ORIGINAL INVESTIGATIONS

Late-onset neutropenia induced by anti-B cell therapy with rituximab in patients with ANCA-associated systemic vasculitis

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In the last decade, anti-neutrophil cytoplasmic antibodies (ANCA)-associated systemic vasculitis (SV) has been treated with the anti-B-cell drug, rituximab (RTM) both for induction and maintenance therapy. One of the problems of the treatment with RTM in patients with ANCA-SV is the risk of late-onset neutropenia (LON), mechanisms of development of which have not been studied enough yet.

Objective: *to evaluate the incidence and outcomes of LON in patients with ANCA-SV treated with RTM.*

Patients and methods. *A retrospective analysis of the register of 140 patients with ANCA-SV who received RTM treatment at the V.A. Nasonova Research Institute of Rheumatology from 2009 to 2021 years. The median duration of RTM treatment was 49 (6–121) months, the median of the total RTM dose was 3.5 (0.5–9.5) grams. The duration of follow-up exceeded 6 months after the first administration of RTM.*

Results and discussion. *LON was detected in 16 (11.4%) patients, of which 6 suffered from Wegener's granulomatosis with polyangiitis (GPA), 4 – microscopic polyangiitis (MPA), 4 – Churg-Strauss eosinophilic granulomatosis with polyangiitis (EGPA) and 2 – undifferentiated ANCA-SV. In 8 (50%) out of 16 patients, LON developed within 2 months after the 1st course of RTM, in the remaining 8 patients, on average, after 10 (4–15.5) months. A lethal outcome was documented in 5 (31.2%) of 16 cases of LON (1 with MPA, 3 with GPA, and 1 with EGPA) on average 2 (1.5–9) months after the 1st course of RTM, at the same time, in 4 patients LON was complicated by pneumonia, including 2 with septic shock, in another 1 case LON was combined with the development of acute myocardial infarction and progression of chronic renal failure. Overall mortality among 140 patients with ANCA-SV treated with RTM was 11.4%, while in cases with a fatal outcome, the frequency of LON reached 31.2%.*

Conclusion. *Thus, LON induced by RTM is a common (11%) and clinically significant consequence of B-cell depletion in patients with ANCA-SV, in every 5th case it is complicated by serious infections (including sepsis in 13%) and accounts for a significant proportion in the structure of lethal outcomes (31.2%).*

Patients treated with RTM require careful monitoring of absolute neutrophil count both during the first months after initiation of anti-B-cell therapy and thereafter. In the combined administration of RTM with cytotoxic drugs (primarily cyclophosphamide) in patients with ANCA-SV, it is necessary to consider the risk of LON developing, secondary immunodeficiency, and infectious complications. During the coronavirus pandemic, one should remember that treatment with interleukin 6 inhibitors used in severe COVID-19 can also be accompanied by neutropenia and requires careful dynamic monitoring of the absolute number of neutrophils in patients with ANCA-SV treated with RTM. It is necessary to inform both patients and physicians of the risk of LON development during the treatment of RTM in ANCA-SV and other rheumatic diseases.

Key words: *neutropenia; rituximab; B cells; antineutrophil cytoplasmic antibodies; vasculitis.*

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Systemic vasculitis associated with antineutrophil cytoplasmic antibodies (AAV) is a group of severe multiple organ diseases requiring aggressive long–term immunosuppressive therapy. In the last decade, progress in the treatment of AAV is primarily associated with the introduction of anti-B-cell therapy with rituximab (RTX) [1], which is a chimeric monoclonal antibody specific to the CD20 antigen expressed on B cells and causing rapid sustained depletion of B lymphocytes, usually lasting from 6 up to 12 months [2, 3]. One of the problems of using RTX in patients with AAV is the risk of developing late onset neutropenia (LON) [1, 4].

LON is defined as a decrease in the absolute neutrophil count $<1.5 \cdot 10^9$/l, which develops 4 weeks after the last infusion of RTX. According to foreign literature, RTX-associated LON is characteristic of patients with lymphomas (CD20+ B-cell lymphoma, mantle cell lymphoma, Burkitt's lymphoma, diffuse large B cell lymphoma); its frequency is 8–27% [4]. In patients with rheumatoid arthritis (RA), which, as well as AAV, is a registered indication for the use of RTX, the frequency of LON against the background of RTX treatment ranges from 1.3% to 3% [5, 6], which may explain insufficient attention of practicing rheumatologists to this problem. Thus, in a large Russian cohort study [3], which included 511 patients with various rheumatic diseases who received RTX therapy, LON developed in 3% of patients with systemic lupus erythematosus (SLE) and was not observed in patients with systemic scleroderma, Sjogren's syndrome, IgG4-related disease, cryoglobulinemia. Meanwhile, according to the results of a retrospective analysis of a Swedish cohort of 209 patients with various rheumatic diseases treated with RTX [5], LON was observed in 20% of SLE cases and

23% of AAV cases. In patients with lymphomas, RTX-associated neutropenia is rarely accompanied by infections and, thus, is not a primary clinically significant problem [4]. At the same time, in rheumatic diseases, including AAV and SLE, a severe course of LON can be complicated by sepsis in a large number of cases (55%) [5].

Objectives. To study the frequency and outcomes of LON in patients with AAV receiving RTX treatment.

Patients and methods. A retrospective analysis of the register of 140 patients with AAV who received RTX as induction or maintenance therapy from 2009 to 2021 at V.A. Nasonova Research Institute of Rheumatology and/or regional rheumatology centers. The study was approved by the Ethics Committee of V.A. Nasonova Research Institute. The decision on the administration of RTX was considered by the commission of V.A. Nasonova Research Institute for the assignment of therapy with genetically engineered biological drugs. The duration of follow-up exceeded 6 months after the first administration of RTX. In all patients, excluding 12 cases of eosinophilic granulomatosis with polyangiitis (EGPA), at various periods of the disease, ANCA hyperproduction was detected by indirect immunofluorescence or by enzyme immunoassay. The median age of patients was 52 (20–81) years; 57% were women. Sixty-three patients were diagnosed with granulomatosis with polyangiitis (GPA) corresponding the classification criteria of the ACR (American College of Rheumatology, 1990) [7]. In 45 patients, the disease was classified as microscopic polyangiitis (MPA) in accordance with the definition of the 2012 Chapel Hill Conciliation Conference (Chapel Hill, USA) [8]. EGPA was diagnosed in 24 patients corresponding the classification criteria of the ACR 1990 [9], 12 of them showed hyperproduction of ANCA. In 8 patients, it was not possible to clarify the nosological affiliation of AAV. The median duration of RTX treatment reached 49 (6–121) months, the median of total dose of RTX was 3.5 (0.5–9.5) g. The first course of RTX in most cases amounted to the total dose of 2 g, repeated courses were carried out using a reduced dose of 500 mg.

Results. A decrease in the absolute neutrophil count $<1.5 \cdot 10^9$/l was observed in 16 (11.4%) out of 140 patients, including 6 cases of GPA, 4 cases of MPA, 4 cases of EGPA and 2 cases of undifferentiated AAV. The frequency of LON in GPA, MPA, EGPA and undifferentiated AAV was 9.5%, 8.9%, 16.6% and 25%, respectively. In 8 out of 16 (50%) patients LON developed rapidly (within 2 months after the first course of RTX), in the remaining 8 cases – on average after 10 (4–15,5) months. In 4 cases RTX was prescribed less than 1 month after intravenous (IV) administration of cyclophosphamide (CP).

Three episodes (18.7%) of LON grade I were detected accidentally in asymptomatic patients during routine monitoring of a complete blood count. In 7 (43.7%) cases LON was accompanied by fever; all patients were prescribed intravenous antibiotics and antifungal drugs, 5 patients were also injected with colony-stimulating factors (filgrastim or molgramostin, one dose in most cases). In cases of LON without fever, colony-stimulating factors were not used, the absolute neutrophil count was restored within 3–30 days. In 3 cases of febrile LON serious infections were diagnosed: destructive pneumonia with septic shock in a patient with GPA 1.5 months after the 1st course of RTX; pneumonia with septic shock in a patient with GPA 2 months after the 1st course of RTX; pneumonia in a patient with MPA 6 months after the 1st course RTX.

Subsequently, 6 patients with an episode of neutropenia continued therapy with reduced doses of RTX (200–500 mg) with good clinical effect, while 5 of them had no adverse reactions. In 1 patient with EGPA, febrile LON was observed twice: 11 months after the 4th course of RTX and 4 months after the 7th course (complicated by uterine bleeding), while the absolute neutrophil count was $0.21 \cdot 10^9$/l and $0.06 \cdot 10^9$/l, respectively. Each time treatment with granulocyte colony stimulating factor (filgrastim) contributed to the normalization of the absolute neutrophil count. Currently, maintenance therapy is successfully carried out by interleukin (IL) 5 antagonist mepolizumab.

There were 5 fatal outcomes out of 16 (31.2%) cases of LON (1 – with MPA, 3 – GPA and 1 – EGPA) on average 2 (1.5–9) months after the 1st course of RTX. In 4 of these patients, LON was complicated by pneumonia (in 2 with septic shock). In another comorbid patient with GPA, the development of LON 2 months after the first course of RTX was combined with acute myocardial infarction and progression of chronic renal failure. In 4 fatal cases, the treatment regimen included the administration of RTX less than 1 month after the intravenous administration of CP.

The total mortality in the group of 140 patients with AAV receiving anti-B cell therapy with RTX was 11.4%, while in 5 (31.2%) out of 16 death cases LON developed. We observed the majority of fatal outcomes of LON (3 cases) in 2013–2014, which we associate with the use of aggressive induction therapy regimens during this period (higher doses of glucocorticoids; in some cases administration of CP in total dose more than 2 g immediately before the introduction of RTX). In addition, in the early years of the introduction of RTX, insufficient informing of patients about the risk of LON led to delayed visits to a physician, delayed blood tests and untimely diagnosis of LON. It should be noted that at the initial stage, the original drug Mabthera was used, while after 2014, it was mainly its biosimilar Acellbia.

Disadvantages of the study include the lack of information about the absolute neutrophil count in 4 patients whose cause of death was COVID-19, especially because treatment with IL6 inhibitors used in a severe course of COVID-19 can also cause neutropenia.

Discussion. Thus, in the present study, the frequency of RTX-associated LON in patients with AAV was 11%, which significantly exceeds the literature data on the frequency of LON in RA (1,3–3%) [5, 6] and is comparable with that in lymphomas (8–27%) [5]. It should also be noted that our patients had a lower incidence of LON compared to the Swedish cohort [5]: 11% and 23%, respectively. The data on the prevalence of LON are probably somewhat underestimated, since patients with a short period of asymptomatic neutropenia may not be taken into account. So, in 20% of cases, LON was detected accidentally during routine monitoring.

The relatively low frequency of LON in this study can be attributed to the use of reduced doses of RTX (500 mg) for repeated courses. High cumulative doses of RTX and previous immunosuppression are regarded as predisposing factors for the development of LON [5, 10]. Thus, according to a large American cohort study, which included 739 patients with various rheumatic diseases treated with RTX, the risk of developing LON increased with combined induction therapy with CP and RTX, compared with patients who were not prescribed CP (relative risk 1.99; 95% confidence interval 1.01–3.92) [10]. When using a combination therapy with RTX and azathioprine, it should be borne in mind that deficiency of the enzyme thiopurin-S-methyltransferase due to mutant alleles of its gene, significantly increases the risk of neutropenia with the use of standard doses of azathioprine. So,

according to Hessels et al. [11], who examined 207 patients with AAV and thiopurin-S-methyltransferase gene deficiency/polymorphism receiving azathioprine maintenance therapy after CP, the frequency of neutropenia was 31%, which, according to the authors, was primarily determined by the combined effect of azathioprine and CP on hematopoiesis. Thus, in genetically predisposed patients, the combination of azathioprine and RTX can theoretically affect the safety of the therapy. Simultaneous use of RTX and methotrexate did not increase the frequency of LON in rheumatic diseases, unlike lymphomas [5].

According to our own data, in all patients with fatal LON, the induction treatment regimen included a combination of CP and RTX; in addition, in more than a half of cases (in 4 out of 7) of rapid development of LON (within 2 months after the use of RTX), RTX was prescribed shortly after CP. The European recommendations for the diagnosis and treatment of AAV published in 2016 emphasize that a routine combination of CP and RTX should be avoided; however, in a severe, life-threatening course of the disease, combined use of RTX and CP in a standard dose for one or several months is possible in order to accelerate the clinical effect [1]. Recommendations for the treatment of AAV recently published by ACR and the Vasculitis Foundation state that in life-threatening AAV refractory to CP or RTX, or with damage to vital organs, physicians should switch to another treatment, rather than combine CP and RTX [12].

Since the arsenal of drugs for effective control of AAV is limited, the question of the possibility of continuing RTX therapy after LON, which is often used in patients with lymphomas, is relevant. As our experience shows, in patients with AAV who have had LON, treatment with RTX can be safely continued with reduced doses and regular monitoring of the patient's condition and the absolute neutrophil count. At the same time, a relapse of LON was noted in 1 out of 6 cases (the patient required the introduction of filgrastim). According to an American study, the incidence of LON was 9.6% (3.2 cases per 100 patient-years), the relapse rate in patients who developed LON was 22% (8.3 cases per 100 patient–years) [10].

Undoubtedly, the risk of developing severe infections, including sepsis, should be taken into account when prescribing RTX after LON [5]. Thus, according to our data, septic shock developed in 2 out of 4 fatal cases of LON. Severe infectious complications are associated with the parallel development of a secondary immunodeficiency condition, while hypogammaglobulinemia may be caused by the administration of both RTX and CP and depends on the cumulative dose of the drugs. During the pandemic of coronavirus infection, it should be taken into account that treatment with IL6 inhibitors used in severe COVID-19 cases may also be accompanied by neutropenia. Thus, according to Roumier et al. [13], in patients with COVID-19 treated with tocilizumab, the frequency of neutropenia reached 35%.

The mechanism and risk factors of the development of RTX-induced LON have not been sufficiently studied. It is assumed that the direct toxic effect of RTX is unlikely, since granulocytes and hematopoietic progenitor stem cells do not express CD20 antigen. Possible mechanisms include features of B cell depletion, delayed maturation of promyelocytes, production of B lymphocyte activation factor (BAFF), ANCA. It is also possible that the formation of autoantibodies binding to the surface of neutrophils or their hematopoietic precursors may lead to neutropenia [14]. So, Voog et al. [15] revealed ANCA in the blood serum of 2 out of 8 patients with LON. However, no ANCA was found in other studies [5, 16–20].

Patients with LON were found to have a longer and deeper depletion of B lymphocytes and lower serum IgM levels than control subjects [5], which is considered a risk factor for relapse of LON. According to our data, patients with AAV who developed LON also had a long (more than 1 year) period of complete depletion of CD19+ B cells in the circulation.

Treatment of RTX can disrupt the balance between granulo- and lymphopoiesis in the bone marrow [5, 17, 19]. In the study of Terrier et al. [17] it was demonstrated that LON can develop as a result of competition of hematopoietic clones. It was noted that RTX can induce proliferation of large granular lymphocytes with the secretion of a large number of Fas and Fas ligand, which leads to apoptosis of mature neutrophils [18]. The development of LON in the early period of B cell repopulation is also associated with the activity of the chemokine stromal cell-derived factor-1 (SDF1), which enhances B cell proliferation and slows down the exit of neutrophils from the bone marrow [19].

In the study of bone marrow in patients with RTX-associated LON, granulocyte maturation stops at the promyelocytic stage [20, 21], which is characteristic of severe hereditary neutropenia of autosomal dominant and recessive inheritance type [22] associated with mutations in HAX1 or ELA2 genes [23]. This allows us to discuss the similarity of RTX-induced LON and severe congenital neutropenia with key mechanisms of delayed maturation of hematopoietic clones. Undoubtedly, further studies of the mechanisms underlying the selective suppression of myelopoiesis in rheumatological patients with LON are needed.

Conclusion. Thus, LON, induced by RTX, is a common (11–23%) and clinically significant consequence of B cell depletion in patients with AAV which is often complicated by serious infections (including sepsis in 13–55%) and may account for a significant share in the structure of deaths (31%). Patients receiving RTX treatment require careful monitoring of the absolute neutrophil count both in the first months after the start of anti-B-cell therapy and later on. When administering RTX in combination with cytostatics (mainly with CP) for patients with AAV, it is necessary to take into account the risk of LON, secondary immunodeficiency, and infectious complications. During the pandemic of coronavirus infection, the risk of neutropenia should be considered when treating severe COVID-19 cases with IL6 inhibitors, which requires careful dynamic control of the absolute neutrophil count in AAV patients receiving RTX. It is necessary to inform both patients and physicians about the risk of developing LON against the background of RTX treatment in AAV and other rheumatic diseases.

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Conflict of Interest Statement

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