing antibodies, Abs was 450/630, cut-off control = 0.0147. All results with $A450/630 \leq 0.05$ were considered negative and > 0.05 were positive, according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS version 24.0 (SPSS Inc., Chicago, IL, USA). The collected information was verified, coded and entered into a computer database for processing, transcoding and analysis. Data is given as mean \pm SD or as median. T-test was used to compare two groups with normal distribution and Mann-Whitney *U* test

was used to compare groups with non-normal distribution. Correlations between data were evaluated by calculating Pearson's or Spearman's correlation coefficients depending on the distribution of the continuous variables. $P < 0.05$ was considered as statistically significant.

RESULTS

In the cross-sectional part of the study 213 patients were observed – 90 males (42.3%) and 123 females (57.7%) and 31 healthy controls, or 244 in total. As a result of the analysis, no significant difference in the age distribution was found between male (47.20 ± 1.45) and female (49.34 ± 1.06) patients ($t = 1.19$; $p > 0.05$). The mean age of the total number of patients (48.00 ± 1.13 ; range 25-71 years) was greater than the age of 31 controls (mean age 36.67 ± 1.77 years; range 22-52 years) ($t = 5.42$; $p < 0.05$) as the control group included younger healthy individuals. Only, the age of the patients suffering from AS did not differ from the age of the control group ($t = 1.71$; $p > 0.05$) (Table 1).

Estimation of the mean values of some demographic and paraclinical data as well as pain intensity assessed by the VAS (Visual Analogue Score) scale and assessment of disability and quality of life by the HAQ-DI (Health As-

Table 1. Age distribution of patients by disease and healthy controls (years, $\bar{x} \pm SD$)

Disease	\bar{x}	N	SD	Max	Min	Range	SE	SE of kurtosis
Control	36.67	31	9.50	52.00	22.00	30.00	1.70	
RA	49.28	121	13.73	74.00	25.00	49.00	1.24	0.437
AS	40.30	50	9.02	66.00	25.00	41.00	1.27	0.662
PsA	55.66	42	11.30	74.00	31.00	43.00	1.74	0.717
Total	48.46	213	13.29	74.00	25.00	49.00	0.910	0.332

Table 2. Demographic, clinical, and functional parameters of patients with RA, AC, and PsA prior to initiation of treatment with TNF- α blockers ($\bar{x} \pm SE$)

Indicators	Control group Patients with	Clinical groups			P_1	P_2	P_3	
		RA (n = 121)	Patients with AS (n = 50)	Patients with PsA (n = 42)				
Age (yrs)	36.6 \pm 1.7	49.2 \pm 1.14	40.0 \pm 1.27	55.6 \pm 1.74	0.001	NS	0.001	
Sex	Women	38.70%	23.96	62%	45.23%	NS	0.04	NS
	Men	61.30%	76.04%	38%	54.76%	NS	0.001	NS
BMI	24.7 \pm 0.40	27.79 \pm 0.23	25.88 \pm 0.41	28.45 \pm 0.51	NS	NS	NS	
Disease duration		11.71 \pm 0.50	9.26 \pm 0.48	12.80 \pm 0.93				
ESR mm	10.67 \pm 0.71	49.28 \pm 1.24	40.30 \pm 1.27	55.66 \pm 1.74	0.001	0.001	0.001	
ESR \geq 28 (%)	0%	98.34%	92%	97.61%	0.001	0.001	0.001	
CRP mg/L	6.64 \pm 0.93	47.67 \pm 1.40	42.74 \pm 1.69	56.59 \pm 1.67	0.001	0.001	0.001	
CRP \geq 10 (%)	25.81%	100%	100%	100%	0.001	0.001	0.001	
Morning stiffness	2.32 \pm 0.52	60.82 \pm 2.90	40.20 \pm 2.69	68.73 \pm 5.08	0.001	0.001	0.001	
VAS (mm)*	3.6 (1-10)	67.07 (31-90)	44.78 (25-90)	66.00 (34-90)	0.001	0.001	0.001	
HAQ-DI *	0.67 (0-1.1)	2.29 (1.0-3.0)	2.3 (1.0-3.0)	2.38 (1.0-3.0)	0.001	0.001	0.001	

The normal distribution of the data is presented as the mean and standard deviation error ($\bar{x} \pm SE$)

* Non-homogeneous data scatter is presented as the median (minimum-maximum)

assessment Questionnaire-Disability Index) index, as well as some other relevant parameters in patients with RA, AC and PsA are presented in **Table 2**.

There are different correlations between the parameters, reflecting the studied demographic indicators of the patients, their functional tests and the values of the clinical laboratory parameters (**Table 3**).

In the longitudinal part of the study, all 213 patients were included again. Patients receiving adalimumab were 121 (56.8%) and those receiving etanercept – 92 (43.2%). The highest number of patients observed was women suf-

fering from RA, who are 59 (48.76% of treated patients with adalimumab and 27.69% of all patients). The lowest number of patients observed were patients suffering from AS, who were 3 (2.47% of treated patients with adalimumab and 1.40% of all patients), as shown in **Table 4**.

At the 6th month (± 3 weeks) from the beginning of treatment, we examined all patients for the presence of drug-induced neutralizing antibodies to both drugs, in the following months the number of patients studied decreased due to discontinuation due to low efficacy, manifestation of side effects (development of tuberculosis, severe pneumo-

Table 3. Correlations between demographics, functional tests, and laboratory parameters at initiation of treatment (using Spearman-Rho correlation)

Indicators	Age	Duration	BMI	ESR	CRP	Morning stiffness	VAS	HAQ-DI
Age								
r	1.000	-0.009	0.020	1.000 **	0.890 **	0.601 **	0.180 **	-0.116
Sig. (2-tailed)		0.897	0.772		0.000	0.000	0.009	0.92
N	213	213	213	213	213	213	213	213
Duration								
r	-0.009	1.000	0.061	-0.009	-0.021	0.072	0.149 *	-0.028
Sig. (2-tailed)	0.897		0.376	0.897	0.761	0.298	0.29	0.689
N	213	213	213	213	213	213	213	213
BMI								
r	0.020	0.061	1.000	0.020	0.021	-0.028	-0.001	-0.025
Sig. (2-tailed)	0.772	0.376		0.772	0.765	0.684	0.988	0.712
N	213	213	213	213	213	213	213	213
ESR mm/h								
r	1.000 **	-0.009	0.020	1.000	0.890 **	0.601 **	0.180 **	-0.116
Sig. (2-tailed)		0.897	0.772		0.000	0.000	0.009	0.092
N	213	213	213	213	213	213	213	213
CRP mg/L								
r	0.890 **	-0.021	0.021	0.890 **	1.000	0.621 **	0.101	-0.120
Sig. (2-tailed)	0.000	0.761	0.765	0.000		0.000	0.140	0.080
N	213	213	213	213	213	213	213	213
Morning stiffness, min								
r	0.601 **	0.072	-0.028	0.601 **	0.621 **	1.000	0.300 **	-0.045
Sig. (2-tailed)	0.000	0.298	0.684	0.000	0.000		0.000	0.517
N	213	213	213	213	213	213	213	213
VAS mm (pain)								
r	0.180 **	0.149 *	-0.001	0.180 **	0.101	0.300 **	1.000	-0.113
Sig. (2-tailed)	0.009	0.029	0.988	0.009	0.140	0.000		0.099
N	213	213	213	213	213	213	213	213
HAQ-DI								
r	-0.116	-0.028	-0.025	-0.116	-0.120	-0.045	-0.113	1.000
Sig. (2-tailed)	0.092	0.689	0.712	0.92	0.80	0.517	0.099	
N	213	213	213	213	213	213	213	213

r: correlation coefficient; ** correlation is significant at the 0.01 level (2-tailed); * correlation is significant at the 0.05 level (2-tailed), * $0.7 < r_s < 0.9$ – high correlation exists; ** $0.5 < r_s < 0.7$ – there is a significant correlation, $0.000 - p < 0.001$.

Table 4. Distribution of patients by disease, sex, medication taken, and percentage relative to the group treated with the respective medication (%)

Disease	Sex	Adalimumab-treated patients	Etanercept-treated patients
RA	Male	20 (16.52%)	9 (9.78%)
	Female	59 (48.76%)	33 (35.86%)
AS	Male	13 (10.74%)	29 (31.52%)
	Female	3 (2.47%)	5 (5.43%)
PsA	Male	10 (8.26%)	9 (9.78%)
	Female	16 (13.25%)	7 (7.63%)

nia or sepsis), at the discretion of the supervising rheumatologist or at the request of the patient himself, as shown in **Table 5**.

Table 5. Distribution of patients by disease and medication taken in dynamics relative to the group treated with the respective drug in (n, %)

Disease	6 months (± 3 weeks)	12 months (± 3 weeks)	24 months (± 3 weeks)
RA treated with adalimumab	79 (37.70%)	63 (33.33%)	51 (32.27%)
RA treated with etanercept	42 (19.71%)	40 (21.16%)	35 (22.15%)
AS treated with adalimumab	16 (7.51%)	16 (8.60%)	10 (15.80%)
AS treated with etanercept	34 (15.96%)	34 (18.27%)	34 (21.51%)
PsA treated with adalimumab	26 (12.20%)	20 (10.75%)	14 (8.86%)
PsA treated with etanercept	16 (7.51%)	16 (8.60%)	14 (8.86%)
Total	213 (100%)	189 (100%)	158 (100%)

Table 6. Distribution of patients treated with biologics versus clinical response monitored in dynamics

Clinical response	Presence of antibodies	6 months	12 months	24 months
Adalimumab treatment				
Patients with good clinical response	At +	1 (0%)	1 (0%)	1 (0%)
	At-	63 (58.88%)	61 (74.39%)	44 (80.00%)
Patients with moderate clinical response	At +	2 (21.42%)	2 (17.64%)	5 (25.00%)
	At-	47 (43.92%)	18 (21.95%)	9 (16.36%)
Patients with poor clinical response	At +	11 (78.58%)	14 (82.36%)	15 (75.00%)
	At-	2 (2.80%)	3 (3.65%)	2 (3.63%)
Etanercept treatment				
Patients with good clinical response	At +	1 (0%)	1 (0%)	1 (0%)
	At-	74 (80.44%)	76 (91.56%)	70 (93.33%)
Patients with moderate clinical response	At +	1 (0%)	2 (28.58%)	1 (12.5%)
	At-	18 (19.56%)	6 (7.22%)	5 (6.67%)
Patients with poor clinical response	At +	1 (0%)	5 (71.42%)	7 (87.5%)
	At-	1 (0%)	2 (1.20%)	1 (0%)

At+: antibodies available; At-: lack of antibodies

At 6 months from initiation of treatment with adalimumab, 14 (11.57%) of the studied patients had drug-induced neutralizing antibodies against the drug, at the end of the first year they were 17 (17.17%), at the end of the second year they were 20 (25.31 %).

At 6 months from initiation of etanercept treatment, none of the studied patients had drug-induced antibodies against the drug; at the end of the first year they were 7 (7.77 %), at the end of the second year they were 8 (9.63%).

We divided the patients into two groups – Group A included patients without available drug-induced neutralizing antibodies against TNF- α blocker, and group B – patients with available antibodies. Because group B included patients with the three diseases (RA, AS and PsA), but their number was small, we summarized all results for patients with good clinical response (DAS-28 \leq 3.2 for patients with RA, BASDAI <2 and ASDAS <1.3 for patients with AS, DAPSA <4-14 for patients with PsA), moderate clinical response (DAS-28 >3.2 but \leq 5.1 for patients with

RA, BASDAI < 4, and ASDAS < 2.1 for patients with AS, DAPSA > 4-14 < 28 for patients with PsA) and poor clinical response (DAS-28 > 5.1 for b I RA, BASDAI > 4.5 and ASDAS > 3.5 for patients with AS, DAPSA > 4-14 < 28 patients with PsA (Table 6).

There is a strong positive correlation between the presence of drug-induced neutralizing antibodies to TNF- α blockers and the available disease activity indices, Pearson Correlation coefficient $r = 0.701$, $p = 0.001$.

Patients with poor clinical response and available drug-induced neutralizing antibodies to TNF- α blockers at 12 months were 82.36%, and patients treated with etanercept 71.42%, the difference between the two groups was non-significant ($U = 0.527$, $p > 0.05$).

Patients with poor clinical response and available drug-induced neutralizing antibodies to TNF- α blockers against adalimumab at 24 months were 75.0%, and in patients treated with etanercept 87.5%, the difference between the two groups was non-significant ($U = 0.623$, $p > 0.05$).

Patients with drug-induced neutralizing antibodies to TNF- α blockers (adalimumab and etanercept) at 6, 12, 24 months had significantly worse clinical response in patients with RA, AC and PsA ($p < 0.05$).

DISCUSSION

The results of the study suggest that 11.5% of adalimumab-treated patients have drug-induced neutralizing antibodies against the drug at 6 months, in the subsequent months this percentage increasing to 17.64% at 1 year and 24.8% at 2 years. Our data is different from that obtained by Moots et al.¹⁹, who reported detecting neutralizing antibodies against adalimumab in 31% and etanercept in 0% at 12 months from initiation of treatment in the first study based on a large number of patients (more than 600). This difference in results may also be due to the fact that Moots et al.¹⁹ included only patients with RA (no AS and PsA patients in contrast to our study) and that 19.0% of these patients had previously received other biological drugs.

Our data is consistent with that of Krickaert and Bartelds et al.²⁸ who detected anti-adalimumab antibodies in 21 patients (17%) during a 28-week treatment, and with the those reported by Moss et al.²⁹, who detected antibodies in 31% of patients treated with adalimumab and in 0 (0%) of patients treated with etanercept monitored for 12 months. Similarly, Hoxha et al.²¹ demonstrated drug-induced neutralizing antibodies against adalimumab in 11/58 (19%) patients at the end of the second year of initiation of treatment. According to the authors these antibodies can be found within 4 weeks after treatment initiation and their number gradually increases ($p < 0.05$); failure of the treatment was observed in 20/58 (34.5%) patients in the end of the reference period and was significantly associated to a large extent with the antibodies formed ($p < 0.05$).

Our results differ from those of Paramarta and Baeten²⁰, who showed serum antibodies to adalimumab in 23% of

patients with AS at the end of 16 weeks of treatment and in 35% at follow-up. According to the authors, these antibodies were "masked" during therapy and were therefore not detected during treatment.

In the relevant literature, the cumulative percentage of drug-induced neutralizing antibodies against etanercept is approximately 6% in patients with rheumatoid arthritis, 7.5% in patients with psoriatic arthritis, 2% in patients with ankylosing spondylitis.³⁰ Our results are similar to the authors that demonstrated that the number of patients developing antibodies against etanercept in longer-term studies (up to 5 years) increases over time, as expected. Due to their transitory nature, the incidence of antibodies detected at any point in the evaluation is usually below 7% in patients with rheumatoid arthritis and patients with psoriasis.³⁰

SUMMARY

Investigation of drug-induced neutralizing antibodies against some of the most commonly used blockers of TNF- α (adalimumab, etanercept) is now routine practice. However, information on the clinical potential of these assays is still scarce and controversial.

In most studies, the presence of drug-induced neutralizing antibodies to TNF- α blockers used to treat RA, AS, and PsA was associated with a reduced therapeutic response evaluated at 6 months, 12 months, and 24 months from treatment initiation. According to our results, patients treated with adalimumab and available neutralizing antibodies had a poor clinical response in 78.58% of patients at 6 months and 82.36% at 12 months, which is consistent with Chen et al.¹² but differs from Paramatra et al.²⁰, who do not establish such a relationship. Our results seem more likely because the biology of neutralizing antibodies involves the formation of immune complexes with the medication taken, and this, according to most authors, leads to accelerated drug clearance.

At 6 months from initiation of etanercept treatment, none of the studied patients had drug-induced antibodies against the drug, at the end of the first year they were 7 (7.77%), at the end of the second year they were 8 (9.63%), this differs from the results of other authors who found significantly higher levels of antibodies against the drug.³⁰

CONCLUSION

The presence of drug-induced neutralizing antibodies to TNF- α blockers such as adalimumab and etanercept have been associated with worse clinical response according to scales and disease activity indices.

The study of drug-induced neutralizing antibodies to TNF- α blockers can be used as reliable biomarker for predicting the effect of the biological treatment and preparation of predictive mathematical models to patients with RA, AS and PsA.

Acknowledgements

The results of the present study are based on project No 19/2014, finished in 2018, and entitled *Drug-induced neutralizing antibodies in patients with rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis treated with TNF- α blockers* and project No DP-P-8278/02/13/2018 – *Study of the immunogenicity of TNF- α blockers for the production of drug-induced neutralizing antibodies in patients with rheumatological diseases.*

REFERENCES

1. Furst D, Schiff M, Roy M, et al. Adalimumab, a fully human anti-tumor necrosis factor-alpha monoclonal antibody, and concomitant standard antirheumatic therapy for the treatment of rheumatoid arthritis: results of STAR (Safety Trial of Adalimumab in Rheumatoid Arthritis). *J Rheumatol* 2003; 30(12):63–71.
2. Genovese M, Mease Ph, Thomson A, et al. Safety and efficacy of adalimumab in the treatment of patients with psoriatic arthritis who have failed a disease modifying antirheumatic drug therapy. *J Rheumatol* 2007; 34(5):1040–50.
3. Boehncke WH, Brembilla NC. Immunogenicity of biological therapies: causes and consequences. *Expert Rev Clin Immunol* 2018; 14(6):513–23.
4. Pan A, Gerriets V. Etanercept. StatPearls [Internet]. Treasure Island (FL); StatPearls Publishing LLC: 2019.
5. Geneva-Popova M, Kraev K, Karalilova R. [Categorising adverse side effects resulting from biologic therapy.] *Medical Magazine* 2017; 38:76–80 [Article in Bulgarian].
6. Kang E, Jin Y, A Tong, et al. Risk of serious infection among initiators of TNF inhibitors plus methotrexate versus triple therapy for rheumatoid arthritis: a cohort study. *Arthritis Care Res (Hoboken)* 2019. doi: 10.1002/acr.24038 [Epub ahead of print].
7. Siljehult F, Årlestig L, Eriksson C, et al. Concentrations of infliximab and anti-drug antibodies in relation to clinical response in patients with rheumatoid arthritis. *Scand J Rheumatol* 2018; 47:345–50.
8. Kennedy NA, Heap GA, Green HD, et al. Predictors of anti-TNF treatment failure in anti-TNF-naive patients with active luminal Crohn's disease: a prospective, multicentre, cohort study. *The Lancet* 2019; 4(5):341–53.
9. Kalden J, Schulze-Koops H. Immunogenicity and loss of response to TNF inhibitors: implications for rheumatoid arthritis treatment. *Nature Reviews Rheumatology* 2017; 13:707–18.
10. Strand V, Balsa A, Al-Saleh J, et al. Immunogenicity of biologics in chronic inflammatory diseases: a systematic review. *BioDrugs* 2017; 31(4):299–316.
11. Benucci M, Li Gobbi F, Meacci F, et al. Antidrug antibodies against TNF-blocking agents: correlations between disease activity, hypersensitivity reactions, and different classes of immunoglobulins. *Biologics* 2015; 9:7–12.
12. Chen DY, Chen YM, Tsai WC, et al. Significant associations of anti-drug antibody levels with serum drug trough levels and therapeutic response of adalimumab and etanercept treatment in rheumatoid arthritis. *Ann Rheum Dis* 2015; 74(3):e16.
13. Radstake T, Svenson M, Eijsbouts A, et al. Formation of antibodies against infliximab and adalimumab strongly correlates with functional drug levels and clinical responses in rheumatoid arthritis. *Ann Rheum Dis* 2009; 68:1739–45.
14. Van Kuijk A, de Groot M, Stapel S, et al. Relationships between clinical response to adalimumab treatment and serum levels of adalimumab and anti-adalimumab antibodies in patients with psoriatic arthritis. *Ann Rheum Dis* 2010; 69(3):624–5.
15. Lloyd SS, Bujkiewicz All, et al. The efficacy of anti-TNF- α therapies when used sequentially in rheumatoid arthritis patients: a systematic review and meta-analysis. *Rheumatology* 2010; 49:2313–21.
16. Vincent F, Morand E, Murphy K, et al. Antidrug antibodies (ADAb) to tumor necrosis factor (TNF) -specific neutralizing agents in chronic inflammatory diseases: a real issue, a clinical perspective. *Ann Rheum Dis* 2013; 72:165–78.
17. Kleinert S, Hans-Peter T, Krause A, et al. Impact of patient and disease characteristics on therapeutic success during adalimumab treatment of patients with rheumatoid arthritis: data from a German noninterventional observational study. *Rheumatol Int* 2012; 32(9):2759–67.
18. Mastroianni A, Minutilli E, Mussi A, et al. Cytokine profiles during infliximab monotherapy in psoriatic arthritis. *Br J Dermatol* 2000; 153:531–6.
19. Moots RJ, Xavier RM, Mok CC, et al. The impact of anti-drug antibodies on drug concentrations and clinical outcomes in rheumatoid arthritis patients treated with adalimumab, etanercept, or infliximab: Results from a multinational, real-world clinical practice, non-interventional study. *PLoS One*. 2017; 2(4): e0175207.
20. Paramarta J, Baeten D. Adalimumab serum levels and antidrug antibodies to adalimumab in peripheral spondyloarthritis: no association with clinical response to treatment or disease relapse upon treatment discontinuation. *Arthritis Res Ther* 2014; 16:R160.
21. Hoxha A, Calligaro A, Tonello M, et al. The clinical relevance of early anti-adalimumab antibodies detection in rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis: A prospective multicenter study. *Joint Bone Spine* 2016; 83(2):167–71.
22. Jani M, Chinoy H, Warren R, et al. Clinical utility of random anti-tumor necrosis factor drug-level testing and measurement of antibody antibodies on long-term treatment response in rheumatoid arthritis. *Arthritis and Rheumatology* 2015; 67(18):2011–9.
23. Van Schouwenburg P, Bartelds G, Hart M, et al. A novel method for the detection of antibodies to adalimumab in the presence of drug reveals hidden immunogenicity in rheumatoid arthritis patients. *J Immunol Methods* 2010; 362(1-2):82–8.
24. Vogelzang E, Kneepkens E, Nurmohamed M, et al. Anti-adalimumab antibodies and adalimumab concentrations in psoriatic arthritis; an association with disease activity at 28 and 52 weeks of follow-up. 2014; 73(12):2178–82.
25. Fransen J, van Riel PLCM. The disease activity score and the EULAR response criteria. *Clin Exp Rheumatol* 2005; 23(39):93–9.
26. Anderson J, Baron G, van der Heijde D, et al. Ankylosing Spondylitis Assessment Group preliminary definition of short-term improvement in ankylosing spondylitis. *Arthritis Rheum* 2001; 44:1876–86.
27. Calin A, Garrett S, Whitelock H, et al. A new approach to defining functional ability in ankylosing spondylitis: the development of Bath Ankylosing Spondylitis Functional Index. *J Rheumatol* 1994; 21:2281–5.
28. Krieckaert C, Bartelds G, Lems W, et al. The effect of immunomodulators on the immunogenicity of TNF-blocking therapeutic monoclonal antibodies: a review. *Arthritis Res Ther* 2010; 12(5):217.
29. Moss A, Brinks V, Carpenter J. Review article: immunogenicity of anti-TNF biologics in IBD – patient role, product and prescriber factors. *Aliment Pharmacol Ther* 2013; 38:1188–97.
30. Reyes-Beltrán V, Delgado G. Anti-drug antibodies in Colombian patients with rheumatoid arthritis treated with Enbrel vs Etaner – Preliminary report. *J Immunotoxicol* 2017; 14(1):103–8.

Антитела, нейтрализующие лекарственные средства против TNF- α -блокаторов, в качестве биомаркеров для оценки эффекта терапии

Красимир Краев¹, Мариела Генева-Попова¹, Величка Попова¹, Станислава Попова¹, Ана Манева², Анастас Баталов¹, Теодора Станкова², Гинка Делчева², Катя Стефанова²

¹ Кафедра пропедевтики внутренних болезней, Факультет медицины, Медицинский университет -Пловдив, Пловдив, Болгария

² Кафедра биохимии, Факультет фармации, Медицинский университет - Пловдив, Пловдив, Болгария

Адрес для корреспонденции: Красимир Краев, Кафедра пропедевтики внутренних болезней, Факультет медицины, Медицинский университет -Пловдив, бул. „Васил Априлов” № 15А, 4002, Пловдив, Болгария; E-mail: kkraev@hotmail.com

Дата получения: 24 августа 2019 ♦ **Дата приемки:** 2 декабря 2019 ♦ **Дата публикации:** 30 июня 2020

Образец цитирования: Kraev K, Geneva-Popova M, Popova V, Popova S, Maneva A, Batalov A, Stankova T, Delcheva G, Stefanova K. Drug-neutralizing antibodies against TNF- α blockers as biomarkers of therapy effect evaluation. Folia Med (Plovdiv) 2020;62(2): 282-9. doi: 10.3897/folmed.62.e39402.

Абстракт

Введение: Терапия блокаторами TNF- α является частью лечения биологическими препаратами, используемыми для контроля воспалительных заболеваний суставов. В последние годы было показано, что индуцированные лекарственными средствами нейтрализующие антитела оказывают негативное влияние на течение процесса заболевания.

Цель: Изучить индуцированные лекарственными средствами нейтрализующие антитела против блокирующих ФНО препаратов, применяемых у пациентов с воспалительными заболеваниями суставов, и их влияние на клиническое течение заболевания.

Материалы и методы: В исследование были включены 121 (56,8%) пациент с ревматоидным артритом, 50 (23,5%) пациентов с анкилозирующим спондилитом, 42 (19,7%) пациента с псориатическим артритом и 31 здоровый контроль того же пола и возраста. Пациенты наблюдались через 0, 6, 12 и 24 месяца после начала лечения блокаторами TNF- α . Демографические данные, показатели жизнедеятельности и параметры воспалительной активности (С-реактивный белок, скорость оседания эритроцитов и показатели активности заболевания) были проанализированы у всех пациентов. Индуцированные лекарственными средствами анти-TNF- α -блокаторы (адалimumаб и этанерцепт) тестировали с помощью ELISA. Статистический анализ был выполнен с SPSS v. 24

Результаты: Вызванные лекарственными средствами нейтрализующие антитела к адалimumабу были обнаружены у 11,57% пациентов через 6 месяцев, у 17,64% через 12 месяцев и у 24,8% через 24 месяца. Индуцированные лекарственными средствами нейтрализующие антитела к этанерцепту не были установлены у пациентов с последующим наблюдением через 6 месяцев, у 7,77% через 12 месяцев и у 9,63% через 24 месяца. Не было сильной положительной корреляции и корреляции Пирсона = 0,701, $p = 0,001$ между присутствием нейтрализующих антител к блокаторам TNF- α и показателями, присутствующими в активности заболевания. Пациенты с плохим клиническим ответом и имеющимися антителами против адалimumаба через 24 месяца составляли 75%, а у пациентов, получавших этанерцепт - 87,50%, разница между двумя группами не достигала значимости ($U = 0,623, p > 0,05$).

Вывод: Индуцированные лекарственными средствами нейтрализующие антитела против блокаторов TNF- α (адалimumаб и этанерцепт) оказывают негативное влияние на течение воспалительного заболевания суставов и могут быть использованы в качестве надёжного биомаркера для оценки эффекта лечения этими препаратами.

Ключевые слова

биомаркеры, воспалительное заболевание суставов, TNF- α блокеры