ORIGINAL INVESTIGATIONS

Characteristics of clinical, laboratory, and immunological manifestations in patients with anticentromere antibody-associated Sjogren's disease

Chaltsev B.D.[1], Vasilyev V.I.[2], Palshina S.G.[1], Torgashina A.V.[1], Sokol E.V.[1], Khvan Yu.I.[1], Rodionova E.B.[3], Safonova T.N.[4], Lapin S.V.[5], Tkachenko O.Yu.[5], Mazing A.V.[5]

[1]V.A. Nasonova Research Institute of Rheumatology, Moscow; [2]MEDSI Clinic, Moscow; [3]OOO «Treatment Center». Moscow; [4]Research Institute of Eye Diseases, Moscow; [5]Acad. I.P. Pavlov First Saint Petersburg State Medical University, Ministry of Health of Russia, Saint Petersburg

[1]34A, Kashirskoe Shosse, Moscow 115522, Russia; [2]3A, Gruzinsky Lane, Moscow 123056, Russia; [3]15/1, Timur Frunze St., Moscow 119021, Russia; [4]11A, Rossolimo St., Moscow 119021; Russia; [5]6-8, Lev Tolstoy St., Saint Petersburg 197022, Russia

Objective: *to study clinical and laboratory features in patients with anticentromere antibody (ACA)-positive Sjögren's disease (SD), as well as the sensitivity of different methods for determination of ACA, and to elaborate an algorithm for differential diagnosis in ACA-positive patients.*

Patients and methods*. The V.A. Nasonova Research Institute of Rheumatology followed up 136 patients who were highly positive for ACA. The investigators used the 2001 Russian criteria for the diagnosis for SD; the 2013 ACR/European League Against Rheumatism (EULAR) criteria for that of scleroderma systematica (SDS); the guidelines of the American Association for the Study of Liver Diseases, the Russian Gastroenterological Association, and the Russian Society for the Study of the Liver for that of primary biliary cholangitis (PBC)/biliary duct epitheliitis in the presence of SD. Lymphomas were diagnosed by biopsies of affected organs according to the WHO classification. SD was diagnosed in 119 patients; SDS in 49 cases (37 with SDS concurrent with SD and 12 with isolated SDS), PBC/biliary duct epitheliitis in 23 (all cases with PBC/biliary duct epitheliitis concurrent with SD and/or SDS); 5 patients were excluded from the investigation. Further analysis included 131 ACA-positive patients. The patients were divided into three groups: SD (n=82 or 62.6%); SD+SDS (n=37 or 28.24%); SDS (n=12 or 9.16%).*

Results and discussion. *Autoantibodies to centromere peptide (CENP) A and CENP-B in the same titers were detected in all ACA-positive patients, regardless of diagnosis. Comparative analysis of three patient groups revealed no statistically significant differences in the frequency of laboratory deviations. The signs characteristic of classical SD (rheumatoid factor (RF)), anti-Ro and anti-La antibodies, leukopenia, higher ESR values, hypergammaglobulinemia, and elevated IgG/IgA levels) were found in a small proportion of patients. The frequency and severity of glandular manifestations did not differ in SD and SD + SDS. PBC/biliary duct epitheliitis was present in 17.5% of ACA-positive patients (in most antimitochondrial antibody-positive cases); no statistically significant differences in its frequency were found between the groups. Other extraglandular manifestations in SD and SD + SDS were identified in a smaller number of patients. All sclerodermic spectrum manifestations were more common in SD and SD + SDS than in BS. Pulmonary arterial hypertension was not diagnosed in any patient from the SD group. MALT lymphomas were detected in 19 ACA-positive patients. Those were present only in BS patients and absent in the SDS group. MALT lymphomas developed in the first 10 years after the onset of SD. The transformation of MALT lymphoma into diffuse large B-cell lymphoma was observed in 2 patients. The main signs of lymphomas in SD patients were persistent parotid salivary gland enlargement, decreased levels of complement C4 and peripheral blood CD19+ cells, as well as cryoglobulinemic vasculitis, serum monoclonal secretion, lymphoid infiltration in the minor salivary glands (a focus score of >4), and severe damage to the salivary and lacrimal glands.*

Conclusion. *ACA-associated SD is an independent disease subtype characterized by an increased risk for SDS, PBC, and MALT lymphomas and by a low frequency of the systemic manifestations and laboratory signs characteristic of classical SD. Regardless of the detected type of antibodies and the presence or absence of extraglandular manifestations, damage to the salivary and lacrimal glands progresses in SD, which often leads to lymphomas; therefore, the therapy that may prevent this complication should be initiated as soon as possible after SD diagnosis. The lymphoproliferation signs identified in this investigation should be taken into account in all ACA-positive patients with SD for the early diagnosis of lymphoid tumors before therapy is prescribed. An algorithm for differential diagnosis in seropositivity for ACA is presented. Determination of autoantibodies to CENP-A and CENP-B does not allow the differential diagnosis in ACA-positive patients.*

Keywords: *anticentromere antibodies; Sjögren's disease; scleroderma systematica; primary biliary cholangitis; lymphoproliferative diseases; MALT lymphoma.*

Contact: *Bogdan Dmitrievich Chaltsev;* ***bodya92@inbox.ru***

For reference: *Chaltsev BD, Vasilyev VI, Palshina SG, et al. Characteristics of clinical, laboratory, and immunological manifestations in patients with anticentromere antibody-associated Sjogren's disease. Sovremennaya Revmatologiya=Modern Rheumatology Journal. 2020;14(4):50–59.* ***DOI****: 10.14412/1996-7012-2020-4-50-59*

Sjogren's disease (pSS) ranks second after rheumatoid arthritis (RA) among systemic rheumatic diseases (RDs) in terms of prevalence in the population [1–3]. Also, among all RDs, pSS is most often a component of polyautoimmune conditions [4], combined with RA, systemic lupus erythematosus (SLE), systemic sclerosis (SSc). At the same time, glucocorticoids, methotrexate, aminoquinolines, azathioprine, leflunomide, sulfasalazine, most widely used in the treatment of RA, SLE, SSc, do not stop the progression of pSS and do not reduce the incidence of lymphoproliferative disorders (LPDs) in pSS patients. [5–7]. Therefore, the detection of pSS in patients with other RDs should certainly be reflected in the choice of optimal therapeutic tactics. Considering the above, optimal diagnosis and treatment of pSS are extremely important in the practice of a rheumatologist. The immunological hallmark of the pSS is anti-Ro/La antibodies, which are detected in 60–80% of cases [8]; however, subtypes of the syndrome have been described in which these autoantibodies may be absent [9–11]. One of these subtypes is pSS associated with anticentromere antibodies (ACA). Despite the fact that this subtype of the disease was first described in the 20th century [12] and occurs in 3–27% of cases of pSS [10], at present, ACA-positive patients are often not screened to exclude pSS due to the fact that the presence of ACA is regarded as a highly specific marker of the limited form of SSc [13, 14]. Seropositivity for ACA is determined by conducting an indirect immunofluorescence reaction (IIF) on Hep-2 cells, in case of ACA-positivity antinuclear factor (ANF) has a centromeric type of fluorescence [15]. To further determine a specific autoantigen for ACA, an enzyme-linked immunosorbent assay (ELISA) or immunoblotting is performed. Eight centromeric ribonucleoproteins with different molecular weights (A, B, C, D, E, F, H, O) [4] and heterochromatin protein 1 (HP1a) can be autoantigens for ACA [16, 17]. The main autoantigen that reacts with almost all ACA-positive sera is peptide B (CENP-B) [15]. Autoantibodies to CENP-A, -B, -C, -D, -E and -O were detected in SSc, to CENP-B, -C, -H and -HP1a – in pSS, to CENP-F – in tumors [10, 16]. In most cases, in patients with ACA-positive SSc, autoantibodies to both CENP-A and CENP-B are simultaneously detected due to the cross-reactivity of the epitopes of these proteins [18]. When comparing the epitope specificity of ACA in patients with pSS and SSc, Gelber et al. showed that sera from patients with pSS and ACA are positive for autoantibodies to CENP-C, while sera from patients with SSc and ACA are positive for autoantibodies to both CENP-B and CENP-C [19]. Tanaka et al. [16] also compared ACA-positive sera from patients with pSS and SSc. In this study, a high frequency of autoantibodies to CENP-B was found in both pSS and SSc. However, autoantibodies to CENP-C and HP1a were found significantly more often in patients with pSS than in SSc, on the basis of which the authors concluded that pSS associated with ACA is a subtype of pSS that does not depend on SSc. However, Baer et al. [10], as well as Gelber et al. [19] note that IIF is highly sensitive to antibodies to CENP-B, but may not detect autoantibodies to other centromere proteins (eg, CENP-C).

The aim of this study was to analyze the clinical, laboratory and immunological characteristics of patients with pSS and ACA, to compare clinical and laboratory manifestations in 3 groups of ACA-positive patients: with isolated pSS, with combination of pSS and SSc, with isolated SSc; to compare clinical and laboratory manifestations in patients with ACA-associated pSS with and without lymphomas, thereby identifying the characteristic signs of lymphomas in this group of patients; to assess the sensitivity of IIF, ELISA and immunoblotting in the determination of ACA, as well as the usefulness of the determination of autoantibodies to CENP-A and CENP-B for differential diagnosis in the group of ACA-positive patients, to develop an algorithm for the differential diagnosis of ACA-positive patients.

Materials and methods

A long-term prospective study included 136 ACA-positive patients (130 women, 6 men), who were followed up at the V.A. Nasonova Research Institute of Rheumatology from 1998 to 2019 every 6–12 months. The inclusion criterion was the presence of a high titer of ACA (more than 3 norms). ACA seropositivity was assessed by IIF on Hep-2 cells, as well as by ELISA (antibodies to CENP-B). Twenty eight of 136 patients underwent immunoblotting with the determination of antibodies to CENP-A and CENP-B.

All patients were examined by a rheumatologist and underwent immunological (antinuclear autoantibodies, rheumatoid factor (RF), C-reactive protein (CRP), concentration of immunoglobulins and C3 / C4 complement components), stomatological (ultrasound examination of salivary glands, stimulated whole saliva flow, sialography, biopsy of the labial salivary glands, biopsy of the parotid salivary glands or submandibular salivary glands in the presence of their significant enlargement) and ophthalmological (stimulated SchirmerXs test, tear break-up time, ocular staining score, ultrasound of the orbits in case of enlarged lacrimal glands) to diagnose glandular manifestations of pSS, as well as X-ray, functional and laboratory examination to diagnose extraglandular manifestations of pSS and concomitant RDs. The diagnosis of pSS was established on the basis of Russian criteria of 2001 [20]. To assess the compliance of patients with international classification criteria, we used the criteria of the pSS ACR 2012 [21] and ACR / EULAR 2016 [22], the criteria of the SSc ACR / EULAR 2013 [23]. Diagnosis of primary biliary cholangitis (PBC) / epitheliitis of the biliary ducts as a manifestation of pSS was carried out based on the assessment of the course of the disease, liver function, the results of antimitochondrial antibody (AMA) detection, as well as morphological examination of liver biopsies, according to the recommendations of the American Association for the Study of Liver Diseases [24], the Russian Gastroenterological Association and the Russian Scientific Liver Society [25]. The diagnosis of MALT lymphoma was established on the basis of histological and immunohistochemical studies, as well as polymerase chain reaction with the determination of the B-cell clonality of biopsies of affected organs, according to the criteria for the diagnosis of hematopoietic tumors of the World Health Organization [26]. During long-term follow-up of 136 ACA-positive patients, pSS was diagnosed in 119 patients, SSc - in 49 (in 37 cases in combination with pSS, in 12 cases isolated SSc), PBC/ epitheliitis of the biliary ducts as a manifestation of pSS – in 23 (in all cases in combination with pSS and/or SSc). One patient with IgG4-related systemic disease with lesions localized in the paranasal sinuses, bladder, urethra, subcutaneous fat, and 4 patients in whom no disease could be diagnosed were excluded from further analysis (Fig. 1).

Thus, 131 ACA-positive patients were included in the further study, the patients were divided into 3 groups: 1) pSS, 2) combination of pSS and SSc, 3) SSc. The group of isolated pSS was the most numerous and included 82 patients (62.6%), the group of patients with a combination of pSS and SSc included 37 patients

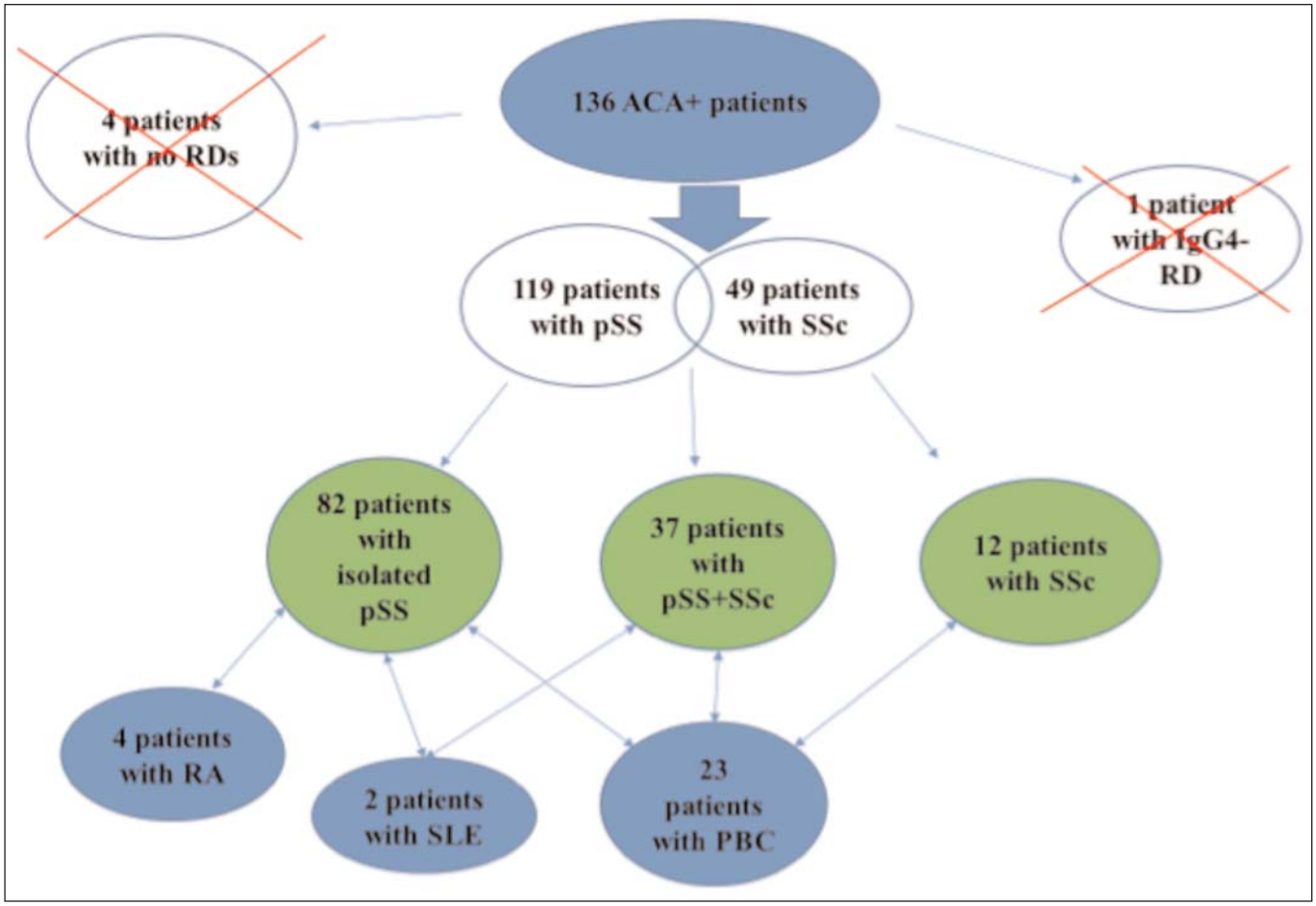

Figure 1. *Diagnoses of ACA-positive patients in this study.*
ACA – anticentromere antibodies, pSS – primary Sjogren's syndrome, SSc – systemic sclerosis, RDs – rheumatic diseases, IgG4-RD – IgG4-related disease, RA – rheumatoid arthritis, PBC – primary biliary cholangitis, SLE – systemic lupus erythematosus.

(28.24%), and the group of isolated SSc – 12 (9.16%). A comparison of clinical, laboratory and immunological manifestations in the above 3 groups was made, the frequency of lymphomas was assessed, and a comparison of clinical and laboratory manifestations in patients with and without lymphomas was made.

Quantitative variables were described by the following parameters: number of patients, arithmetic mean (M), standard deviation from the arithmetic mean (δ), 25th and 75th percentiles, median. Qualitative variables were described as absolute and relative frequencies (percentages). Differences were considered statistically significant at $p < 0.05$.

For quantitative variables, a normal distribution test was performed. To evaluate the results obtained, we used: Person's χ^2-test (analysis of contingency tables), unpaired Student's t-test. If the samples from the variables did not correspond to the normal distribution law, nonparametric tests were used: Mann-Whitney U-test, Kruskal-Wallis test. Spearman's correlation analysis was used to determine the mutual influence of the indicators. The calculations were performed on a personal computer using Microsoft Excel and Statistica 10 for Windows (StatSoft Inc., USA).

Results

To assess the significance of the determination of antibodies to centromere proteins A and B in the differential diagnosis of ACA-positive patients, 28 of them underwent immunoblotting with the determination of autoantibodies to CENP-A and CENP-B. Twenty six of these 28 patients had pSS, SSc was diagnosed in 7 patients, signs of PBC / epitheliitis of the biliary ducts as a manifestation of pSS were also detected in 7 patients, in 2 patients no signs of RDs were detected, in 1 of whom, with dynamic observation, Raynaud's phenomenon subsequently developed. All patients according to IIF on Hep-2 cells had highly positive ANF values (from 1: 2560 to 1: 10240), in all cases ANF had centromere fluorescence, in 6 cases there was also mitochondrial fluorescence, of which AMA positivity upon ELISA was identified only in 3 cases. All patients were seropositive for antibodies to CENP-B by ELISA. When conducting immunoblotting with the determination of antibodies to CENP-A and CENP-B, all 28 patients, regardless of the diagnosis, were highly positive for both.

In all three study groups (pSS, pSS + SSc, SSc), women predominated, the age of patients with pSS and pSS + SSc was slightly higher than in patients with SSc (49.7 + 11.7, 47.5 + 10.9 and 39.2 + 9.9 years respectively). The median duration of pSS before detection of LPDs in the first two groups did not differ and amounted to 8.5 years.

All patients were seropositive for ANF (Tab. 1). Leukopenia, RF, anti-Ro antibodies, anti-La antibodies, increased ESR,

Table 1. Frequency of laboratory signs.

Sign	pSS (n=82)	pSS+SSc (n=37)	SSc (n=12)
ANF hep-2 (≥ 1/320)	82 (100%)	37 (100%)	12 (100%)
AMA	16 (27%)	9 (36%)	2 (50%)
RF	18 (21.95%)	11 (29.73%)	1 (8.33%)
Anti-Ro	29 (35.37%)	8 (21.62%)	0 *
Anti-La	7 (8.54%)	3 (8.11%)	0
Anti-RNP70	0	3 (16.67%)	1 (11%)
Anti-Scl70	0	0	0
Low C3	7 (8.54%)	4 (11.43%)	0
Low C4	10 (12.2%)	9 (25.71%)	1 (12.5%)
Hypergammaglobulinemia	14 (17%)	2 (5.41%)	1 (8.3%)
Increased IgG	10 (12.2%)	1 (3%)	0
Increased IgM	20 (24.39%)	8 (32%)	2 (40%)
Increased IgA	14 (17%)	4 (12.5%)	1 (20%)
Increased CRP	6 (7.32%)	6 (16.22%)	0
Cryoglobulinemia	5 (12.5%)	2 (10.53%)	0
Monoclonal secretion	4 (12.5%)	2 (20%)	-
Anemia	7 (8.5%)	5 (13.5%)	2 (16.7%)
Leukopenia	8 (9.7%)	2 (5.4%)	0
Thrombocytopenia	6 (7.3%)	1 (2.7%)	1 (8.33%)
Increased ESR	12 (14.6%)	6 (16.22%)	4 (33%)

ANF – antinuclear factor; RF – rheumatoid factor; AMA – antimitochondrial antibodies, CRP – C-reactive protein, ESR – erythrocyte sedimentation rate;
* - $p<0,05$ compared to pSS.

hypergammaglobulinemia, and increased IgG / IgA levels characteristic of "classical" pSS were found in a minority of patients. Comparative analysis of laboratory signs revealed no significant differences in the three groups, with the exception of the absence of anti-Ro and anti-La antibodies in the SSc + ACA group.

When analyzing the glandular manifestations of pSS, there were no significant differences between pSS and pSS + SSc. The severity of labial salivary gland lymphohistiocytic infiltration in these patients did not differ either. 76.5% and 79.4% of patients from the pSS group, respectively, and 61.1% of patients from the pSS + SSc group met the international classification criteria for the pSS ACR 2012 and ACR / EULAR 2016. Severe dysfunction of the salivary / lacrimal glands and significant changes in sialography and biopsy of the labial salivary glands in pSS and pSS + SSc were observed with the same frequency. Biopsy of significantly enlarged salivary glands made it possible to diagnose LPDs in all 24 cases: MALT tissue in 5 and development of MALT lymphomas in 19 patients. There were no significant differences in the frequency of detection of LPDs in pSS and pSS + SSc. Severe lymphoid infiltration (>4 foci) was most common in patients with LPDs. In pSS + ACA, all glandular manifestations were detected significantly more often than in SSc. Although 41.7% of patients with SSc complained of dry mouth, and 25% noted dry eyes, objective signs of damage to the salivary and lacrimal glands were not detected in any of them. Labial biopsy was not performed in patients from the SSc group, therefore, the assessment of their compliance with the international classification criteria of the pSS, for the use of which this procedure is necessary, was not carried out. MALT lymphoma of the salivary glands, which was diagnosed in the pSS + ACA group in 17% of patients, was not found in patients from SSc group.

In all groups PBC was detected with the same frequency, some cases of PBC were interpreted by us as epitheliitis of the biliary ducts as a manifestation of pSS. Other systemic manifestations often detected in "classical" pSS, such as damage to the peripheral nervous system, hypergammaglobulinemic purpura, kidney damage, were diagnosed in isolated cases (Tab. 2). Interstitial lung disease (ILD), as well as arthritis and arthralgias, were significantly more frequently diagnosed in patients with pSS + SSc. In patients with SSc, ILD, pericarditis, and arthritis were found significantly more often than in pSS; there were no significant differences in the frequency of PBC and other extraglandular manifestations.

Signs, conditionally attributed by us to changes in the "sclerodermic spectrum", were significantly more frequent in pSS + SSc and SSc than in pSS. Puffy fingers, sclerodactyly, proximal scleroderma, digital ulcers / scars were not detected in any patient from the pSS group. Raynaud's phenomenon was detected in only a third of them, in 77% of cases, it was accompanied by capillaroscopic changes of the scleroderma type, which were also detected in 3 patients with pSS without Raynaud's phenomenon. All patients with pSS had a mild course of Raynaud's phenomenon without digital ulcers or scars. ILD was detected in a minority of patients, while in the pSS + SSc and SSc groups it was significantly more frequent than in the pSS group (16.2%, 18.8% and 2.8%, respectively). Pulmonary arterial hypertension (PAH) was not diagnosed in any patients of the pSS group, while in the pSS + SSc group PAH was detected in 18.9% of cases, in the SSc group – in 8.3%. Thus, none of the patients from the pSS group met the SSc classification criteria ACR / EULAR 2013.

Table 2. Frequency of extraglandular manifestations.

Sign	pSS (n=82)	pSS+SSc (n=37)	SSc (n=12)
PBC/ epitheliitis of the biliary ducts as a manifestation of pSS	13 (15.9%)	8 (21.6%)	2 (16.6%)
AIH	3 (3.6%)	1 (2.7%)	0
Neuropathy	1 (1.2%)	2 (5.4%)	0
Lymphadenopathy	3 (3.6%)	1 (2.7%)	0
Kidney damage	2 (2.4%)	1 (2.7%)	0
ILD	2 (2.78%)	6 (16.2%) *	2 (18%) *
Pleuritis	1 (1.2%)	1 (2.7%)	0
Pericarditis	2 (2.4%)	4 (10.8%)	4 (33%) *
Cryoglobulinemic vasculitis	5 (6.1%)	1 (2.7%)	0
Hypergammaglobulinemic purpura	1 (1.2%)	0	0
Arthritis	5 (6.1%)	8 (21.6%) *	4 (33%) *
Arthralgias	23 (28%)	23 (62.1%) *	6 (50%)
RA	4 (4.9%)	0 *	0
AIT	7 (30.4%)	2 (40%)	-

PBC – primary biliary cholangitis, AIH – autoimmune hepatitis, ILD – interstitial lung disease, RA – rheumatoid arthritis, AIT – autoimmune thyroiditis; * - $p<0.05$ compared to pSS.

MALT lymphomas were diagnosed in 19 ACA-positive patients: in 14 (17%) from the pSS group and in 5 (13.5%) from the pSS + SSc group, there were no lymphomas in the SSc group. Transformation of MALT lymphoma into diffuse large B-cell lymphoma (DLBCL) was detected in 2 patients (1 with pSS and 1 with pSS + SSc). Comparative characteristics of patients with lymphomas and without them are given in Table 3. In patients with lymphomas, persistent enlargement of the parotid salivary glands, decreased C4 level, decreased CD19 + cells in the peripheral blood, cryoglobulinemic vasculitis, lymphohistiocytic infiltration of the labial salivary glands (>4 foci), as well as severe xerostomia and hypolacrimia were significantly more often detected in comparison with patients without lymphoma. RF and anti-Ro antibodies were present in only a quarter of patients with lymphomas and its frequency did not differ from that in the group of patients without lymphomas. The frequency of detecting increased levels of immunoglobulins in the study groups did not differ either. Only 20% of lymphomas were secreting, and the frequency of detecting monoclonal secretion in the groups with and without lymphomas did not differ. Anemia, leukopenia, thrombocytopenia, increased ESR, hypergammaglobulinemia occurred in the study groups with the same frequency. In patients with lymphomas, recurrent parotitis and lymphadenopathy were slightly more frequent, but these differences did not reach statistical significance. No differences in the frequency of such extraglandular manifestations as Raynaud's phenomenon, arthritis and arthralgia, pleuritis / pericarditis, neuropathy, kidney damage, hypergammaglobulinemic purpura were found.

Discussion

The low detection rate of immunological and laboratory parameters characteristic of the "classical" variant of pSS in pSS associated with ACA leads to underdiagnosis of this subtype of the disease. According to our study, only 79.4% and 61.1% of patients, respectively, in ACA-positive groups pSS and pSS + SSc, met the classification criteria of pSS ACR / EULAR 2016, while 100% of patients met the Russian classification criteria [20]. Also, in the present study, when analyzing the immunological parameters, no significant differences allowing for differential diagnosis were found between the groups, which indicates the need of examining all ACA-positive patients to exclude pSS. For this, it is advisable not only to determine standard immunological parameters, but also to conduct a complete list of stomatological and ophthalmological tests used to diagnose pSS.

According to research data, the risk of developing a limited form of SSc in the group of patients with ACA-positive pSS is 25% [9, 16]. According to our study, out of 119 patients with pSS and ACA, 37 were diagnosed with limited SSc, and more than a third of patients from the group of isolated pSS (36.7%) had Raynaud's phenomenon with capillaroscopic changes of the scleroderma type, which means an increased risk of developing limited SSc in the future [27]. In our study, not a single case of PAH was detected in the group of patients with isolated pSS, while in the pSS + SSc group, PAH was detected in 18.9% of cases. ILD in our study in the pSS + SSc and SSc groups occurred significantly more often than in the pSS group (in 16.2%, 18.8% and 2.8% of cases, respectively). Thus, patients with ACA-positive pSS require long-term follow-up and alertness regarding the development of SSc signs, including PAH and ILD.

Also, in the present study, in 26% of patients with ACA during long-term follow-up, cholestatic liver diseases were diagnosed, among which there were both PBC, in some cases accompanied by progression to cirrhosis of the liver, and mild cases of epitheliitis of the biliary ducts as a manifestation of pSS. Other extraglandular manifestations characteristic of "classical" pSS (kidney and lung damage, neuropathy, hypergammaglobulinemic purpura) were detected in a minority of cases. Thus, the main systemic manifestations in the group of patients with pSS and ACA were signs of a limited form of SSc (most often Raynaud's phenomenon with capillaroscopic changes of the scleroderma type)

Table 3. Comparative characteristics of clinical and laboratory manifestations in pSS patients with and without lymphomas.

Sign	pSS+lymphoma (n=19)	pSS without lymphoma (n=100)
RF	5 (26.3%)	24 (24%)
Anti-Ro	5 (26.3%)	32 (32%)
Anti-La	0	10 (10%)
Low C3	3 (15.8%)	8 (8.16%)
Low C4	8 (42.1%)	11 (11.2%) *
Increased IgG	1 (5.3%)	10 (10.4%)
Increased IgM	5 (26.3%)	23 (24.2%)
Increased IgA	4 (21%)	14 (14.7%)
Increased CRP	3 (15.8%)	9 (9%)
Cryoglobulinemia	3 (18.75%)	4 (9.3%)
Monoclonal secretion	3 (20%)	3 (11%)
Anemia	0	12 (12%)
Leukopenia	2 (10,5%)	8 (8%)
Thrombocytopenia	1 (5.3%)	6 (6%)
Increased ESR	1 (5.3%)	17 (17%)
Hypergammaglobulinemia	2 (10.5%)	14 (14%)
Decreased CD19 + cells in the peripheral blood	10 (100%)	0 *
Lymphohistiocytic infiltration of the labial salivary glands > 4 foci	5 (71.4%)	15 (33%) *
Neuropathy	1 (5.3%)	2 (2%)
Raynaud's phenomenon	8 (42.1%)	59 (59%)
Recurrent parotitis	6 (31.6%)	15 (15%)
Hypolacrimia 3 st.	14 (73.7%)	45 (45%) *
Xerostomia 3 st.	15 (83.3%)	39 (41%) *
Lymphadenopathy	2 (10.5%)	2 (2%)
Kidney damage	0	3 (3%)
Pleuritis	0	2 (2%)
Pericarditis	1 (5.3%)	5 (5%)
Cryoglobulinemic vasculitis	3 (15.8%)	3 (3%) *
Hypergammaglobulinemic purpura	0	1 (1%)
Arthritis	2 (10,5%)	11 (11%)
Arthralgias	9 (47.4%)	37 (37%)
Persistent enlargement of the parotid salivary glands	17 (89.5%)	17 (17%) *

RF – rheumatoid factor, CRP – C-reactive protein, ESR – erythrocyte sedimentation rate; * – $p<0.05$.

and PBC / epithelitis of the biliary ducts as a manifestation of pSS.

Despite the peculiarities of the course of ACA-positive pSS and a low frequency of RF detection (in the present study, 22% and 29.7% in pSS and pSS + SSc, respectively), a high incidence of salivary MALT lymphomas was revealed in the first 10 years after the onset of the disease as in the case of "classical" pSS (in the present study, 17% and 13.5% with pSS and pSS + SSc, respectively). The main signs of lymphomas in patients with pSS and ACA were: persistent enlargement of the parotid salivary glands, decreased levels of the C4-complement and CD19 + cells in the peripheral blood, cryoglobulinemic vasculitis, lymphohystiocitic infiltration of the labial salivary glands (more than 200 cells in focus), as well as severe xerostomia and hypolacrimia. The above-mentioned predictors of lymphomas should be taken into account in the diagnostic search in all ACA-positive patients with pSS for early diagnosis of lymphoid tumors. Previously, the signs of lymphomas and their morphological / immunomorphological subtypes in the group of patients with ACA-positive pSS were not widely covered, the descriptions were sporadic [28, 29]. In

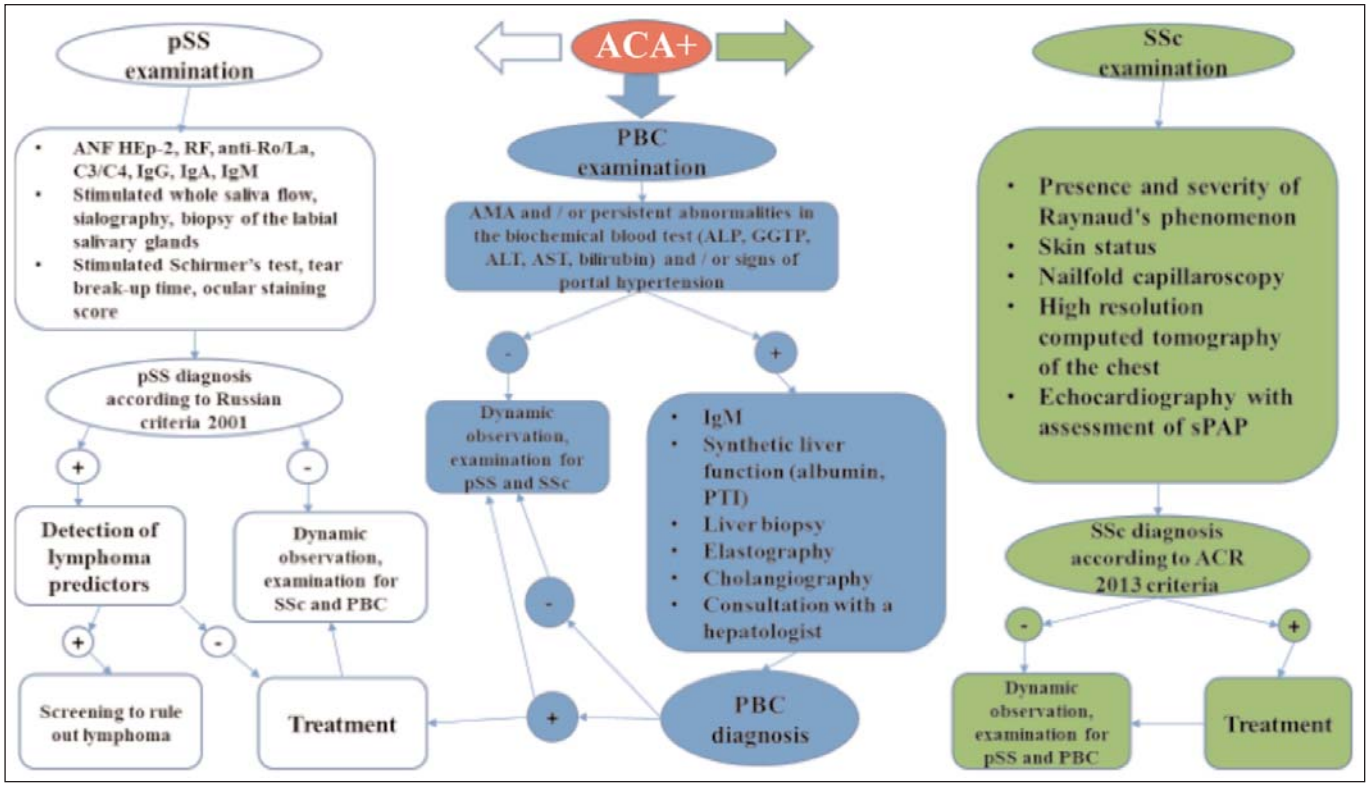

Figure 2. *Algorithm for differential diagnosis of a patient with anticentromere antibodies.*
ACA – anticentromere antibodies, pSS – primary Sjogren's syndrome, SSc – systemic sclerosis, PBC – primary biliary cholangitis, ANF – antinuclear factor; RF – rheumatoid factor; AMA – antimitochondrial antibodies, ALP – alkaline phosphatase, GGTP – gamma-glutamyltranspeptidase, ALT – alanine aminotransferase, AST – aspartate aminotransferase, PTI – prothrombin index, sPAP – systolic pulmonary artery pressure

numerous works devoted to the study of pSS [30–41], persistent enlargement of the parotid salivary glands, a decrease in the level of the C4-complement, cryoglobulinemic vasculitis were regarded as the strongest predictors of lymphoma development, which is also confirmed in this study. On the contrary, a decrease in the number of CD19 + cells in the peripheral blood was not previously considered in the literature as a predictor of the onset of lymphoma in pSS; in the present study, all patients with lymphomas had this feature, in contrast to patients without it. Probably, a decrease in the number of CD19 + lymphocytes in the peripheral blood in B-cell lymphomas may be associated with the intensive proliferation of these cells in the target organs and, as a consequence, their decrease in the peripheral blood. Previously, we considered an increase in IgM levels in the "classical" variant of pSS as one of the predictors of lymphomas; in the present study, however, the IgM levels in patients with and without lymphomas did not differ, which is probably due to the fact that some patients were diagnosed with PBC / epitheliitis of the biliary ducts as a manifestation of pSS, in which there is also an increase in the level of IgM. The secreting nature of lymphomas was noted only in 20% of patients in our study, which indicates the need for biopsy of persistently enlarged salivary glands to exclude lymphoma even in the absence of monoclonal secretion according to immunochemical studies of blood and urine. According to international recommendations for the treatment of pSS [3, 42–44], in glandular forms symptomatic agents should be used to stimulate the secretion of saliva and tears, and systemic therapy in the absence of extraglandular manifestations is not required. It should be noted that, according to our data, the course of pSS in patients with lymphomas was characterized by minimal systemic manifestations and low immunological activity, but severe glandular manifestations with the development of late stages salivary and lacrimal gland damage with high lymphoid infiltration of the labial salivary glands, which led to the development of MALT-lymphomas, and in two cases their subsequent transformation into DLBCL. Thus, in pSS, regardless of the detected type of antibodies (anti-Ro / La antibodies, RF, ACA or others) and the presence of extraglandular manifestations, lesions of the salivary and lacrimal glands progress, which often leads to the development of LPDs in the first ten years from the onset of the disease, which means that therapy that can prevent the development of this complication should be initiated immediately after the diagnosis of pSS is established.

In the present study, the sensitivity of IIF, ELISA and immunoblotting in relation to the determination of ACA was comparable, and the determination of autoantibodies to CENP-A and CENP-B did not allow for differential diagnosis between pSS, SSc and PBC. Both types of autoantibodies in the same titers were detected in all examined patients, regardless of the diagnosis. The limitation of our study was the impossibility of determining autoantibodies to CENP-C and HP1a, which, according to several studies [10, 16, 19], may become a useful tool for differential diagnosis between pSS and SSc in patients with seropositivity for ACA.

Thus, the high frequency of combinations of pSS, SSc, and PBC in ACA-positive patients is beyond doubt. Such polyautoimmune conditions can cause difficulties in diagnosis and in the center of this differential diagnostic triangle are ACAs, which requires a more detailed study of ACA-positive patients with a multidisciplinary thorough examination of them for the aforementioned conditions. Below, we present a simple algorithm for the differential diagnosis of ACA-positive patients, which, we hope, will help rheumatologists in their daily clinical practice (Fig. 2). This algorithm includes mandatory examination and follow-up of ACA-positive patients to exclude pSS, SSc and PBC, as well as hematological examination of patients with pSS for the presence of LPDs, if predictors of their development are found. The use of this algorithm will make it possible to diagnose pSS, SSc, PBC and LPDs in the early stages and to more correctly treat these patients.

REFERENCES

1. Reksten TR, Jonsson MV. Sjogren's syndrome an update on epidemiology and current insights on pathophysiology. *Oral Maxillofac Surg Clin North Am*. 2014 Feb; 26(1):1-12. doi: 10.1016/j.coms.2013.09.002.
2. Thomas E, Hay EM, Hajeer A, Silman AJ. Sjogren's syndrome: a community based study of prevalence and impact. *Br J Rheumatol*. 1998 Oct;37(10):1069-76. doi: 10.1093/rheumatology/37.10.1069.
3. Vivino FB, Bunya VY, Massaro-Giordano G, et al. Sjogren's syndrome: An update on disease pathogenesis, clinical manifestations and treatment. *Clin Immunol*. 2019 Jun;203: 81-121. doi: 10.1016/j.clim.2019.04.009. Epub 2019 Apr 29.
4. Amador-Patarroyo MJ, Arbelaez JG, Ruben D, et al. Sjögren's syndrome at the crossroad of polyautoimmunity. *J Autoimmun*. 2012 Sep;39(3):199-205. doi: 10.1016/j.jaut.2012.05.008. Epub 2012 Jun 30.
5. Васильев ВИ. Болезнь Шёгрена: клинико-лабораторные, иммуноморфологические проявления и прогноз. Автореф. дисс. докт. мед. наук. Москва; 2007. 45 с. [Vasil'ev VI. Sjogren's disease: clinical and laboratory, immunomorphological manifestations and prognosis. Autoref. diss. doct. med. sci. Moscow; 2007. 45 p.]
6. Brito-Zeron P, Ramos-Casals M, EULAR-SS task force group. Advances in the understanding and treatment of systemic complecations in Sjogren's syndrome. *Curr Opin Rheumatol*. 2014 Sep;26(5):520-7. doi: 10.1097/BOR.0000000000000096.
7. Gottenberg JE, Ravaud P, Puechal X, et al. Effects of hydroxychloroquine on symptomatic improvement in primary Sjögren syndrome: the JOQUER randomized clinical trial. *JAMA*. 2014 Jul 16;312(3):249-58. doi: 10.1001/jama.2014.7682.
8. Fayyaz A, Kurien BT, Hal Scofield R. Autoantibodies in Sjögren's Syndrome. *Rheum Dis Clin North Am*. 2016 Aug;42(3): 419-34. doi: 10.1016/j.rdc.2016.03.002. Epub 2016 Jun 21.
9. Nakamura H, Kawakami A, Hayashi T, et al. Anticentromere antibody-seropositive Sjögren's syndrome differs from conventional subgroup in clinical and pathological study. *BMC Musculoskelet Disord*. 2010 Jul 1;11:140. doi: 10.1186/1471-2474-11-140.
10. Baer AN, Medrano L, McAdams-DeMarco M, Gniadek TJ. Anticentromere antibodies are associated with more severe exocrine glandular dysfunction in Sjögren's syndrome: Analysis of the Sjögren's International Collaborative Clinical Alliance cohort. *Arthritis Care Res (Hoboken)*. 2016 Oct;68(10):1554-9. doi: 10.1002/acr.22859. Epub 2016 Sep 16.
11. Tinazzi E, Patuzzo G, Lunardi C. Autoantigens and Autoantibodies in the Pathogenesis of Sjögren's Syndrome. In: Sjögren's Syndrome. Elsevier; 2016. P. 141-156.
12. Vlachoyiannopoulos PG, Drosos AA, Wiik A, Moutsopoulos HM. Patients with anticenromere antibodies, clinical features, diagnoses and evolution. *Br J Rheumatol*. 1993 Apr;32(4):297-301. doi: 10.1093/rheumatology/32.4.297.
13. Villalta D, Imbastaro T, Di Giovanni S, et al. Diagnostic accuracy and predictive value of extended autoantibody profile in systemic sclerosis. *Autoimmun Rev*. 2012 Dec; 12(2):114-20. doi: 10.1016/j.autrev.2012.07.005
14. Ferri C, Valentini G, Cozzi F, et al. Systemic sclerosis: demographic, clinical and serologic features and survival in 1012 Italian patients. *Medicine (Baltimore)*. 2002 Mar; 81(2):139-53. doi: 10.1097/00005792-200203000-00004.
15. Ананьева ЛП, Александрова ЕН. Аутоантитела при системной склеродермии: спектр, клинические ассоциации и прогностическое значение. Научно-практическая ревматология. 2016;54(1):86-99. [Anan'eva LP, Aleksandrova EN. Autoantibodies in systemic scleroderma: spectrum, clinical associations, and prognostic value. *Nauchno-prakticheskaya revmatologiya = Rheumatology Science and Practice*. 2016;54(1):86-99. (In Russ.)].
16. Tanaka N, Muro Y, Suzuki Y, et al. Anticentromere antibody-positive primary Sjögren's syndrome: Epitope analysis of a subset of anticentromere antibody-positive patients. *Mod Rheumatol*. 2017 Jan;27(1): 115-21. doi: 10.1080/14397595.2016.1176327. Epub 2016 May 10.
17. Katano K, Kawano M, Koni I, et al. Clinical and laboratory features of anticentromere antibody positive primary Sjögren's syndrome. *J Rheumatol*. 2001 Oct;28(10): 2238-44.
18. Hudson M, Mahler M, Pope J, et al; Canadian Scleroderma Research Group. Clinical Correlates of CENP-A and CENP-B Antibodies in a Large Cohort of Patients with Systemic Sclerosis. *J Rheumatol*. 2012 Apr; 39(4):787-94. doi: 10.3899/rheum.111133.
19. Gelber AC, Pillemer SR, Baum BJ, et al. Distinct recognition of antibodies to centromere proteins in primary Sjogren's syndrome compared with limited scleroderma. *Ann Rheum Dis*. 2006 Aug;65(8):1028-32. doi: 10.1136/ard.2005.046003. Epub 2006 Jan 13.
20. Насонов ЕЛ, редактор. Российские клинические рекомендации. Ревматология. Москва: ГЭОТАР-Медиа; 2017. 464 с. [Nasonov EL, editor. *Rossiiskie klinicheskie rekomendatsii. Revmatologiya* [Russian clinical guidelines. Rheumatology]. Moscow: GEOTAR-Media; 2017. 464 p.].
21. Shiboski SC, Shiboski CH, Criswell LA, et al; Sjögren's International Collaborative Clinical Alliance (SICCA) Research Groups. American College of Rheumatology Classification Criteria for Sjögren's Syndrome: A Data-Driven, Expert Consensus Approach in the SICCA Cohort. *Arthritis Care Res (Hoboken)*. 2012 Apr;64(4):475-87. doi: 10.1002/acr.21591.
22. Shiboski CH, Shiboski SC, le Seror R, et al; International Sjogren's Syndrome Criteria Working Group. 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjogren's Syndrome: A Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. *Arthritis Rheumatol*. 2017 Jan;69(1): 35-45. doi: 10.1002/art.39859. Epub 2016 Oct 26.
23. Van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum*. 2013 Nov;65(11):2737-47. doi: 10.1002/art.38098. Epub 2013 Oct 3.
24. Lindor KD, Bowlus CL, Boyer J, et al. Primary Biliary Cholangitis: 2018 Practice Guidance from the American Association for the Study of Liver Diseases. *Hepatology*. 2019 Jan; 69(1):394-419. doi: 10.1002/hep.30145. Epub 2018 Nov 6.
25. Ивашкин ВТ, Широкова ЕН, Маевская МВ и др. Клинические рекомендации Российской гастроэнтерологической

ассоциации и Российского общества по изучению печени по диагностике и лечению холестаза. Российский журнал гастроэнтерологии гепатологии колопроктологии. 2015;2:41-57.
[Ivashkin VT, Shirokova YeN, Mayevskaya MV, et al. Clinical Guidelines of the Russian Gastroenterological Association and the Russian Society on Study of the Liver on Diagnostics and Treatment of Cholestasis. *Russian Journal of Gastroenterology, Hepatology, Coloproctology.* 2015;25(2):41-57. (In Russ.)].
26. Swerdlow SH, Campo E, Harris NL, et al, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th edition. IARC: Lyon; 2017. 523 p.
27. Tektonidou M, Kaskani E, Skopouli FN, Moutsopoulos HM. Microvascular abnormalities in Sjögren's syndrome: nailfold capillaroscopy. *Rheumatology (Oxford).* 1999 Sep; 38(9):826-30. doi: 10.1093/rheumatology/38.9.826.
28. Baldini C, Mosca M, Della Rossa A, et al. Overlap of ACA-positive systemic sclerosis and Sjögren's syndrome: a distinct clinical entity with mild organ involvement but at high risk of lymphoma. *Clin Exp Rheumatol.* Mar-Apr 2013;31(2):272-80. Epub 2013 Jan 18.
29. Gulati D, Kushner I, File E, Magrey M. Primary Sjogren's syndrome with anticentromere antibodies – a clinically distinct subset. *Clin Rheumatol.* 2010 Jul;29(7):789-91. doi: 10.1007/s10067-009-1359-9. Epub 2010 Jan 19.
30. Retamozo S, Brito-Zeron P, Ramos-Casals M. Prognostic markers of lymphoma development in pSjS. *Lupus.* 2019 Jul;28(8): 923-36. doi: 10.1177/0961203319857132. Epub 2019 Jun 19.
31. Sutcliffe N, Inanc M, Speight P, Isenberg D. Predictors of lymphoma development in primary Sjogrens syndrome. *Semin Arthritis Rheum.* 1998 Oct;28(2):80-7. doi: 10.1016/s0049-0172(98)80040-1.
32. Ioannidis JP, Vassiliou VA, Moutsopoulos HM. Long-term risk of mortality and lymphoproliferative disease and predictive classification of primary Sjogren's syndrome. *Arthritis Rheum.* 2002 Mar;46(3): 741-7. doi: 10.1002/art.10221.
33. Theander E, Henriksson G, Ljungberg O, et al. Lymphoma and other malignancies in primary Sjogren's syndrome: A cohort study on cancer incidence and lymphoma predictors. *Ann Rheum Dis.* 2006 Jun;65(6):796-803. doi: 10.1136/ard.2005.041186. Epub 2005 Nov 10.
34. Tzioufas AG, Boumba DS, Skopouli FN, Moutsopoulos HM. Mixed monoclonal cryoglobulinemia and monoclonal rheumatoid factor cross-reactive idiotypes as predictive factors for the development of lymphoma in primary Sjogren's syndrome. *Arthritis Rheum.* 1996 May;39(5):767-72. doi: 10.1002/art.1780390508.
35. Baimpa E, Dahabreh IJ, Voulgarelis M, Moutsopoulos HM. Hematologic manifestations and predictors of lymphoma development in primary Sjogren syndrome: Clinical and pathophysiologic aspects. *Medicine (Baltimore).* 2009 Sep;88(5):284-93. doi: 10.1097/MD.0b013e3181b76ab5.
36. Solans-Laque R, Lopez-Hernandez A, Bosch-Gil JA, et al. Risk, predictors, and clinical characteristics of lymphoma development in primary Sjogren's syndrome. *Semin Arthritis Rheum.* 2011 Dec;41(3):415-23. doi: 10.1016/j.semarthrit.2011.04.006. Epub 2011 Jun 12..
37. Baldini C, Pepe P, Luciano N, et al. A clinical prediction rule for lymphoma development in primary Sjögren's syndrome. *J Rheumatol.* 2012 Apr;39(4):804-8. doi: 10.3899/jrheum.110754. Epub 2012 Feb 15.
38. Johnsen SJ, Brun JG, Gøransson LG, et al. Risk of non-Hodgkin's lymphoma in primary Sjögren's syndrome: A population-based study. *Arthritis Care Res (Hoboken).* 2013 May;65(5):816-21. doi: 10.1002/acr.21887.
39. Quartuccio L, Isola M, Baldini C, et al. Biomarkers of lymphoma in Sjogren's syndrome and evaluation of the lymphoma risk in prelymphomatous conditions: Results of a multicenter study. *J Autoimmun.* 2014 Jun;51: 75-80. doi: 10.1016/j.jaut.2013.10.002. Epub 2013 Nov 11.
40. Risselada AP, Kruize AA, Bijlsma JW. Clinical features distinguishing lymphoma development in primary Sjogren's syndrome: A retrospective cohort study. *Semin Arthritis Rheum.* 2013 Oct;43(2):171-7. doi: 10.1016/j.semarthrit.2013.03.001. Epub 2013 May 7.
41. Fragkioudaki S, Mavragani CP, Moutsopoulos HM. Predicting the risk for lymphoma development in Sjogren syndrome: An easy tool for clinical use. *Medicine (Baltimore).* 2016 Jun;95(25):e3766. doi: 10.1097/MD.0000000000003766.
42. Mariette X, Criswell LA. Primary Sjögren's Syndrome. *N Engl J Med.* 2018 Mar 8;378(10):931-939. doi: 10.1056/NEJMcp1702514.
43. Chen X, Wu H, Wei W. Advances in the diagnosis and treatment of Sjogren's syndrome. *Clin Rheumatol.* 2018 Jul;37(7): 1743-1749. doi: 10.1007/s10067-018-4153-8. Epub 2018 May 26.
44. Stefanski AL, Tomiak C, Pleyer U, et al. The Diagnosis and Treatment of Sjögren's Syndrome. *Dtsch Arztebl Int.* 2017 May 26; 114(20):354-361. doi: 10.3238/arztebl.2017.0354.

Received/Reviewed/Accepted
24.07.2020/19.09.2020/23.09.2020

Conflict of Interest Statement

The investigation has not been sponsored. There are no conflicts of interest. The authors are solely responsible for submitting the final version of the manuscript for publication. All the authors have participated in developing the concept of the article and in writing the manuscript. The final version of the manuscript has been approved by all the authors.

Chaltsev B.D. https://orcid.org/0000-0003-4188-3578
Vasilyev V.I. https://orcid.org/0000-0002-1425-8622
Palshina S.G. https://orcid.org/0000-0003-3389-7064
Torgashina A.V. https://orcid.org/0000-0001-8099-2107
Sokol E.V. https://orcid.org/0000-0002-2191-9361
Khvan Yu.I. https://orcid.org/0000-0003-2314-1466
Safonova T.N. https://orcid.org/0000-0020-4601-0904
Lapin S.V. https://orcid.org/0000-0002-4998-3699
Tkachenko O.Yu. https://orcid.org/0000-0002-1479-6551
Mazing A.V. https://orcid.org/0000-0002-3055-6507