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DETERMINATION OF ANTINUCLEAR ANTIBODIES BY IMMUNOBLOTING TO CLARIFY THE IMMUNOLOGICAL CHARACTERISTICS OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND SJOGREN'S SYNDROME

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Objective: to study the immunological characteristics of patients with systemic lupus erythematosus and Sjogren's syndrome by determining antinuclear antibodies using immunoblotting.

Materials and methods. We observed 69 patients whose average age was 38.9 years [23.2-62.9], of which 63 (91.30%) were women and 6 (8.69%) men. BMI was 27.3 kg/m2 [21.8-49.2]. Inclusion criteria: age from 18 to 70 years, presence of a reliable diagnosis. To study the diagnostic value of determining the ANA profile, patients were divided into 3 groups: 1st group – 15 patients with systemic lupus erythematosus (SLE), 2nd – 21 patients with the disease and Sjögren's syndrome (SS), 3rd (control) group – 33 patients with osteoarthritis. The control group was comparable to the study groups by gender and age.

Results. The determination of anti-SS-A in SLE has good quality (area under the ROC curve - 0.66). A cut-off value was determined with 79.6% specificity and 53.3% sensitivity. Anti-RNP/SM, anti-Sm, anti-dsDNA and anti-HI were somewhat less sensitive (30%), with a specificity level of 91% for anti-dsDNA and anti-Sm and 100% for anti-RNP/Sm and anti -HI. The most informative diagnostic tests for the disease and Sjogren's syndrome are anti-Ro-52 recombinant (sensitivity 57.1%, specificity 96%), anti-SS-Anative (sensitivity 52.4%, specificity 86%). The determination of anti-Ro-52 in SS is of good quality, which confirms the value of the area under the ROC curve (>0.7). The optimal cut-off value corresponded to 99.6% specificity and 57.1% sensitivity. Somewhat less sensitive (28.6%) were anti-Sm (specificity - 92%), anti-dsDNA (specificity - 92%) and anti-RIB (specificity 100.0%).

Findings. The laboratory tests studied, as a rule, had high specificity, but rather low sensitivity. The most specific tests for diagnosing SLE are antibodies to the antigens RNP/Sm, SS-Anative, antibodies to histones, for SS - anti-SS-Anative, anti-Ro-52 recombinant, anti-RIB.

Keywords: systemic lupus erythematosus, Sjögren's syndrome, immunoblotting, antinuclear antibodies.

Introduction. Rheumatic diseases are a huge economic and social burden and, according to WHO recommendations, the study of their prevalence, morbidity, mortality and prevention should be an integral part of national programs for maintaining public health and the basis for planning medical care [1]. The pathogenesis of rheumatic diseases is based on the interaction of environmental and genetic factors. The most studied genetic factors are human leukocyte antigens (HLA), specific haplotypes of which are reliably associated with a specific diagnosis. Thus, haplotypes HLA-DRB1*03:01 and *15:01 are genetic risk factors for systemic lupus erythematosus in the European population, and PTPN22 occurs not only in systemic lupus erythematosus, but also in other rheumatic diseases, in particular rheumatoid arthritis (RA) [8]. Systemic rheumatic diseases have a heterogeneous clinical phenotype, which complicates their clinical diagnosis and requires the active use of laboratory and instrumental research methods. Experts emphasize the need for early diagnosis of SLE, but recent studies confirm that patients with SLE still face late diagnosis of the disease (on average 2 years from the onset of symptoms) [4].

The advantages of laboratory research methods are the objectivity of the data obtained on the nature of the immunopathological process and autoimmune diseases, the possibility of using them for diagnosis, assessing disease activity, determining prognosis, identifying damage to individual organs, choosing a treatment method and monitoring the effectiveness of therapy [2, 9].

The basis for the nosological diagnosis of systemic rheumatological diseases are immunological studies [3]. Immunofluorescent determination of antinuclear antibodies (ANA) is the standard laboratory examination of patients with systemic rheumatological diseases [6]. ANAs are a class of antibodies that bind to cellular components in the nucleus, DNA, RNA, and nucleic acid-protein complexes [9].

Traditional methods for studying ANA are screening methods that assess the presence of ANA in blood serum, without specifying the specifics (indirect immunofluorescence method on tissue sections of rats or mice). In recent years, new methods have emerged to determine the type of ANA a patient has. These include the ELISA method, used to determine a large number of autoantibodies, requiring the simultaneous use of several test systems (up to 20) [1, 5].

One of the most common methods is to determine the entire ANA profile simultaneously using immunoblotting. This method allows you to detect antibodies to autoantigens: Sm, RNP/Sm, SS-A(60 kDa), SS-A(52 kDa), SS-B, Scl-70, PM-Scl, PCNA, CENT-B, dsDNA/ Histone/ Nucleosome, RibP, AMA-M2 and Jo-1. It is believed that the method can be used for ANA screening [10], but the value of this method has not been sufficiently studied and requires clarification.

Objective: to study the immunological characteristics of patients with systemic lupus erythematosus and Sjogren's syndrome by determining antinuclear antibodies using immunoblotting.

Materials and methods. The study was conducted on the basis of the Federal State Budgetary Institution “Research Institute of Clinical and Experimental Rheumatology named after. A.B. Zborovsky", Volgograd. We observed 69 patients whose average age was 38.9 years [23.2-62.9], of which 63 (91.30%) were women and 6 (8.69%) men. BMI was 27.3 kg/m2 [21.8-49.2]. Inclusion criteria: age from 18 to 70 years, presence of a reliable diagnosis.

Diagnoses were made based on generally accepted clinical guidelines [8].

To study the diagnostic value of determining the ANA profile, patients were divided into 3 groups: 1st group – 15 patients (14 (93.3%) women, 1 (6.7%) man) with systemic lupus erythematosus (SLE), 2- y – 21 patients (19 (90%) women and 2 (10%) men) with Sjögren’s disease and syndrome (SS), 3rd (control) group – 33 patients (30 (91%) women and 3 (9%) men) with osteoarthritis. All groups were comparable to each other by gender and age.

Diagnoses were made based on generally accepted clinical guidelines [3, 6, 7].

When performing the work, a set of reagents was used to determine IgG antibodies to nuclear antigens by immunoblotting (EUROLINEANAProfile 3 (IgG), cat. no. DL 1590-1601-3), with which the following types of antibodies were determined:

RNP - antibodies to the protein components of the small nuclear nucleotide U-1-RNA;

Sm - antibodies to U1-, U2-, U4-ribonucleoproteins;

SS-A native - antibodies to proteins associated with RNA Y1-Y5 in spliceosomes;

Ro-52 recombinant – antibodies to recombinant antigen (52 kDa protein);

SS-B - antibodies to RNA polymerase-3 associated protein

Scl-70 - antibodies to DNA topoisomerase 1;

PM-Scl100 – antibodies to the recombinant antigen PM-Scl;

Jo-1 - antibodies to histidine-tRNAsynthetase,

CENPB-anticentromere B antibodies;

PCNA - antibodies to proliferating cell nuclear antigen,

Table 1

Frequency of detection of antinuclear antibodies in rheumatic diseases

Antibodies	Groups of patients according to the main diagnosis			Reliability, $\chi 2$; p
	SLE (n=15)	SS (n=21)	Control group (n=33)	
RNP/Sm	5(30)	0(0)	0(0)	13.2; p<0.0001
Sm	5(30)	6(28.6)	0(0)	14.0; p=0.001
SS-A native	8(53.3)	11(52.4)	0(0)	28.19; p<0.0001
Ro-52 recombinant	2(13.33)	12(57.1)	0(0)	25.81; p<0.0001
SS-B	2(13.33)	4(19.0)	0(0)	6.88; p=0.017
Scl-70	0(0)	0(0)	0(0)	-
PM-Scl100	0(0)	2(9.52)	0(0)	3.28; p=0.134
Jo-1	0(0)	3(14.3)	0(0)	5.02; p=0.034
Centromere B	0(0)	0(0)	0(0)	-
PCNA	0(0)	0(0)	0(0)	-
dsDNA	5(30)	6(28.6)	0	13.97; p=0.001
Nucleosomes(NUC)	3(20)	2(9.52)	0(0)	6.24; p=0.025
HI	5(30)	0(0)	0(0)	13.16; p<0.0001
Ribosomal-P-protein (RIB)	0(0)	6(28.6)	0(0)	11.78; p<0.0001
AMA-M2	0(0)	0(0)	0(0)	-

dsDNA - antibodies to double-stranded DNA,

NUC – antibodies to nucleosomes,

HI – antibodies to histones,

RIB – antibodies to ribosomal protein P,

AMA-M2 - antimitochondrial antibodies.

Statistical calculations were performed using the STATISTICA 10.0 program. The threshold value at the cut-off point for detecting SLE and SS was determined by determining the point of the highest value of the intersection of sensitivity and specificity.

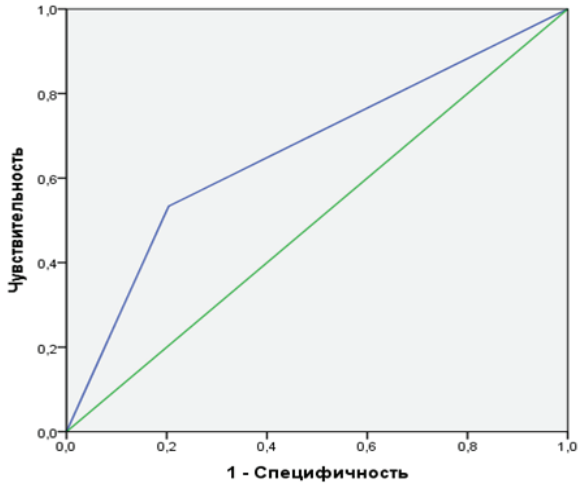
Results. We investigated the frequency of detection of ANA in rheumatic diseases, the results are presented in Table 1.

Table 1 shows that autoantibodies to the extractable nuclear antigens RNP/Sm, which are considered the traditional criterion for diagnosing SLE, were detected in 30% of patients with SLE. Sm antibodies to U1-, U2-, U4-ribonucleoproteins were determined in patients with SLE and SS. In groups 1 and 2, anti-SS-A native was detected significantly more often (53.3 and 57.1%, respectively). Recombinant anti-Ro-52 was detected in half of the patients with SS. Anti-SS-B was detected statistically more often in patients with SS compared to group 3. In 3 patients with SLE and 2 with Sjögren's disease, anti-NUC was detected. Anti-HI was detected significantly more often (30%) in the group of patients with SLE.

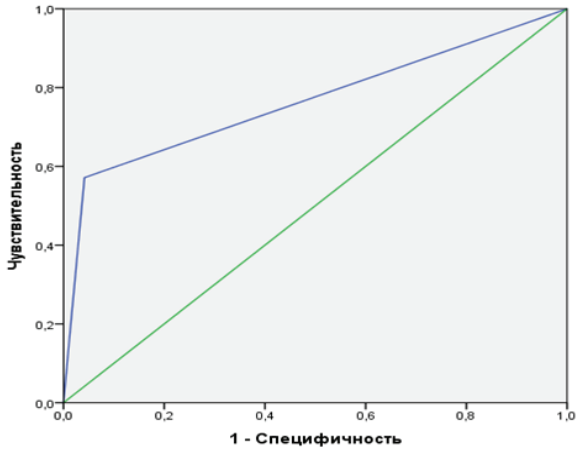
Next, we determined the sensitivity and specificity of laboratory tests for SLE and SS, which made it possible to identify the most optimal method that is most suitable for diagnosing a specific nosology. The sensitivity of the test is determined by a formula that shows the proportion of reliable diagnostic indicators in patients with a given disease. Specificity is determined by the percentage of significantly negative indicators among obviously healthy individuals. The results are presented in Table 2.

Table 1 shows that autoantibodies to the extractable nuclear antigens RNP/Sm, which are considered the traditional criterion for diagnosing SLE, were detected in 30% of patients with SLE. Sm antibodies to U1-, U2-, U4-ribonucleoproteins were determined in patients with SLE and SS. In groups 1 and 2, anti-SS-A native was detected significantly more often (53.3 and 57.1%, respectively). Recombinant anti-Ro-52 was detected in half of the patients with SS. Anti-SS-B was detected statistically more often in patients with SS compared to group 3. In 3 patients with SLE and 2 with Sjögren's

a



b



Roc curve characterizing the diagnostic value of anti-SS-A in SLE (a) and anti-Ro-52 in SS (b)

Table 2

Sensitivity and specificity of antibody tests

Antibodies	SLE		SS	
	Sensitivity	Specificity	Sensitivity	Specificity
RNP/Sm	30	100	0	94
Sm	30	91	28.6	92
SS-A native	53.3	82	52.4	86
Ro-52 recombinant	13.3	82	57.1	96
SS-B	13.3	94	19.0	97
Scl-70	-	100	-	100
PM-Scl100	-	97	9.52	100
Jo-1	0.0	96	14.3	100
CENP B	-	100	-	100
PCNA	-	100	-	100
dsDNA	30	91	28.6	92
NUC	20	97	9.52	95
HI	30	100	28.6	93
RIB	-	91	28.6	100
AMA-M2	-	100	-	100

Table 3

Main descriptive characteristics of the ROC curve characterizing the diagnostic value of anti-SS-A in SLE and anti-Ro-52 in SS

	Анти-SS-A for SLE	Анти- Ro-52 for SS
Area under the ROC curve	0.665	0.765
Standard error	0.084	0.071
Confidence interval	0.499-0.830	0.65-0.905

disease, anti-NUC was detected. Anti-HI was detected significantly more often (30%) in the group of patients with SLE.

Next, we determined the sensitivity and specificity of laboratory tests for SLE and SS, which made it possible to identify the most optimal method that is most suitable for diagnosing a specific nosology. The sensitivity of the test is determined by a formula that shows the proportion of reliable diagnostic indicators in patients with a given disease. Specificity is determined by the percentage of significantly negative indicators among obviously healthy individuals. The results are presented in Table 2.

According to Table 3, the anti-SS-A laboratory test for SLE is of good quality (area under the ROC curve - 0.66). We determined the cut-off threshold value with 79.6% specificity and 53.3% sensitivity. The laboratory test for anti-Ro-52 in SS is of good quality, which is confirmed by the value of the area under the ROC curve (>0.7). The optimal cut-off value corresponded to 99.6% specificity and 57.1% sensitivity.

Thus, using additional methods of statistical analysis, we were able to confirm the previously calculated sensitivity and specificity of anti-SS-Anative for the diagnosis of SLE and anti-Ro-52 for SS.

Conclusions. In our study, the main markers of autoimmune connective tissue diseases were studied using immunoblotting.

In the groups of patients with SLE and SS, anti-SS-A native was observed significantly more often (53.3 and 52.4%, respectively). Antibodies to histones were detected significantly more often with a frequency of 30% in the group of patients with SLE. It can be assumed that extractable nuclear antigens have the greatest diagnostic value in SLE, antibodies to the SS-A and Ro-52 antigens - in SLE and Sjögren's disease (syndrome), and antinuclear antibodies to histones are more characteristic of patients with SLE.

The laboratory tests studied, as a rule, had high specificity, but rather low sensitivity. The most specific tests for diagnosing SLE are antibodies to the antigens RNP/Sm, SS-Anative, antibodies to histones, for SS - anti-SS-Anative, anti-Ro-52 recombinant, anti-RIB. Thus, these tests are optimally used to confirm a specific nosological diagnosis in a patient with an already identified rheumatological disease, however, for screening studies in order to identify rheumatological pathology in the population, these tests are not rational to use.

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