ORIGINAL INVESTIGATIONS

Sequential therapy with rituximab and belimumab in patients with systemic lupus erythematosus

Mesnyankina A.A.[1], Solovyev S.K.[1], Nikishina N.Yu.[1], Aseeva E.A.[1], Demidova N.V.[1], Nasonov E.L.[1,2]

[1]V.A. Nasonova Research Institute of Rheumatology, Moscow; [2]Department of Rheumatology, Institute of Professional Education, I.M. Sechenov First Moscow State Medical University (Sechenov University), Ministry of Health of Russia, Moscow

[1]34A, Kashirskoe Shosse, Moscow 115522, Russia; [2]8, Trubetskaya St., Build. 2, Moscow 119991, Russia

Objective: *to determine the efficiency of sequential (combined) therapy with rituximab (RTM) and belimumab (BLM) in patients with active systemic lupus erythematosus (SLE).*

Patients and methods. *Twelve patients with true SLE having moderate-to-high activity were followed up. Six of them were noted to have skin and articular manifestations and 6 had kidney damage, vasculitis. The patients took RTM at 500–2000-mg doses, with 6-methylprednisolone as premedication, whereupon they were prescribed BLM according to the standard regimen of 10 mg/kg once monthly. The follow-up period was 1 year. At baseline and every three months after RTM administration, the efficiency and tolerability of therapy were evaluated, the concentrations of autoantibodies and complement components was estimated, and the dose of oral glucocorticoids (GCs) was recorded.*

Results and discussion. *During combined therapy with the biological agents (BAs), there was a considerable clinical and laboratory improvement: reductions in disease activity (median (Me) SLEDAI-2K scores were 12 [9.5; 17] at baseline and 2 [2; 6] at Visit 4), the Me concentrations of anti-double-stranded DNA (anti-ds-DNA) antibodies, 101 [39; 250] and 28 [6; 112] U/ml, respectively; those of complement component 3 (C3), 0.44 [0.39; 0.59] and 0.83 [0.81; 0.87] g/L, respectively; and those of complement C4, 0.06 [0.031; 0.1] and 0.16 [0.15; 0.18] g/l, respectively). Most patients received the medium and low doses of oral GCs as initiating therapy. During the year, the dose of GCs was reduced by more than a quarter and they could be completely discontinued.*

Conclusion. *Combined biological therapy with RTM and BLM is a promising treatment for active SLE. The use of this regimen promotes a rapid and effective reduction in disease activity, normalization of laboratory markers of SLE (anti-ds-DNA antibody and complement C3 and C4 levels), and decreases in the dose of oral GCs and, as a consequence, in the risk of irreversible organ damages.*

Keywords: *systemic lupus erythematosus; combined biological therapy; rituximab, belimumab.*

Contact: *Anna Aleksandrovna Mesnyankina;* ***a.a.mesnyankina@gmail.com***

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Systemic lupus erythematosus (SLE) is a severe systemic autoimmune disease characterized by impaired regulation of the immune response with hyperproduction of organ-specific autoantibodies to various components of the cell nucleus, leading to the development of inflammation and progressive tissue damage [1].

Recently, due to the emergence of new data on the pathogenesis and nature of the disease, as well as the introduction of targeted drugs, the tactics of treating SLE are being revised. Special attention is paid to the ultimate goals of therapy – achieving remission of the disease, normalizing the quality of life, and preventing the development of irreversible organ damage [2, 3]. In most patients, it was difficult to achieve these goals due to insufficient effectiveness, toxicity, and long-term consequences of traditional methods of SLE treatment, primarily long-term use of high- and medium-dose glucocorticoids (GCs), as well as immunosuppressants [4, 5]. Currently, an active search is underway for new treatment regimens for SLE that allow maximum dose reduction and even discontinuation of oral GCs [6]. The effectiveness and prospects of using various genetically engineered biological drugs (GEBD), including those used for other rheumatic diseases, are being studied [7].

In the development of immune inflammation in SLE, disorders of innate and acquired immunity play a role: activation of monocytes/macrophages, dendritic cells, neutrophils, natural killers; hyperproduction of various interleukins, interferon (IFN), BLyS (B-Lymphocyte Stimulator, also known as B-Cell-Activating Factor, BAFF), disturbance of the balance between suppressor regulatory T-lymphocytes and pathogenic effector T-helper cells, etc. [8–10]. There is more and more data on the significance of NETs (neutrophil extracellular traps) (a form of cell death which is accompanied by the formation of neutrophil extracellular traps) in maintaining SLE activity. NETs act on plasmocytoid dendritic cells (PDCs) via intracellular recognition receptors TPR7, TPR9. PDCs, in turn, produce IFN type 1. It is believed that reducing their number allows to avoid the presentation of their own antigens, which may be crucial for maintaining tissue homeostasis and regulating the course of SLE [10–12].

B-lymphocytes also play an important role in the progression and maintenance of SLE activity. In their development, they go through certain stages, transforming into B-cells of memory and plasma cells. Activated lymphocytes participate in cytokine synthesis, antigen presentation to T-lymphocytes, and secrete various autoantibodies [8]. In addition, it is important to increase the

concentration of BLyS, which is one of the ligands of the tumor necrosis factor family that stimulates activation and increases the survival of B cells. An increase in the concentration of BLyS in the blood serum of patients with SLE is associated with the activity, relapse of the disease, and an increase in the number of autoantibody-secreting plasma cells [13–15].

Accordingly, anti-B-cell targeted therapy was proposed as a new rational approach to the treatment of the disease. The emergence and implementation of GEBD, in particular, rituximab (RTX) and belimumab (BLM), contributed to effective and rapid reduction of SLE activity and decreased the need for GCs.

RTX is a monoclonal antibody (AB) to CD20 that causes B-cell depletion. Open clinical studies have proven the effectiveness of this drug in real clinical practice [16–18]. However, in two randomized trials involving patients with lupus nephritis, the effectiveness of RTX was not confirmed, so it is not registered for the treatment of SLE and is currently used off-label [19, 20].

BLM is a human monoclonal antibody directed against the B-lymphocyte stimulator (BLyS), the first GEBD that effectively reduces the activity of mild to moderate SLE. It is a human recombinant monoclonal antibody (IgG1?) that prevents the interaction of BlyS with cellular receptors of autoreactive B-lymphocytes, thereby reducing B-cell hyperreactivity and survival of autoreactive B-lymphocyte clones [21, 22]. The use of BLM leads to a decrease in SLE activity, normalization of blood immunological parameters, in particular, C3-, C4-complement components, and anti-dsDNA [23, 24].

It should be noted that only certain B cells that have membrane markers on the CD20 surface, namely naive B cells and memory B cells, are sensitive to RTX [25]. Such therapy leads to rapid and almost complete depletion of circulating B cells; however, a fairly large number of them remain in the tissues [26]. In addition, the plasma level of BlyS increases several times in 3–4 months after RTX use, and this can contribute to survival, B-lymphocyte repopulation, and subsequent exacerbation of SLE, which has been demonstrated in several studies [27, 28].

BLM, by blocking BlyS, affects transient, naive B cells and plasma cells, as well as B cells of the marginal zone [29]. According to W. Stohl et al. [23], BLM did not significantly affect the patient's levels of antibodies to anti-pneumococcal and tetanus toxoid, which may be important for assessing the risk of infectious complications when prescribing the drug.

A treatment regimen with consistent use of GEBD can contribute to a more effective reduction of disease activity, depletion of tissue and circulating autoreactive B-lymphocytes, reduction of autoantibody levels, and, by blocking BlyS, can prevent rapid recovery of the B-lymphocyte population and reduce the risk of SLE exacerbation [23, 28, 30]. A decrease in the BLyS concentration is also observed with the use of high doses of GCs [31]. Therefore, adding BLM to the therapy can significantly reduce the need for GCs.

The assumption of the effectiveness of combination therapy is further supported by case descriptions in patients with SLE, lupus nephritis, and Sjogren's syndrome [32–34]. Previously, we reported successful use of combined therapy with GEBD in 3 patients with SLE [35].

Currently, clinical studies [7, 36] study the combined therapy with BLM and RTX for the skin-joint form of SLE (BLISS-BELIEVE), SLE (BEAT Lupus), SYNBloSe.

Table 1. Characteristics of patients with SLE

Characteristic	Value
Age, years, Me [25th; 75th percentiles]	29 [20; 35]
Female / male, n	11/1
Average duration of the	10
disease, years	
SLEDAI-2K, points Me [25; 75 percentiles]	12 [9.5; 17]
Activity, SLEDAI-2K, n:	
2-nd degree	3
3-d degree	9
DI SLICC/ACR ≥1, n (%)	6 (50)
Damage to organs and systems, n (%):	
Lupus nephritis	4 (33)
Involvement of the peripheral nervous system	1 (8)
Vasculitis	3 (25)
Skin lesion	6 (50)
Involvement of mucous membranes	6 (50)
Arthritis	8 (67)
Serositis	2 (17)
Hematological disorders	8 (67)
Previous treatment with, n (%):	
No	4 (33)
Glucocorticoids	8 (67)
PT with glucocorticoids	8 (67)
PT with cyclophosphamide	3 (25)
Mycophenolate mofetil	4 (33)
Methotrexate	2 (17)
Antimalarial drugs	5 (42)
Intravenous immunoglobulin	5 (42)

Note. SLEDAI-2K – Systemic Lupus Erythematosus Disease Activity Index, the 2000 modification; SLICC/ACR DI – Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; PT – pulse therapy; CP – cyclophosphamide; MPM – mycophenolate mofetil; MTX – methotrexate; IVIG – intravenous immunoglobulin.

Patients and methods. The study included 12 patients (11 women and 1 man) with a reliable diagnosis of SLE of high and moderate activity (Table 1).

Six patients had mainly skin-joint manifestations, 4 had lupus nephritis and 3 – vasculitis. Four patients with the onset of SLE had not previously received treatment for the underlying disease, the rest took GCs at a dose of 5 to 90 mg/day; in 5 cases cytostatics were used. Initially, 6 patients had irreversible organ damage, mainly due to damage to the visual organ (retinal angiopathy, cataract), and the development of avascular necrosis.

The use of GEBD was due to the high activity of the disease, the ineffectiveness of GCs and cytotoxics, as well as the presence of concomitant diseases that limited the use of the standard therapy. Patients with SLE were given combination therapy: initially, RTX was administered at a dose of 500–2000 mg with premedication with 6-methylprednisolone from 0.25 g to 1 g intravenously, and then BLM was prescribed according to the standard scheme (10 mg/kg once per month). The observation period was one year. Eight patients started receiving BLM after 1–4 months, and 4 patients – 5–7 months after RTX

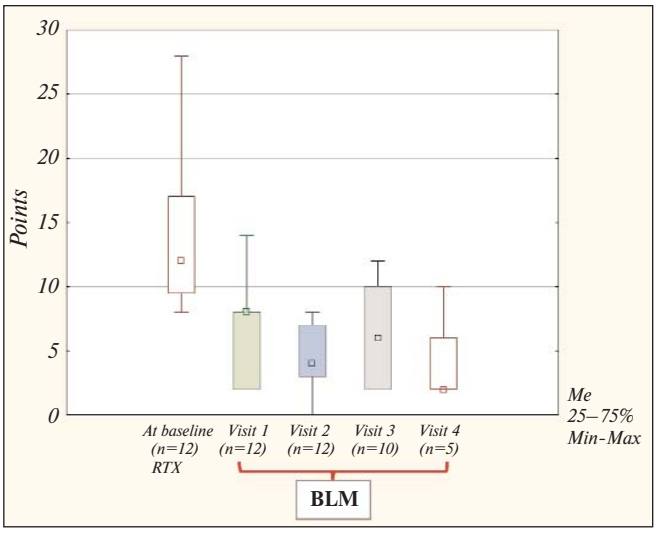

Fig. 1. *Dynamics of SLEDAI-2K*

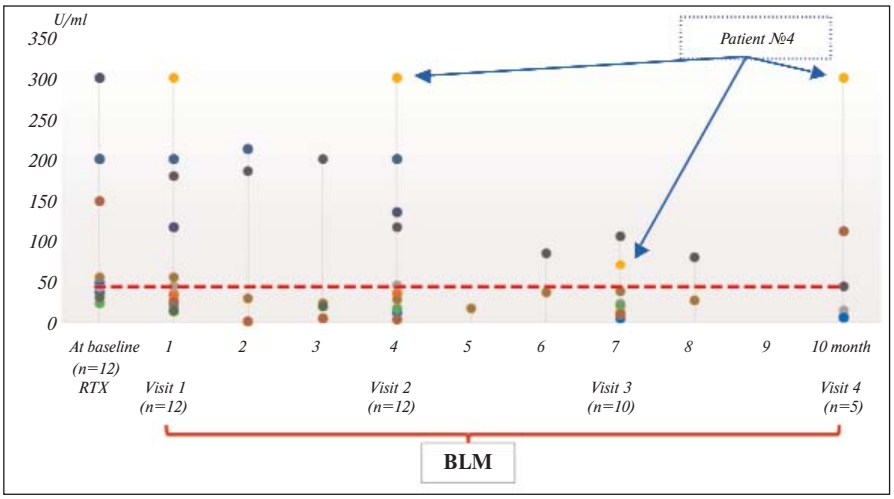

Fig. 2. *Dynamics of anti-dsDNA*

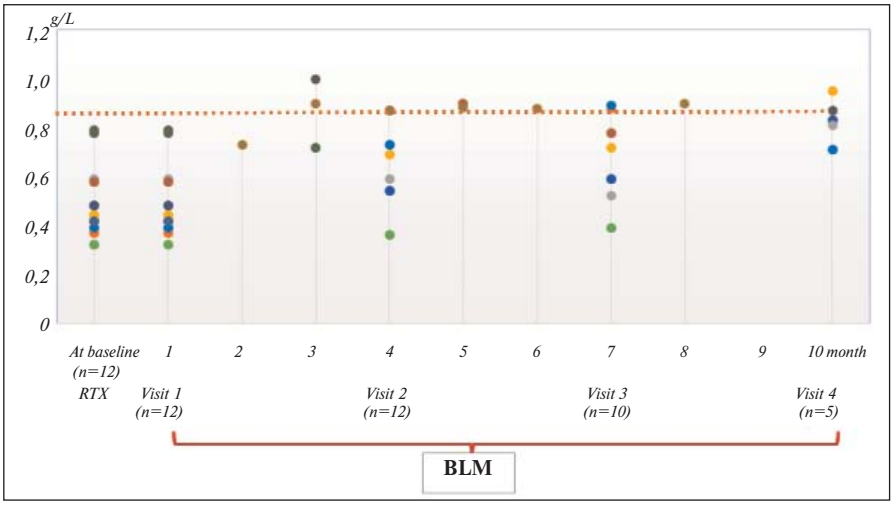

Fig. 3. *Dynamics of the level of C3 complement component*

administration. Assessment of the disease activity and laboratory blood parameters was performed at the time of the first BLM administration (session 1). Subsequent control visits were carried out once every 3 months. Visit 2 took place 4 months after the start of BLM treatment (n=12), visit 3 – 7 months later (n=10), and visit 4 – 10 months after the start of the BML therapy (n=5). Some patients were able to conduct an interim analysis of laboratory and clinical manifestations of SLE.

To evaluate the results, we used the SLEDAI-2K activity index, the SFI exacerbation index (SELENA Flare Index; moderate, severe exacerbation), and the SRI response index (SLE Responder Index), DI SLICC/ACR (damage index developed by Systemic Lupus International Collaborating Clinics/American College of Rheumatology).

Statistical processing of the results was performed using the program Statistica 7.0 (StatSoft, USA). The results are presented as median (Me), 25th; 75th percentiles. Methods of descriptive statistics were used.

The Study Protocol was approved by the Ethics Committee of the Nasonova Research Institute of Rheumatology. All patients signed an informed consent to participate in the study.

Results. Against the background of RTX therapy, in 8 out of 12 patients a clinical and immunological response was achieved. Initially, before the introduction of RTX, Me SLEDAI2K was 12 [9.5; 17] points, by the 1st infusion of BLM – 8 [2; 8] points. Against the background of the subsequent BLM infusions, a decrease and/or preservation of a low degree of disease activity was achieved in 10 patients, mainly by the 3rd and 6th months of BLM treatment. By session 2, the SLEDAI2K Score was 4 [3; 7], by session 3 – 6 [2; 10], and by session 4 – 2 [2; 6]. One patient (No. 5), 9 months later, despite a decrease in the disease activity (SLEDAI-2K decreased from 9 to 4 points), had a relapse of SLE due to a delay in BLM infusion. In another patient (No. 3) with a torpid course of the disease, the presence of common skin rashes, mucosal lesions and immunological disorders, insufficient effectiveness of therapy was observed. However, the area of skin rashes was reduced, enanthema was stopped, and the dose of GC, which initially and for many years had been ⩾15 mg/day, was reduced. The dynamics of SLEDAI-2K in patients is shown in Fig. 1.

During the treatment, there was a gradual decrease in the level of anti-dsDNA. Initially, before the use of RTX, Me anti-dsDNA was 101 [39; 250] U/ml, and at the time of visits 1, 2, 3 and 4 – 38 [23; 147], 34 [13; 134], 20 [9; 38] and 28 [6; 112] U/ml, respectively (Fig. 2). Me of C3 component of complement increased from 0.44 [0.39; 0.59] to 0.68 [0.5; 0.88] g/L by visit 1 and to 0.83 [0.81; 0.87] g/L

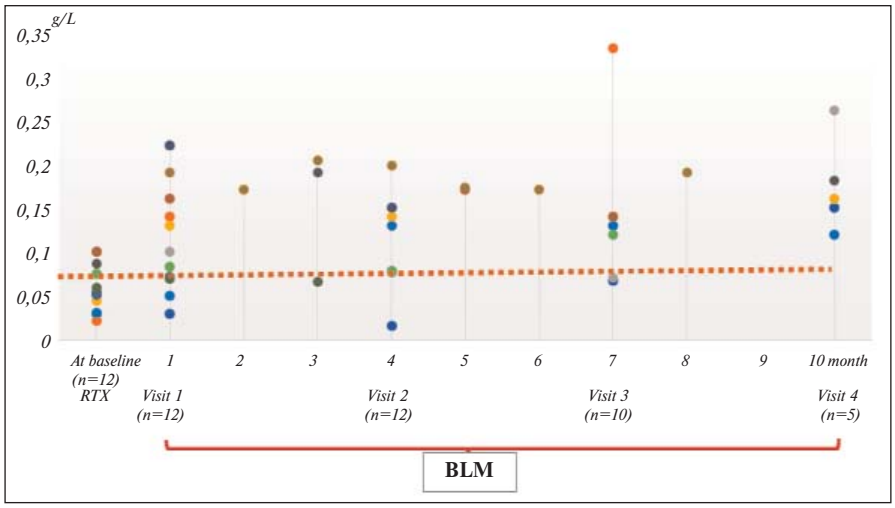

Fig. 4. *Dynamics of the level of C4 complement component*

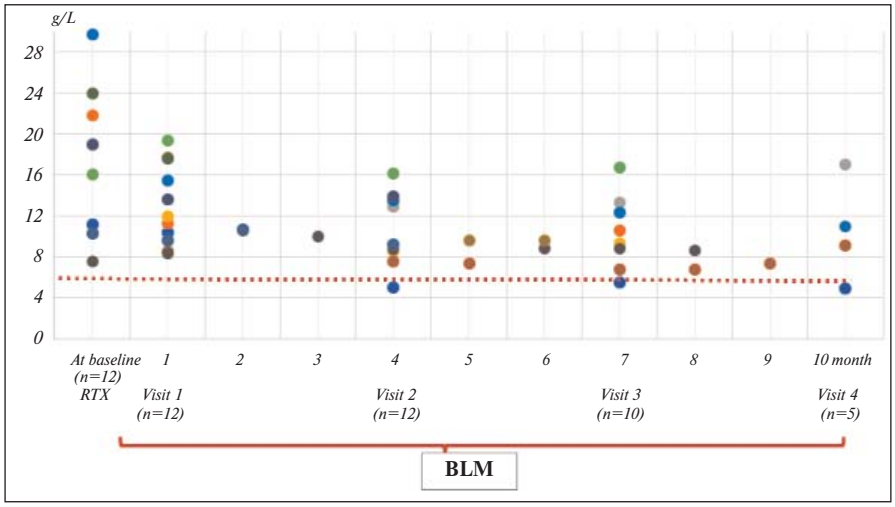

Fig. 5. *Dynamics of IgG level*

by visit 4 (Fig. 3); Me of C4 component of complement increased from 0.06 [0.031; 0.1] to 0.1 [0.07; 0.16] and 0.16 [0.15; 0.18] g/L, respectively (Fig. 4). Patient No. 4 received only 5 BLM infusions. Two months after the last administration of GEBD, she had a significant increase in the concentration of anti-dsDNA, but no exacerbation of SLE was recorded.

In patients with SLE, treatment with GEBD led to a decrease in IgG content. Initially, before the use of RTX, Me IgG level was 17.5 [10; 22.9] g/L, and by visits 1 and 4 – 12.8 [10; 17.6] and 11 [9.1; 11] g/L, respectively; but in general, IgG levels remained within the normal range during the treatment (Fig. 5). There was also a decrease in Me IgM concentration from 2.3 [1.1; 2.6] g/L initially to 0.71 [0.5.1; 0.9] and 0.5 [0.35; 0.55] g/L at the time of visits 1 and 4, respectively. The IgA concentration remained within the normal range, and its Me was initially 2.3 [0.9; 3.9] g/L, and by visits 1 and 4 – 2.7 [2.1; 3.92] and 2.2 [1.35; 3,4] g/L, respectively. Despite this dynamics, no severe infections were registered in patients.

One of the goals of combined therapy with RTX and BLM was to reduce the dose of oral GCs as much as possible. Initially, 10 patients with high and moderate disease activity received a prednisolone equivalent dose of GCs – 2.5 to 15 mg/day (Table 2). Two patients were an exception. One patient (No. 8) with vasculitis, peripheral nervous system and kidney damage already received GCs (60 mg/day) before admission to the clinic and, accordingly, before the start of combination therapy. Another patient (No.1) with vasculitis took prednisolone 20 mg/day. Both patients required pulse therapy with cyclophosphamide (CP), which was replaced with mycophenolate mofetil (MMF) in patient No. 8. Taking into account kidney damage and vasculitis, 4 patients received MMF during the entire follow-up.

After the addition of BLM therapy, the dose of oral GCs was reduced by more than a quarter in 7 patients receiving medium and high doses of GCs. In 3 patients, the dose did not change and was 2.5–5 mg/day. In 1 patient, GC was completely canceled, and in 1 (No. 12), GC was not used during the follow-up. They did not have any exacerbations. Patient No. 8, with an initially high dose of GC, by the end of the study received ? of a tablet of a GC per day, and she had a complete clinical and laboratory remission.

In 6 out of 8 patients previously treated with GCs, DI ranged from 1 to 5 points. However, no new organ damage was detected in any case during the observation period.

Discussion. Currently, the combination therapy of RTX and BLM is a promising scheme for the treatment of SLE. By 2014, isolated cases of its successful use in patients with SLE and Sjogren's disease were described [32, 33]. Thus, T. Kraaij et al. [33] presented data on the use of combination therapy in a 32-year-old patient with progressive lupus nephritis. The authors drew attention to the ineffectiveness of immunosuppressive therapy, which included MMF, GCs. After RTX administration, a partial response was achieved, and there was a decrease in proteinuria from 9 to 3.5 g/day. After 7 months, BLM therapy was started. In connection with the development of adverse events (nausea, tremors, loss of body weight) MMF and GCs were cancelled. After 18 months, the patient was on BLM monotherapy, remission was achieved, the levels of anti-dsDNA, C3-, C4-complement components were normalized, and the number of circulating B cells was reduced.

In recent years, major clinical studies have been conducted to evaluate this treatment regimen. Thus, the BLISS-BELIEVE Protocol [36] provides for the use of RTX in patients with SLE with a further transition to BLM in the subcutaneous form at a dose of 200 mg/week. However, this study can only include patients with lesions of the skin, joints, and mucous membranes in the absence of changes in vital organs, which limits the possi-

Table 2. Dose of oral GCs in dynamics, mg (prednisolone equivalent)

Patient No	At baseline (before the introduction of RTX)	Visit 1 (the first introduction of BLM)	Visit 2	Visit 3	Visit 4	Dynamics
1	20	20	15	15	13.75	↓
2	7.5	5	5	5		↓
3	15	15	15	13.75	10	↓
4	5	5	5	5	5	=
5	10	10	10	10	7.5	↓
6	15	10	8.75	7.5		↓
7	5	5	5			=
8	60	7.5	10	2.5	1.25	↓↓↓
9	10	2.5	0	0		↓↓↓
10	10	10	10	5		↓
11	2.5	2.5	2.5	2.5		=
12	0	0	0			=

bility of studying the effectiveness of combination therapy for more severe manifestations of the disease.

T. Kraaij et al. [37] present data of 11 patients with SLE refractory to standard therapy, 10 of whom had kidney damage. On the background of combined application of RTX and BLM in this group, a marked reduction of disease activity was achieved with the improvement of immunological blood parameters (the level of antibody to dsDNA, C3-, C4-components of complement), reducing and maintaining low numbers of B cells. In 11 patients with lupus nephritis a response to therapy was observed. It should be noted that the patients received immunosuppressive therapy. Later, an equally interesting report was published about a decrease in the concentration of NETs after sequential administration of GEBD, which can also have a beneficial effect on cellular homeostasis [12]. Data on the incidence of infectious complications in 16 patients with SLE who received a combination of RTX and BLM were also presented. Only 1 of them developed a serious infection that required hospitalization. Four patients had mild forms (upper and lower respiratory tract lesions, urinary infection, etc.), 2 of them had a significant decrease in IgG levels (up to 2.5–3.4 g/L), which was associated with concomitant immunosuppressive therapy [38].

In 2018, R. Gualtierotti et al. [34] reported successful use of RTX and BLM in 3 patients with SLE. The study demonstrated an increase in BlyS concentration after RTX administration and a significant decrease after BLM administration. These patients were able to achieve a long-term remission, and subsequently reduce the dose or cancel oral GCs.

In our work, sequential therapy with RTX and BLM was effective in most patients with SLE. It provided a decrease in both the clinical and immunological activity of the disease by the time of the first BLM administration, as well as a further increase in the clinical effect during the follow-up. In addition, the combined use of GEBD made it possible to use medium and low doses of GCs as an initiating therapy for exacerbation of SLE, followed by their reduction. Of course, it is currently impossible to speak about a complete rejection of cytostatic therapy in the presence of indications; patients with vital organ damage should be prescribed immunosuppressive drugs. In the present follow-up, 2 patients with lupus nephritis and vasculitis received CP and 4 patients – MMF. During the observation period, we did not detect any new irreversible organ damage.

No serious infections were detected in our patients during the entire study period. IgG levels remained within the normal range in most cases. In 1 patient (No. 1) with vasculitis, who additionally required CP pulse therapy on a monthly basis, there was a decrease in IgG concentration in dynamics, which was also observed by T. Kraaij et al. [38], but this patient did not show signs of infectious complications.

Currently it is impossible to assess the effectiveness of the therapy depending on the duration of the interval between the introduction of RTX and the administration of BLM due to the small number of patients, differences in the disease activity and severity of affection of organs and systems. At the same time, literature data on an increase in the concentration of BlyS 3–4 months after the introduction of RTX may be the basis for an earlier start of anti-BlyS therapy (BLM). Since an increase in BLyS is associated with an early exacerbation [28], it is advisable to start treatment with BLM in the first 3 months after the last RTX administration, which can prevent an exacerbation of SLE. The

duration of BLM use, in our opinion, should be more than a year. Such therapy can help achieve and maintain remission, but more research is needed to determine its optimal duration.

Taking into account the available data, it is of great interest to study the concentration of BLyS at different stages of sequential therapy with GEBD in order to identify potential candidates for such therapy and determine the optimal time for administration of BLM.

Conclusion. Thus, although to date the combination of GEBD has not been widely used in other diseases, there are serious reasons to study the combined therapy with BLM and RTX in SLE. The persistent activity of the disease, which, despite the use of modern treatment methods, is observed in many patients, justifies conducting such studies. Their results may change the current treatment paradigm and allow patients with SLE to stop taking conventional, often toxic drugs.

Consistent use of RTX and BLM can contribute to:

– rapid and effective reduction of disease activity;

– normalization of laboratory markers of SLE (the level of anti-dsDNA, C3-, C4-complement components);

– reducing the dose of oral GCs and, as a result, the risk of irreversible organ damage.

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Mesnyankina A.A. https://orcid.org/0000-0001-5411-7317
Solovyev S.K. https://orcid.org/0000-0002-5206-1732
Nikishina N.Yu. https://orcid.org/0000-0002-4160-7218
Aseeva E.A. https://orcid.org/0000-0002-1663-7810
Demidova N.V. https://orcid.org/0000-0003-3065-4235
Nasonov E.L. https://orcid.org/0000-0002-1598-8360