

THE EXPERIENCE OF APPLICATION OF APPARATUS “AKLIDES” IN KAZAKHSTAN FOR THE DIAGNOSIS OF SYSTEMIC AUTOIMMUNE DISEASES

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Abstract

The main methods of laboratory diagnosis of autoimmune diseases (AD) are: the reaction of indirect immunofluorescence, enzyme immunodetection and immunoblotting. There are direct and indirect reaction of immunofluorescence. The method of direct immunofluorescence is used to detect Ig deposits and complement factors in biopsy samples of skin and kidneys. The reaction of indirect immunofluorescence is used to detect Ab in the blood serum and other biological fluids. It should be noted that this method requires very high qualification of the technician. The disadvantages of this method are the subjective account of the results and the complexity, lack of standardization of substrates, reagents, microscope. Computer-based systems for interpreting cell fluorescence tests contribute to the standardization and improvement of reproducibility of the reaction of indirect immunofluorescence in the determination of autoantibodies in patients with rheumatoid diseases.

The blood serum of 17 patients were studied with this method for the presence of the following autoantibodies: Antinuclear Antibodies (ANA), Cytoplasmic antineutrophil antibodies (cANCA), Perinuclear Antineutrophil Antibodies (pANCA). The studies were conducted on the AKLIDES apparatus made by company “Medipan GmbH”, Germany. Modern methods of serological diagnosis RIF were applied in Kazakhstan for the first time to assess serological activity of SLE. Currently in Kazakhstan there is a need for accurate diagnostics of autoimmune rheumatic diseases. The correct diagnosis with early stages of this disease will stop the process of disease progression and improve life quality of patients.

Keywords

autoimmune diseases, laboratory diagnosis, indirect immunofluorescence reaction, autoimmune antibodies, immunoblot.

Қазақстанда «Аклидес» аппаратын жүйесі аутоиммундық ауруларды анықтауда қолдану тәжірибесі

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Аңдатпа

Аутоиммунды ауруларды лабораториялық диагностикалаудың негізгі әдістері: жанама иммунофлюоресценция реакциясы, ферментативті иммунды анықтау және иммуноблоттау. Иммунофлюоресценцияның тікелей және жанама реакциялары бар. Тікелей иммунофлюоресценттік әдіс терінің және бүйректің биопсиясындағы Ig шөгінділерін және комплемент факторларды анықтау үшін қолданылады. Қан сарысуындағы және дененің басқа сұйықтықтарындағы антидене анықтау үшін қолданылады. Айта кету керек, бұл әдіс маманның жоғары біліктілігін талап етеді. Бұл әдістің кемшіліктері - нәтижелер мен күрделіліктің субъективті қарастырылуы, субстраттардың, реагенттердің, микроскоптардың стандартталуының болмауы. Жасушалық флуоресценттік тестілерді интерпретациялауға арналған компьютерлік жүйелер ревматоидті аурулары бар науқастарда аутоантиденелерді анықтауда жанама иммунофлюоресценция репродукциясын стандарттауға және жақсартуға көмектеседі.

Бұл әдіспен 17 науқастың қан сарысуында келесі аутоантиденелер бар екендігі тексерілді: Антиядролық антиденелер, Цитоплазмалық антинейтрофилді антиденелер, Перинуклеарлы антинейтрофты антиденелер. Зерттеу «Medipan GmbH», Германия шығарған AKLIDES аппаратында жүргізілді. жанама иммунофлюоресценция реакциясы серологиялық диагностикасының заманауи әдістері Қазақстанда алғаш рет жүйелі қызыл жіңішке эритематоз серологиялық белсенділігін бағалау үшін қолданылды. Қазіргі уақытта Қазақстанда аутоиммунды-ревматикалық аурулардың нақты диагнозы қажет. Аурудың бастапқы кезеңдерінде дұрыс диагноз қою аурудың дамуын тоқтатады және пациенттердің өмір сапасын жақсартады.

Түйін сөздер

аутоиммунды аурулар, зертханалық диагностика, жанама иммунофлюоресценттік реакция, аутоиммундық антиденелер, иммуноблот.

Опыт применения аппарат «Аклидес» в Казахстане для диагностики системных аутоиммунных заболеваний

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Аннотация

Основными методами лабораторной диагностики аутоиммунных заболеваний являются: реакция непрямой иммунофлуоресценции, ферментативная иммунодетекция и иммуноблоттинг. Существуют прямые и непрямые реакции иммунофлуоресценции. Метод прямой иммунофлуоресценции используется для выявления отложений Ig и факторов комплемента в биоптатах кожи и почек. Реакция непрямой иммунофлуоресценции используется для обнаружения антител в сыворотке крови и других биологических жидкостях. Следует отметить, что этот метод требует очень высокой квалификации специалиста. Недостатками данного способа являются субъективный учет результатов и сложности, отсутствие стандартизации субстратов, реагентов, микроскопа. Компьютерные системы для интерпретации клеточных флуоресцентных тестов способствуют стандартизации и улучшению воспроизводимости. Реакция непрямой иммунофлуоресценции при определении аутоантител у пациентов с ревматоидными заболеваниями.

Сыворотка крови 17 пациентов была исследована этим методом на наличие следующих аутоантител: Антинуклеарные антитела, Цитоплазматические антинейтрофильные антитела, Перинуклеарные антинейтрофильные антитела. Исследования проводились на аппарате AKLIDES производства фирмы «Medipan GmbH», Германия. Современные методы серологической диагностики реакция непрямой иммунофлуоресценции впервые были применены в Казахстане для оценки серологической активности системной красной волчанки. В настоящее время в Казахстане существует необходимость в точной диагностике аутоиммунных ревматических заболеваний. Правильный диагноз с ранними стадиями этого заболевания остановит процесс прогрессирования заболевания и улучшит качество жизни пациентов.

Introduction

Autoimmune diseases (AD) affect 5-7% of the world population, more common in women than in men, and usually at a young age. AD develops when antibodies interact with their own antigens, thereby destroying cells and tissues, which carry these antigens. A vicious circle appears: more autoantibodies – a greater tissue damage – greater exposure of internal antigens – more autoantibodies. Proteins, nucleic acids, phospholipids, sugars, steroids, etc. may serve as autoantigens. Mechanism of cells and tissues autoimmune destruction is the same as naturally in adaptive immunity. Occurred autoimmune process, usually is chronic and leads to long-term tissue damage, whereas the autoimmune reaction is constantly maintained by tissue antigens [1, 2].

Autoimmune diseases (AD) are the third leading cause of chronic diseases in developed countries after cardiovascular and oncological conditions [3].

In the AD progression, both cellular and humoral mechanisms of the immune system play a significant role. Probably, autoimmune cell reactions underlie the basis of AD pathogenesis. However, their estimation, namely the calculation of autoreactive T cells, today is only experimentally possible and does not have independent clinical significance. At the same time, the detection of auto-antibodies is widely used for early and differential diagnosis of

AD, and in some cases can be used for predicting the course of disease and monitoring the effectiveness of therapy [4].

The main methods of laboratory diagnosis of autoimmune diseases (AD) are: indirect immunofluorescence (IIF), enzyme immunodetection and immunoblotting. There are direct and indirect immunofluorescence. The method of direct immunofluorescence is used to detect Ig deposits and complement factors in biopsy samples of skin and kidneys. The RIF is used to detect Ab in the blood serum and other biological fluids. It should be noted that this method requires very high qualification of the technician. The disadvantages of this method are the subjective account of the results and the complexity, lack of standardization of substrates, reagents, microscope. Computer-based systems for interpreting cell fluorescence tests contribute to the standardization and improvement of reproducibility of RIF in the determination of autoantibodies in patients with rheumatoid diseases (rd).

Study methods

The blood serum of 17 patients was studied by the method of RIF for the presence of the following autoantibodies: ANA, cANCA, pANCA. All 17 patients were women, average age is 34 years, age range varied from 15 to 50 years (Table 2).

ОБ АВТОРАХ

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Ключевые слова

аутоиммунные заболевания, лабораторная диагностика, непрямая реакция иммунофлуоресценции, аутоиммунные антитела, иммуноблот.

The studies were conducted on the AKLIDES apparatus made by company "Medipan GmbH", Germany. It provides: automatic screening; quantification; archivation of results; the reproduction and reflection of the samples in the reports; the image of appearance in a live mode; connection to the laboratory information management system (LIMS).

Used test systems: AKLIDES ANA plus REF4065 made by company "Medipan GmbH", Germany, for 120 definitions – a test of indirect immunofluorescence for an automatic separation of antibodies into nuclear and cytoplasmic antigens (ANA) in the serum of human blood. AKLIDES ANA plus – a set of reagents for the qualitative and semi-quantitative separation of antibodies into antigens in the cell nucleus and in the cytoplasm of cells HEp-2 in human blood serum by automatic evaluation of the test results. Titer ≤ 80 – negative result. Titer ≥ 80 – positive result.

Systemic rheumatic inflammatory diseases: systemic lupus erythematosus (SLE) and its variants, progressive systemic sclerosis (PSS), primary Sjogren syndrome, dermatomyositis, Sharp's syndrome (mixed connective tissue disease – (MCTD) and rheumatoid arthritis (RA) are characterized by the emergence of a number of autoantibodies directed against components of the cell nucleus and cytoplasm. These antibodies can be used as markers to determine the aspect of the disease, and as an active parameter [5, 6, 7, 8, 9].

Immunofluorescence test using the cell line Hep-2 is a sensitive screening test for the determination of antinuclear antibodies (ANA). Recognizing individual samples of fluorescence, this test provides an opportunity to talk about the fundamental underlying antigens and diseases associated with them [10, 11, 12].

AKLIDES ANA plus is a test of indirect immunofluorescence for the qualitative and semi-quantitative determination of ANA by the automatic evaluation of the test results.

In the first step of the reaction antibodies of the diluted patient sample or control serum specifically react with antigens of cells HEp-2, fixed on the objects carrier. After 30 minutes of incubation at a room temperature, unreacted components are removed by rinsing.

In the second step unreacted and coupled antibodies specifically react with non-human antibodies (IgG and specific light chain), conjugated with fluorescein isothiocyanate (FITC). After an incubation

period of 30 minutes held at a room temperature has finished, the free conjugate molecules are removed from immune complexes in the solid phase by subsequent washing.

In accordance with the histological arrangement of antigens in the cells HEp-2 there are special samples of fluorescence conditions. After covering and placing objects under the fluorescent microscope (excitation wavelength is 490 nm, emission is wavelength 520 nm), they will be transcribed by an automated measurement system.

Also a test-system AKLIDES of cANCA, REF4060 was used made by company "Medipan GmbH", Germany, for 60 definitions – indirect immunofluorescence analysis for the detection of IgG antibodies to neutrophil cytoplasmic antigens (ANCA) in human serum. A titer of ≤ 20 – negative result. Titer ≥ 20 – positive.

This test system is a kit of reagents for qualitative and semi-quantitative determination of IgG antibodies to antigens in the cytoplasm of neutrophil granulocytes (APSA) in human serum by immunofluorescence on human granulocytes, fixed with ethanol and automated evaluation of results with the purpose of diagnosis of systemic vasculitis (SV).

The pathogenesis of SV is expressed in inflammatory processes of the walls of blood vessels (veins and arteries) and morphological changes. Clinical aspect is expressed through general symptoms: lethargy, fever and weight loss. In the further course of the disease clinical symptoms vary depending on the types of vessels.

Serological diagnosis of SV is based on the determination of antineutrophilic cytoplasmic antibodies (ANCA) by the indirect immunofluorescence on granulocytes, fixed in ethanol. Depending on the sample of fluorescence, there are cytoplasmic ANCA (cANCA) and perinuclear ANCA (pANCA). Ethanol fixation destroys membranes of granules in the cytoplasm of granulocytes; positively charged proteins tend to negatively-charged nucleus. Antibodies are reliably determined to antigens in the cytoplasm (cANCA) by cytoplasmic fluorescence. However, (peri)nuclear fluorescence is caused by antibodies and affects the structures of the cell nucleus; therefore, the pANCA antibodies shall be confirmed by granulocytes, fixed in formalin. On this substrate the separation of the antigen does not occur, ANCA reactions are observed only in the cytoplasm.

Table 1.
Granulocytes, fixed with
ethanol and formalin.

	Granulocytes, fixed with ethanol	Granulocytes, fixed with formalin
cANCA	cytoplasmic	cytoplasmic
pANCA	perinuclear	cytoplasmic
ANA	(peri)nuclear	(peri)nuclear (reduced fluorescence)

For immunofluorescence pattern of cANCA proteinase 3 (PR3) is identified as the main antigen, here we are talking about a serine-proteinase 29 kDa. PR3 autoantibodies are described as pathognomic to the Wegener disease. The pattern of pANCA, mainly, is based on the reaction with myeloperoxidase (MPO), 146 kDa protein. Antibodies MPO are usually found in a number of vasculites, such as microscopic form of angiopathy, Churg-Strauss syndrome and polyarteritis nodosa. Other target antigens of the antibodies of cANCA and pANCA are found in patients with heterospecific ulcerative colitis or primary sclerosing cholangitis.

The AKLIDES cANCA represents indirect immunofluorescence analysis for the qualitative and semi-quantitative determination of ANCA on granulocytes fixed with ethanol with an automated evaluation of the results.

At the first stage of the reaction, the antibodies in diluted patient samples or control serum specifically react with antigens on human granulocytes, fixed on the object carriers. Unbound components after 30 minutes of incubation at a room temperature are removed at the stage of rinsing.

IgG with light chains are bounded with fluorescein-isothiocyanate (FITC). Excess conjugate molecules after 30 minutes of incubation at a room temperature, are removed from the attached immune complexes by washing.

After covering object carriers in an automated process are transcribed under a fluorescent microscope (excitation wavelength is 490 nm, emission wavelength is 520 nm). In accordance with the histological structure of the antigens in the cells a specific pattern of fluorescence is determined (cytoplasmic, perinuclear).

Also the test-system AKLIDES pANCA, REF4072 was used made by company "Medipan GmbH", Germany, for 60 definitions – indirect immunofluorescence analysis for the detection of IgG antibodies to neutrophil cytoplasmic antigens (ANCA) in human serum. A titer of ≤ 20 – negative result. Titer ≥ 20 positive.

AKLIDES pANCA is a kit of reagents for (qualitative and semi-quantitative) determination of IgG antibodies to antigens in the cytoplasm of neutrophil granulocytes (ANCA) in human serum using indirect immunofluorescence on ethanol fixed human granulocytes for the purpose of systemic vasculitis (SV) diagnosis. The analysis is used to confirm positive results for pANCA on granulocytes fixed with ethanol. The principle of the method and its stages are similar to those in the set of AKLIDES cANCA.

Research results

Laboratory testing is one of the main methods of the objectification of many of AD, especially au-

toimmune rheumatic diseases (ARD), which are characterized by the variety of clinical manifestations [13, 14]. With early diagnosis of ARD thoughtful clinical examination of the patient has leading importance, which allows to eliminate other causes of symptoms and to suspect the diagnosis of AD.

Today, in autoimmune diagnostics two main approaches to laboratory testing are widely used: indirect reaction of immunofluorescence (RIF) on cells or tissue sections and immunometrics solid-phase methods, which include enzyme immuno-detection, radioimmunoassay, chemiluminescent and fluorescent analyses, as well as line/dot blotting and various options for analysis on immuno-reactive microparticles, which allow the selective testing with individual autoantigens [15]. Although solid-phase immunodeficiency method lends itself more easily to automation, the use of its results for screening is limited. This is due to the fact that the method of RIF with fixed cells allows to identify antinuclear antibodies (ANA) on "biological multiparametric chip", which allows to detect a lot more potential autoantigens than all available tests with purified or recombinant single antigens [16, 17]. Therefore, in accordance with international recommendations for initial ANA screening RIF should be used, in the case of testing using other clinical methods laboratory is required to provide the information about those single autoantigens that were used during the screening. A new promising direction is the integration of RIF and immunometric methods for combining the screening and confirmatory tests in one reaction field. To determine APLA, RF and anti-CCP immunometric methods are mainly used, but to identify some families of autoantibodies such as ANA and ANCA, the main screening method is RIF. Due to the identifying a maximum autoantibodies spectrum, RIF is the "gold standard" for the ANA detection in case of suspected ARD in accordance with the recommendations of experts of the European League against Rheumatism (EULAR) and the American College of Rheumatology (ACR) [18,19]. Despite the comparative ease, quickness and cheapness the main disadvantage of RIF is its subjectivity, since until recently, the test result was assessed by a doctor of clinical laboratory diagnostics. It was obstructing the comparison of results from different laboratories, the interpretation of titles and types of RIF luminescence. At the same time, quantitative instrumental measurement of the autoantibody level can be extremely important for assessing a course of the disease on the background of the conducted therapy. As far as the RIF is an obligatory method of screening, attempts were made to standardize this study with the help of automated microscopy and the use of computer programs of image recognition. In recent years an

Table 2.

The data of patients whose serum was investigated

Diagnosis	n%	Age	Sex
SLE	41% (7 patients)	Xav – 43y.	fem
SLE?	18% (3 patients)	Xav – 37y.	fem
Wegener disease	6% (1 patient)	Xav – 55y.	fem
Diagnosis not made	35% (6 patients)	Xav – 40y.	fem
	100% (17 patients)	Xav – 44y.	

objective and partly automated system was developed for accounting of autoantibodies detection results using RIF. The transition to instrumental evaluation of the RIF results can be considered as a component of measures to ensure quality as a part of international requirements for accreditation. Corresponding rooms, analytical equipment, qualified personnel with regular specialized advanced training, internal and external activities for quality assurance are just some of the central points from the requirements list for optimal autoimmune diagnosis. The main prerequisites for the standardization of RIF were methods of semi-automatic analysis of immunofluorescent images which formed the basis of a number of commercially available analytical systems for the RIF results interpretation [20]. An important feature of such systems is that despite the fact that the estimation of the signal is performed on the levels of fluorescence that are not sufficient for laboratory specialist subjective evaluation, but the resulting images allow us to avoid informational losses and to provide the best dynamic signal analysis [21]. Using image analysis systems we can automatically identify and classify different types of ANA, ANCA and detect anti-dsDNA antibodies [22, 23, 24]. Digital assessment of the immunofluorescence level, by means of calibration using test microparticles, allows to standardize the method of RIF [25, 26]. New developments in the field of automatic analysis of immunofluorescent images allow us to reach the previously unattainable analytical parameters of RIF, providing its comparison with other analytical techniques, including solid-phase immunometric analysis. It is important to emphasize that the proposed software and hardware solutions provide a sufficient degree of standardization only in compliance with the exact protocol, which includes the correct dilutions of serum and used immunofluorescent antisera, high quality of biological substrates used in RIF, as well as the time and conditions of the reagents incubation.

Currently, semi-automatic systems for the immunofluorescent images analysis are at the initial stage of introduction to the standard diagnostic practice. Although the use of software and hardware complexes is still relatively expensive and, furthermore, requires detailed exploration by the laboratory staff, however, their use leads to rapid development of ARD diagnostics by obtaining accurate and standardized results, the quality of which can compete with the quality of expert laboratories [27, 28, 29].

As a result of the analysis of the problem and the literature data on its solution the set task is to apply modern serological diagnostic method of RIF to assess serological activity of SLE using the apparatus AKLIDES made by the company "Medipan GmbH".

Epithelial finite cell line of the human larynx adenocarcinoma HEp-2 is widely used in the determination of antinuclear factor by indirect fluorescence method. HEp-2 cells are a very convenient substrate for laboratory studies, as they have large nuclei and grow on the glass in a single layer. ANA are detected during the bounding of intracellular antigens with cells HEp-2.

Depending on the evaluation results of the illumination type further tactics of patient treatment and appointment of additional research are being developed, clarifying the range of ANA.

In the studied group of patients there were identified diagnoses, which are shown in the Table 2.

The data of the fluorescence intensity obtained on the device AKLIDES when determining the fluorescence intensity of antinuclear antibodies (ANA), cANCA, pANCA in the sera of patients with rheumatic diseases was expressed in the minuses and pluses, and had a numeric expression: results were defined as negative (-), border (+/-), weakly positive (+), positive (++), strongly positive (+++), very strongly positive (++++). (Tables 2,3,4; figures 1,2,3).

Table 3.

Comparison of the fluorescence intensity of antinuclear antibodies (ANA) in sera of patients with rheumatic diseases

n%	-	+/-	+	++	+++	++++
-	6 (35,3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
+/-	0 (0)	1 (5,9)	0 (0)	0 (0)	0 (0)	0 (0)
+	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
++	0 (0)	0 (0)	0 (0)	1 (5,9)	0 (0)	0 (0)
+++	0 (0)	0 (0)	0 (0)	0 (0)	3 (17,6)	0 (0)
++++	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (35,3)

n%	-	+/-	+	++	+++	++++
-	7 (41,2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
+/-	0 (0)	3 (17,6)	0 (0)	0 (0)	0 (0)	0 (0)
+	0 (0)	0 (0)	2 (11,8)	0 (0)	0 (0)	0 (0)
++	0 (0)	0 (0)	0 (0)	2 (11,8)	0 (0)	0 (0)
+++	0 (0)	0 (0)	0 (0)	0 (0)	2 (11,8)	0 (0)
++++	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5,9)

In particular, during the definition of ANA in the patients' blood seras: in 6 cases (35,3%) negative results revealed; in 1 case (5,9%) – border result; 1 patient (5,9%) – positive result; 3 patients (17,6%) – strongly positive results; in 6 (35,3%) – a very positive results. In turn, in the study of cANCA in the patients' blood seras: in 7 (41,2%) the negative results were determined; 3 patients (17,6%) – border results; 2 (11,8%) – weakly positive results; 2 patients (11,8%) – positive results; 2 patients (11,8%) strongly positive results; in 1 patient (5,9%) there was a very positive result. Also during the study of pANCA in the patients' blood seras: in 13 (76,5%) – negative results; in 1 (5,9%) – border results; 2 (11,8%) – weakly positive results; in 1 (5,9%) – a positive result.

As can be seen from table 6 and figure 4, statistical quantitative criteria characterizing measures of central tendency and dispersion of variational series of antinuclear (ANA), antineutrophilic cytoplasmic ANCA (cANCA) and perinuclear ANCA (pANCA) antibodies indicate that antineutrophil cytoplasmic (ANCA) antibodies and cytoplasmic ANCA (cANCA) antibodies are approximately distributed according to normal Gauss-Laplace distribution law (asymmetry = 1,04-1,62; kurtosis = 0,65-1,1, respectively) with averages within $585,5 \pm 153,7$ (ANCA) and $222,3 \pm 88,9$ (cANCA). In contrast to the above variables, the variation series characterizing the quantitative distribution of perinuclear ANCA (pANCA) antibodies, have not been distributed by a normal distribution law (asymmetry = 3,3; kurtosis = 11,3), which implies the use of a number of nonparametric tests such as the median = 3 and interquartile range = 7 as evaluation measures of central tendency and dispersion of the considered variational series.

n%	-	+/-	+	++	+++	++++
-	13 (76,5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
+/-	0 (0)	1 (5,9)	0 (0)	0 (0)	0 (0)	0 (0)
+	0 (0)	0 (0)	2 (11,8)	0 (0)	0 (0)	0 (0)
++	0 (0)	0 (0)	0 (0)	1 (5,9)	0 (0)	0 (0)
+++	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
++++	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 4.

The comparison of fluorescence intensity of IgG antibodies to antigens in the cytoplasm of neutrophil granulocytes (cANCA) in seras of patients with rheumatic diseases.

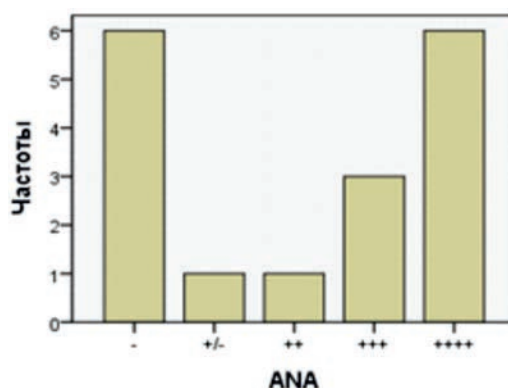


Fig. 1

Frequency of the fluorescence intensity during the determination of the fluorescence intensity of antinuclear antibodies (ANA) in seras of patients with rheumatic diseases. Translation of elements: Частоты – Frequencies.

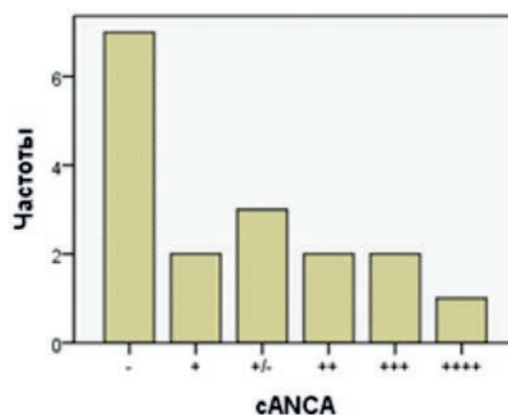


Fig. 2

Frequency of the fluorescence intensity of IgG antibodies to antigens in the cytoplasm of neutral granulocytes (cANCA) in seras of patients with rheumatic diseases. Translation of elements: Частоты – Frequencies.

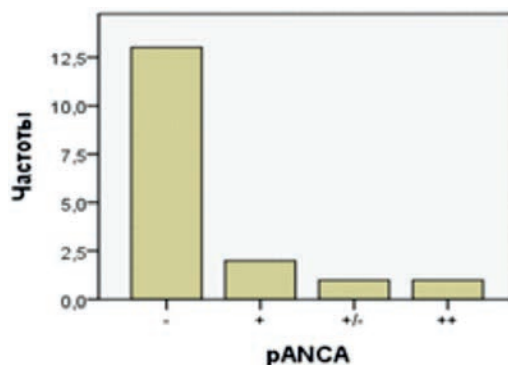


Fig. 3

Frequency of the fluorescence intensity of pANCA in seras of patients with rheumatic diseases. Translation of elements: Частоты – Frequencies.

Table 5.

Comparative table of the intensity of fluorescence of pANCA in seras of patients with rheumatic diseases

Table 6.
Quantitative characteristics of antinuclear (ANA), antineutrophilic cytoplasmic ANCA (cANCA) and perinuclear ANCA (pANCA) antibodies

			Statistics	Std. fault
ANA	Average		585,4476	153,70158
	95% confidence interval for the average	Lower limit	259,6148	
		Upper limit	911,2804	
	5% truncated average		531,8957	
	Median		545,5000	
	Dispersion		401611,004	
	Std. deviation		633,72786	
	Minimum		,00	
	Maximum		2134,83	
	Range		2134,83	
	Interquartile range		1014,95	
	Assymetry		1,036	,550
	Kurtosis		,651	1,063
pANCA	Average		18,4118	9,81822
	95% confidence interval for the average	Lower limit	-2,4019	
		Upper limit	39,2255	
	5% truncated average		11,4020	
	Median		3,0000	
	Dispersion		1638,757	
	Std. deviation		40,48157	
	Minimum		,00	
	Maximum		163,00	
	Range		163,00	
	Interquartile range		7,00	
	Assymetry		3,274	,550
	Kurtosis		11,325	1,063
cANCA	Average		222,2647	88,91090
	95% confidence interval for the average	Lower limit	33,7820	
		Upper limit	410,7474	
	5% truncated average		189,5719	
	Median		17,0000	
	Dispersion		134387,504	
	Std. deviation		366,58901	
	Minimum		,00	
	Maximum		1033,00	
	Range		1033,00	
	Interquartile range		317,00	
	Assymetry		1,617	,550
	Kurtosis		1,114	1,063

Discussion of obtained results

Thus, for the first time in Kazakhstan a survey on the apparatus AKLIDES was performed to determine autoimmune antibodies in order to clarify the diagnoses of the patients. New system AKLIDES provides the automatic reading of the images of IIF and includes software algorithms for the mathematical description of the indirect immunofluorescence of autoantibodies picture. Such a device allows to clarify the diagnosis of rheumatoid diseases during the screening and previously divide not organ-specific autoantibodies within the routine diagnostics of systemic autoimmune dis-

eases. Automatic system reduces financial costs, brings down the value of cellular IIF analyses and contributes to the reduction of interlaboratory analyses dispersion on the autoantibodies. At suspicion on systemic lupus erythematosus (SLE), Sjogren's syndrome, scleroderma, mixed connective tissue disease, autoimmune hepatitis, juvenile chronic arthritis and a number of other ARD, it is recommended to define antinuclear antibodies (ANA). High diagnostic value of their identification allows to include the ANA identification into the classification criteria of SLE and autoimmune hepatitis [30]. Diagnosis of rheumatoid arthritis

is based on the study of antibodies against cyclic citrullinated polypeptides and rheumatoid factor (RF). Repeated venous or arterial thromboses and pregnancy complications are caused by the appearance of antiphospholipid antibodies (APLA). In systemic vasculitis the anti-neutrophilic cytoplasmic antibodies (ANCA) are primary laboratory marker [31, 32, 33]. At suspicion on rapidly progressive glomerulonephritis detection of ANCA is the only extra test in the ARD diagnosis [34, 35]. The content of the majority of autoantibodies is the highest at the onset or relapse of AD on the background of high clinical activity.

Currently in Kazakhstan there is an urgent need for accurate diagnostics of autoimmune rheumatic diseases. So, correct diagnosis in the early stages of this disease will really help to stop the process of the disease progression and to improve life quality of patients [36].

Conclusions

1. Laboratory autoimmune diagnostics from a purely scientific investigation method is transforming into an independent section of clinical laboratory diagnostics.
2. The current state of knowledge and technological innovations, particularly in the area of digital analysis of immunofluorescence images, allow to provide the necessary standardization and harmonization of autoantibodies detection results.
3. Although the use of software and hardware complexes is still relatively expensive and, furthermore, requires detailed exploration by the laboratory staff, however, their use leads to rapid development of ARD diagnostics by obtaining accurate and standardized results, the quality of which can compete with the quality of expert laboratories.

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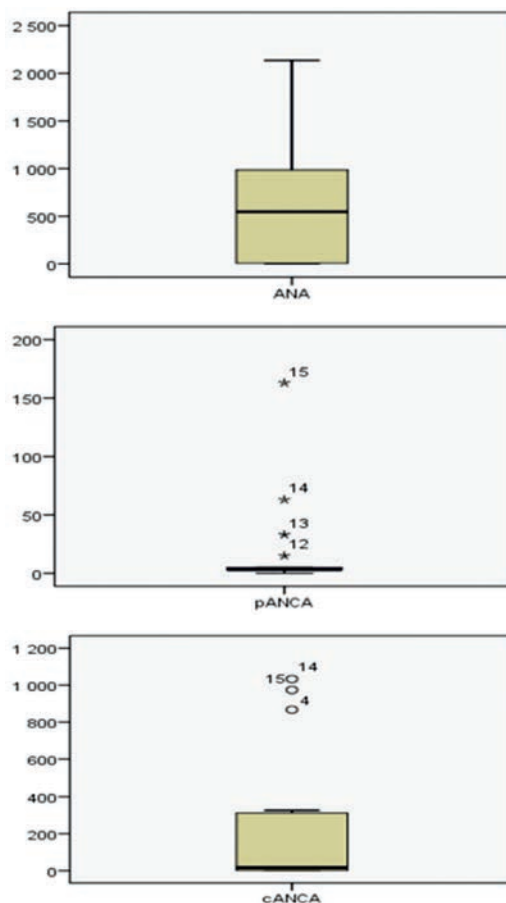


Figure 4. Indicators of central tendency and dispersion of antinuclear (ANA), antineutrophilic cytoplasmic ANCA (cANCA) and perinuclear ANCA (pANCA) antibodies.

Note: *, 0 – pop-up values of the variational series.

4. For the first time in Kazakhstan a survey on the apparatus AKLIDES was performed to determine autoimmune antibodies in order to clarify the diagnoses of the patients.
5. New system AKLIDES provides the automatic reading of the images of IIF and includes software algorithms for the mathematical description of the indirect immunofluorescence of autoantibodies picture.

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