RESEARCH ARTICLE

Arterial and Venous Thrombosis as a Symptom of Antiphospholipid Syndrome – A Noticeable Analysis from the Past.

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ABBREVIATIONS:

aAPA
-asymptomatic antiphospholipid antibodies
ACA
-anticardiolipin antibodies
ACCP
-American College of Chest Physicians
APA
-antiphospholipid antibodies
APCR
-activated protein C resistance
β_2-GPI
-β2-glikoprotein-I
CT
-computed tomography
DVD
-deep-vein thrombosis
ECAT
-External quality Control of diagnostic Assays and Tests
INR
-international normalized ratio
LAC
-lupus anticoagulant
LMW
-low molecular weight heparin
PAPS
-primary antiphospholipid syndrome
PE
-pulmonary embolisim
USG
-ultrasonography
NMR
-nuclear magnetic resonance
SLE
-systemic lupus erythematosus
VTE
-Venous thromboembolic disease

Abstract

Antiphospholipid syndrome (APS), defined as combination of venous and/or arterial thrombosis as well as obstetric complications with antiphospholipid antibodies presence in blood, is an example of acquired thrombophilia. The thrombotic episodes in the APS course are highly recurrent, with an increasing incidence with years after secondary anticoagulant prophylaxis cessation.

In between 2005-2011 a study was conducted in Poznań, Poland, with the aim to find a correlation between actual APS diagnostic guidelines (criteria from Sydney'2006) and the clinical feature of thrombosis in this syndrome with coexistence of inherited thrombophilia and risk factors for cardio-vascular disease included. Additionally, a selection of the highest thromboembolic risk group was made among asymptomatic patients with antiphospholipid antibodies (APA) detected to compare with APS patients. An association between a type of laboratory test confirming the longtime APA presence and thrombotic risk was analyzed as well.

The follow up had lasted for meanly 47 months and included 75 patients (50 females and 25 males) at the mean age of 43, divided into three groups (25 persons each): I – with asymptomatic APA, IIA – with arterial episodes in the history and IIB – with past venous complications in the course of APS. The majority of them comprised persons with primary APS or with asymptomatic APA (aAPA) - without any other autoimmune diseases.

The laboratory tests included: lupus anticoagulant (LA) according to the 3-step procedure recommended by ISTH (International Society on Thrombosis and Hemostasis), anticardiolipin (ACA) – of IgG and IgM class and anti-β2-glycoprotein I (aβ 2 GPI) – of IgG class, both with the cut-off value of 99 percentile. D-dimer and fibrinogen concentration, protein C and antithrombin activity, activated protein C resistance, free protein S concentration, factor VIII were further analyzed.

Among comorbidities and risk factors for venous and arterial thrombosis, there was significantly more frequent incidence of autoimmune diseases in the asymptomatic group of people compared to patients after thromboembolic episodes.

Most often the aAPA presence was confirmed in the LA tests – the majority of positive results appeared among patients with asymptomatic course and with past venous thrombosis as well and less frequently - in the group of arterial episodes in the history.

Any significant differences were found in the reference to the incidence of thrombotic episodes in the retrospective assessment of symptomatic patients and prospective – in the whole investigated group, although the time of their occurrence after beginning of observation period almost doubled in people with past thromboembolic episodes, comparing to the earlier asymptomatic persons.

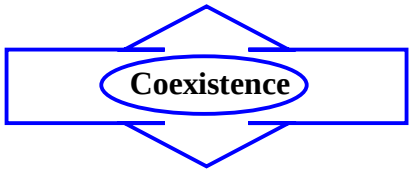
1. Introduction

Venous thromboembolic disease (VTE), including deep-vein thrombosis (DVT) and pulmonary embolism (PE), as its severe complication, is associated with substantial morbidity and mortality, causing a very serious social problem in highly developed countries. Based on a total population estimate of 310 million inhabitants of 6 EU countries (France, Germany, Italy, Spain, Sweden, UK) in the VITAE study, 465 thousands could have developed DVT, 295 thousands – PE and 370 thousands could die[1]. The incidence of VTE rises up with age, and in people at the age of ≥ 80 reaches 500/100 000 per year, of which 70% includes cases at the age of > 60. In the US, 600 thousands of patients annually experience VTE, and one third of them – die[2]. These data are of considerable social importance and clearly indicate a very high need of VTE prevention, diagnosis and treatment.

Spontaneous VTE is connected in more than 50% of cases with congenital or acquired thrombophilia and in 20-25% - with antiphospholipid antibodies (APA)[3], as the second one example. Their presence induce a very high thrombotic risk, associated with both: venous (mostly – in 2/3 of cases) and arterial events, creating - together with obstetric complications - clinical criteria of antiphospholipid syndrome (APS, Fig. 1), together with laboratory ones, revised by the experts in Sydney'2006[4].

Clinical criteria

- ≥ 1thrombotic events (venous and/or arterial and/or small vassel thrombosis)
- and/or pregnancy morbidity



Laboratory criteria
(on 2 or > occasions at least 12 weeks apart)

- Lupus anticoagulant (LA) presence in palsma (ISTH guidelines[5,6])
[phospholipid responsive clotting tests prolongation]
- and/or APA plasma presence in medium or high titer, and/or > 99th percentile
(e.g. ACA or anti-β2-GPI – in standardized ELISA, IgG and/or IgM isotype)

Figure 1 (Fig. 1)

This autoimmune systemic disease with a spectrum of (predominantly thrombotic) clinical manifestations, is caused by autoantibodies directed against phospholipid-binding plasma proteins [mostly β2-glycoprotein-I (consisted of V domains and considered, if associated with LA activity, as a target with the highest thrombogenic potential [7,8]) or prothrombin], and not - against phospholipids (PL) alone, participating in blood coagulation process (Figure 1)[9]. APA are very heterogenous group of autoantibodies of IgG, IgM rarely – IgA (the last one probably not pathogenic) class, complementary to plasma proteins with the ability of binding negatively charged phospholipids (PL), such as: cardiolipin,

phosphatidylglycerol, phosphatidylserine, phosphatidylinositol etc. (Fig. 2).

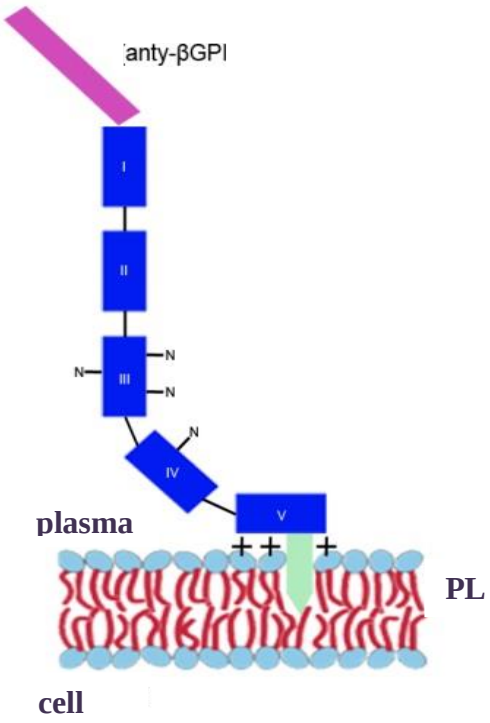


Figure 2. The schematic structure of β2-glycoprotein I (β2-GPI)

APS, on the base of comorbidity, can be categorized as secondary – if other autoimmune disease exists [in 30-50% cases – systemic lupus erythematosus (SLE)] or as primary (primary antiphospholipid syndrome, PAPS) – if doesn't. According to the experts' opinion this classification isn't justified due to lack of differences in the clinical picture and thromboembolic events in both of them [10].

The thrombotic episodes in the APS course are highly recurrent, with predilection into the previous localization and increasing incidence with years after secondary anticoagulant prophylaxis cessation (reaches up 30% annually and 78% after 8 years of treatment discontinuation)[11].

Confirmation of antiphospholipid antibodies presence in blood isn't always clear-cut. A healthy population, developing clinically silent APA - without any thrombosis, obstetric complications nor other disorders (asymptomatic antiphospholipid antibodies – aAPA) has been described (ACA in 2-5% of middle-aged people, in 1-10% of pregnant females and in 50% of persons at the age > 70; LA – in 1% of healthy individuals and 7-65% in SLE patients)[12,13].

Between 2005-2011 a study was conducted in Poznań, Poland, with the aim to find a correlation between APS diagnostic guidelines (Sydney'2006 criteria) and the clinical feature of thrombosis in this syndrome with coexistence of inherited thrombophilia and risk factors for cardio-vascular disease included. Additionally, a selection of the highest thromboembolic risk group was made among asymptomatic patients with antiphospholipid antibodies (APA) detected to compare with APS patients. An association between a type of laboratory test confirming the longtime APA

presence and thrombotic risk was analyzed as well.

2. Patients and methods

Patients and study design

The follow up period was lasting from 2005 till 2011 and included 75 adult patients, admitted to the hospital due to either thrombophilia suspicion or APTT prolongation. Based on the APA presence confirmation (Fig. 1), all of them later on were categorized into three groups (25 persons each): I – with asymptomatic APA (only laboratory criterion of APS fulfilled) and II – symptomatic, further split into two subgroups: IIA – with arterial episodes in the history and IIB – with past venous complications in the course of APS (Fig. 3.).

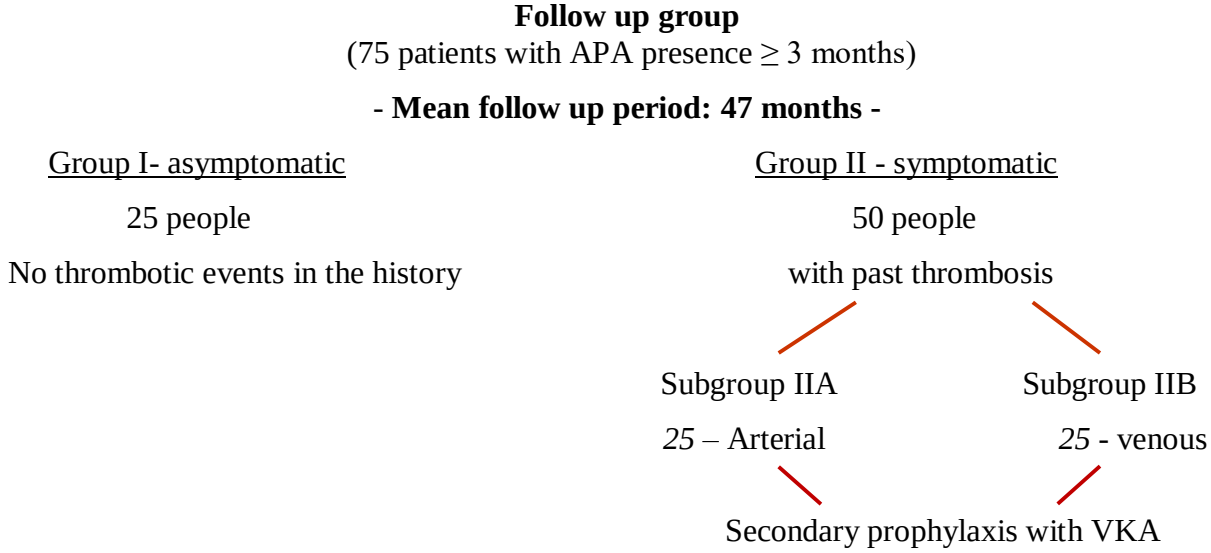


Figure 3. The study design

The whole II group underwent at least one thrombotic event, no earlier than 4 weeks before and no later than 5 years before APA presence recognition[4], confirmed by objectively recognized imaging methods (e.g.: 5 DOPPLER usg, CT scan, NMR imaging). Assignment to each of the symptomatic subgroup took place based on the initial location of thromboembolic event. In patients from the whole II group secondary prophylaxis was implemented, using vitamin K antagonists, under control of the international normalized ratio (INR) value to target 2-3. In the perioperative period or during diagnostic procedure, LMWH (low molecular weight heparin) was administered sc in the higher prophylactic dose instead, according to the guidelines of American College of Chest Physicians (ACCP), current then. In the asymptomatic group any anticoagulants were routinely applied, except occasional cases, e.g. – LMWH sc in the lower prophylactic dose while oral contraception, etc. The research methodology for the study included also a questionnaire survey - the investigated population was also interviewed/examined for comorbidities and vascular risk factors, such as: cardiovascular diseases, dyslipidemia, diabetes, obesity, smoking, thrombosis in the family history.

Laboratory studies
– performed with commercially available reagents and assays -

(over time in 2 consecutive assays, at least 12 weeks apart)[4]

Lupus anticoagulant (LA) was detected based on coagulation methods, according to the 3-step procedure recommended by International Society on Thrombosis and Hemostasis (ISTH)[6,14,15]. They included sequentially:

1. screening - prolongation of a phospholipid responsive clotting assay in at least two, using different principles, tests (e.g. activated partial thromboplastin time – aPTT, performed with silica as activator in low concentration of plant phoshplipids [PATHROMTIN SL (SIMENS)] and dilute Russels' Viper Venom Test – dRVVT, considered as strong test, highly specific for LA detection), especially in patients at high risk of thrombosis;
2. mixing studies - evidence of inhibition demonstrated as aPTT assay with different, reagent – DADE ACTIN FSL (SIEMENS), enriched in phosphatides of soybeans and a rabbit brain, with ellagic acid as an activator. In accordance with worked out by ISTH subcommittee[6] guidelines, the mixing procedure was run by adding the index plasma to equal portion of pooled normal plasma and repetition of the coagulation test later on. The result was positive, if the difference between aPTT of the mixture was > 50%, than the resultant aPTT of mixing tests, performed with plasma from healthy subjects and pooled normal plasma. Of course, lack of a specific inhibitor of any coagulation factor had to be ruled out;
3. confirmation tests, essential in the diagnosis of LA as an evidence of phospholipid-dependence: shortening or correction of the initially prolonged clotting time in a phospholipid-dependent assay by the addition of excessive phospholipids with exercising two simplified reagents: LA1-screening/LA2-confirmation for detection of LA in one-stage clotting tests (DADE BEHRING). Directed activation of factor X, initiated by dilute Russel's viper venom present in LA1, after 1 min. of incubation with 0,1ml of patient' at the temperature of 37^0C, was measured as screening clotting time, prolonged by LA antibodies. LA2 confirmation test was run in the same to LA1 manner, only with different reagent, containing a high PL concentration in order to counteract with the LA antibody and correct the clotting time. The final results were presented as a ratio = LA1 screening test/LA2 confirmation clotting time. The value > 1,3 shows LA presence and is weekly, medium or strongly expressed, if the LA ratio is respectively: 1,3-1,5; 1,5-2; > 2,0.

All the coagulation assays were carried out in a device called FIBRINTIMER II (Dade Behring).

Enzyme-linked immunosorbent assays (ELISA) were performed as commercially available microplate tests to measure levels of anticardiolipin antibodies (ACA) – of IgG and IgM class (BIOSYSTEMS, SPAIN) and anti-β_2-glycoprotein 1 antibodies (anti-β_2-GPI) – of IgG class (INNOVA Diagnostics, USA). ELISA results were expressed respectively in: G antiphospholipid units (GPL) and M antiphospholipid (MPL) units for ACA and standard IgG anti-aβ_2GP1 units (SGU) based on a calibration curve, according to the manufacturer's instructions, both with the cut-off value of 99 percentile [4,5,6, 14,15].

APA profiles of used APA tests were created, according to the revised classification criteria for APS from Sydney in 2006[4], in order to include the studied patients, based on the positivity for single or multiple APA assay, into one of the following categories:

I. Present more than one laboratory criteria (in any combination)

IIa. LA present alone

IIb. ACA present alone

IIIc. aβ_2GP1 – antibody present alone

Assessment towards other kinds of thrombophilia, with difficult to rule out

possible PL or phospholipid-binding plasma proteins engagement in the pathomechanism of the arising process was also initially *performed with commercially available assays*. Among them were further analyzed: antithrombin activity in the fully automated immunoturbidimetric assay (IL TEST™ ANTITHROMBIN, USA), protein C automated, quantitative measurement in enzyme linked fluorescent assay (ELFA; (VIDAS®Protein C, bioMERIEUX, FRANCE/ USA), activated protein C resistance (APCR) functional detection in screening, coagulation test – manual method (Proc®AcR, SIEMENS, GERMANY) and free protein S plasma concentration in ELISA microplate test (HELENA BioSciences Europe, GREAT BRITAIN).
The results of molecular assays towards factor V Leiden or polymorphism G20210 of prothrombin gene presence were the data taken from the patients' medical history (confirmed in the medical files).

3. Statistical analysis

The statistical analysis was performed using the STATISTICA 10,0 statistics package (StatSoft; POLAND) or the StatXact 3,0 program (Cytel Software Corporation; USA).
The collected parameters were analyzed to determinate statistical differences in patients' clinical features or to find the strength of dependency between assessed features. The ones derived from an interval scale were compared with Student's test, and in case of failure to meet required assumptions (e.g.: the normality of distribution for Shapiro-Wilk test or the unity of variance for Levene test) – the non-parametric tests were applied.
Pairwise comparisons of groups were made by Mann-Whitney test, and for more than two of them – by Kruskal-Wallis test. If the statistically significant difference was reached, the investigated groups were further analyzed in the "post-hoc" tests (e.g. Dunn test) to determine homogenous groups.
Comparing the dependency between specific features in the nominal scale, the chi-square test of independence was applied. In the case of low or zero cardinality, in at least one of the observed groups, the exact Fisher test or Fisher-Freeman-Hulton test was used, as appropriate.
The comparison of percentage difference between studied groups was made by a test for proportion.
All the tests were analyzed at the significance level $\alpha = 0,05$ (p value less than 0,05).

4. Results

The study group included 75 patients (50 females and 25 males) at the mean age of 43 (between 19-85 years old) during follow up period between 2005 till 2011 - meanly for 47 months (± 14,8 SD: 13-80 months). Based on a medical history and medical records the laboratory criterion was confirmed in all the patients. Later on, depending on the presence or absence of APS clinical symptoms, they were classified into of three groups (25 persons each): I, IIA and IIB respectively – as has been described already (Fig. 3). Next, the whole study group underwent assessment, according to differences in clinical features and laboratory values in order to specify the clinical picture of each subgroup and try to answer the question, whether there is a stigma determining a clinical course (venous, arterial or asymptomatic) of the disease (Table 1).

Table 1. Patients' clinical characteristics.

Clinical features	**Group I** (n 25)	**Subgroup IIA** (n 25)	**Subgroup IIB** (n 25)
Age (mean ± SD)	46±19,6	41±14,37	42±17,16

Gender	18F, 7 M	19 F, 6M	13F, 12M
Venous thrombosis:	-	8:	25:
Deep vein thrombosis – lower limbs	-	*5*	*20*
Pulmonary embolism	-	*1*	*10*
Atypical location:	-	*2:*	*5:*
- venous sinuses of the brain and DVT of lower limbs	-	*1*	-
- common jugular, subclavian and axillary vein	-	*1*	-
- central vein of the retinae	-	-	3
- multiorgan thrombosis	-	-	1
-mitral valve thrombosis	-	-	1
Arterial thrombosis:	-	25:	-
ischemic stroke	-	*24*	-
ischemic stroke and myocardial infarction (3x – age <47 years)	-	*1*	-
Mixed thrombosis (arteriovenous)	-	8	-
Family history of VTE	4	3	7
Family history of arterial events	6	11	5
Smoking	4	10	8
Cardiovascular diseases:	8:	10:	6:
Hypertension	*5*	*4*	*4*
pulmonary hypertension secondary to PE	-	-	*1*
ischeamic heart disease	*1*	*3*	*1*
valvular defects	*2*	*1*	-
valvular defects and the cardiologic X syndrome	-	*1*	-
peripheral atheriosclerosis	-	*1*	-
Dyslipidemia	5	7	7
Diabetes	-	-	-
Obesity	3	5	4
Other autoimmune diseases:	9:	4:	2:
rheumatoid arthritis	*1*	-	-
SLE	-	*1*	-
lupus-like syndrome	-	*1*	-
autoimmune haemolytic anemia	*2*	-	*1*
Addison-Biermer's anemia	*1*	-	-
Hashimoto's disease	*1*	*2*	*1*
Sjögren's syndrome	*1*	-	-

mixed connective tissue disease	*2*	-	-
primary immune thrombocytopenia	*1*	-	-
Trombophilia:	**3:**	**5:**	**6:**
activated protein C resistance (aPCR)	*1*	*0*	*2*
factor V Leiden	-	*1*	*1*
polymorphism G20210A of the prothrombin gene	-	*1*	-
protein C deficiency	-	-	*1*
protein S deficiency	*1*	-	*1*
antithrombin deficiency	*1*	-	-
aPCR + factor V Leiden	-	*2*	*1*
aPCR + protein C deficiency	-	-	*1*
aPCR + protein S deficiency	-	*1*	-

The study population was homogenous in terms of sex and age – the differences of statistical significance were not indicated in any of the described groups, although people with aAPA had the highest mean of age (46 ± 19,6 SD - Table1).

The majority of 75 followed up patients didn't present with any other autoimmune disease (Table 1). Among comorbidities and risk factors for venous and arterial thrombosis, there was significantly more frequent incidence of autoimmune diseases in the asymptomatic group compared to the symptomatic one, and the main cause of such a difference was in the less frequent occurrence of this these disorders within the IIB subgroup (p = 0,0168). The patients with the past, arterial episodes and the asymptomatic ones were comparable in terms of this parameter (Chart 1).

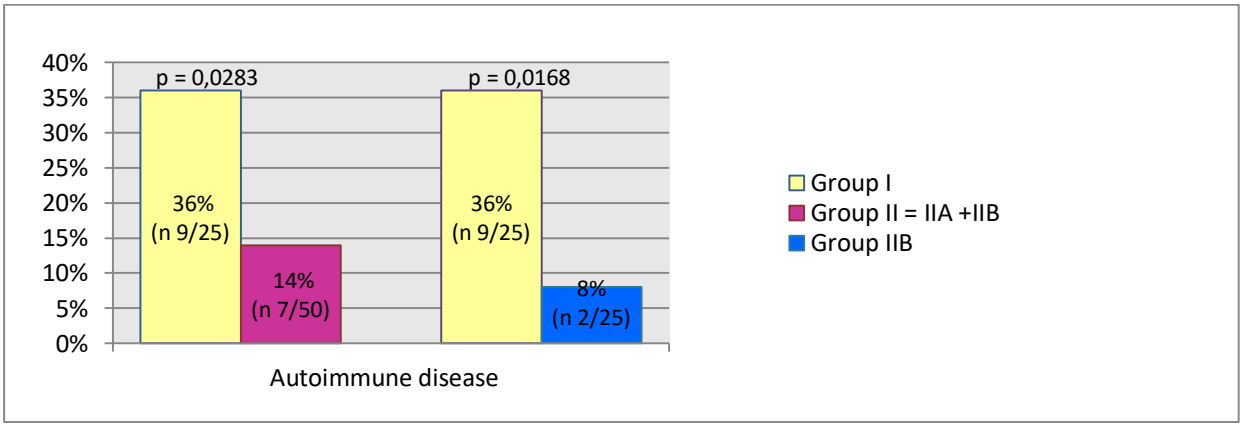


Chart 1. The incidence of autoimmune diseases in the selected study groups.

Assessment of recognized risk factors for venous thrombosis (an oral contraception, active neoplastic disease, the deficiency of protein C, S and antithrombin, the APC-r; pregnancy, VTE in the family history of first degree relatives) and arterial one (hypertension, diabetes, dyslipidemia, obesity, smoking) didn't indicate any of studied groups as burdened with a higher number of these factors.

Entering the follow up, only a part of followed up patients presented with a recurrent course of thromboembolic episodes in the medical history. Unfortunately, the recurrent feature didn't appear significantly more often within any of the observed, symptomatic subgroups (Table 2).

Table 2. Positive medical history towards recurrent thromboembolic episodes within the symptomatic patients while entering the observation.

Investigated subgroup	Patients with recurrent course of thromboembolic events in the past history n (%)	p
IIA (arterial thrombosis)	14 (58,3)	ns
IIB (venous thrombosis)	11 (44,0)	

LA was the laboratory assay, detecting APA presence the most frequently, but the statistical significance was reached only within the aAPA group (Chart 2).

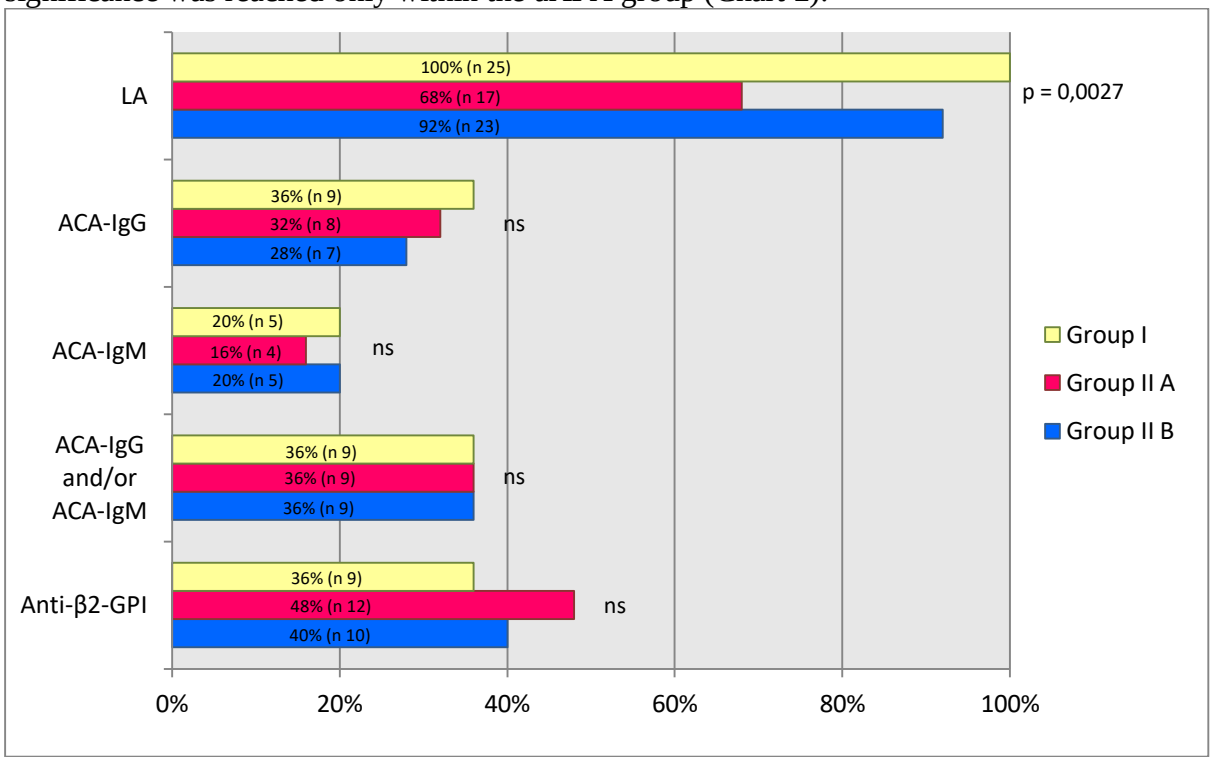


Chart 2. The incidence of APA assays in the observed groups.

Focusing the analysis on the II – symptomatic group and comparing selected parameters between the subgroups IIA vs IIB, it was demonstrated, that the LA phenomenon appeared significantly more often within the patients with venous thrombotic episodes in the past history (p = 0,0033) and probably therefore the only significant difference in the reference of LA frequency from a perspective asymptomatic vs symptomatic subjects was shown only between the I group and the IIA. The patients with venous episodes and the aAPA ones were comparable in this regard (Chart 3).

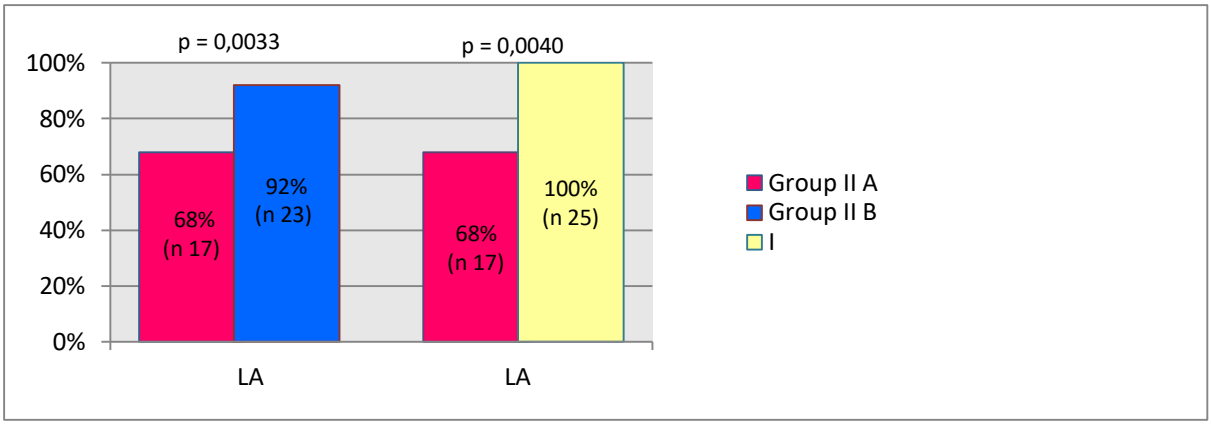


Chart 3. The incidence of LA in the selected study groups.

Any significant differences were found in the reference to the frequency of the APA presence detection by any other laboratory test.

The APA profiles of the implemented APA laboratory tests were created, according to the revised classification criteria for APS from Sydney in 2006[4], in order to classify the studied patients, based on the positivity for single or multiple APA[4,15 (14)].

Table 3. The incidence of the respective APA profiles in the investigated groups; I class: present more than one laboratory criteria (in any combination); IIa class - LA present alone; IIb class - ACA present alone; IIc class: aβ_2GP1 – antibody present alone; Miyakis et al.[4]

APA profile based on the laboratory criteria	Group I n (%)	Group IIA n (%)	Group IIB n (%)	p
I	10 (40)	9 (36)	10 (40)	ns
IIa	15 (60)	12 (48)	14 (56)	
IIb	0	1 (4)	1 (4)	
IIc	0	3 (12)	0	

None of these profiles was present significantly more frequent in the analyzed groups. Nevertheless, it is worth of emphasizing, that the IIc profile was seen only in patients with past arterial episodes and that the IIb profile didn't appear in the asymptomatic ones at al. (Table 3). The statistical significance was also not obtained while comparing the symptomatic (II = IIA + IIB) and the asymptomatic groups in the reference to the frequency of each APA profile. Only the luck of the IIb and IIc profiles was observed within the asymptomatic patients.

Any significant differences were found in the reference to the incidence of thrombotic episodes in the retrospective assessment of symptomatic patients and prospective – in the whole investigated population, although they were observed prospectively in each of the study group (I, IIA, IIB – in 2,7 and 4 patients respectively) and the time of their occurrence after entering the observation almost doubled in people with past thromboembolic episodes, comparing to the earlier asymptomatic ones (Table 4).

Table 4. The incidence of thrombosis in the prospective observation within the investigated groups (statistical significance and essential dependencies in terms of the risk of recurrence in each of the group - not reached; the data not shown) and the time of observation, needed to develop the thrombotic events.

Thrombosis after entering the study	**Group I (25) n (%)**	**Group IIA (25) n (%)**	**Group IIB (25) n (%)**	**p**
YES	2 (8)	7 (28)	4 (16)	ns
Thrombosis after entering the study	**Time to thrombosis Group I (months ± SD)**	**Time to thrombosis Group II (IIA+IIB) (months ± SD)**		**p**
YES	22±5,66	40,8±12,38		0,0363

Among the symptomatic subjects, the recurrence occurred mostly in the vascular bed of the primary localization, except for 4 (2%) persons: 2 of them experienced an ischemic stroke after initial venous episode, while the subsequent 2 patients underwent thrombosis of the same vascular bed, but of different topography (1 case - DVT of two different lower limbs, 2 case – PE after DVT. In the asymptomatic group – one episode affected arterial, cerebrovascular system and the other one – deep veins of the lower limb.

There wasn't found any correlation between thrombosis occurrence after the study initiation and the APA profiles (defined above), although the assessment was conducted in a multivariate manner. However, the essential observation was made, that the thromboembolic episodes, confirmed within earlier asymptomatic group, occurred only in patients with the I class of APA profiles (2 out of 25 patients) and that additional thrombophilia was detected in these patients (the protein S deficiency – one case and the APCR – the second case).

The question, weather the additional thrombophilia coexistence, such as: the deficiency of antithrombin, protein C and S and acquired APCR rises the risk of the thrombosis recurrence was tried to be answered. It was shown, that the protein S deficiency is the only risk factor for recurrent course of the disease [over 13 times (OR 13,31; confidential interval, CI: 1,093-162,631)]. Due to the low representation of patients with additional prothrombotic defects, a new concept of combined thrombophilia was created for the statistical assessment of the influence of this phenomenon on the thrombosis course. The prospective analysis of patients with APA and the thrombophilia coexistence, defined as combined of natural anticoagulants deficiency and/or activated protein C resistance demonstrated 5-fold rise of thrombotic episode recurrence risk (Table 5).

Table 5. The incidence of the thrombosis episodes in the prospective analysis in patients with thrombophilia
(↓ b. C i/lub S i/lub ↓ AT i/lub aPCR).

Thrombophilia (↓ b. C i/lub S i/lub ↓ AT i/lub aPCR)	**Thrombosis in the observation (13) n (%)**	**No thrombosis in the observation (62) n (%)**	**p**
YES	5(38,4)	7 (11,2)	0,0151

The next investigation step taken, was an effort to find a dependency between the APA profile, according to the diagnostic guidelines current then[4,14] and the clinical picture of APS. Unfortunately, any of the mentioned profiles, didn't occur significantly more often in each of the investigated group, didn't correlate with comorbidities, cardiovascular risk factors, positive family history of arterial and thrombosis events in first degree relatives nor did predispose to thrombotic complications in the prospective assessment.

While working on the study material, an observation was made that the APA profile varied personally with time and that the positive APA test performed initially didn't always confirm the APA presence on the second occasion (at least 3 months after their first appearance). This phenomenon was demonstrated in over 25% of all followed up subjects (26 patients – 34,6%), and the further analysis (in different correlations: symptomatic vs asymptomatic group, IIA vs IIB group and each of them vs asymptomatic one) always indicated this trend at the level over 20%. In this respect, groups IIA vs IIB and IIA vs I varied significantly (Table 6). In 6 of the asymptomatic patients spontaneous vanishing of APA was confirmed after approximately 34 months of follow up.

Table 6. The incidence of the heterogeneity phenomenon over time in APA assays performed twice, at least 3 months apart.

Other APA test positive on 2nd performance	**Group I (25) n (%)**	**Group IIA (25) n (%)**	**Group IIB (25) n (%)**	**p**
YES	7 (28)	14 (56)	5(20)	IIA vs IIB: 0,0087 IIA vs I: 0,0448 IIB vs I: ns

5. Discussion

The antiphospholipid syndrome is a very heterogeneous disease. Although it's enough to diagnose APS based on only one laboratory and one clinical criterion, it is a challenge for physicians of many different specialties. The essential symptom – thrombosis, may arise in a vessel of any caliber and sometimes – diminish or even stop blood distribution within several (at least three) organs at once, as in the catastrophic course (catastrophic antiphospholipid syndrome – CAPS) [16]. Thus, this disease can reveal a whole range of symptoms, far beyond the diagnostic guidelines and probably therefore one of polish experts in this field considered APS as magnifying glass of medicine [17].

The recognition problems are increased by the fact, that in adults the disease can occur at any age, regardless of gender, what was shown also in our observation. The differences of statistical significance were not indicated in any of the described groups (I,IIA, IIB), although people with aAPA had the highest mean of age (respectively 46 vs 41 in the IIA group and vs 42 in the IIB - Table1). The mean age of all the patients was 43 (between 19-85 years old) – as in most publications on the subject. Among them it was hard to find epidemiological data on APS and aAPA especially. More precise information were obtained from international registry established in 2000 year, dedicated to the catastrophic antiphospholipid syndrome – a very rare form of the disease (approx. 1% of APS), occurring between the ages of 7 and 70, more often in females [16,17,18]. In our study there was only one patient with CAPS in the past history at the age of 23, and prospectively we didn't observe such a severe course in any of followed up.

In 2007 Pengo V. et al compared APS patients with past arterial (28 people) and venous (29 people) episodes, of the same APA profile (positive in LA, ACA and anti-β_2-GPI presence, with the similar potential and titer of APA). They showed significantly higher number of arterial risk factors in the group with past arterial episodes. The number of venous risk factors was low in this group and this was seen as the cause of no VTE in patients with arterial episodes in the history [19]. Against the initial assumption, the connection between the arterial episode occurrence, as a part of atherosclerosis process, and the higher titer of antibodies against oxLDL/β_2-GPI complexes (oxLDL - oxidized low density lipoprotein) was not shown.

Unlike Pengo V. et al,in our analysis the significant differences between the observed groups in terms of arterial and venous risk factors of vascular episodes were not shown. However, these parameters are very individual and difficult to compare directly, especially in the separate study populations. It means, that our population was homogenous or had too low cardinality in the respect to the mentioned parameters to reach the level of significance or - both. It needs to be underlined, that our observation included patients with various APA laboratory profiles, and in the Pengo V. et al. analysis the triple APA positivity was the study inclusion criterion [19].

Neither it was shown, that any of the evaluated, symptomatic groups had significantly more nor a special type of comorbidities (except for autoimmunological diseases). In other words, an unique kind of clinical stigma, selecting APA patients with precise clinical course of the disease (e.g. venous, arterial thrombo-embolic complications nor asymptomatic process) was not gained. Other insights presented Danowski et al. in 2009 [20], analyzing 122 subjects, divided into 3 groups: with the primary antiphospholipid syndrome, with APS secondary to SLE and with SLE plus APA coexistence, but without past thrombosis and pregnancy complications. They demonstrated more frequent, thrombotic complications in patients with APS secondary to SLE, than - with primary form of the disease and that the thrombosis risk factors vary between people with arterial and venous episodes in the history. The first ones were associated with hypertriglyceridemia, inherited thrombophilia, the IgG titer > 40 GPL and LAC presence, while the second – with arterial hypertension and hyperhomocysteinemia. However, this analysis focused on another classification criterion into the study groups – the APS form and not – the initial thrombosis localization in the vascular bed (arterial vs venous).

Among our followed up patients only the ones with aAPA presented with more frequent autoimmunologic disease coexistence, comparing to the symptomatic group (IIA and IIB). There were observed not only the connective tissue disorders, such as: the rheumatoid artritis, the Sjögren's syndrome, the mixed connective tissue disease, but also: the Addison-Biermer's anemia, the Hashimoto's disease, the primary immune thrombocytopenia, autoimmunological anemia. This phenomenon indicates a greater predisposition of patients from the I group to the primary autoantibody generation, with a wide, pathophysiological spectrum, and the immunoglobulins directed against phospholipid-binding plasma proteins may be just one sign of such predisposition and don't have to induce the thrombotic events at all, especially if they are β_2-GPI (more precisely – the fragment gly-40-arg-43 of the I domain in this glycoprotein) independent or if they are of low avidity [17, 21, 22, 23, 24, 25, 26].

Any significant differences were found in the reference to the incidence of thrombotic episodes in the retrospective

assessment of symptomatic patients nor – in the prospective observation, in the whole investigated group. According to the similar incidence of past thromboembolic complications in the IIA and IIB groups (58% vs 44%), which underwent the same kind of secondary, anticoagulant prophylaxis (described already), the past, recurrent episodes simply couldn't be the subject of significant differences. It's hard to find out, whether the patients' compliance with medical recommendations for secondary prophylaxis was good and optimal, prior to the study enrollment. Probably indeed, the incidence of thrombotic recurrence is similar in both groups, independently from their initial localization and the latter shouldn't be considered a predictor of the future clinical course. In fact, right away at the beginning of the follow up, there was a category of 8 (4%) patients extracted, with the arterial-venous thrombosis episodes. Based on the arterial localization of their last event, which appeared within the time frame required by the diagnostic criteria from Sydney'2006 and induced APA detection, they were classified into the IIA group.

In turn, in a prospective assessment, thrombosis was confirmed in the groups: I, IIA and IIB – respectively in 2,7 and 4 patients, after mean observation time 22; 40,8 and 40,75 months respectively. Among the symptomatic ones, thrombotic complications usually were secondary to the inefficient anticoagulant therapy (described already): mostly if INR was < 2,0 or during uncontrolled treatment cessation, also while shifting the prophylaxis from oral anticoagulants into LMWH or vice versa. In most symptomatic patients the recurrence appeared in the vascular bed of the primary origin, however this regularity wasn't confirmed in 4 (2%) of them: in 2 people an ischemic stroke took place, after initial venous episode, and in the next 2 ones – within the same vascular bed, but of a various location (in the 1st case – subsequent DVT of different lower limbs, in the 2nd one – a pulmonary embolism after earlier DVT). To the similar conclusions on the location of recurrent thrombosis, not always the same as the initial one, came Pengo et all., during prospective analysis of the APS patients with the triple positivity APA profile (LA, ACA i anti-β_2-GPI)[27].

Among the patients from the I group, the prospective observation confirmed multifocal ischemic changes of the brain in 1 case and lower limb DVT - in the 2 one. It needs to be emphasized, that both of them were classified to the I class profiles, presenting the triple positivity within the APA assays (LA, ACA and anti-β_2-GPI) and that in these patients additional thrombophilia was confirmed (the protein S deficiency in one of them and APCR – in the other), but this tendency didn't reach the statistical significance.

In turn, the time of the thrombotic events occurrence after entering the observation period almost doubled in the symptomatic people, compared to the earlier asymptomatic ones (40,8 m-cy ± 12,38SD vs 22 m-ce ±5,66SD vs; p = 0,0363) – mostly without any anticoagulant prophylaxis (only 4 of them were on aspirin – 75mg daily and the next 4 – on LMWH temporally in the perioperative period). As already mentioned, these patients belonged to the I class profile, based on the results of performed APL assays (with LA, ACA and anti-β_2-GPI presence), suggesting a high need of the longtime, primary prophylactic treatment in them, or temporal one – in the case of increased prothrombotic risk in the asymptomatic patients classified to the IIa, IIb and IIc profiles. A strategy of preemptive prophylaxis in the aAPA patients seemed unclear. In 2009 Metijan A. and Lim L. reviewed the literature and found only 7 trials referring to the subject [28]. Most of them included patients with SLE – a disease independently associated with thrombotic events [20, 29, 30, 31]. According to the APLASA prospective study, after one year of

observation only 3 – out of 98 – experienced thromboembolic complications, although they were on aspirin – 81mg daily. Paradoxically, the placebo group didn't present any thrombotic episodes, but mostly this symptomatic patients had additional prothrombotic or cardiovascular risk factors [32].

There were several options of primary anticoagulant prophylaxis, proposed to use in the aAPA. It was shown, that in such patients aspirin or LMWH administration during the higher thrombotic risk period (e.g. surgery or the other cause of immobilization) reduces this risk [33]. In turn, in the SLE patients with APA presence, the combined therapy with aspirin and hydroxychloroquine was recommended to reduce the frequency of potential thrombotic events [30,34,35].

In the light of ambiguous reports, the best solution would be the proposal of individualized approach to the initial prothrombotic risk assessment in such patients and switching on the preemptive anticoagulant prophylaxis during a temporary increase in this risk, as well as the elimination of additional cardiovascular risk factors, e.g. smoking and dyslipidemia [28]. Maybe the APA profile consideration (for instance the triple positivity in the APA assays), would be one of the essential elements in an individualized approach to this patients?

The study groups were also analyzed for a potential influence of thrombophilia (confirmed at the study enrollment) on the clinical course. In the prospective assessment of the patients with the persistence (at least 3 months) APA presence, a 13-fold increase in the thrombosis recurrence risk was shown in patients with the additional protein S deficiency (OR 13,31; CI 1,093 - 162,631; the results not presented). This risk, compared to the general population of Europe with the isolated, heterozygous protein S deficiency, was additionally 3-fold grater in relation to the venous thrombosis [36,37]. However, given that this deficiency was confirmed only in 2 out of all the followed up patients and the very wide confidence interval, one has to take into account a large error of such an estimate and the need to verify these data in a larger study population.

While taking past histories and collecting the data from the medical records, an observation was made, that there were patients with combined deficiency of natural anticoagulants – a phenomenon rather very rare as an inherited defect. In the prospective analysis of patients with APA and the thrombophilia coexistence, defined as combined deficiency of natural anticoagulants (antithrombin and/or the protein C and/or the protein S) and/or activated protein C resistance, demonstrated 5-fold rise of thrombotic episode recurrence risk (OR 4,911; CI 1,252 - 19,263), during mean time of observation approx. 47 months. Any of these coagulation disorders analyzed separately in the prospective assessment, didn't significantly increased the recurrence risk of thrombosis, suggesting rather weak influence of inherited thrombophilia on the clinical course of APS and a quite probable, acquired origin of these combined deficiencies, secondary to APA presence.

To the opposite conclusions came Danowski et all. They demonstrated 7-fold increase of the thrombosis appearance risk in the APA patients with congenital thrombophilia, but didn't specify of this hypercoagulable disorder [20].

So, it has to be admitted, that in the case of persistent APA presence, the clinical course is very individual, and may depend on congenital and acquired risk factors, life style, nutrition habits, which taken together (in combination with the APA profile) determine the risk characteristics of an individual. Probably therefore, the antipfospholipid antibodies, complementary to plasma proteins with the ability of binding negatively charged phospholipids of the coagulation system (sometimes pathologically modified by hereditary or acquired disorders), affect

different hemostatic processes, exercising plasma substances, endothelium, blood cells and microparticles, etc. As the effect of these interactions, a specific clinical picture may arise, superimposed, as it were, on the initial, biological state, of which a laboratory test tube is only an imperfect substitute [38].

Most often the APA presence was confirmed in the LA tests – the majority of positive results appeared among patients with the asymptomatic course and with the venous thrombosis in the past, but the significance level was reached only within the aAPA subjects [group I: n = 25 (100%) - p = 0,0027; group IIA: n = 17 (68%); group IIB: n = 23 (92%)], what probably was induced by the methods of selecting the patients – they were referred for further diagnostics based on the APTT prolongation, without any hemorrhagic diathesis.

There were not any significance differences in the frequency of the positive LA assays between the I and IIB group, because in fact the group IIA, compared to the asymptomatic one, was the main reason of this significance. It means, that the LA phenomenon rather predisposes to the venous thrombosis events, what is consistent with Danowski's et al.' observation[20] and paradoxically – to the asymptomatic clinical course. This paradox can be explained. First of all, false positives need to be taken into account, especially in the elderly and also – cases with low inhibitory potential of LA [39], what may lead to irrelevant prolongation of coagulation times and, as result – to misinterpretation. Thus, a critical approach to the lab tests performance, according to the 3-step, sequential procedure recommended by ISTH[6,14,15] may significantly increase their reliability. Additionally, the possibility of non-pathogenic, often transient and secondary to various infections APA uprising should be taken into account. These antibodies - so-called - the β_2-GPI-independent ones – are capable of direct binding to the cardiolipin or – to the Vth domain (instaed of the Ist one), as in the case of leprosy or atopic dermatitis [40,41,42], which also may induce the LA phenomenon. They can be detected only in the LA assays, because the ELISA tests, exercised in the APS diagnostics, use completely different epitopes – with the potency of inducing pathogenic antiphospholipid antibodies: within the Ist domain (and not – the Vth ones) of β_2-glycoprotein I and - within the complex of cardiolipin bound to the protein cofactor in the form of β_2-GPI. According to Pengo et al. [39,42,43], the isolated LA presence doesn't have to be associated with thrombosis or pregnancy complications, what could explain the highest frequency of this assay positive results among our asymptomatic patients. The authors even suggested another disease entity distinction, based on other than anti-β_2-GPI inhibitors, responsible for LA phenomenon, not always inducing thrombosis risk[39]. To the similar conclusions came de Groot et al. after the Leiden study completion. Among the patients with LA positivity, they confirmed, that after anti-β_2-GPI and anti-prothrombin antibodies presence exclusion, the thrombotic risk is low (odds ratio: 1,3; 95% CI: 0,3 - 6,0) [19,44].

From the other hand, in the IIB w group of our study (with venous events in the past history), LA was present similarly. Besides, Galli M. in her meta-analysis demonstrated, in contrast to the ACA positivity, the LA presence association with the risk of thrombosis[45], independently from its type and location. However, it has to be taken into account, that this analysis included data published between 1988-2000, when the anti-β_2-GPI assay performance wasn't routinely practiced. Also, based on the presented data, it isn't clear, if all the LA positive patients were ACA-negative at the same time. Thus, there is uncertainty about the isolated character of LAC in this meta-analysis[38,45]. Can these discrepancies be explained, by following the APA profile in our study? AS

shown in the table 6, the percentage of isolated LAC presence was the highest in the asymptomatic patients (60%), in which, neither ACA nor anti-β2-GPI exclusive presence wasn't demonstrated at all – their occurrence was always combined with LAC, creating together the I category of laboratory diagnostic criteria[4] (40% in the I observed group). From the prospective view, there were thrombotic events in 2 patients with this profile, observed after approx. 22 months from the study enrollment, the 8 remaining ones continued the asymptomatic course until the end of the follow-up period (approx. 47 months). It's hard to answer the question, why? In the literature the reports of 2 β_2-GPI conformations can be found: the closed, unbound one – a circular plasma structure with shape maintained by interaction between the I and V domains of β_2-GPI and an "activated", open one. In vitro, by changing pH and salt concentration, it was possible to convert the one conformation into another and back, what was detected beased on X-ray crystallography, electron microscopic images, differential trypsin digestion profiles, surface plasmon resonance binding studies of the affinity of recombinant domains for each other, and functional studies that compare the anticoagulant effects of the conformations[46,47]. In vivo, e.g. as a result of interactions with the anionic phospholipids, non-covalent bonds between the domains I and V can be disrupted in order to insert the hydrophobic loop of the latter (with positively charged residues) within the hydrophobic part of the PL bilayer (Fig.4[47]).

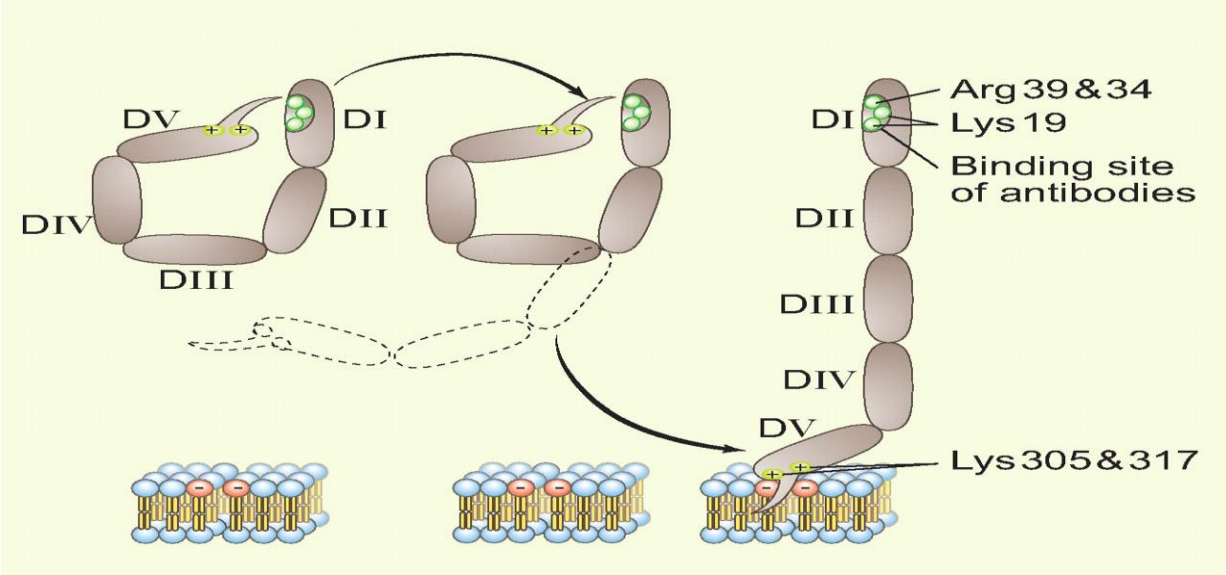


Figure 4. The convertion of the one β_2-GPI conformation into another - an adaptation of Ağar et al.[47].

Agar et al. presented that APA recognize only the open form of β_2-GPI, in the other words – after interaction with the anionic PL, because the affinity of human anty-β_2-GPI antibodies by themselves seemed to weak, to compete with domain V for domain I binding. The presence of these PL enables the conformation conversion to increase the affinity of autoantibodies for the domain I epitopes and to trigger prothrombotic mechanisms induced by APA, such as: endothelial damage (usually acquired one), tissue factor expression and so on (Fig. 6)[46,47]. In vitro, the occurrence of β_2-GPI in a particular conformation form may depend on purification methods of this glycoprotein and

as a consequence - finally may affect the laboratory diagnostics of APS[47]. For example, an assay performed with the closed variant may produce false negative results. Of course, a separate issue is the cause of autoantibodies generation, directed against the I domain, among others - a hereditary background[46]. Maybe that's why, in our so called asymptomatic patients, a predisposition to a primary autoantibody formation in general (not only antiphospholipids) occurred? It can be assumed, that under unknown conditions, patients with the symptomatic course revealed their congenital tendency to produce prothrombotic antibodies, complementary to the fragment gly-40-arg-43 of the I domain within β_2-GPI.

Returning to the study, it's worth mentioning, that the APA profile in the symptomatic patients, was comparable in the IIA and IIB groups, besides the exclusive presence of anti-β_2-GPI in patients with past arterial thrombotic events (12%). The isolated LA phenomenon was observed in the lower percentage in this group, than in the asymptomatic one, and the frequency of the combined APA presence (the I category profile) was comparable with the I group. In turn, the positive ACA titer was observed more frequently – 4%, both, in the IIA and IIB groups. It's worth noting, that the APA profile was more heterogeneous within the patients with past arterial and venous events, but wasn't specific for any of them (maybe except for isolated presence of anti-β_2-GPI in the IIA group). It would mean the luck of correlation between the APA category and the thrombosis location in the vascular bed, what may reflect by contradictory reports in the literature current than dedicated to LA as a risk factor for thrombosis. Danowski et al. associate this phenomenon rather with venous thrombotic events[20], while Urbanus at al. and Linnemann et al.[48,49] - with arterial ones. This approach would make the clinical course dependent on biological conditions (congenital and acquired) of each individual, what can support the fact of 8 patients group (4%) selection at the study enrollment, with both – arterial and venous - events in the past history. Not all of them represented the same APA profile – in 5 ones LA was detected, in the 3 last – more than one positive APA assay, in any combination. Additionally, it has to be emphasized, that any significant differences were found in the frequency of the APA profile between the observed groups. Also, any of these profiles predisposed significantly to the thromboembolic events in the prospective assessment of the all followed up patients, although in the previously asymptomatic group these complications appeared only among patients with the I APA profile. Also, neither of the comorbidities nor the risk factors identified in the patients' history and based on the medical records correlated significantly with the APA category, according to the laboratory diagnostic criteria current then.

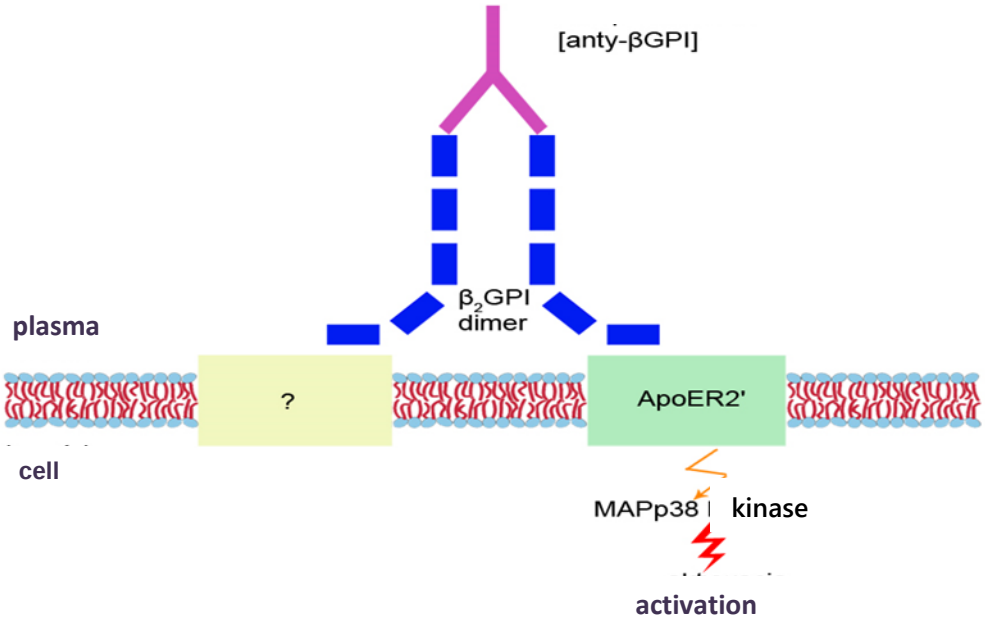
Contrary to the obtained results, many published reports indicate an association of the detected APA profile (LA and/or ACA and/or anti-β_2-GPI – the I category according to the guidelines[4]) with the thrombosis risk. As Swadźba et al. demonstrated, each kind of these antibodies significantly rises the prothrombotic risk up, but the simultaneous, triple positivity of all these assays in one person increases this risk much more (OR 5,63; CI 2,82 - 11,24)[29]. However, in these study patients a secondary to another comorbidity APS was mostly recognized (the so called PAPS was found only in approx. 10% of them. Our analysis mostly included people with primary presence of APA (the secondary APS patients – with additional autoimmunological disease - constituted 20% of all those observed and 12% of the asymptomatic ones).

Similarly, Ruffatti et al. claimed, that the triple positivity of tests performed in one person correlates with higher thrombotic risk

and pregnancy loss, than in case of incorrect results of two or one of these assays[50]. The researchers showed, that patients with this APA profile tend to higher titers of anti-β_2-GPI expressed in SGU, what indicates a role of these antibodies in the pathogenesis pathway of APS and it's clinical presentation[50,51]. This observation is consistent with the thesis of Pengo et al.[39,42,52] [(38,42,52)], according to which the highest risk of the thromboembolic events and pregnancy complications represent patients with the simultaneous, triple positivity APA profile, in sufficiently high titers (LA and ACA-IgM > 40MPL or ACA-IgG > 40GPL and anti-β_2-GPI > 99 percentile) [39,42,52] [(38,42,52)]. Such a combination of the APA assays results, give the basis for a certain APS recognition in the symptomatic patients, as an autoimmunological disease in fact, induced by one kind of autoantibodies – to β_2-glikoproteinie I, causing positivity of both: the LA and ELISSA tests. Researchers proved, that anti-β_2-GPI, obtained by plasma affinity chromatography from the APS patients, can induce LA activity in normal plasma[53] and positivity of ACA measurement in the ELISA assays[41]. According to the authors, the isolated LA, ACA or anti-β_2-GPI presence associated with the clinical criteria of APS create other than the antiphospholipid syndrome disease entity, because these autoantibodies vary from those described earlier – the pathogenic anti-β_2-GPI[41,52].

The prothrombotic action of β_2-GPI influenced by antiphospholipid antibodies may be related to a dimeric form of this glycoprotein, generated after binding of one APA particle to two particles of β_2-glycoprotein I (Fig. 5).

Figure 5. Dimeric form of β_2-GPI generated after complementary antiphospholipid antibody binding (β_2-GPI - β2-glycoprotein-I, PL – phospholipids)



The resulting complex has a much stronger affinity to the plasma PL (approx. 35 fold higher than a monomer)[21,54,55].

On the contrary to Pengo et al. our observation included patients with often lower than 40MPL/GPL ACA titer, based on

the value of 99th percentile of healthy population[4], determined in our local laboratory – respectively: ≤ 10,4 MPL and ≤ 11,3 GPL and ≤ 19,9 SGU for anti-β_2-GPI. This could be the source of significant discrepancies, observed between the obtained results and the reports from the world literature. From the other hand, we aimed in the objective observation of all the patients with the persistent (≥ 3 months) APA presence, and not only those with the high prothrombotic risk in the APS course.

It's worth of emphasizing, that the APA profile varied personally with time, what may lead to the probability of misinterpretation and further consequences. For example, by limiting the second APA determination only to the assays positive 3 months earlier, it's easy to conclude the temporal APA presence instead of the full-blown APS and inaccurately discontinue the anticoagulant treatment. We demonstrated, that the kind of APA confirmed ≥ 3 months after their initial detection differed from the one found at the beginning of the observation in almost 35% (26 patients – 34,6%) out of 75 people included to the study. Estimating this phenomenon in all the created groups (I,IIA, IIB), it's presence was always confirmed at the level of ≥ 20%. The variability of the results rather shouldn't be explained by low reliability of the determinations, because our hemostasis laboratory is certified by the ECAT (External quality Control of diagnostic Assays and Tests) Foundation, participating every year in the international program of external quality assessment in the field of thrombosis and hemostasis. Thus, for reliable diagnosis, second laboratory assessment (after 12 months) should include whole panel of tests used for their initial detection and recommended for the APS recognition.

Spontaneous vanishing of APA, confirmed in our 6 patients after approximately 34 months of follow up is consistent with suggested above, dynamic status of the APA profile, raising further questions. It's hard to answer, how to deal with such patients, whether to repeat the APA assays and how often to do so and if the anticoagulant prophylaxis can be discontinued. Such a problem is the only one of many doubts induced by the APA presence. In order to control patients without the APS diagnostic criteria fulfilled, a definition of the „probable or seronegative" APS was formulated[4] This term includes cases of APA other than included in the revised classification criteria (such as against: ACA and β_2-GPI – both of IgA class, prothrombin, phosphathidyloserine, phosphathidyloethanolamine) and unusual for the definite APS clinical symptoms (e.g.: cardiac valve disease, neurological manifestations, livedo reticularis, nephropathy, thrombocytopenia, Raynaud's phenomenon)[4,17]. These situations may induce serious therapeutic problems, and long lasting anticoagulation - adverse events (e.g. spontaneous bleeding etc.) in patients without any indications for such treatment. From the other hand, skipping the therapy for example due to misclassification to the asymptomatic group can result in tragic effects [56]. In the own observation, conducted in cooperation with The Clinic of General and Vascular Surgery in Poznań, Polnad, we confirmed APA presence in 5, out of 15, patients with peripheral artery, ischemic changes of upper and lower limbs [57]. These changes were associated initially with thrombangiitis obliterans (TAO), especially in young, smoking men. Probably, the final and optimal answer could be given by the histopathologists, indicating presence of thrombosis without the inflammation features within the vessel wall[4].

Summarizing, the pathogenesis of APS and sequentially – the clinical course determinants, irrespective of the vessel location, probably will remain a mystery, as an effect of to many prothrombotic mechanisms, subcellular, cellular and tissue

structures involved in this disease. Among them can be mentioned: Cell activation process and high expression of tissue factor, the annexin 5 crystallization, the protein C system activation, fibrinolysis with the annexin A2 engagement as a mediator and a prothrombotic phenotype stimulation and adhesive interactions, the complement activation (Fig. 6 – an adaptaion of Erkan D, Lockshin MD.)[58].

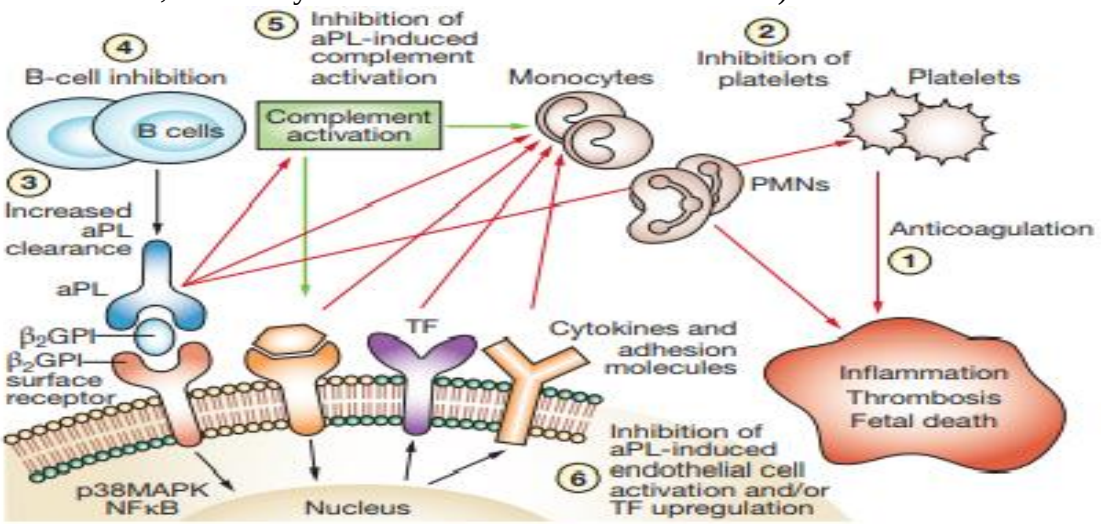


Fig. 6 Proposed mechanism of aPL-mediated thrombosis. Cell membranes neutral PC phospholipids (green circles), which are the major constituent of the outer layer of inactivate cells, and negatively charged PS phospholipids (orange circles), which migrate from the inner to the outer cell membrane during activation or apoptosis of platelets and endothelial cells. Dimeric β_2GPI then binds to PS (via β_2GPI surface receptors, such as apoER2', annexin A2, or Toll-like receptors) and aPL bind to β_2GPI, activating the complement system and leading to the generation of C5a, which induces expression of adhesion molecules (e.g. ICAM1), cytokines (e.g. IL-1, IL-6, IL-8) and TF, and activation of monocytes, PMN cells and platelets, which subsequently results in the release of proinflammatory mediators (such as TNF or VEGFR1) and the prothrombotic state. Both NFκB and p38MAPK could have a role in the thrombotic intracellular signaling cascade. The numbers on the figure (1–6) represent the stages at which the effects of aPL can be inhibited; these also correspond to the targets shown in Table 1. Abbreviations: aPL, antiphospholipid antibodies; apoER2', apolipoprotein E receptor 2'; β_2GPI, β_2-glycoprotein-I; ICAM1, intracellular adhesion molecule 1; IL, interleukin; NFκB, nuclear factor κB; PC, phosphatidylcholine; PMN, polymorphonuclear; PS, phosphatidylserine; p38MAPK, p38 mitogen-activated protein kinase; TF, tissue factor; TNF, tumor necrosis factor; VEGFR1, vascular endothelial growth factor receptor 1.

The recent reports from recent years reveal more and more facts to facilitate the disease biology perception, especially at the very early stage of pathogenesis. The acquired character of APS and it's different course in the group of similar - in terms of sex, age and race patients- suggests dependence of the final clinical picture on individual biological conditions, determining (together with the APA positivity category) the full risk profile of an organism.

Our results shouldn't serve as a base to reflect the estimated dependencies in the general population. The recruitment criteria, due to significant difficulties in obtaining the investigation material, were limited to the patients with clinical and/or laboratory proven coagulation disorders and therefore could be influenced by a typical, selection error.

Further investigations, especially prospective, are needed in patients recruited to the proposed groups, with the APA profile

classification included, but in much higher number. Based on a long recruitment period and the enrollment limitation just mentioned, a multicenter cooperation should be established.

6. Conclusions

1. Autoimmune diseases occurred significantly more often in the asymptomatic patients compared to the ones with APS. No differences were found in terms of other comorbidities or arterial and venous thrombotic risk factors.

2. Among the previously asymptomatic group a tendency to thrombotic complications occurred only in patients with the I APA profile according to the revised classification criteria for definite APS. Therefore the primary, anticoagulant prophylaxis should be considered in such patients.

3. Coexistence of APA and inherited thrombophilia may further increase the prothrombotic risk of a single defect presence, what was shown in the respect to the natural anticoagulant deficiency and/or activated protein C resistance.

4. The most sensitive laboratory assay, confirming the APA presence was LA.

5. The APA profile may vary personally with time, as demonstrated in 1/3 of all investigated persons. Therefore, for reliable diagnosis, the second laboratory assessment (after 12 months) should include the whole panel of assays, recommended for the APS recognition.

In some patients the spontaneous vanishing of initially detected APA was confirmed.

6. Further, especially prospective, investigations are needed in patients recruited to the proposed groups, but in much higher number and with the APA profile classification inclusion. Based on a long recruitment period and enrollment limitations, a multicenter cooperation should be established.

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