THE EFFICIENCY OF BIOLOGICAL THERAPY AND THE FEATURES OF HUMORAL IMMUNITY IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Objective: to investigate the effect of various biological agents (BAs), including combined treatment with rituximab (RTM) and belimumab (BLM), on the activity of systemic lupus erythematosus (SLE) and to evaluate their efficacy and impact on some parameters of humoral immunity.
Subjects and methods. BAs were prescribed to 54 patients with a reliable diagnosis of SLE with high and medium activity according to SLEDAI-2K; 40 of them received RTM, 7 – BLM; 7 – combined therapy with RTM and BLM. Clinical and laboratory examinations were made in all the patients at the time of their inclusion and then every 3 months during a year. The results were assessed using SLEDAI-2K, BILAG index, Lupus Erythematosus National Assessment (SELENA)-SLEDAI Flare index (SFI) (a moderate, severe exacerbation), and SLE Responder Index (SRI).
Results and discussion. At 3, 6, and 12 months after start of therapy, the use of BAs in all the patients resulted in a disease activity reduction. It was statistically significant ($p < 0.00001$) in the RTM group; and no statistical analysis was carried out in the BLM and RTM+BLM groups due to the small numbers of patients. At the same time, there was a progressive decrease in the levels of anti-double-stranded DNA (ds-DNA) antibodies (Abs) and an increase in the concentration of the complement fractions C3 and C4 in the RTM and RTM+BLM groups ($p < 0.05$) at one-year follow-up. After 12 months of therapy with BAs, there was a decrease in IgG ($p < 0.02$) and IgM ($p < 0.03$) levels; but overall it remained within the reference ranges. Prior to therapy, irreversible organ damages were recorded in 23 (42.6%) of the 54 patients. The increased damage index at 12 month was observed only in patients receiving RTM, which is probably due to the use of higher-dose glucocorticoids.
Conclusion. All three methods of therapy with BAs in SLE patients demonstrated good efficiency shown as a significant decrease in clinical and laboratory activity measures that were assessed by SLEDAI-2K and the levels of anti-ds-DNA and complement components C3 and C4. The decrease in immunoglobulin levels did not go beyond the reference values. Therapy with BLM and RTM+BLM allowed for managing patients with the low and average doses of oral glucocorticoids, which contributed to the reduction of not only the activity, but also risk of irreversible organ damages.
Keywords: systemic lupus erythematosus; treatment; rituximab; belimumab; biological agents.
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Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease of an unknown etiology characterized by hyperproduction of autoantibodies to various cell components with immunoinflammatory damage of tissues and visceral organs [1].

Impairment of a complex process of interaction between various components of congenital and acquired immunity underlies the disease resulting in abnormal activation of T- and B-cells with further hyperproduction of autoantibodies. Recently a large body of new evidence was obtained concerning the role of different subpopulations of B and T-lymphocytes, cytokines, dendritic cells, interferon (IFN)-1, etc. in SLE development [2]. The data available provide an insight into peculiarities of disease development and progression, determine development of new medicinal products. Measurement of antibodies (Abs) secreted by plasmacytes (PC) plays an important role in SLE diagnosis, evaluation of activity, prognosis, and therapeutic efficacy [3,4]. The most informative parameter is Abs to double-stranded (ds)-DNA. Currently they serve as the principal diagnostic marker of SLE and their serum levels correlate with the disease activity [5]. Ab level to ds-DNA and complement components is used for SLE assessment including by SLEDAI2K index. Furthermore, in addition to the clinical signs it is taken into account for therapeutic efficacy evaluation [5,6].

Ab level is affected by various treatment methods including long-term administration of high and ultrahigh doses of glucocorticoids (GCs), immunosuppressive agents and biologic medicines such as rituximab (RTM) and belimumab (BLM) [5,7,8,9].

GCs serve as a basis for SLE therapy increasing survival and drastically reducing early lethality of SLE subjects from their introduction in clinical practice [10]. However, need in high and ultrahigh doses of GCs, pulse therapy (PT) at the initiation and relapses of the disease as well as long-term administration of medium doses to maintain remission, inappropriate prescription of high doses results in serious adverse reactions (ADRs) associated with the product effect on musculoskeletal system, homeostasis, endocrine, cardiovascular systems, etc. GC treatment combined with the disease activity, frequent relapses and sometimes severe SLE progression promote increased risk of irreversible organ damage

[11,12]. Meanwhile cytostatic therapy increased the risk of severe viral and bacterial infections thus largely limiting its use for SLE treatment [13,14].

Therefore, measures to prevent vital organ impairment and irreversible organ damage, achieving remission, maintaining stable SLE course and minimizing GC dose are of key importance. These issues were partly solved by biologics development [15,16].

Currently two biologics are available for SLE therapy: RTM, monoclonal Ab to CD20 causing depletion of B-cells, and BLM, monoclonal Abs preventing interaction between BlyS with cell receptors of autoreactive B-lymphocytes. Efficacy of these products was verified in subjects with severe SLE refractory to conventional therapy [17–19]. RTM provided relatively rapid and long-term reduction in disease activity associated with stable decrease in anti-ds-DNA antibody levels and normalized level of complement components [17,20].

By affecting BLyS, BLM reduces B-cell hyperresponsiveness thus reducing survival of autologous reactive B-lymphocyte clones and therefore Ab synthesis further reducing SLE activity [21]. Furthermore, such therapy allows gradual GC dose reduction with minimal risk of the disease exacerbation and prevents GC-associated ADRs [22]. However, BLM compared to RTM has slower effect and, based on the recommendations, is not utilized in subjects with active vital organ lesions [23].

Currently, strong focus is made on consecutive administration of the two biologics using RTM to assure rapid effect and further administration of BLM to maintain low activity and achieve remission. Overlapping mechanisms of action of the products should promote suppression of a large group of B-lymphocyte subpopulations minimizing their activation, B-cell hyperresponsiveness and, therefore, reducing levels of a broad range of autoantibodies [23–24]. Another important factor justifying such combination are trial findings verifying that after 3–4 month of RTM treatment BlyS plasma levels tend to increase several times [21,25], while high doses of GCs or BLM lead to reduction of its level. These results suggest that co-administration of RTM and BLM in subjects with high and moderate disease activity would ensure both fast suppression of activity and retain the effect achieved by BLM preventing from early SLE relapses without administration of high GC doses [26].

The purpose of this study was to investigate effects of RTM and BLM on SLE progression, evaluate their efficacy and their specific effects on several humoral immunity parameters.

Materials and methods:

The study enrolled 54 subjects with verified diagnosis of highly or moderately active SLE according to SLEDAI-2K (table 1). The reason for biologic agents prescription in 44 subjects was lack of efficacy of high doses of GCs, cyclophosphane (CP) and other cytotoxic products, and in 10 subjects with SLE – high disease activity.

Forty subjects received RTM at 500–2000 mg. At repeated visits (after 3, 6, 9 months) 15 subjects underwent a scheduled RTM administration at 500–1000 mg. Seven subjects received BLM at 10 mg/kg body weight monthly. They predominantly had skin, joint and mucosa lesions. Other 7 subjects received combination therapy with RM and BLM. RTM was administered at dose of 500–1000 mg, and three months later BLM was prescribed by standard scheme of 10 mg/kg once monthly for 8 months. No repeated RTM courses were performed during follow-up in this group (table 2).

All subjects received conventional therapy including immunosuppressive products, GCs, and at biologics initiation

Table 1. Characteristics of the subjects included in observation.

Parameter	RTM, n–40	BLM, n–7	RTM+BLM, n–7
Age, years Me [25th; 75th percentiles]	36 [26.5; 45]	34 [30; 34]	21 [20; 29]
Gender, F/M, n	37/3	7/0	6/1
Disease duration, n		-	
- 0-5 years	27	5	4
- > 6 years	13	2	3
SLEDAI-2K, score, Me [25th; 75th percentiles]	16 [11; 20]	8 [8; 12]	10 [9; 16]
SLEDAI-2K, n		-	
- Activity 2 degree	5	4	2
- Activity 3 degree	35	3	5
BILAG total Me [25th; 75th percentiles]	18 [14; 25].	14.5 [9; 17].	17 [10; 18].
Organ and system damage, n (%):		-	
- Lupus nephritis	16 (40)	0	1 (14.2)
- Neurolupus	6	0	0
- Vasculitis	8 (20)	0	1 (14.2)
- Skin damage	14 (35)	5 (71)	3 (42)
- Mucosa membranes	15 (37.5)	3 (42)	4 (57)
- Arthritis	19 (47)	5 (71)	4 (57)
- Serositis	15 (37.5)	1 (14.2)	1 (14.2)
- Hematological abnormalities	18 (45)	5 (71)	5 (71)
SLICC/DI >1, n (%)	17 (42.5)	2 (28.6)	4 (57)
Previous therapy, n (%):		-	
- not performed	5 (12.5)	1 (14.2)	3 (42)
- GC <30 mg/day	9 (22.5)	2 (28.6)	0
- GC ≥30 mg/day	26 (65)	4 (57)	4 (57)
- GC PT	21 (52.5)	4 (57)	4 (57)
- CP PT	15 (37.5)	0	1 (14.2)
- MMF	6 (15)	1 (14.2)	3 (42)
- antimalarial products	24 (60)	3 (42)	3 (42)
- IVIG	4 (10)	1 (14.2)	3 (42)
- GEBP (RTM)	5 (12.5)	1 (14.2)	2 (28.6)

Notes: PT – pulse therapy, IVIG – intravenous immunoglobulin, CP – cyclophosphane, MMF – mycophenolate mofetil, GC glucocorticoids.

Table 2. Repeated RTM doses in group 1 subjects at visits after treatment initiation, n

Visit, after	Repeated RTM infusions	
	500 mg	1000 mg
3 months	2	1
6 months	6	4
9 months	2	4
12 months	1	1

49 (90.7%) subjects received PT with 6-methylprednisolone at doses of 0,25 to 3 g. In subjects receiving RTM TM, oral GC dose varied from 50 to 5 mg/day calculated as prednisolone. Eight of them received ⩾30 mg/day (high doses), and Me was 32.8 [30; 42.5] mg/day. Generally, mean dose of GC dose in the group of subjects receiving RTM was 15 [10; 20] mg/day. The subjects on BLM therapy with no severe organ lesions received medium and low GC doses. The subjects receiving combination therapy with RTM + BLM received medium and low GC doses from 20 to 5 mg/day. Some patients due to vital organ damage received cytostatic products including CP (short course), mycophenolate mofetil (MM) and methotrexate.

At enrollment and every 3 months within one year the subjects underwent a routine examination typical for SLE management: hematology, biochemistry, urinalysis and immunological examination (measurement of Abs to ds-DNA, C3c, C4 components, IgG, IgA, IgM). Chest X-ray, abdominal ultrasound examination, echocardiography (Echo-CG) were performed where applicable. SLEDAI-2K, BILAG, SFI (moderate, severe flare) and SRI values were assessed over time. SLICC damage index (DI) was determined at enrollment and 12 months after biologic initiation.

At baseline, before biologic initiation 23 subjects out of 54 (42.6%) had irreversible organ damage – SLICC DI was >0 (1–5 points).

Statistical processing was performed using Statistica 7.0 (StatSoft, USA) including non-parametric analysis methods. To compare the two groups Mann-Whitney test was used for parameters with non-normal distribution; the results were presented as median [Me] [25–75 percentile]. Descriptive statistics was also applied. Statistical significance was determined as $p<0.05$.

Results:

By month 3 of observation during GEBP therapy all subjects showed reduced disease activity, by month 6 median SLEDAI-2K score was 4 [2; 8] in RTM group, 6 [4; 8] in BLM group and 4 [2; 8] in RTM + BLM group. By month 12 4 [2; 4], 4 [2; 4], 4 [2; 4] scores were obtained, respectively. Similar results were obtained for BILAG score (by month 6 – 1 [0; 9], 8 [1; 9], 2 [0; 9] and 12 – 1 [0; 8], 1 [1; 5], 1 [0; 9]) in RTM, BLM and RTM + BLM, respectively (Fig.1).

Mathematical processing of SLEDAI-2K changes over time in BLM and RTM + BLM groups was not performed due to small number of subjects; however, decreased activity was distinctly observed at all control time points.

Evaluation of changes in immunological blood parameters in subjects receiving RTM and RTM + BLM revealed gradual reduction in titers of Abs to ds-DNA and increased levels of C3c, C4 throughout 1-year follow-up period (changes in Abs to ds-DNAK, C3, C4 – $p<0.05$ for subjects treated with RTM). However, the subjects treated with RTM + BLM showed slower increase in the levels of complement components. At enrollment levels of complement components in these patients were twice as low as that in subjects receiving RTM and was 0.44 [0.37; 0.48] g/L and 0.69 [0.52; 0.69] g/L for C3c, 0.045 [0.03; 0.75] g/L and 0.09 [0.06; 0.14] g/L for C4. Nevertheless, by month 12 all subjects had increased C3c level (to 0.83 [0.71; 0.88] g/L and 0.92

Figure 1. Changes in SLEDAI2K score over time in RTM, BLM and combination therapy groups, (*p<0,00001)

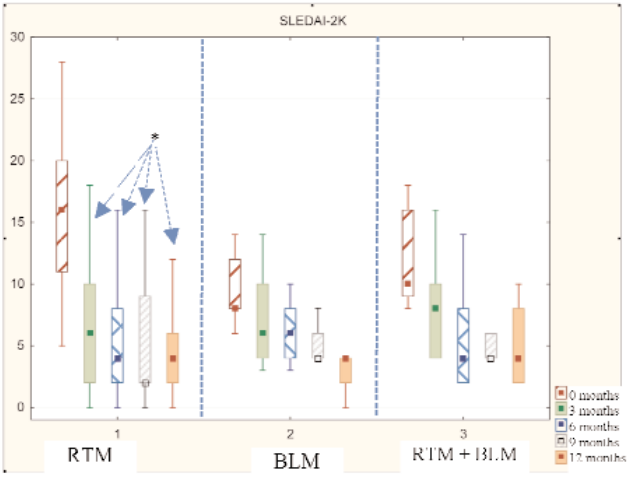
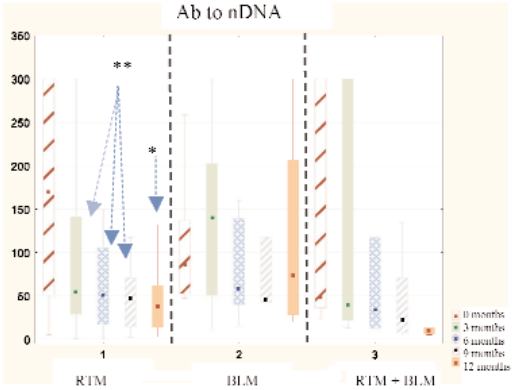
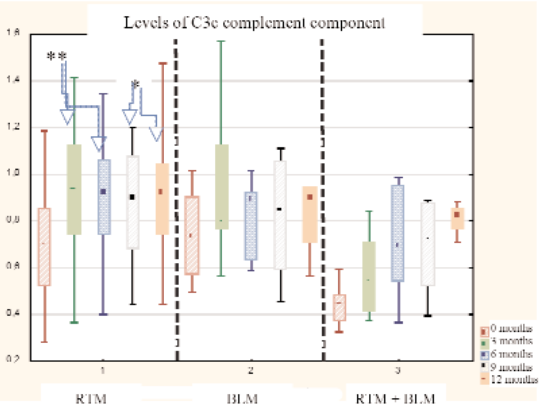
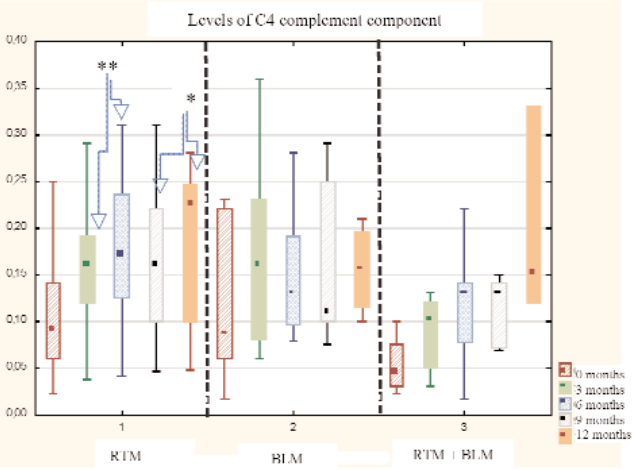


Figure 2. Changes in level of Abs to ds-DNA, C3c, C4 complement components over time during RTM, BLM and combination therapy, (*p<0.02; **p<0.0007)



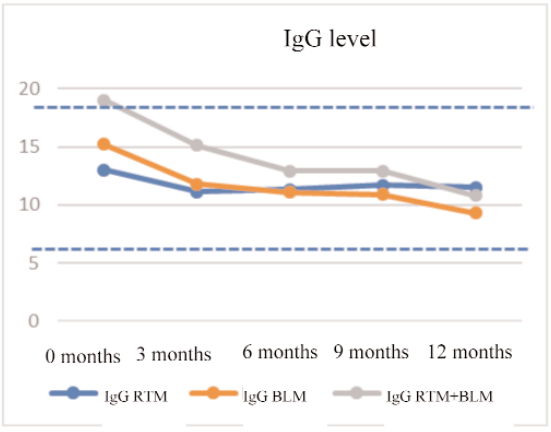


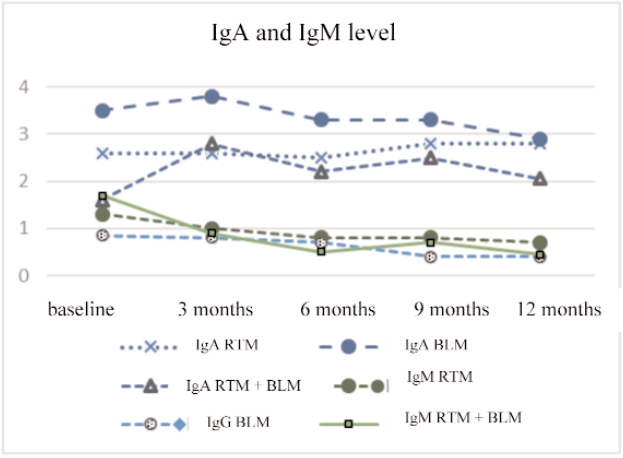


[0.74; 1.06] g/L); C4 (0.15 [0.12; 0.33] g/L and 0.25 [0.1; 0.24] g/L. (fig. 2).

In BLM group reduced level of Ab to ds-DNA was also observed throughout 1-year follow-up period. Changes were flexuous with periods of increased and decreased titers of Abs to ds-DNA and levels of complement components, however, by month 12 distinct positive changes of these parameters were revealed. One female subject in BLM group had retained SLE activity (SLEDAI-2K- 8–6 points) due to skin and mucosa lesions, high immunological activity within 9-month follow-up period. Prior to enrollment she received methotrexate (lack of efficacy, increasing levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), azathioprine (lack of efficacy), MM (lack of efficacy, allergic reaction), CP PT (lack of efficacy, increasing levels of ALT/AST), PT with 6-methylprednisolone, dose of oral GCs was 15 mg daily (calculated as prednisolone) for a long period of time), while attempts to reduce GC doses induced SLE exacerbation. Combination RTM + BLM therapy in this subject improved clinical SLE symptoms (reduced skin eruptions, oral mucosa enanthem), normalized levels of anti-ds-DNA antibodies, increased levels of C3, C4 complement components and reduced dose of oral GCs to 7.5 mg/day by month 12 of the follow-up without new exacerbations.

Figure 3. Changes in IgG, IgA, IgM levels over time during RTM, BLM and combination therapy, Me g/L.





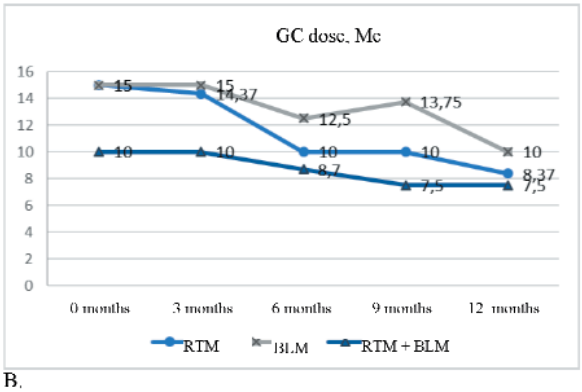
Biologics affected levels of immunoglobulins of various classes In all SLE subjects biologic therapy reduced IgG levels (baseline – 13.15 [9.8; 15.9] g/L, 15.2 [13.2; 19.1] g/L, 19 [16.1; 21] g/L, in 12 months – 11 [8.7; 14.7] g/L ($p<0.02$), 9.3 [9.8; 11] g/L, 10.8 [7.75; 14] g/L, respectively, for RTM, BLM and RTM + BLM), however median of this parameter remained within normal range (fig. 3). Decreased IgM levels was also revealed in all groups (baseline – 1.3 [0.8; 2.3] g/L, 0.85 [0.55; 0.9] g/L, 1.7 [1; 6] g/L, in 12 months 0.7 [0.4; 1.3] g/L ($p<0.03$), 0.4 [0.3; 1.2] g/L, 0.45 [0.3; 0.55] g/L for subjects receiving RTM, BLM and RTM + BLM, respectively) (fig. 3). Despite significantly reduced immunoglobulin levels, incidence of infections was low. Thirteen out of 54 subjects (24%) exhibited signs of infections (predominantly herpes, bronchitis, less frequently urinary tract infection, one subject had pneumonia), 10 of them received RTM, 2 BLM, 1 – combination therapy. Only four of these subjects showed reduced IgG or IgM levels. IgA levels in all groups were normal.

During the first 3 months GC therapy remained almost unchanged in all groups. By month 6 the dose of oral GCs was decreased by almost 25% in subjects from RTM group receiving high and medium GC doses at baseline as well as in BLM and combination groups. By month 12 oral GC doses in all three groups were not exceeding 10 mg. In RTM group the subjects required additional therapy due to SLE exacerbation at months 6, 9 and 12 with repeated courses of RTM in 8 subjects (20%), GC PT in 2 (5%), CP PT in 1 (2.5%), addition of MM in 1 subject, while oral GC dose remained unchanged.

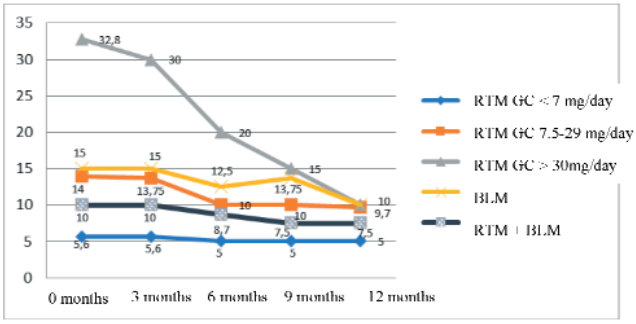
Fig. 4B displays changes in oral GC doses over time during RTM therapy. These subjects were divided into 3 subgroups based on the baseline GC dose (high, medium, low). Changes in GC dose over time in subjects receiving BLM and RTM +BLM are also demonstrated. These findings evidence that combination therapy allowed to maintain low GC dose (fig. 4).

Figure 4. Changes in oral GC dose over time, Me

A.



B.



Notes: 4A. Changes in GC dose over time in RTM, BLM and RTM + BLM groups. 4B. Changes in GC dose over time in subjects receiving RTM, BLM and RTM + BLM therapy. Subjects receiving RTM were divided into 3 subgroups according to the baseline GC dose (high, medium, low). Me values are presented.

Table 3. Development of irreversible organ damage during observation period. Baseline GC dose.

	SLICC (DI)						Baseline oral GC dose, n		
	Baseline		12 months						
	Me	n	*Me*	n					
				total	Newly diagnosed organ damage	DI increase	High doses	Medium doses	Low doses
RTM	*2*	**17**	*2.5*	**18**	**1**	**6**	4	14	-
BLM	*1*	2	*1*	2	-	-	-	2	-
RTM + BLM	*1*	4	*1*	4	-	-	-	3	1

Notes: median DI values were calculated for each group in subjects with irreversible organ damage at baseline and by month 12. The right part of the table shows the dose of oral GCs at enrollment of subjects with DI.

In RTM group increased number of subjects with signs of irreversible changes was recorded by month 12 as well as increased DI value in subjects with pre-existing organ lesions. This was probably associated with therapy using high and medium GC doses (table 3). No SLICC DI increase was reported in BLM and RTM + BLM groups.

Discussion:

At the beginning of XXI century a new trend in SLE therapy was developed, i.e. targeted therapy with biologics [27,28]. The main target of these products in SLE is B-lymphocytes [29]. Several open-label clinical trials demonstrated that anti-B-cellular therapy in SLE patients including subjects with lupus nephritis was safe and effective [30,31]. Despite that fact that RTM has not been approved for SLE therapy, ACR and EULAR /ERA-EDTA, APP and other national rheumatology associations recommend RTM for SLE subjects with lack of efficacy of conventional therapy [30,32,33]. Another biologic is BLM, efficacy of which was confirmed in large-scale clinical studies. It is officially approved for SLE therapy. RTM and BLM have different mechanisms of action, though having a shared target, i.e. B-lymphocytes, and play the key role in the current SLE treatment [34]. Our study provided the data concerning RTM and BLM and their combination administration, described their effects on humoral immunity and the disease progression and specified the proposed future directions of biologic treatment in SLE.

Biologic therapy in SLE subjects was effective reducing both clinical and immunological disease activity after 3 months of treatment and further increasing clinical effect and reducing immunological activity throughout the remaining follow-up period. RTM and BLM vary in terms of the time to achieve clinical effect and impact on immunological parameters. Our findings verify good efficacy of BLM in subjects with joint, skin, mucosa lesions and high immunological activity. BLM therapy gradually reduced levels of Abs to ds-DNA and increased serum complement levels. Such effects were more pronounced by month 12. Meanwhile positive changes in clinical SLE manifestations were reported as soon as by month 3 with further effect increment being in line with our earlier findings [35,36] and results of BLM administration in real clinical practice. Thus, according to Collins CE et al. [22], BLM treatment in 501 SLE subjects ensured slower development of clinical effect after 6-12 months of therapy.

Subjects administering RTM and combination therapy showed higher SLE activity as compared to those receiving BLM monotherapy. RTM rapidly reduced activity, levels of Abs to ds-DNA, increased levels of C3 and C4 complement components. Our data are in line with the results provided by S. Iwata et al. [37] using RTM for treatment of 63 SLE subjects refractory to conventional therapy. The authors reported rapid and long-term (1 year) reduction in activity, (SLEDAI and BILAG $p< 0.0001$), level of Abs to ds-DNA (from 35.7 to 9.4 units/mL), increased complement levels (from 39.2 to 46.5 units/mL) and reduced prednisolone doses ($p< 0.0001$). Multivariant analysis performed by the authors revealed predictors of RTM effect - high level of Abs to ds-DNA at baseline and short SLE duration. Our data concerning combination of RTM+BLM are of special interest. The literature available provided two publications describing the results of RTM + BLM combination therapy. Thus, Kraaij T. et al. [38] specified two clinical cases where the subjects with highly active SLE and prevailing lupus nephritis with proteinuria > 8 g/day received RTM infusion with subsequent administration of BLM. The treatment reduced proteinuria to the level < 1.5 g/day, increased levels of C3, C4 complement components, reduced levels of Abs to ds-DNA and maintained low levels of autoreactive B-cells. Throughout further 12-month of follow-up period minimal activity of the disease was retained (SLEDAI2K 6–4 points). E. Simonetta et al. [39] also reported that consequent administration of RTM and BLM in a female subject with lupus nephritis ensured more effective suppression of SLE activity by inhibiting BLyS using BLM in association with B-cell depletion achieved after RTM administration. A recently published article by Gualtierotti R. et al. [40] also demonstrated an excellent result of combination therapy with RTM+BLM in 3 subjects with SLE. Currently two prospective clinical trials are ongoing (NCT02260934; NCT02284984) aimed to determine efficacy of sequential administration of RTM and BLM in SLE subjects.

Biologic therapy affects serum immunoglobulin levels. The study by L. Watson et al. [20] revealed significant reduction in IgG, IgM levels after one RTM course and significant IgA level decrease after several courses. Long-term BLM therapy was also associated with reduced immunoglobulin levels. The study by Merrill JT et al. [41] revealed larger reduction in IgM levels rather than IgG and IgA levels, and further their median values continuously decreased. The subjects also exhibited

expressed hypogammaglobulinemia, however, conclusive evidence confirming association of such changes with high risk of severe infections was not obtained and the treatment was continued. In our study RTM also affected IgG and IgM levels, however, in most cases they were within reference values. The largest impact on IgM level was observed with BLM. Concentrations of the study parameters reduced to lower limit of normal in BLM and combination groups observed by month 12 of the follow-up. Nevertheless, only 4 subjects with low Ig levels had infections and one of them had pneumonia, while the remaining 9 subjects with signs of infection had normal peripheral blood IgG, IgA, IgM levels.

A key feature of combination therapy was ability of managing the subjects at medium and low GC doses throughout the whole observation period from biologics initiation. Among the subjects receiving such therapy only one case of SLE exacerbation was reported which was associated with BLM infusion delay by 2 weeks. Another positive factor affecting the choice of therapeutic approach was ability to minimize risk of irreversible organ lesions. No DI increase was reported in our study in BLM or combination groups. Bruce IN et al. [42] also reported that BLM therapy was associated with low incidence of irreversible organ lesions and low risk of its increase in subjects with pre-existing DI within long follow-up period.

Therefore, sequential administration of RTM and BLM in patients with high clinical and laboratory disease activity, with vital organ damage allows to achieve both rapid response and maintain the effect with the trend for further decrease in the disease activity. Furthermore, the study results allow to suggest that combination therapy minimizes incidence of exacerbations and risk of irreversible organ damage due to low and medium GC maintenance doses.

Study transparence:

The study was not sponsored. The authors are fully responsible for the final version of the manuscript for printing.

Declaration of financial and other relations:

All authors were involved in development of the article concept and manuscript preparation. The final version was approved by all authors. The authors did not obtain a fee for the article.

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