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THE INFLUENCE OF TOCILIZUMAB IMMUNOGENICITY MARKERS ON THE EFFECTIVENESS OF TREATING RHEUMATOID ARTHRITIS

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◆ ***Purpose.*** The study assesses the impact of tocilizumab immunogenicity markers on clinical response to conducted treatment in patients with rheumatoid arthritis.

Materials and methods. A total of 17 patients with the confirmed diagnosis of RA receiving tocilizumab therapy for more than 1 year were enrolled into the study. Blood serum samples were collected once every six months before every drug injection during 2.5 years of treatment. The concentration of antibodies to tocilizumab and level of tocilizumab was determined using the ELISA. Additionally, DAS28 values were measured at the first and the last patient visit during the course of study, whereas levels of C-reactive protein (CRP), white blood cells, platelets, rheumatoid factor, and circulating immune complexes were only measured at the last examination.

Results. Positive correlations between the antibodies to tocilizumab and the last point DAS28 values were found, as well as a negative correlation of tocilizumab level and the level of DAS28.

Conclusions. The data obtained indicate a significant effect of serum levels of tocilizumab, as well as of the concentration of antibodies to tocilizumab on the effectiveness of RA treatment. A routine study of these biomarkers might be useful for individualizing treatment approaches for RA patients and determining the causes of tocilizumab resistance.

◆ **Keywords:** tocilizumab; immunogenicity; rheumatoid arthritis.

ВЛИЯНИЕ МАРКЕРОВ ИММУНОГЕННОСТИ ТОЦИЛИЗУМАБА НА ЭФФЕКТИВНОСТЬ ЛЕЧЕНИЯ РЕВМАТОИДНОГО АРТРИТА

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◆ ***Цель исследования*** — оценить влияние маркеров иммуногенности тоцилизумаба на эффективность лечения пациентов с ревматоидным артритом.

Материалы и методы. В исследование были включены 17 пациентов с критериально подтвержденным ревматоидным артритом, получавших генно-инженерную терапию тоцилизумабом в сочетании с метотрексатом или в варианте монотерапии. У всех пациентов в течение 2,5 года лечения тоцилизумабом раз в полгода производили забор крови непосредственно перед следующим введением данного препарата. Концентрацию антител к тоцилизумабу и уровень препарата определяли с помощью иммуноферментного анализа. Активность ревматоидного артрита оценивали по индексу DAS28. Наряду с этим определяли

содержание С-реактивного белка, лейкоцитов, тромбоцитов, ревматоидного фактора и циркулирующих иммунных комплексов.

Результаты. Была выявлена прямая корреляционная связь между уровнем антител к тоцилизумабу с DAS28 (в последней точке исследования) и количеством лейкоцитов. При этом уровень в крови тоцилизумаба обратно зависел от уровня DAS28.

Заключение. Полученные данные указывают на значительное влияние сывороточного уровня препарата, а концентрации антител к нему — на терапевтический эффект терапии ревматоидного артрита. Представленные биомаркеры могут помочь при прогнозировании эффективности терапии ревматоидного артрита, а также способствовать выявлению причин резистентности к тоцилизумабу.

◆ **Ключевые слова:** тоцилизумаб; иммуногенность; ревматоидный артрит.

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by symmetrical damage to the peripheral joints causing destruction and ankylosis, as well as the involvement of various organs and tissues in the pathological process. Nowadays, RA is an important problem of internal medicine because of its high prevalence (from 0.5% to 1%) in the population, frequent occurrence in working-age people, and quite fast onset disability [1]. Significant progress in understanding the pathogenesis of this disease has resulted in the development and introduction into clinical practice of genetically engineered biological drugs (GIBDs) capable of blocking the cytokines involved in RA development. Interleukin-6 (IL-6) plays an important role in RA pathogenesis as it stimulates the synthesis of acute phase proteins and pro-inflammatory cytokines and participates in the activation of osteoclasts, which causes the formation of an erosive process in the joints.

During the first clinical studies, to assess the effectiveness and safety of various GIBDs, it was revealed that resistance to GIBD is formed in 30–40% of RA patients already at the initial stages of therapy. These patients had primary resistance to GIBD, which was detected during the first 12 weeks of therapy. The causes of primary resistance are still not fully understood, but it was found that the response level to GIBD during the first 12 weeks of treatment was inversely correlated with body mass index, disease duration, smoking, and RA activity degree [2, 3].

Over time, the so-called secondary resistance to GIBD can occur in RA patients, which is formed in the case of a fairly high efficiency of therapy with biological drugs and accompanied by an increase in RA activity and a progressive destructive process in the joints. It should be noted that secondary resistance is registered on average in 30% of RA patients who have been receiving long-term GIBD treatment. Most researchers attribute the development of secondary resistance to a decrease in the GIBD serum concentration because of the increased degradation of protein molecules by the reticuloendothelial system and the antibody formation to GIBD capable of neutralizing the biological effects of this group of drugs [4]. Most often, the paratope of neutralizing antibodies are epitopes of GIBD binding to its ligand. Neutralizing antibodies not only inhibit the binding of the drug to the target molecule by forming immune complexes but also accelerate the clearance of GIBD [5]. In addition to neutralizing antibodies, binding antibodies can develop in GIBD-receiving patients, which can be synthesized to any part of the GIBD molecule [6].

In 2010, the drug tocilizumab (TOC) was licensed, which is an IL-6 receptor inhibitor, which is a monoclonal antibody that specifically binds the membrane and soluble form of the IL-6 receptor. Like other GIBDs, TOC has shown high efficiency in reducing the degree of RA inflammatory activity and slowing the progression of the erosive process in the joints [7].

This **work aimed** to study the effect of TOC immunogenicity on the treatment efficiency of RA patients.

Materials and methods

The study included 17 patients with a confirmed diagnosis of RA according to the American College of Rheumatology/European League Against Rheumatism criteria of 2010 and who received TOC for 2.5 years at the center for treatment with GIBD of the E.E. Eichwald Clinic. It should be noted that the TOC combination therapy with methotrexate was prescribed to eight patients of this group and TOC monotherapy to

nine RA patients. Ten patients received TOC at a dose of 400 mg intravenously once a month, five received 600 mg, and two received 360 mg.

All patients agreed to the sampling of biological material and the use of personal data [8].

Initially, as well as 6 and 18 months before the next GIBD administration, blood was sampled to determine the level of TOC and antibodies to it, as well as the level of blood serum C-reactive protein (CRP), leukocytes, platelets, rheumatoid factor, and circulating immune complexes. In addition, before the start and completion of the study, the RA activity index was evaluated using the Disease Activity Score 28 (DAS28). Point 0 is used as the control group.

The concentration of antibodies to TOC (ImmunoGuide, Turkey) was determined using an indirect noncompetitive variant of enzyme-linked immunosorbent assay (ELISA) based on the reaction of anti-GIBD antibodies binding to the adsorbed preparations on the bottom of the well. All manipulations and measurements were performed following the manufacturer's instructions.

An antibody neutralization test was performed to confirm the binding specificity of the antibodies to GIBD. Stepwise dilutions of TOC at a concentration of 100, 10, and 1 μg/mL were incubated with a positive control for the presence of antibodies to this drug. This enabled to analyze the reaction inhibition of an excess of GIBD antigen. After that, the samples were examined by ELISA for the presence of antibodies to TOC.

GraphPad Prism 6.0 program was used for statistical processing. Depending on the type of normal distribution, parametric or nonparametric methods for estimating samples were used. The relationship between the two variables was investigated using the Spearman rank correlation method. The significance level for all statistical tests was less than 0.05.

Results

To confirm the binding specificity of antibodies to TOC, a neutralization reaction was performed. High concentrations of TOC (100 μg/mL), incubated with positive samples, caused the binding of antibodies to the drug, which in turn inhibited the chromogenic reaction during ELISA. With an increase in TOC concentration, the activity of chromogenic reaction decreased, and at the maximum concentration, it was completely absent. The results of the study are presented in Table.

Reaction of neutralization for tocilizumab
Реакция нейтрализации для тоцилизумаба

Drug concentration (μg/mL)	Tocilizumab
	Activity of chromogenic reaction (signal density index)
0	121
1	50
10	12
100	No reaction

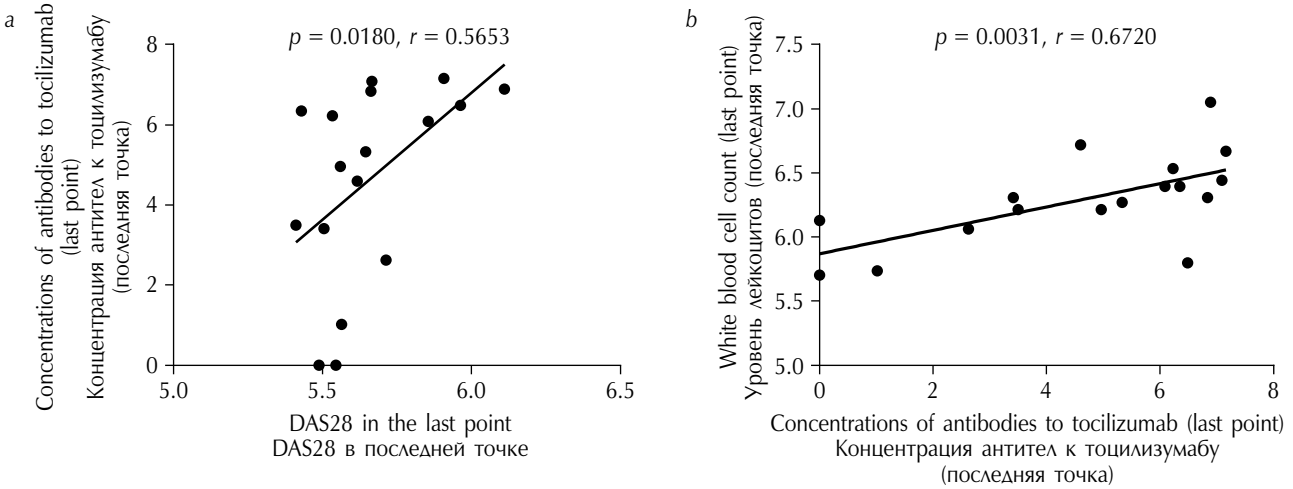

Fig. 1. Correlation between concentration of anti-tocilizumab antibodies with DAS28 (*a*) and number of leucocytes (*b*)

Рис. 1. Корреляция между концентрацией антител к тоцилизумабу с DAS28 (*a*) и количеством лейкоцитов (*b*)

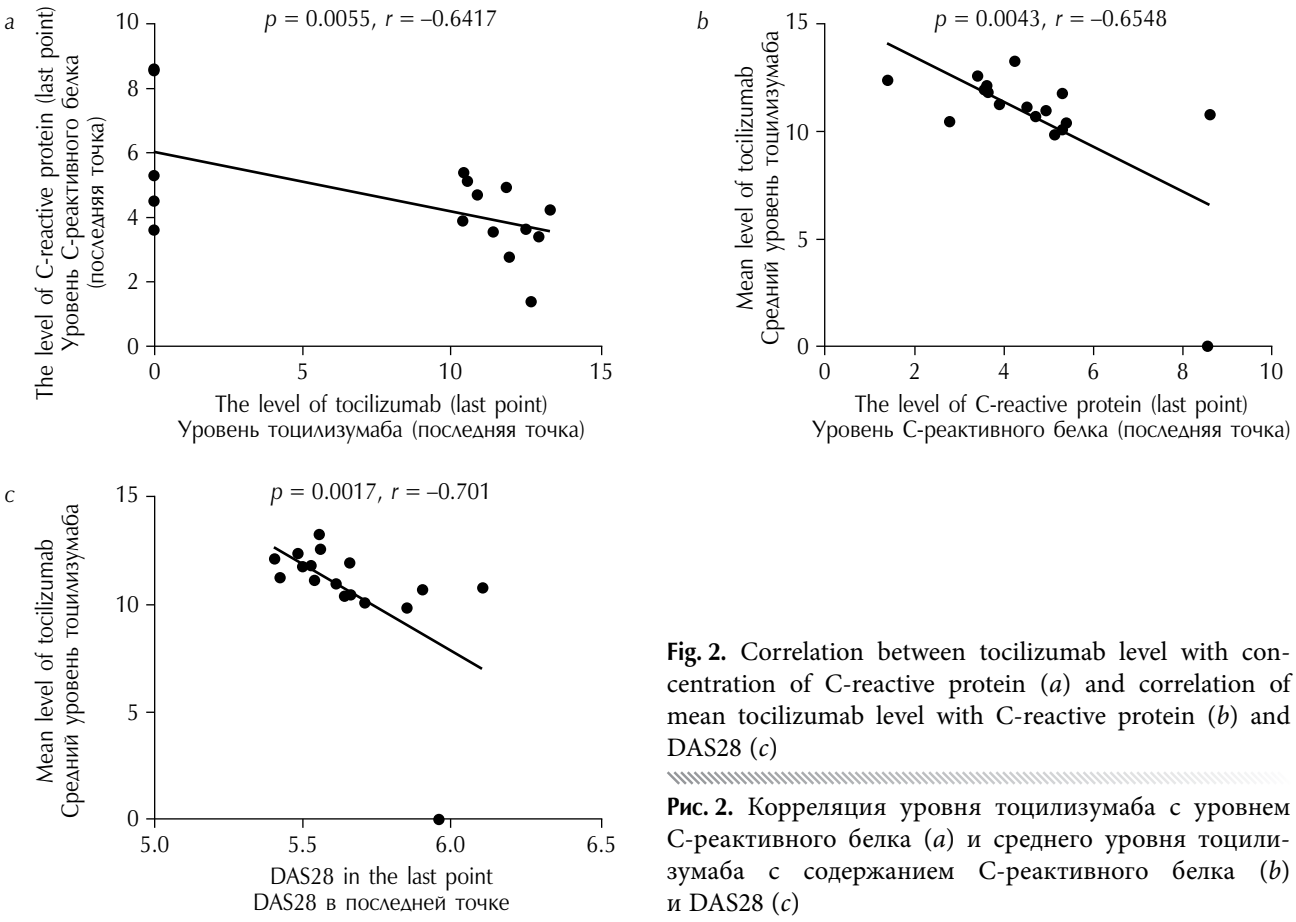

Fig. 2. Correlation between tocilizumab level with concentration of C-reactive protein (*a*) and correlation of mean tocilizumab level with C-reactive protein (*b*) and DAS28 (*c*)

Рис. 2. Корреляция уровня тоцилизумаба с уровнем С-реактивного белка (*a*) и среднего уровня тоцилизумаба с содержанием С-реактивного белка (*b*) и DAS28 (*c*)

The results of the study showed that at the last observation point, antibodies to TOC were detected in most RA patients (70.5% of cases). For this reason, it was important to establish the relationship between the concentration of TOC and the levels of antibodies to it, as well as laboratory parameters and RA activity according to the DAS28 indices at the beginning of therapy and the last visit. It turned out that the examined patient group had a direct correlation between the anti-TOC level and the DAS28 index at the last point of the study ($r = 0.5653$, $p = 0.0180$), as well as the leukocyte number ($r = 0.6720$, $p = 0.0031$). An inverse correlation was established between the TOC blood level in RA patients at the last point of the study and CRP ($r = –0.6417$, $p = 0.0055$). In addition, an inverse correlation was revealed between the average TOC level for the entire study period and the DAS28 index at the last point ($r = 0.701$, $p = 0.0017$), as well as CRP indices ($r = –0.6548$, $p = 0.0043$). The results are presented in Figures 1 and 2.

It should be noted that in this study, the relationship between GIBD and the antibody levels to TOC was not possible to identify. The fact that the content of TOC in the blood of the patients examined, as well as the level of antibodies to them did not differ significantly in the groups of RA patients who received the combination therapy with methotrexate or the TOC monotherapy, was no less interesting.

Discussion

GIBDs used in rheumatology over the past 20 years have enabled not only to achieve RA remission much more often and improve patients' quality of life but also to reduce the disability incidence in working-age patients. However, when using the GIBD therapy often, secondary resistance occurs in RA patients; as a result, the treatment efficiency decreases, and the patient has to be shifted to other GIBDs or synthetic targeted drugs. Secondary resistance to GIBD is mainly caused by a decrease in the drug serum level or the formation of neutralizing antibodies to it. Anti-GIBD antibodies are synthesized to a foreign site of a monoclonal immunoglobulin molecule,

which is the variable region of the Fab fragment binding the target molecules. This, in turn, leads to not only an increased drug elimination rate by the reticuloendothelial system but also a decrease in the GIBD biological activity due to the block of binding of therapeutic immunoglobulin to the target molecule [5]. However, the maximum clinical effect of GIBD remains at a high level in blood serum, which is determined immediately before the administration of the next drug dose.

In this study, in 70.5% of patients taking TOC, an increased concentration of anti-TOC antibodies was detected after 2.5 years of therapy. At the same time, the cited research materials differ significantly from previously published data indicating a low level of TOC immunogenicity (up to 2%), which can be explained not only by a heterogeneous sample of patients but also by the use of unequal methods for determining antibodies to TOC [9]. Thus, most test systems for determining the level of therapeutic immunoglobulins are based on the specific binding of the serum drug to a target molecule adsorbed on the bottom of the well of an ELISA plate. Other methods include the adsorption steps on the bottom of the ELISA plate of monoclonal antibodies specific for the variable region of the immunoglobulin under study. Thus, with reference to the methodological aspects, the GIBD serum level indicates not only the drug concentration but also the bioavailability of the functionally significant region of the protein molecule. Antibodies synthesized against GIBD block this region, which is manifested by a decrease in GIBD concentration. Its high concentrations, in turn, completely saturate the titer of anti-GIBD antibodies, which can also affect the study results.

The study demonstrated that the anti-TOC antibody levels correlated directly with DAS28 and CRP indices and the leukocyte count at the last point. For real clinical practice, it seems important that the TOC level, assessed immediately before the next injection of the drug, can become one of the markers for monitoring the effectiveness of RA treatment. This is confirmed by the high correlation values of the average TOC level and the DAS28 and CRP indices, as well as the CRP concentration at the last point ($r = -0.701$, $p = 0.0017$ and $r = -0.6548$, $p = 0.0043$, respectively). Moreover, the high elimination rate of TOC can be explained by the synthesis of neutralizing antibodies to this drug.

Thus, the data obtained suggest that the increase in RA activity in the examined group of patients at the last point of the study depended on a decrease in the blood serum TOC concentration and an increase in the antibody levels to this GIBD. Moreover, it can be assumed that the decrease in the efficiency of RA treatment depends directly on the low level of TOC before the next administration of this drug and the high level of formed antibodies to TOC. The use of these two markers in real clinical practice will help not only to personalize approaches to RA treatment but also to identify the causes of resistance to this GIBD.

Conflict of interest. The authors declare no conflict of interest.

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