ISPITIVANJE POVEZANOSTI IZMEĐU VRSTE I TITRA ANTIFOSFOLIPIDNIH ANTITELA SA KLINIČKIM MANIFESTACIJAMA ANTIFOSFOLIPIDNOG SINDROMA

ORIGINALNI RAD

ORIGINAL ARTICLE

INVESTIGATION OF THE RELATION BETWEEN THE TYPE AND TITER OF ANTIPHOSPHOLIPID ANTIBODIES AND THE CLINICAL MANIFESTATIONS OF ANTIPHOSPHOLIPID SYNDROME

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SAŽETAK

Uvod: Antifosfolipidni sindrom (AFLS) je autoimuno oboljenje, koje se manifestuje arterijskim ili venskim trombozama i/ili spontanim pobačajima udruženim sa perzistentno povišenim nivoom antifosfolipidnih antitela. Do sada nije jasno uočena povezanost nivoa i vrste autoimunih antitela i kliničkih manifestacija AFLS-a, koje variraju od blagih poremećaja koagulacije do životno ugrožavajućih stanja.

Cilj: Cilj rada je ispitivanje povezanosti vrste i titra antifosfolipidnih antitela sa najčešćim kliničkim manifestacijama AFLS-a.

Materijali i metode: Retrospektivnom studijom je obuhvaćeno 32 pacijenta sa potvrđenim laboratorijskim nalazom povišenih antifosfolipidnih antitela, koji su praćeni u Kabinetu za hemofiliju Univerzitetskog kliničkog centra Srbije, u periodu od 1. juna 2017. do 31. decembra 2018. godine. Podaci o pacijentima preuzeti su iz njihove medicinske dokumentacije. Analizirani su osnovni demografski podaci, vrsta i titar antifosfolipidnih antitela, odnosno njihova udruženost sa prisutnim kliničkim manifestacijama AFLS-a, uz korišćenje standardnih statističkih metoda.

Rezultati: Između osoba koje su imale kliničke manifestacije antifosfolipidnog sindroma i osoba koje su imale antifosfolipidna antitela, ali nisu imale klinički ispoljenu bolest, nije bilo značajne razlike u pogledu zastupljenosti pozitivnog nalaza lupusnog antikoagulansa, antikardiolipinskih i anti-beta-2-glikoprotein-I antitela. Procenat osoba sa istovremenom pozitivnošću dva ili sva tri antifosfolipidna antitela je bio isti u obe grupe ispitanika.

Zaključak: Suprotno ranijim publikacijama, naša studija nije dokazala povezanost titra antifosfolipidnih antitela i pojave kliničkih manifestacija AFLS-a. Simptomatski i asimptomatski pacijenti se nisu značajno razlikovali po vrsti i učestalosti povišenih antifosfolipidnih antitela. Ovakvi rezultati ukazuju da je za ispoljavanje kliničkih manifestacija antifosfolipidnih antitela potrebno i prisustvo drugih, još uvek malo poznatih faktora.

Ključne reči: antifosfolipidni sindrom, tromboza, spontani pobačaj, antifosfolipidna antitela

ABSTRACT

Introduction: Antiphospholipid syndrome (APS) is an autoimmune disorder manifested by arterial or venous thromboses and/or spontaneous abortions associated with a persistently elevated level of antiphospholipid antibodies. To date, no clear relationship has been established between levels and types of autoimmune antibodies and clinical manifestations of APS, which can range from mild coagulation disorders to life-threatening conditions.

Aim: The aim of this study is to examine the relation between antiphospholipid antibody type and titer and the most common clinical manifestations of APS.

Materials and methods: The retrospective study included 32 patients with a confirmed laboratory finding of elevated antiphospholipid antibodies, who came for follow-up examinations to the Hemophilia Unit of the University Clinical Center of Serbia, between June 1, 2017 and December 31, 2018. Data on patients were taken from their medical records. Basic demographic data, type and titer of antiphospholipid antibodies, and their association with the present clinical manifestations of APS were analyzed using standard statistical methods.

Results: There was no significant difference regarding the frequency of positive results for lupus anticoagulant, anti-cardiolipin, and anti-beta-2-GP-I antibodies, between the symptomatic and asymptomatic group. The percentage of persons with simultaneous positivity for two or all three antiphospholipid antibodies was the same in both groups of subjects.

Conclusion: As opposed to previous studies, our study did not demonstrate a correlation between the titer of antiphospholipid antibodies and the clinical manifestations of APS. Symptomatic and asymptomatic patients did not significantly differ in the frequency of elevated antibodies. These results indicate that the presence of other factors, which are as yet little-known, is necessary for the clinical manifestations of APS.

Key words: antiphospholipid syndrome, thromboses, spontaneous abortions, antiphospholipid antibodies

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UVOD

Antifosfolipidni sindrom (AFLS) je sistemska autoimuna bolest koja se odlikuje stalnim prisustvom povišenih vrednosti antifosfolipidnih antitela u krvi, što je udruženo sa pojavom tromboembolijskih komplikacija ili komplikacija u vezi sa trudnoćom [1]. Antifosfolipidna antitela su heterogena grupa autoantitela koja su usmerena na različite proteine koji se vezuju za fosfolipide. Do sada je pokazano da su, iz grupe antifosfolipidnih antitela, od patogenetskog značaja lupusni antikoagulans (LA), antikardiolipinska antitela (ACLA), kao i anti-beta-2-glikoprotein-I (aβ2GP-I) antitela, IgG i IgM klase [2]. Prisustvo ovih antitela se dokazuje različitim laboratorijskim metodama, pri čemu se lupusni antikoagulans određuje koagulacionim metodama, a antikardiolipinska i anti-beta-2-glikoprotein-I antitela imunoesejima, najčešće ELISA metodom.

Po kliničkim manifestacijama, antifosfolipidni sindrom je veoma heterogena bolest i može se ispoljiti na različite načine. Prema revidiranim Sapporo kriterijumima, da bi se postavila dijagnoza antifosfolipidnog sindroma, neophodno je prisustvo bar jednog kliničkog kriterijuma, odnosno vaskularne tromboze ili neuspeha trudnoće, i jednog laboratorijskog kriterijuma, što podrazumeva perzistiranje povišenih vrednosti jednog ili više antifosfolipidnih antitela u vremenu dužem od 12 nedelja [3].

Antifosfolipidni sindrom je najčešći uzrok stečene trombofilije i odgovoran je za oko 15% svih slučajeva tromboze dubokih vena, za jednu trećinu svih slučajeva infarkta mozga kod osoba mlađih od 50 godina, kao i za oko 10% – 15% svih slučajeva ponavljanog gubitka ploda [4]. Međutim, pored tromboze i akušerskih komplikacija, antifosfolipidna antitela mogu biti udružena i sa čitavim nizom drugih kliničkih manifestacija, kao što su trombocitopenija, ali i dermatološke ili neurološke manifestacije, koje ne ulaze u kriterijume za postavljanje dijagnoze antifosfolipidnog sindroma.

Antifosfolipidna antitela se mogu dokazati i kod malog procenta zdravih osoba, bez ikakvih kliničkih manifestacija antifosfolipidnog sindroma. Prema podacima iz literature, učestalost pozitivnih antikardiolipinskih antitela u zdravoj odrasloj populaciji je 1% – 5%, a učestalost pozitivnog lupusnog antikoagulansa je 0% – 4% [5]. Antifosfolipidna antitela se mogu javiti u okviru drugih autoimunih oboljenja, kao što su sistemske bolesti vezivnog tkiva, posebno često kod bolesnika sa sistemskim lupusnim eritematodesom (SLE). U jednoj studiji, od 1.000 bolesnika sa antifosfolipidnim sindromom, 36% je istovremeno imalo i SLE [6]. Ukoliko je antifosfolipidni sindrom prisutan kod bolesnika bez drugih autoimunih oboljenja, govori se o primarnoj formi bolesti, a u suprotnom je u pitanju sekundarni antifosfolipidni sindrom.

INTRODUCTION

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by a persistent presence of increased levels of antiphospholipid antibodies in the blood, which is associated with the development of thromboembolic complications or pregnancy-related complications [1]. Antiphospholipid antibodies are a heterogenous groups of antibodies directed towards different proteins that bind to phospholipids. So far, it has been shown that, out of the antiphospholipid group of antibodies, the following have pathogenetic significance: lupus anticoagulant (LA), anticardiolipin antibodies (ACLA), and anti-beta-2-glycoprotein-I (aβ2GP-I) antibodies belonging to the IgG and IgM classes [2]. The presence of these antibodies is proven with different laboratory methods – the lupus anticoagulant is detected with coagulation methods, while anticardiolipin and anti-beta-2-glycoprotein-I antibodies are detected with immunoassays, most commonly the ELISA method.

With regard to clinical manifestations, antiphospholipid syndrome is quite a heterogenous disease and can manifest in various forms. According to the revised Sapporo criteria, in order to establish the diagnosis of antiphospholipid syndrome, it is necessary that at least one clinical criterion is present, i.e., vascular thrombosis or pregnancy loss, as well as one laboratory test criterion, which entails the persistence of elevated levels of one or more antiphospholipid antibodies over a period of more than 12 weeks [3].

Antiphospholipid syndrome is the most frequent cause of acquired thrombophilia and is responsible for around 15% of all cases of deep vein thrombosis, for one third of all cases of cerebral infarction in persons under 50 years of age, as well as for around 10% – 15% of all cases of repeated pregnancy loss [4]. However, in addition to thrombosis and obstetric complications, antiphospholipid antibodies may be associated with a series of other clinical manifestations, such as thrombocytopenia, and also dermatological or neurological manifestations, which do not enter the criteria for establishing the diagnosis of antiphospholipid syndrome.

Antiphospholipid antibodies can also be proven in a small percentage of healthy people, without any clinical manifestation of antiphospholipid syndrome. According to data found in literature, the frequency of anticardiolipin antibody positivity in the healthy population is 1% – 5%, while the frequency of positive lupus anticoagulant is 0% – 4% [5]. Antiphospholipid antibodies may occur within other autoimmune diseases, such as systemic connective tissue disorders, particularly in patients with systemic lupus erythematosus (SLE). In a study including 1,000 patients with antiphospholipid syndrome, 36% of them also had SLE [6]. If antiphospholipid syndrome is

Mehanizam nastanka tromboze ili akušerskih komplikacija kod bolesnika sa antifosfolipidnim antitelima još uvek nije jasan, mada se pretpostavlja da aktiviranje trombocita, inhibicija fibrinolize, aktiviranje endotelnih ćelija, inhibicija prirodnih antikoagulantih sistema ili aktiviranje komplementa, mogu imati važnu ulogu u patogenezi bolesti [7]. Takođe, malo su poznati faktori koji utiču na način kliničkog ispoljavanja antifosfolipidnog sindroma. Prepoznavanje ovih faktora je od velike važnosti u identifikovanju asimptomatskih osoba sa antifosfolipidnim antitelima, koje imaju povećani rizik od pojave tromboze ili od akušerskih komplikacija.

U ovom radu ispitivana je veza između vrste i titra antifosfolipidnih antitela i kliničkih manifestacija antifosfolipidnog sindroma, kod osoba kod kojih su ova antitela povišena u dužem vremenskom periodu i koje se ambulantno kontrolišu u Kabinetu za hemofiliju, Klinike za hematologiju Univerzitetskog kliničkog centra Srbije (UKCS), u Beogradu.

MATERIJALI I METODE

U studiju je uključeno 32 pacijenta, koji su, u periodu od 1. juna 2017. do 31. decembra 2018. godine, dolazili na kontrolni pregled u Kabinet za hemofiliju UKCS-a, zbog perzistentno povišenih vrednosti antifosfolipidnih antitela. Podaci o pacijentima su prikupljeni retrospektivnom analizom iz elektronske dokumentacije i istorije bolesti. Svi pacijenti su imali potvrđeno visok nivo barem jednog od ispitivanih antifosfolipidnih antitela: lupusni antikoagulans (LA), antikardiolipinska antitela (ACLA), odnosno anti-beta-2-glikoprotein-I (aβ2GP-I). Od 32 pacijenta, 22 pacijenta su imala i potvrđenu dijagnozu AFLS-a, prema revidiranim Sapporo kriterijumima, dok je bilo 10 asimptomatskih nosilaca povišenih antitela. Vrednosti za ACLA i aβ2GP-I određivane su laboratorijskim testovima iz krvi, ELISA metodom, dok je LA određivan koagulacionom metodom po ISTH kriterijumu za laboratorijsku dijagnostiku lupusnog antikoagulansa [8]. U zavisnosti od procene kliničkih kriterijuma, bolesnici su podeljeni u dve grupe: u prvoj grupi su bili bolesnici koji su imali kliničke kriterijume za AFLS (tromboza ili akušerska komplikacija) dok su drugu grupu činile osobe bez tromboze ili akušerskih komplikacija (asimptomatski nosioci).

Standardnim statističkim metodama ispitivana je učestalost povišenih antifosfolipidnih antitela u grupama simptomatskih i asimptomatskih ispitanika. Za našu studiju, pozitivnim antifosfolipidnim antitelima se smatrala vrednost iznad gornje granice normalnog opsega laboratorije u kojoj su nalazi rađeni. U analizu su uključene demografske karakteristike ispitanika, vrsta i titar antifosfolipidnih antitela, kao i kliničke manifestacije AFLS-a. Grupa simptomatskih ispitanika je obuhvatila pacijente sa arterijskim ili venskim trombozama, kao i pacijente sa akušerskim komplikacijama. Na kraju,

present in patients without other autoimmune diseases, this is then considered the primary form of the disease, while in cases where the patient also suffers from another autoimmune disorder, it is considered as secondary antiphospholipid syndrome.

The exact mechanism of the occurrence of thrombosis or obstetric complications in patients with antiphospholipid antibodies is as yet unclear, although it is believed that platelet activation, fibrinolysis inhibition, endothelial cell activation, the inhibition of natural anticoagulant systems, or complement activation, may play an important part in the pathogenesis of the disease [7]. Also, little is known of the factors which affect the way that antiphospholipid syndrome is clinically manifested. Recognizing these factors is of great significance in identifying asymptomatic individuals with antiphospholipid antibodies, as they are at increased risk of thrombosis or obstetric complications.

In this paper, we analyze the link between the type and titer of antiphospholipid antibodies and the clinical manifestations of antiphospholipid syndrome, in patients in whom the level of these antibodies had been elevated for a longer period of time and who have been regular patients of the Hemophilia Unit of the Clinic for Hematology of the University Clinical Center of Serbia, in Belgrade.

MATERIALS AND METHODS

The study included 32 patients, who came to the Hemophilia Unit of the UCCS for follow-up examinations, between June 1, 2017 and December 31, 2018, because of persistently elevated antiphospholipid antibody levels. The data on the patients were collected through retrospective analysis of their electronic medical records and case histories. All patients had a confirmed elevated level of at least one of the observed phospholipid antibodies: lupus anticoagulant (LA), anticardiolipin antibodies (ACLA), or anti-beta-2-glycoprotein-I (aβ2GP-I). Of the 32 patients, 22 patients had a confirmed diagnosis of APS, according to the revised Sapporo criteria, while there were 10 asymptomatic carriers of elevated antibodies. ACLA and aβ2GP-I values were determined with laboratory blood tests, using the ELISA method, while LA was determined with the coagulation method, according to the ISTH criteria for laboratory diagnostics of the lupus anticoagulant [8]. Depending on the estimation of the clinical criteria, patients were divided into two groups: the first group was made up of patients who fulfilled the clinical criteria for APS (thrombosis or obstetric complication), while the second group was composed of individuals without thrombosis or obstetric complications (asymptomatic carriers).

Standard statistical methods were used to investigate the frequency of elevated antiphospholipid

međusobno su ispitivane grupe pacijenata sa najčešćim kliničkim manifestacijama AFLS-a, prema polu i uzrastu.

Statistička analiza je rađena u programu IBM SPSS 23 (IBM Corporation, New York, USA). Deskriptivni statistički parametri su prikazani kao aritmetička sredina ± standardna devijacija ili medijana (min. – maks.), a učestalost kategorijalnih varijabli kao broj (%). Za analizu numeričkih podataka korišćen je t-test, Man-Vitnijev test sume rangova i Kruskal-Valisov test. Razlika učestalosti kategorijalnih podataka testirana je Fišerovim testom tačne verovatnoće. Nivo statističke značajnosti je postavljen na $p < 0,05$.

REZULTATI

Demografski podaci

U studiju je uključeno 32 pacijenta, od toga 8 (25%) muškog pola i 24 (75%) ženskog pola. Prosečna starost svih pacijenata uključenih u studiju je bila 43,5 ± 11,3 godina (raspon: 25 – 71). Prosečna starost pacijenata muškog pola je iznosila 50,6 ± 12,3 godine (raspon: 35 – 71), dok je prosečna starost pacijentkinja bila 41,2 ± 10,2 godine (raspon: 25 – 69). Pacijenti muškog pola sa AFLS-om su bili statistički značajno starijeg životnog doba, u odnosu na ženski pol ($p = 0,039$).

Povezanost demografskih podataka i kliničkih manifestacija AFLS-a

Polna distribucija pacijenata nije pokazala značajnu razliku između pacijenata sa kliničkim manifestacijama AFLS-a i asimptomatskih nosilaca ($p = 0,380$), a razlika nije postojala ni u pogledu starosnog doba ($p = 0,360$). Kliničko-demografske karakteristike pacijenata, prema proceni kliničkih kriterijuma, prikazane su u Tabeli 1.

Kliničke manifestacije AFLS-a

Od svih pacijenata uključenih u studiju, 22 (68,8%) pacijenta su imala potvrđenu dijagnozu antifosfolipidnog sindroma, dok je 10 (31,2%) pacijenata bilo bez tromboze ili akušerske manifestacije (asimptomatski nosioci). Kod pacijenata sa potvrđenom dijagnozom AFLS-a, 13 (59,1%) pacijenata je imalo trombozu, a 7 (31,8%) pacijentkinja je imalo neku od akušerskih manifestacija. Samo dva (9,1%) pacijenta su imala više od jedne

antibodies in the symptomatic and asymptomatic groups. For the purpose of our study, a positive antiphospholipid antibody finding was considered to be any finding above the upper reference limit of the laboratory where the test had been performed. Demographic characteristics of the subjects, the type and titer of antiphospholipid antibodies, as well as the clinical manifestations of APS were included in the analysis. The group of symptomatic subjects included patients with arterial or venous thrombosis, as well as patients with obstetric complications. Finally, groups of patients with the most frequent clinical manifestations of APS were analyzed amongst each other, by sex and age.

Statistical analysis was performed using the IBM SPSS 23 software (IBM Corporation, New York, USA). Descriptive statistical parameters were presented as the mean ± standard deviation or the median (min – max), while the frequency of the categorical variables was expressed in numbers (%). The t-test, Mann-Whitney rank sum test, and the Kruskal-Wallis test were used for the analysis of numerical data. The difference in the frequency of categorical data was tested with Fisher's exact test. The level of statistical significance was set at $p < 0.05$.

RESULTS

Demographic data

The study involved 32 patients, of whom 8 (25%) were male and 24 (75%) were female. The average age of all of the patients involved in the study was 43.5 ± 11.3 years, (range: 25 – 71). The average age of the male patients was 50.6 ± 12.3 years (range: 35 – 71), while the average age of the female patients was 41.2 ± 10.2 years (range: 25 – 69). Male patients with APL were statistically significantly older, as compared to female patients ($p = 0.039$).

Relationship between demographic data and clinical manifestations of APS

The sex distribution of the patients did not show a significant difference between the patients with clinical manifestations of APS and asymptomatic carriers ($p = 0.380$), and there was also no difference with respect to age ($p = 0.360$). The clinical and demographic

Tabela 1. Kliničko-demografske karakteristike ispitanika prema proceni kliničkih kriterijuma

Table 1. Clinical and demographic characteristics of patients according to clinical criteria assessment

	Simptomatski / *Symptomatic*	Asimptomatski / *Asymptomatic*
Broj (%) / *Number (%)*	22 (68.75%)	10 (31.25%)
Pol, n (%) / *Gender, n (%)*		
Muški / *Male*	7 (31.8%)	1 (10.0%)
Ženski / *Female*	15 (68.2%)	9 (90.0%)
Prosečna starost (godine) / *Average age (years)* Aritmetička sredina ± SD / *mean ± SD*	42.3 ± 11.1	46.3 ± 12.0

Tabela 2. Distribucija kliničkih manifestacija AFLS-a među pacijentima uključenim u studiju

Table 2. Distribution of clinical manifestations of APS among patients included in the study

	Tromboza / *Thrombosis*	Akušerske manifestacije / *Obstetric manifestations*	Kombinovane manifestacije / *Combined manifestations*
Broj (%) / *Number (%)*	13 (59.1%)	7 (31.8%)	2 (9.1%)
Pol, n (%) / *Gender, n (%)*			
Muški / *Male*	7 (53.8%)	0 (0.0%)	0 (0.0%)
Ženski / *Female*	6 (46.0%)	7 (100.0%)	2 (100.0%)
Starost (godine) / *Age (years)* medijana (opseg) / *median (range)*	47.0 (25 – 71)	38.0 (28 – 41)	41.5 (35 – 48)

kliničke manifestacije AFLS-a. Kod pacijenata sa trombozom, 7 (53,38%) je bilo muškog, a 6 (46,0%) ženskog pola. Starosno doba pacijenata sa dijagnostikovanim AFSL-om nije se značajno razlikovalo prema kliničkim manifestacijama (p = 0,333). U Tabeli 2 se mogu videti kliničko-demografske karakteristike ispitanika prema kliničkim manifestacijama AFLS-a.

Učestalost prisustva i vrednosti titra antifosfolipidnih antitela

Najveći broj pacijenata je imao povišen LA, njih 25 (78,1%). Povišena ACLA IgG i IgM antitela je imalo po 11 (34,4%) pacijenata. Povišene vrednosti aβ2GP-I IgG je imalo 8 (25%) pacijenata, a povišene vrednosti aβ2GP-I IgM 12 (37,5%) pacijenata. Vrednosti titra antifosfolipidnih antitela nisu se značajno razlikovale između pacijenata koji su imali kliničke manifestacije (tromboze i akušerske manifestacije) i asimptomatskih nosilaca. Prikaz vrednosti titra antifosfolipidnih antitela je dat u Tabeli 3.

Učestalost pozitivnih nalaza pojedinačnih vrsta antifosfolipidnih antitela i prisustva kliničkih manifestacija antifosfolipidnog sindroma

Poređenjem simptomatskih sa asimptomatskim osobama, ni za jednu vrstu antifosfolipidnih antitela nije ustanovljena povezanost između učestalosti

characteristics of patients, according to the assessment of clinical criteria, are shown in Table 1.

Clinical manifestations of APS

Of all the patients involved in the study, 22 (68.8%) patients had a confirmed diagnosis of antiphospholipid syndrome, while 10 (31.2%) patients were without thrombosis or obstetric manifestations (asymptomatic carriers). In patients with confirmed APL diagnosis, 13 (59.1%) patients had thrombosis, while 7 (31.8%) female patients had an obstetric manifestation of APL. Only two (9.1%) patients had more than one clinical manifestation of APL. In patients with thrombosis, 7 (53.38%) were male and 6 (46,0%) were female patients. The age of the patients with confirmed diagnosis of APL did not significantly differ in relation to clinical manifestations (p = 0.333). Table 2 shows clinical and demographic characteristics of the subjects, according to the clinical manifestations of APL.

The frequency of the presence of antiphospholipid antibodies and the values of their titer

The greatest number of patients had an elevated LA, 25 (78.1%) of them. Elevated ACLA IgG and IgM antibodies occurred in 11 (34.4%) patients. Elevated levels

Tabela 3. Prosečne vrednosti titra antifosfolipidnih antitela
Vrednosti titra antifosfolipidnih antitela prema kliničkim kriterijumima

Table 3. Mean antibody titer of antiphospholipid antibodies
Values of antibody titer of antiphospholipid antibodies, by clinical criteria

	Tromboza / *Thrombosis*	Akušerske manifestacije / *Obstetric manifestations*	Asimptomatski / *Asymptomatic*	*p* vrednost / *p value*
LA medijana (opseg) / *median (range)*	1.8 (1.2 – 2.6)	1.7 (0.9 – 2.6)	1.7 (1.2 – 2.6)	0.634
Acla IgG medijana (opseg) / *median (range)*	15.8 (0.5 – 21.7)	8.4 (0.4 – 120.0)	31.4 (0.3 – 161.4)	0.578
Acla IgM medijana (opseg) / *median (range)*	9.0 (1.5 – 200.2)	45.5 (1.5 – 90.2)	19.8 (1.7 – 84.7)	0.634
aβ2GP-I IgG medijana (opseg) / *median (range)*	7.0 (0.2 – 360.0)	1.3 (0.7 – 118.0)	17.6 (0.6 – 100.0)	0.526
aβ2GP-I IgM medijana (opseg) / *median (range)*	11.8 (1.8 – 374.8)	88.0 (0.4 – 200.0)	13.2 (0.1 – 162.7)	0.968

Tabela 4. Učestalost pozitivnih nalaza određenih antifosfolipidnih antitela u grupi simptomatskih i asimptomatskih ispitanika

Table 4. Frequency of positive findings of certain antiphospholipid antibodies in the group of symptomatic and the group of asymptomatic subjects

	Simptomatski / *Symptomatic*	Asimptomatski / *Asymptomatic*	*p* vrednost / *p value*
Broj / *Number*	22	10	
LA	16 (72.7%)	9 (90.0%)	0.387
Acla IgG	8 (36.4%)	6 (60.0%)	0.266
Acla IgM	11 (50%)	5 (50.0%)	1.000
aβ2GP-I IgG	7 (31.8%)	4 (40.0%)	0.703
aβ2GP-I IgM	11 (50%)	4 (40.0%)	0.712

pozitivnog nalaza i kliničke manifestacije bolesti. Takođe, titar antikardiolipinskih antitela i aβ2GP-I antitela je bio sličan kod simptomatskih i asimptomatskih osoba. Učestalost pozitivnog nalaza različitih tipova antitela kod pacijenata sa klinički manifestnim AFLS-om i asimptomatskih nosilaca, prikazana je u Tabeli 4.

Povezanost kliničkih manifestacija AFLS-a i sveukupne pozitivnosti testova

Od svih pacijenata uključenih u studiju, potvrđeno je da su 22 (68,75%) pacijenta ispoljavala kliničke manifestacije AFLS-a. Među simptomatskim pacijentima, skoro polovina (45,45%) je imala samo jedan pozitivan test, svaki peti (18,18%) pacijent je imao dva pozitivna testa, a svaki treći (36,37%) sva tri pozitivna testa. U skladu sa rezultatima, nađena je povezanost na nivou statističkog trenda između ispoljavanja kliničkih manifestacija AFLS-a i pozitivnosti dva imunološka testa ($p = 0,096$). Za detaljan prikaz videti Tabelu 5.

DISKUSIJA

Antifosfolipidna antitela, koja su osnovni laboratorijski kriterijum za antifosfolipidni sindrom, veoma su heterogena grupa autoantitela, jer se vezuju za različite fosfolipide i dokazuju se različitim laboratorijskim metodama [9]. Lupusni antikoagulans se ispoljava produženjem fosfolipid-zavisnih testova koagulacije, dok se antikardiolipinska i anti-beta-2-glikoprotein-I antitela dokazuju imunološkim metodama. Prema ranijim literaturnim podacima, prisustvo sve tri grupe antitela uzrokuje izrazitu sklonost ka trombozama ili dovodi do neuspeha trudnoće. Međutim, u opštoj populaciji

of aβ2GP-I IgG were present in 8 (25%) patients, while elevated levels of aβ2GP-I IgM were registered in 12 (37.5%) patients. The antiphospholipid antibody titer values did not significantly differ between patients with clinical manifestations of antiphospholipid syndrome (thrombosis and obstetric manifestations) and asymptomatic carriers. The antiphospholipid antibody titer values are shown in Table 3.

The frequency of positive findings of individual types of antiphospholipid antibodies and the frequency of the presence of clinical manifestations of antiphospholipid syndrome

The comparison of symptomatic and asymptomatic persons did not find a connection between the frequency of a positive finding and the frequency of clinical manifestation of the illness, for any of the individual types of antiphospholipid antibodies. Also, the anticardiolipin antibody titer and the aβ2GP-I antibody titer were similar in symptomatic and asymptomatic subjects. The frequency of a positive result for different types of antibodies in patients with clinically manifest APL and asymptomatic carriers is shown in Table 4.

Relationship between clinical manifestations of antiphospholipid syndrome and overall test positivity

It was confirmed that, of all the patients involved in the study, 22 (68.75%) patients exhibited clinical manifestations of APL. Amongst symptomatic patients, almost a half of them (45.45%) had only one positive test, every fifth patient (18.18%) had two positive tests, while

Tabela 5. Učestalost pozitivnosti na jednu, dve ili sve tri vrste antifosfolipidnih antitela

Table 5. Frequency of positivity to one, two, or three types of antiphospholipid antibodies

	Simptomatski / *Symptomatic*	Asimptomatski / *Asymptomatic*	*p* vrednost / *p value*
Jedan pozitivan test / *One test positive*	10 (45.45%)	1 (10.0%)	0.248
Dva pozitivna testa / *Two tests positive*	4 (18.18%)	5 (50.0%)	0.096
Tri pozitivna testa / *Three tests positive*	8 (36.37%)	4 (40.0%)	1.000

se antifosfolipidna antitela mogu dokazati i kod malog procenta potpuno zdravih osoba, što ukazuje na kompleksnu patogenezu tromboze, odnosno akušerskih komplikacija u antifosfolipidnom sindromu. Ukoliko je prisustvo antifosfolipidnih antitela dominantan mehanizam u nastanku ovih komplikacija, moglo bi se pretpostaviti da vrsta, odnosno titar antifosfolipidnih antitela bitno utiču na njihovo ispoljavanje. Međutim, studije koje su ispitivale povezanost između vrste antifosfolipidnih antitela i pojave kliničkih manifestacija AFLS-a nisu obezbedile definitivan zaključak i neretko su imale kontradiktorne rezultate [10].

U našem radu je ispitivana povezanost između prisustva različitih vrsta antifosfolipidnih antitela i kliničkih manifestacija koje ulaze u kriterijume za dijagnozu antifosfolipidnog sindroma. Prisustvo antifosfolipidnih antitela je retrogradno analizirano kod ukupno 32 osobe, od kojih su 22 imale kliničke manifestacije antifosfolipidnog sindroma, dok 10 osoba, i pored jasno dokazanog prisustva antifosfolipidnih antitela u ponovljenom testiranju, nisu imale nikakve znake tromboze ili akušerskih komplikacija.

Iako se radilo o maloj grupi ispitanika, naše ispitivanje je obuhvatilo osobe sa obe manifestacije antifosfolipidnog sindroma, odnosno osobe sa trombozom i one sa akušerskim komplikacijama, ali i posebno interesantnu grupu osoba koje su imale antifosfolipidna antitela ali ne i kliničke manifestacije AFLS-a (asimptomatske osobe). Treba naglasiti da se radi o osobama koje se duže vreme, neretko i po nekoliko godina, redovno kontrolišu u tercijarnoj, univerzitetskoj bolnici, pri čemu je, uglavnom u nekoliko navrata, perzistentno potvrđivano prisustvo antifosfolipidnih antitela, što značajno umanjuje mogućnost greške u izboru bolesnika prema dijagnozi, koju kod antifosfolipidnog sindroma nije uvek jednostavno uspostaviti.

U našem ispitivanju nije ustanovljeno da postoji razlika u učestalosti prisustva lupusnog antikoagulansa kod osoba koje su imale trombozu ili akušerske komplikacije (simptomatske osobe) u odnosu na asimptomatske osobe sa perzistentno prisutnim antifosfolipidnim antitelima. Veoma slični rezultati su dobijeni kada su u pitanju antikardiolipinska i anti-beta-2-glikoprotein-I antitela IgG i IgM klase. Drugim rečima, zastupljenost svakog od ispitivanih antifosfolipidnih antitela bila je slična u simptomatskoj i asimptomatskoj grupi, što ukazuje na to da su za kliničke manifestacije antifosfolipidnog sindroma, pored prisustva antifosfolipidnih antitela, od važnosti i neki drugi, još uvek nedovoljno poznati, faktori. Poznato je da, pored antifosfolipidnih antitela, na pojavu kliničkih manifestacija AFLS-a utiču i infekcija, imobilizacija, tumori, inflamatorna stanja i drugi činioci [11].

every third patient (36.37%) had all three positive tests. In keeping with the results, a connection at the level of a statistical trend was found between the occurrence of clinical manifestations of APL and two positive immunological tests ($p = 0.096$). For details, see Table 5.

DISCUSSION

Antiphospholipid antibodies, which are the basic laboratory criterion for antiphospholipid syndrome, are a very heterogenous group of antibodies, since they bind with different phospholipids and are detected with different laboratory methods [9]. The lupus anticoagulant is detected by prolonged phospholipid-dependent coagulation tests, while anticardiolipin and anti-beta-2-glycoprotein-I antibodies are detected with immunological methods. According to previously published data, the presence of all three groups of antibodies causes a pronounced susceptibility to thrombosis or leads to failed pregnancy. However, in the general population, antiphospholipid antibodies can also be detected in a small percentage of completely healthy individuals, which reflects the complex pathogenesis of thrombosis, i.e., of obstetric complications, in antiphospholipid syndrome. If the presence of antiphospholipid antibodies is the dominant mechanism in the development of these complications, one could assume that the type and titer of antiphospholipid antibodies significantly affect their manifestation. However, studies investigating the connection between the type of antiphospholipid antibodies and the occurrence of clinical manifestation of APL did not provide a definitive conclusion and often had contradictory results [10].

In our study, the relation between the presence of different types of antiphospholipid antibodies and the clinical manifestations that enter into the criteria for diagnosing antiphospholipid syndrome, was investigated. The presence of antiphospholipid antibodies was retrospectively analyzed in a total of 32 individuals, of whom 22 had clinical manifestations of antiphospholipid syndrome, while 10 persons, despite clearly proven presence of antiphospholipid antibodies on repeat tests, had no signs of thrombosis or of obstetric complications.

Although the group of subjects was small, our study included persons with both of the manifestations of antiphospholipid syndrome, i.e., persons with thrombosis and persons with obstetric complications, but also an especially interesting group of individuals, who had the presence of antiphospholipid antibodies but displayed no clinical manifestations of APL (asymptomatic persons). It needs to be emphasized that, in a number of cases, the persons in question had been regularly followed up for a longer period of time, sometimes during a period of several years, at a tertiary health institution

Naši rezultati nisu u potpunom skladu sa ranijim publikacijama, koje su pokazale da je rizik od arterijske ili venske tromboze najveći kod osoba sa prisutnim lupusnim antikoagulansom (rizik povećan 11 puta), a manji kod osoba sa pozitivnim antikardiolipinskim antitelima u srednjem i visokom titru (rizik povećan 1,6 puta) [12].

Međutim, treba istaći da su, u pomenutoj meta-analizi, analizirane studije koje su ispitivale učestalost lupusnog antikoagulansa i antikardiolipinskih antitela u grupi bolesnika sa trombozom i poredile rezultate sa zdravom kontrolnom grupom, dok je u našem ispitivanju pristup bio drugačiji, jer je zastupljenost antitela analizirana kod osoba koje su već zadovoljavale laboratorijske kriterijume za antifosfolipidni sindrom. Zbog toga što je naša studija drugačije osmišljena od pomenutih studija, naši rezultati ukazuju na to da izostanak kliničkih manifestacija nije striktno vezan za prisustvo, odnosno odsustvo određene vrste antifosfolipidnih antitela. Na osnovu našeg ispitivanja, nije moguće reći da li je izostanak patogenetskog efekta antifosfolipidnih antitela u vezi sa samim antitelima ili je rezultat karakteristika osobe kod koje su antitela prisutna bez ispoljavanja kliničkih manifestacija. U svakom slučaju, može se zaključiti da se fenomen "tolerancije" na prisustvo antifosfolipidnih antitela javlja u podjednakoj meri za sve tri vrste antitela, odnosno da ovaj fenomen nije specifičan za jednu vrstu antitela.

U našem ispitivanju, titar antikardiolipinskih i anti-beta-2-glikoprotein-I antitela je generalno bio sličan kod simptomatskih osoba i osoba koje nisu imale kliničke manifestacije AFLS-a. Ovo nije u skladu sa podacima iz literature, koji govore da visina titra antifosfolipidnih antitela korelira sa rizikom od pojave tromboze, odnosno rizikom od pojave akušerskih komplikacija [13]. Isto tako, u svojem ispitivanju nismo uočili da postoji razlika u pozitivnosti na dva ili tri antitela kod iste osobe, između simptomatske i asimptomatske grupe, što je saopšteno u ranijim publikacijama [14]. Ovakav nalaz je verovatno vezan za postavku naše studije, gde su u ispitivanje uključene asimptomatske osobe sa već poznatim prisustvom antifosfolipidnih antitela.

Naša studija je obuhvatila relativno mali broj ispitanika sa pozitivnim antifosflipidnim antitelima, što značajno ograničava mogućnost generalizacije rezultata, ali se njena prednost ogleda u tome što je prisustvo antitela dokazano u nekoliko navrata. Rezultati ukazuju na to da između simptomatskih i asimptomatskih osoba nema razlike u učestalosti pojedinih vrsta antitela, što upućuje na važan zaključak da određeni broj osoba sa antifosfolipidnim antitelima ima zaštitne mehanizme, koji ih štite od pojave kliničkih manifestacija antifosfolipidnog sindroma. Identifikovanje ovih

– teaching hospital, during which time, on several occasions, the persistent presence of antiphospholipid antibodies had been proven, which significantly decreases the possibility of mistake in the selection of patients, according to the diagnosis, which, in phospholipid syndrome, is not always established easily.

Our study did not determine the presence of a difference in the frequency of the presence of lupus anticoagulant in persons who had thrombosis or obstetric complications (symptomatic individuals), as compared to asymptomatic individuals with a persistent presence of antiphospholipid antibodies. Very similar results were obtained with respect to anticardiolipin and anti-beta-2-glycoprotein-I antibodies of the IgG and IgM class. In other words, the presence of each of the observed antiphospholipid antibodies was similar in the symptomatic and asymptomatic groups, which indicates that, for clinical manifestations of antiphospholipid syndrome, in addition to the presence of antiphospholipid antibodies, other, as yet not sufficiently know factors, are also significant. It is known that, in addition to antiphospholipid antibodies, infection, immobilization, tumors, inflammatory states, and other factors, affect the development of clinical manifestations of APL [11].

Our results did not completely correspond with earlier publications, which showed the risk of arterial and venous thrombosis to be the greatest in persons with the presence of lupus anticoagulant (risk increased by 11 times), and to be lesser in persons with positive anticardiolipin antibodies with moderate and high titers (risk increased by 1.6 times) [12].

However, it needs to be highlighted that, in the abovementioned meta-analysis, the analyzed studies investigated the frequency of the lupus anticoagulant and the frequency of anticardiolipin antibodies in a group of patients with thrombosis and compared the results with a healthy control group, while our study had a different design, since the presence of antibodies was analyzed in persons already fulfilling the laboratory criteria for antiphospholipid syndrome. Because of the different design of our study, as compared to the abovementioned studies, our results indicate that the absence of clinical manifestations of APL is not strictly linked to the presence, i.e., absence of a certain type of antiphospholipid antibody. Based on our analysis, it is not possible to conclude whether the absence of a pathogenetic effect of the antiphospholipid antibodies is related to the antibodies themselves or whether it is the result of the characteristics of the individual in whom the antibodies are present, without exhibiting clinical manifestations. In any case, it can be concluded that the phenomenon of "tolerance" of the presence of antiphospholipid antibodies occurs equally frequently

mehanizama bilo bi od velike važnosti, jer bi potencijalno otvorilo mogućnost za uspešniju prevenciju kliničkih komplikacija kod osoba sa prisutnim antifosfolipidnim antitelima. S druge strane, na osnovu rezultata našeg ispitivanja, proizilazi da nije moguće, na osnovu vrste antifosfolipidnih antitela predvideti klinički tok, kod osobe kod koje su ova antitela ustanovljena u asimptomatskoj fazi.

ZAKLJUČAK

Naša studija nije dokazala povezanost između titra antifosfolipidnih antitela i pojave kliničkih manifestacija AFLS-a. Simptomatski i asimptomatski pacijenti se nisu razlikovali značajno po učestalosti povišenih antitela. Ipak, postojala je povezanost, na nivou statističkog trenda, između pojave simptoma AFLS-a i pozitivnosti dva imunološka testa.

Sukob interesa: Nije prijavljen.

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for all three types of antibodies, i.e., this phenomenon is not specific to any one type of antibody.

In our study, the titer of anticardiolipin and anti-beta-2-glycoprotein-I antibodies was generally similar in symptomatic patients and individuals without clinical manifestations of APL. This does not correspond with data that can be found in literature, which indicate that the level of antiphospholipid antibody titer correlates with the risk of thrombosis, i.e., the risk of the occurrence of obstetric complications [13]. Also, in our study, we did not find a difference in positivity to two or three antibody types in the same person, between the symptomatic and asymptomatic groups, which has been reported in previous publications [14]. Such a finding is probably linked to the design of our study, where asymptomatic individuals with already confirmed presence of antiphospholipid antibodies were involved.

Our study included a relatively small number of subjects with positive antiphospholipid antibodies, which significantly limits the possibility of generalization of results, but its advantage is reflected in the fact that antibody presence was confirmed several times over. The results indicate no difference between symptomatic and asymptomatic test subjects with respect to the frequency of individual types of antibodies, which leads to an important conclusion that a certain number of persons with antiphospholipid antibodies has protective mechanisms, which protect against the occurrence of clinical manifestations of antiphospholipid syndrome. Identifying these mechanisms would be very important, as it could potentially open the possibility of more effective prevention of clinical complications in persons with a presence of antiphospholipid antibodies. On the other hand, based on the results from our study, it transpires that it is not possible, on the basis of the type of antiphospholipid antibodies, to predict the clinical development in a person in whom these antibodies were detected in the asymptomatic phase.

CONCLUSION

Our study did not prove the relationship between the antiphospholipid antibody titer and the occurrence of clinical manifestations of APL. Symptomatic and asymptomatic patients did not differ significantly with respect to the frequency of elevated antibodies. However, there was a link, at the level of a statistical trend, between the manifestation of APL symptoms and two positive immunological tests.

Conflict of interest: None declared.

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