Modern diagnostic approaches

for early detection of antiphospholipid syndrome

Enfoques diagnósticos modernos para la detección temprana del síndrome antifosfolípido

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Abstract

Currently, Antiphospholipid syndrome is a multidisciplinary problem, as it is one of the causes of death and disability of patients. Cardiovascular diseases occupy a leading position among the causes of mortality. The reproductive function of women determines not only the quality of their life and offspring, but also the health and quality of life of the nation. Based on a comprehensive assessment of the results of clinical, laboratory and instrumental studies, general and distinctive features of the primary and secondary Antiphospholipid syndrome are shown, on which their differential diagnosis is based. The use of a multiplex test - immunoblotting will reliably reveal the primary Antiphospholipid syndrome.

Keywords: primary antiphospholipid syndrome, immunoblotting, antibodies, autoimmune diseases, diagnosis, clinical manifestations of APS.

Resumen

En la actualidad, el síndrome antifosfolípido es un problema multidisciplinario, ya que es una de las causas de muerte y discapacidad de los pacientes. Las enfermedades cardiovasculares ocupan un lugar destacado entre las causas de mortalidad. La función reproductiva de la mujer determina no solo la calidad de su vida y su descendencia, sino también la salud y la calidad de vida de la nación. Basado en Evaluación integral de los resultados de estudios clínicos, de laboratorio e instrumentales, se muestran los rasgos generales y distintivos del síndrome antifosfolípido primario y secundario, en los que se basa su diagnóstico diferencial. El uso de una prueba múltiple: la inmunotransferencia revelará de manera confiable el síndrome antifosfolípido primario.

Palabras clave: síndrome antifosfolípido primario, inmunotransferencia, anticuerpos, enfermedades autoinmunes, diagnóstico, manifestaciones clínicas del SAF.

Introduction

Pathologies of the cardiovascular system, both in the country and in the world, still occupy the first place among the causes of disability and mortality. From the literature, in the pathogenesis of myocardial infarction there are cases of its occurrence in patients

who do not have signs of pronounced atherosclerosis, and the presence of antibodies to phospholipids. The frequency of recurrences of such thrombosis occurred precisely in young people up to 55 years old and, according to various sources, is 19-29% per year. More often APS affects young, able-bodied patients, including young pregnant women. Reproductive function is the most important integral indicator of a woman's health and determines not only the quality of her life and offspring, but also the health and quality of life of a nation[1].

The medical and social significance of the problem of not carrying a pregnancy, its influence on the indices of perinatal

morbidity and mortality, the reproductive health of the female population puts research in this area among the most important tasks of modern fundamental and clinical medicine[2]. Cases of uncarried pregnancy among women have now reached 27-42%, and without adequate therapy, fetal death is observed in 90-95% of women with autoantibodies to phospholipids. Without treatment, the risk of losing a subsequent pregnancy in women with APS reaches 80-90%. However, there is immune thrombocytopenia and/or neurological disorders (ischemic stroke, seizures, chorea, visual and peripheral neuropathy, myelopathy). Thus, APS is a multidisciplinary problem.

According to the latest data, the diagnosis of APS is reliable with the obligatory combination of clinical signs with laboratory markers. Clinical criteria include thrombosis of a vessel of any caliber and localization (venous and/or arterial, or microvasculature) and obstetric pathology (fetal loss syndrome). Specifications regarding some clinical manifestations have been made: thrombocytopenia, reticular liver disease, valvular heart defects, nephropathy and neurological manifestations, which are included in the diagnostic criteria for APS[3]. Laboratory markers of APS are antiphospholipid antibodies (APLA), with lupus anticoagulant (LA) being the most informative, moderate or high antibodies to cardiolipin titers of G or M isotypes (at least in two studies separated by at least 12 weeks' intervals) and to P2 glycoprotein-1 (anti-p2-GP-1)[4]. The use of the SLICC/ACR damage index in patients with SLE showed that APS was a reliable predictor of a more severe course of the underlying disease. SAPS is described in terms of systemic lupus erythematosus (SLE)[5-8].

Despite significant advances in the study of the clinical picture and laboratory diagnosis of APS and the progress in the study of the pathogenesis and treatment caused by the action of antiphospholipid antibodies, the primary APS remains an open question. There are insufficient data on the diagnostic and prognostic value of clinical and laboratory parameters in this pathology, according to the treatment carried out to the mechanisms of its development[9-12].

Purpose of research is to study the features of antibody Genesis in patients with primary antiphospholipid syndrome (PAPS) and develop proposals for improving the diagnosis and treatment of PAPS.

The intensive development of clinical immunology allows a new approach to the evaluation of the participation of autoimmune reactions in the implementation of these changes. Antiphospholipid syndrome (APS) was first described by G. R. V. Hughes and others co-authors in 1986 and attracted the attention of clinicians in various fields of medicine[13-17]. Since then, the number of published works on the study of the APS has been steadily increasing.

In the pathogenesis of APS, the leading role belongs to organospecific autoantibodies reacting with antigenic determinants of phospholipids-antiphospholipid antibodies[18]. The family of phospholipids-antiphospholipid antibodies include a patient group of antibodies: antibodies that cause false positive reaction of Wasserman (reagins); antibodies that contribute *in vitro* to inhibit phospholipid-dependent coagulation reactions - lupus anticoagulant (LA); antibodies that react with immobilized solid phase cardiolipin, with other negatively charged or neutral phospholipids or complex phospholipids and various protein molecules that are detected by radioimmunological or immunoenzymometric methods[19-22].

The phospholipids-antiphospholipid antibodies detection is associated with the development of a clearly defined symptom complex characterized by a triad of clinical and laboratory signs: recurrent venous or arterial thrombosis with localization in any part of the bloodstream, obstetric pathology in the form of habitual miscarriage and intrauterine fetal death, hematological disorders (thrombocytopenia, hemolytic anemia)[23].

APS was first described in the framework of systemic lupus erythematosus (SLE), but then it was found that the relationship between phospholipids-antiphospholipid antibodies hyperproduction and thrombotic disorders is universal, and observed in the absence of reliable clinical and serological criteria for this or any other leading disease. For the definition of new nosological forms have been suggested the term primary APS (PAPS)[24]. Today it is considered competent to allocate and other forms of APS-catastrophic, seronegative, APS in patients with lupus-like conditions[25].

Clinical and morphological studies indicate that the basis of APS is a kind of vasculopathy associated with thrombotic and/or occlusive vascular lesions. The spectrum of clinical manifestations of antiphospholipid vasculopathy is no less diverse than in other universal form of vascular pathology - systemic vasculitis. At the same time, unlike vasculitis or atherosclerosis, there are no marked inflammatory or degenerative changes in the vascular wall, which emphasizes the nosological independence of the APS[26].

In General, the analysis of the totality of the currently available facts allows to consider the APS as a unique model of autoimmune thrombotic vasculopathy, the study of which is essential to decipher the relationship between such fundamental pathological processes as atherosclerosis, vasculitis, blood clotting disorders and the immune system. At the same time, the diagnosis and treatment of APS is based on serological, hemostasiological tests, little attention is paid to the immunological markers of this pathology, the role of infections in the development and maintenance of the pathological process in APS is insufficiently studied.

The role of etiological factors, triggering mechanisms, including at the initial stages of APS formation is not clearly defined; morphological markers of the disease are poorly studied, effective schemes of APS treatment are not formed.

As you know, the diagnosis of APS is extremely difficult, many clinical features are described, but the real frequency of most of these manifestations is unknown. There are other questions: what are the associative relationships the main features of the APS, what is the risk of development or transformation in different clinical situations, as the association with SLE, or age at the beginning of the disease may modify the severity of the disease and identify some specific subgroups of APS. Thus, there is a need to solve the above-mentioned

problems, which is important for a comprehensive examination of patients in order to clarify the pathogenesis and more complete and early diagnosis of different variants of APS and determine the optimal tests for monitoring therapy and creating effective correction schemes.

The underlying causes of antiphospholipid syndrome are unknown. Meanwhile, studied and identified factors predisposing to increased levels of antibodies to phospholipids. Thus, the transient increase of antiphospholipid antibodies is observed against the background of viral and bacterial infections (hepatitis C, HIV, infectious mononucleosis, malaria, infectious endocarditis, etc.). High titers of antibodies to phospholipids are found in patients with systemic lupus erythematosus, rheumatoid arthritis, Sjogren's disease, scleroderma, nodular periarteritis, autoimmune thrombocytopenic purpura[27-30].

Hyperproduction of antiphospholipid antibodies can be observed in malignant tumors, taking medicines (psychotropic drugs, hormonal contraceptives, etc.), cancellation of anticoagulants. There is evidence of a genetic susceptibility to an increased synthesis of antibodies to phospholipids in individuals carrying HLA DR4, DR7, DRw53 antigens and in relatives of patients with antiphospholipid syndrome. In general, the immunobiological mechanisms of the development of the antiphospholipid syndrome require further study and clarification[29].

Depending on the structure and immunogenicity, there are "neutral" (phosphatidylcholine, phosphatidylethanolamine) and "negatively charged" (cardiolipin, phosphatidylserine, phosphatidyl inositol) phospholipids. The class of antiphospholipid antibodies that react with phospholipids include lupus anticoagulant, antibodies to cardiolipin, beta2-glycoprotein-1-cofactor-dependent antiphospholipids, etc[25-28].

Taking into account the etiopathogenesis and course, the following clinical and laboratory variants of the antiphospholipid syndrome are distinguished:

- primary - there is no connection with any background disease capable of inducing the formation of antiphospholipid antibodies;
- secondary - antiphospholipid syndrome develops on the background of another autoimmune pathology;
- catastrophic - acute coagulopathy, occurring with multiple thrombosis of internal organs;
- The APS-negative variant of the antiphospholipid syndrome, in which the serological markers of the disease (Antibodies to cardiolipin and lupus anticoagulant) are not detected.

According to modern views, antiphospholipid syndrome is an autoimmune thrombotic vasculopathy. In APS, the lesion can affect vessels of different caliber and localization (capillaries, large venous and arterial trunks), which causes an extremely diverse range of clinical manifestations, including venous and arterial thrombosis, obstetric pathology, neurological, cardiovascular, skin disorders, thrombocytopenia. The most frequent and typical signs of antiphospholipid syndrome are recurrent venous thrombosis: thrombosis of the superficial and deep veins of the lower extremities, hepatic veins, portal vein of the liver, retinal veins. In patients with antiphospholipid syndrome, repeated episodes of pulmonary artery thromboembolism, pulmonary hypertension, superior vena cava syndrome, Budd-Chiari syndrome, adrenal insufficiency may occur. Venous thrombosis with antiphospholipid syndrome develops 2 times more often arterial. Among the latter, thrombosis of cerebral arteries prevails, leading to transient ischemic attacks and ischemic stroke. Other neurological disorders may include migraine, hyperkinesis, convulsive syndrome, neurosensory hearing loss, ischemic neuropathy of the optic nerve, transverse myelitis, dementia, mental disorders[15,16].

The defeat of the cardiovascular system in antiphospholipid syndrome is accompanied by the development of myocardial infarction, intracardiac thrombosis, ischemic cardiomyopathy, arterial hypertension. Valve damage to the heart valves is often noted, ranging from minor regurgitation detected by an echocardiogram to mitral, aortic, tricuspid stenosis or insufficiency. As part of the diagnosis of antiphospholipid syndrome with cardiac manifestations, a differential diagnosis with infective endocarditis, myxoma of the heart is required[7,8].

Renal manifestations may include both minor proteinuria and acute renal failure. Gastrointestinal organs in antiphospholipid syndrome occur hepatomegaly, gastrointestinal bleeding, occlusion of mesenteric vessels, portal hypertension, spleen infarction. Typical lesions of the skin and soft tissues are represented by reticular livedo, palmar and plantar erythema, trophic ulcers, gangrene of the fingers; musculoskeletal system - aseptic necrosis of the bones (femoral head). Hematologic signs of antiphospholipid syndrome are thrombocytopenia, hemolytic anemia, hemorrhagic complications[14-16].

APS is often detected in connection with obstetric pathology in the female sex: repeated spontaneous abortion at various times, intrauterine growth retardation, fetoplacental insufficiency, gestosis, chronic hypoxia of the fetus, preterm labor. When conducting pregnancy in women with antiphospholipid syndrome, an obstetrician-gynecologist should consider all possible risks[2,3].

Antiphospholipid syndrome is diagnosed on the basis of clinical (vascular thrombosis, aggravated obstetric history) and laboratory data. The main immunological criteria include the detection in plasma of medium or high titers of Ig to cardiolipin Ig class IgG and IgM and lupus anticoagulant twice within six weeks. The diagnosis is considered reliable when combining at least one of the main clinical and laboratory criteria[7]. Additional laboratory signs of the antiphospholipid syndrome include false positive RW, a positive Coombs reaction, an increase in the titer of antinuclear factor, rheumatoid factor, cryoglobulins, antibodies to DNA. The study also shows a complete blood count, platelet count, biochemical blood test, coagulogram[11-13].

Pregnant women with antiphospholipid syndrome need to monitor indicators of blood coagulation, conduct dynamic ul-

trasound of the fetus and dopplerography of uteroplacental blood flow, cardiography. To confirm the internal thrombosis of the internal organs, Ultrasonic Dopplerography of the vessels of the head and neck, vessels of the kidneys, arteries and veins of the extremities, ocular vessels, etc. is performed. Changes in the cusps of the heart valves are detected during the echocardiogram. Differential-diagnostic measures should be aimed at eliminating the syndrome of disseminated intravascular coagulation, hemolytic-uremic syndrome, thrombocytopenic purpura, etc. Given the polyorganism of the lesion, the diagnosis and treatment of the antiphospholipid syndrome require the combined efforts of physicians of various specialties: rheumatologists, cardiologists, neurologists, and their specialists, and they will work in the field of non-phospholipid syndrome and other[12].

The main goal of antiphospholipid syndrome therapy is the prevention of thromboembolic complications. Regime moments provide for moderate physical activity, refusal to remain stationary for a long time, to engage in traumatic sports and long flights[14-17]. Women with antiphospholipid syndrome should not be prescribed oral contraceptives, and before planning pregnancy it is necessary to consult an obstetrician-gynecologist. Pregnant patients during the entire period of gestation are shown taking small doses of glucocorticoids and antiplatelet agents, the introduction of immunoglobulin, heparin injections under the control of hemostasiogram indicators[5]. Drug therapy for antiphospholipid syndrome may include the administration of indirect anticoagulants (warfarin), direct anticoagulants (heparin, nadroparin calcium, enoxaparin sodium), antiplatelet agents (acetylsalicylic acid, dipyridamole, pentoxifylline)[18,19]. Prophylactic anticoagulant or antiplatelet therapy most patients with antiphospholipid syndrome is carried out for a long time, and sometimes for life. In the catastrophic form of the antiphospholipid syndrome, administration of high doses of glucocorticoids and anticoagulants, holding plasmapheresis, transfusion of fresh frozen plasma, etc. are shown[11].

Timely diagnosis and preventive therapy can avoid the development and recurrence of thrombosis, as well as hope for a favorable outcome of pregnancy and childbirth. In case of secondary antiphospholipid syndrome, it is important to monitor the course of the main pathology, prevention of infections. Prognostically unfavorable factors are the combination of antiphospholipid syndrome with SLE, thrombocytopenia, a rapid increase in the titer of antibodies to cardiolipin, persistent arterial hypertension. All patients diagnosed with “antiphospholipid syndrome” should be monitored by a rheumatologist with periodic monitoring of serological markers of the disease and hemostasiogram indicators.

Materials and Methods

As a target group, cardiological (300) and patients with obstetric pathology of the patient (with 2 and more cases of not carrying of a pregnancy) were identified (50). The control group consisted of 50 people with SLE (secondary APS). All patients are covered by clinical examination, general clinical and biochemical laboratory tests (CBC, C-reactive protein, coagulation, determination of blood to the APL antibodies, antibodies to cardiolipin of IgG or IgM classes; lupus anticoagulant; antibodies to β2-glycoprotein-1 (IgG, IgM); functional and instrumental methods (ECG/ daily Holter ECG monitoring) were used, Echocardiography/Doppler-Echocardiography, brain MRI, stress test and ultrasound sonography, the determination of the levels antibodies to cardiolipin and antibodies to prothrombin, lipid profile) as well as the study of autoimmune antibodies using an automated diagnostic system AKLIDES®.

1. Antiphospholipid syndrome (PAPS and SAPS) is characterized by clinical pathogenetic polymorphism, which determines various clinical subtypes of the disease.
2. The leading role in the pathogenesis of APS is played by systemic immune circulatory disorders with the development of thrombotic vasculopathy and the formation of secondary visceropathy.
3. Patients with primary and secondary APS differ in the frequency of development of a number of clinical manifestations: patients with primary APS with a high level of lupus anticoagulant have a high risk of formation of mesh livedo, miscarriage, recurrent thrombophlebitis, ocular ischemia, pulmonary hypertension, autoimmune thyroiditis, polyneuropathy. In patients with secondary APS, the reverse pattern was established. In the clinical manifestations of secondary APS is dominated by the syndromes, typical for the underlying disease: nephritis, coupled with high anticoagulant activity. In persons with the primary form of the disease there is an early debut of Raynaud’s syndrome, mesh livedo and burdened heredity.
4. When analyzing the pathology of the cardiovascular system in persons with primary APS, compared with the secondary, vegetations on the heart valves, insufficiency and/or stenosis of the mitral, aortic heart valves, pulmonary hypertension, manifestations of intra-cardiac thrombosis were more often found. In secondary APS, changes in heart valves were much less common, clinical manifestations of the underlying disease - arterial hypertension of renal Genesis and associated cardiomyopathy-prevailed. In APS with a high level of lupus anticoagulant, strong associations with the development of following clinical symptoms were noted: thickening, fibrosis and calcification of the valves and walls of the heart, mitral valve insufficiency and/or stenosis, cardiomyopathy, hypertension.
5. Patients with primary and secondary APS had significant differences in the frequency and localization of vascular lesions of the Central nervous system: the primary form of APS was dominated by ischemic stroke, transient disorders of cerebral circulation, migraine, mental disorders,

encephalopathy and convulsive syndrome, which significantly exceeded this pathology in secondary APS. The increased content of lupus anticoagulant in persons with secondary APS is associated with the risk of migraine, stroke, mental disorders and encephalopathy.

6. Established Association of increased fibrinogen content, gray-mucoid, C-reactive protein, cholesterol, white blood cells, accelerate the rate of sedimentation of erythrocytes in peripheral blood with the risk of development of secondary APS; patients with primary APS detected the opposite relationship.
7. The state of the blood coagulation and anticoagulation system in patients with primary and secondary forms of APS is characterized by similar disorders. Discovered a "hidden" hyper aggregation of platelets and leukocytes, inhibition of fibrinolysis, tendency to a deficiency of antithrombin-III and the prevalence of normo-and hypo coagulation. In the secondary form of APS, a high degree of hyperfibrinogenemia, thrombocytopenia and an increased content of antithrombin-III were noted.
8. Antiphospholipid syndrome revealed similar and multidirectional disorders of the immune system. All patients had serological markers of APS, the content and activity of subpopulations of T - and b- lymphocytes did not differ from the reference values. In patients with secondary APS, serological markers of SLE (LE-cells, rheumatoid factor, antibodies to native DNA), elevated Values of f-c dependent phagocytosis by neutrophils were more often detected. In patients with secondary APS with a high content of lupus anticoagulant, in contrast to patients with low content, an increase in CD4+ T-lymphocytes and a decrease in CD20+ b-lymphocytes of peripheral blood were revealed.
9. In APS (on the bioptates of the musculoskeletal flap), the leading event of morphogenesis is endotheliopathy of the microcirculatory bed, the essence of which is the permanent processes of degradation and regeneration of endotheliocytes and compensatory proliferative reactions of perivascular cells. As a result, there are secondary metabolic changes - dystrophy and atrophy of the epidermis, disorganization and subsequent fibrosis of connective tissue, focal atrophy of skeletal myocytes.
10. In secondary APS associated with systemic lupus erythematosus, primary and most pronounced changes were found in endothelial associations of periglomerular arterioles. Among game-root item the earliest signs of alteration shall also be recorded in the population of endothelial cells that is accompanied by compensatory proliferation of mesangial, the overproduction of mesangial Mat-RIX and metaplasia of the podocytes. Biosynthetic reactions (according to radioautography in vitro) reflect structural and functional heterogeneity of endothelial cells caused by a combination of damage and regeneration processes.

Antiphospholipid syndrome as a systemic autoimmune disease with damage to the internal organs can manifest itself as a clinical picture of cardiovascular, rheumatological, neurological, nephrological and obstetric diseases, which requires a General practitioner and specialists' knowledge of this new pathology. The recommended clinical and laboratory criteria lead to the timely diagnosis of primary and secondary forms of APS. Determination of the level of lupus anticoagulant as a marker of alterative and autoimmune disorders is leading to characterize the pathological process and prognosis. To correct, the APS recommended antiplatelet agents (acetylsalicylic acid, ticlopidine), anticoagulants and immunosuppressive therapy for secondary APS[8-13].

It is important for the practitioner to know that early diagnosis and pathogenetic correction lead to the prevention of severe complications of APS (lesions of the cardiovascular system, CNS, kidneys, obstetric pathology, etc.). Identification of risk indices in patients with APS allows to rank the signs of their contribution to the development of PAPS and SAPS, as well as to calculate the total individual risk of transformation of PAPS and SAPS[23,24].

The diagnosis of APS is based on certain combinations of clinical signs and titers of antiphospholipid antibodies (Table 1).

There are the following main forms of APS:

- APS in patients with a reliable diagnosis of SLE (secondary APS);
- APS in patients with lupus-like manifestations;
- primary APS;
- catastrophic APS (acute disseminated coagulopathy / vasculopathy) with acute multiorgan thrombosis;
- other microangiopathic syndromes (thrombotic thrombocytopenic purpura); HELLP syndrome (hemolysis, increased liver enzymes, decreased platelet count, pregnancy); DIC syndrome;
- seronegative APS.

The course of APS, the severity and prevalence of thrombotic complications are unpredictable and, in most cases, do not correlate with changes in titers antiphospholipid antibodies and SLE activity (with secondary APS). In some patients, APS is manifested predominantly by venous thrombosis, in others - by stroke, in the third - by obstetric pathology or thrombocytopenia. It is believed that about half of patients with APS suffer from the primary form of the disease. However, the question of the nosological independence of the primary APS is not completely clear. There is evidence that the primary APS can sometimes be a variant of the onset of SLE. On the contrary, in some patients with classic SLE in the debut, further signs of APS may come to the fore.[27-30]

Table 1. Diagnostic criteria for APS.

Clinical	Lab
Venous thrombosis	IgG Antibodies to cardiolipin (moderate/ high titer)
Arterial thrombosis	IgM Antibodies to cardiolipin (moderate/ high titer)
Habitual miscarriage	Positive lupus anticoagulant test
Thrombocytopenia	

Note. To make a diagnosis of APS, you must have at least one (any) clinical and one (any) laboratory sign; antiphospholipid antibodies should be detected at least twice within 3 months.

The prevalence of APS in the population is unknown. Antibodies to cardiolipin are found in serum in 2–4% (in high titer - less than in 0.2% of patients), more often older than younger age[29,30]. Antiphospholipid antibodies are sometimes found in patients with inflammatory, autoimmune and infectious diseases (HIV infection, hepatitis C, etc.), in patients with malignant tumors, while taking medication (oral contraceptives, psychotropic drugs, etc.). The disease often develops at a young age than in the elderly, it is described in children and even in newborns. In the general population, APS is more commonly detected in women. However, among patients with primary APS there is an increase in the proportion of men. Clinical manifestations of APS develop in 30% of patients with lupus anticoagulant and in 30–50% of patients with moderate or high levels of IgG and Antibodies to cardiolipin. antiphospholipid antibodies were found in 21% of young patients who had myocardial infarction, and in 18–46% of stroke patients, in 12–15% of women with recurrent spontaneous abortions, and in about one third of patients with systemic lupus erythematosus. thrombosis increases to 60 - 70%, and in their absence decreases to 10 - 15%[7-11].

Since the basis of vascular pathology in APS is non-inflammatory thrombotic vasculopathy, affecting vessels of any caliber and localization, from capillaries to large vessels, including the aorta, the spectrum of clinical manifestations is extremely diverse. The APS describes the pathology of the central nervous system, cardiovascular system, impaired renal function, liver, endocrine organs, and the gastrointestinal tract (GIT). Placental thrombosis of blood vessels tends to associate the development of some forms of obstetric pathology (Table 2).

Table 2. The main clinical manifestations of APS

Arterial occlusion	Gangrene of the extremities, stroke, aortic occlusion, heart attacks of internal organs
Venous occlusion	Peripheral venous thrombosis, venous thrombosis of internal organs, including Budd-Chiari syndrome, portal vein thrombosis and adrenal insufficiency
Miscarriage of pregnancy	Recurrent unexplained spontaneous abortions in the first trimester or loss of the fetus in the second or third trimester; HELLP syndrome.
Hematologic complications	Thrombocytopenia, Coombs-positive hemolytic anemia, thrombotic microangiopathic hemolytic anemia
Skin manifestations	Livedo reticularis, leg ulcers and others.
Neurological (non-stroke related)	Chorea, convulsions, cerebral ischemia, multiple sclerosis syndrome, migraine
Renal impairment	Renal failure, Arterial hypertension
Heart damage	Valvular heart disease, myocardial infarction, intracardiac thrombosis
Bone disorders	Aseptic necrosis, transient osteoporosis (?)
Catastrophic APS	Renal failure with hypertension, pulmonary insufficiency, neurological disorders, respiratory distress syndrome, peripheral gangrene

A characteristic feature of APS is the frequent recurrence of thrombosis. It is noteworthy that if the first manifestation of APS was arterial thrombosis, then in the majority of patients arterial thrombosis was observed, and in patients with first venous thrombosis venous recurrences recur[29].

Venous thrombosis is the most common manifestation of APS. Blood clots are usually localized in the deep veins of the lower extremities, but often in the hepatic, portal veins, superficial and other veins. Recurrent embolism from the deep veins of the lower extremities to the lungs, sometimes resulting in pulmonary hypertension, is characteristic. APS (more often primary than secondary) is the second most common cause of Budd-Chiari syndrome. Thrombosis of the central adrenal vein can lead to adrenal insufficiency[13-15].

Thrombosis of intracerebral arteries, leading to stroke and transient ischemic attacks, is the most common localization of arterial thrombosis in APS. Recurrent ischemic microstrokes sometimes occur without bright neurological disorders and can manifest convulsive syndrome, multi-infarction dementia (resembling Alzheimer's disease), mental disorders. A variant of APS is Sneddon syndrome. This concept includes recurrent cerebral vascular thrombosis, reticular living, as well as arterial hypertension (AH). Other neurological disorders, including migraine headaches, epileptiform seizures, chorea, transverse myelitis, which, however, cannot always be associated with vascular thrombosis, are described. Sometimes neurological disorders in APS resemble those in multiple sclerosis[30].

One of the frequent cardiac signs of APS is damage to the heart valves, which varies from minimal disturbances detected only during echocardiography (slight regurgitation, thickening of the valve leaflets), to severe heart defects (stenosis or mitral insufficiency, rarely aortic or tricuspid valves). Some patients quickly develop very severe valve damage with vegetation caused by thrombotic layers, indistinguishable from infective endocarditis. Vegetations on the valves, especially if they are combined with hemorrhages in the subungual bed and fingers in the form of "drumsticks", impede differential diagnosis with infective endocarditis. The development of cardiac blood clots that mimic myxoma of the heart has been described. Coronary artery thrombosis is one of the possible localizations of arterial thrombosis associated with the synthesis of antiphospholipid antibodies[15-19]. Another form of coronary pathology in APS is acute or chronic recurrent thrombosis of small intramyocardial coronary vessels that develops in the absence of signs of inflammatory or atherosclerotic lesions of the main branches of the coronary arteries. It is believed that this process may lead to myocardial pathology resembling cardiomyopathy with signs of regional or general myocardial contractility and left ventricular hypertrophy. Frequent complication of APS is hypertension, which can be labile, often associated with reticular liver disease and damage to the cerebral arteries in the framework of Sneddon syndrome, or stable, malignant, manifested by symptoms of hypertensive encephalopathy[24,25]. The development of hypertension in APS can be attributed to many causes, including renal vascular thrombosis, renal infarction, abdominal aortic

thrombosis ("pseudocarctation") and intraglomerular renal thrombosis. The relationship between hyperproduction of antiphospholipid antibodies and the development of fibromuscular dysplasia of the renal arteries is noted. Kidney damage in APS is associated with intraglomerular micro thrombosis and is defined as "renal thrombotic microangiopathy". It is believed that the glomerular micro thrombosis is the cause of the subsequent development of glomerulosclerosis, leading to impaired renal function[28-30].

A rare complication of APS is thrombotic pulmonary hypertension associated with both recurrent venous embolism and local (in situ) pulmonary vascular thrombosis. When examining patients with primary pulmonary hypertension, we found an increase in the level of antiphospholipid antibodies only in patients with veno-occlusive disease and thrombosis of pulmonary vessels[28]. Several patients with primary APS have been described, in whom the lung lesion was characterized by alveolar hemorrhages, pulmonary capillaritis and microvascular thrombosis, up to the development of a "shock" lung[9-11]. One of the most characteristic signs of APS is obstetric pathology: habitual miscarriage, recurrent spontaneous abortions, fetal death, pre-eclampsia. Among women with APS, the incidence of obstetric pathology reaches 80%. Fetal loss can occur at any time during pregnancy, but more often in the first trimester than in the second and third. In addition, the synthesis of antiphospholipid antibodies is associated with other forms of obstetric pathology, including late gestosis, preeclampsia and eclampsia, fetal intrauterine growth retardation, preterm labor. The development of thrombotic complications in newborns from mothers with APS has been described, which indicates the possibility of transplacental transmission of antiphospholipid antibodies[7,8].

Skin lesions in APS are characterized by a variety of clinical manifestations, such as reticular liver, skin ulcers, pseudo vascular and vasculitic lesions[16,17]. An increase in the level of antiphospholipid antibodies in Dego's disease, a very rare systemic vasculopathy, manifested by common skin thrombosis, the central nervous system and the gastrointestinal tract has been described. A typical hematological sign of APS is thrombocytopenia. Usually, the number of platelets decreases moderately (70 000 - 100 000/mm3) and does not require special treatment. The development of hemorrhagic complications is rarely observed and, as a rule, is associated with a concomitant defect of specific blood coagulation factors, renal pathology, or an overdose of anticoagulants. Often, Coombs-positive hemolytic anemia is observed, Evans syndrome (a combination of thrombocytopenia and hemolytic anemia) is less common[23-27].

Results and Discussion

The statistical analysis of the clinical picture, immunological status, biochemical parameters, hemostasis and other laboratory data in the studied patients was carried out. Thus, among the main clinical manifestations and pathophysiological indicators of higher risk paths, compared to SAPS obtained for the miscarriage of pregnancy, recurrent thrombophlebitis, ocular ischemia, migraine, pulmonary hypertension, thickening, fibrosis/calcification of valves and walls of the heart, pathology of the mitral valve in the form of its insufficiency or stenosis, autoimmune thyroiditis II degree, increased cholesterol levels, low-level al-globulin, leukocyte-platelet of hyper aggregation; in addition, PAPS is characterized by an earlier manifestation of clinical manifestations of APS (migraine and Raynaud's syndrome) and burdened heredity.

While high risk indices of SAPS with moderate and high levels of lupus anticoagulant were established for nephritis, encephalopathy, hypertension, hyperfibrinogenemia, seromucoid, hyperleukocytosis, acceleration of erythrocyte sedimentation rate, increase in C-reactive protein, hypercholesterolemia, monocyte activity index, LE-cell content, rheumatoid factor and antibodies to native DNA.

Common methods of hemostatic system investigation (Activated partial thromboplastin time, prothrombin time) are not informative, and in most cases, they are uninformative for thrombophilia diagnosis[6,7]. Thrombocytopenia occurs in 27.7% of cases of primary APS and 22.4% of cases of secondary APS ($p<0.05$).

1. *Changes in functional immunological tests, so-called non-territorial specific tests, in patients with PAPS:*

- the presence of Antibodies to cardiolipin, antibodies to P2-glycoprotein 1, antibodies to phosphatidylethanolamine and lupus anticoagulant;
- the presence of anticardiolipin antibodies and lupus anticoagulant in some cases, was not observed (seronegative patients); patients with negative tests for lupus anticoagulant and anticardiolipin antibodies had antibodies to the other sub-group of phospholipids (antiphosphotyrosine, 5 antiphosphorylcholine, antiphosphotyrosine, antiphosphotyrosine, antiphosphatidylethanolamine, antibodies to phosphatidic acid) or proteins of the cofactors (protein C and S, (32-glycoprotein I, prothrombin, annexin V, high and low molecular weight kininogens). In these cases, the diagnosis of PAPS is doubtful.
- increased C-reactive protein was observed in most patients;

  patients were found to accelerate the rate of erythrocyte sedimentation and increase the activity of serum ACT and ALT;
- thrombocytopenia was characterized by a decrease in the number of platelets less than 150x109/l;
- in patients with acute myocardial infarction, there was an increase in IgG titer than in those with stable angina, as well as in those over 70 years;
- in patients with acute myocardial infarction with Q wave, the percentage of Increased IgG titer is 2.3 times higher than in patients with AMI without Q wave;

blood IgM levels were within normal range;

- at the level of IgG-Antibody to cardiolipin more than 40 GPL there were cases of acute cerebrovascular disorder in the studied patients;
- low hemoglobin levels and erythrocyte counts in patients with APS were predictors of thrombosis;
- mainly in men older than 38-40 years revealed valvular heart disease;
- recurrent occurrence of neurological manifestations of the conclusions of a neurologist;
- PAPS was detected in young people up to 55 years old, mainly in women;
- focal lesions of the white matter of the brain were observed according to MRI data.

It should be noted that the studied patients are mainly aged 31-69 years (Table 3).

Table 3. Distribution of patients by age and sex.

Age (years)	Women (n=190)		Men(n=210)	
	abs	%	abs	%
20-30	-	-	-	-
31-40	23	12%	58	27%
41-50	82	43%	72	35%
51-60	58	31%	51	24%
61-70	27	14%	29	14%
71-82	-	-	-	-

The duration of the underlying disease was: a) to 3 years = 28% (n=112); b) 4-5 years= 51% (n=204); C) more than 5 years = 21% (n=84).

2. The next criterion was the detection of antiphospholipid antibodies by indirect immunofluorescence using an automated diagnostic system AKLIDES®.

It should be noted that in the pathogenesis of APS the leading role belongs to organ-specific antiphospholipid antibodies (APA), reacting with antigenic determinants of phospholipids. Due to the fact that the interaction of antibodies with prothrombinase complex, altering the balance of Pro - and anticoagulant factors (anticoagulation□hypercoagulability), the manifestation of hypercompleteness and the occurrence of thrombosis was observed more on the background of venous congestion[8,9]. All antibodies, depending on the spectrum and degree involved in the clinical manifestation of the state, accompanied by a violation of microcirculation in blood vessels, including skin, kidney changes, etc. For the diagnostic value of APS, it is recommended to use significant amounts of antibodies, while small values that are considered to be low-specific can occur in healthy people (autoantibodies to phospholipids, ribonucleoproteins, cathepsin and elastase), which provide homeostasis of transport forms of phospholipids in the blood, autoantibodies to the RNA complex interfere with transcription processes, by changing the activity of tRNA transport and enzymes involved in the recognition of the nucleotide sequence.

It is shown that autoantibodies formation is a physiological reaction of regulation of metabolic processes activity in which substances with antigenic properties participate. Thus, the paper presents the pathogenetic concept of APS as a systemic autoimmune disorder characterized by a combination of arterial and venous thrombosis with damage to internal organs (heart, CNS, lungs, liver, kidneys, muscles, skin) and hyperproduction of antiphospholipid antibodies with the leading pathogenetic role of lupus anticoagulant, thrombophilia and endothelial dysfunction.

The study on AKLIDES® revealed: 32% with strokes have primary APS, 10-13% - with deep thrombosis, 40% - in women with APS[3-6]. At first glance, we keep within the global statistics, however, if we consider only the high titles according to international recommendations, the primary APS is abs. We faced with a practice where there is no highly positive data, the tests were dissimilar, with high titers single. Low positive values should be taken into account, if only high positive values are used, the syndrome will not be detected and the diagnosis of PAPS is not set.

While at SLE it is detected, and the primary one seems to be absent due to the lack of high values. It is very important to use mixing tests, which are often not used in the laboratory and confirmation tests, the detection of phospholipids really, i.e. to expand the spectrum of antibodies, because the more positive results of the study, the higher the probability of diagnosis of APS. A similar situation was first discovered in Germany[8,9] and now in Russia[10], but has not followed the widespread introduction of alternatives into practice. This is the first time we have talked about this in our country. So, in our case, there is a vicious circle of doubtful diagnosis: we have thrombosis, test it, we cannot give immunosuppressive therapy, since there is no clear value, but there are clinical manifestations. Consequently, the question arose about the choice of *a new highly sensitive laboratory examination* method that delimits the pathology and physiological state of the organism. The problem is that the plastic is negatively charged and the DNA is negatively charged. Therefore, most T-systems are unsuitable for research, as antiphospholipid antibodies are directed to negatively charged phospholipids, therefore, poorly absorbed on plastic. Hydrophobic huge fat-soluble parts are simply repelled from the tablet-this is one of the problems of low-positive results, insufficient density of enzyme immunoassays.

The alternative is to use immunoblot as a variant of magnetic surfaces. The advantages are that the sorption does not occur on the tablet, but on ANA-hydrophobic cellulose membranes, where the molecules sit down, and not on the tablet. The advantage is that tariff membranes help to Orient and obtain a series of dense antigen-tested samples. We conducted some tests by immunoblot and found that at low titers revealed primary APS. Cutting off high titers, at values >40 GPL and contributed to the diagnosis of APS. Given the persistence of antibodies, the optimal timing of repeated studies is recommended after 12 days.

Thus, the use of such multiplex tests and the range of examination allows to identify reliably the primary APS.

Conclusion

The following regularities are revealed:

1. In patients with coronary heart disease, there is an increase in at titer to phospholipids.

2. The highest level of antibodies to phospholipids is detected in patients with acute myocardial infarction.

3. Patients with acute myocardial infarction have a positive correlation between increased titer of antibodies to phospholipids and blood lymphocytes and negative blood platelet concentration.

4. In surgical patients with PAPS, the titer of antibodies to cardiolipin is significant in diagnostic and prognostic terms, compared with lupus anticoagulant.

Systematic analysis of clinical, laboratory, immunological and pathomorphological manifestations of APS allowed to determine the correlation between them and to identify significant prognostic markers. The increased correlation of immunological parameters with the risk of PAPS development was established. For the first time, on the basis of a comprehensive assessment of the results of clinical, laboratory and instrumental studies, the General and distinctive features of PAPS and SAPS, on which the differential diagnosis of these two diseases is based, are shown.

Clinical and pathogenetic polymorphism of APS determines a differentiated approach to effective therapy.

In high-risk groups of primary APS, the development of the thrombotic and obstetric complications are shown an early screening of disorders method of immunoblot that allows the practitioner to quickly identify the thrombophilia, the timely initiation of pathogenetically reasonable prophylaxis, as thrombotic and obstetric complications.

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