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Investigation of potential correlations between the antiphospholipid syndrome and some parasitary and bacterial infections in Romanian patients

Marian Ghervan[1]*, Irina Teodorescu[2], Tatiana Roșca[3], Valentina Uscătescu[4]

ABSTRACT

The association of the antiphospholipid syndrome (APS) with some parasitary and bacterial infections was investigated on a statistically significant group of 6,657 patients with various vasculary disorders (ocular and neurological), from district clinics of Bucharest and throughout the country. Patients were investigated in the ambulatory service of the Clinic of Neurosurgery of Saint Pantelimon Emergency Hospital, Bucharest, over a period of six years (2004-2009). Most of them (96.4 %) were diagnosed with antiphospholipid syndrome, in the Clinic of Hematology, Fundeni Clinical Institute, Bucharest, using specific blood tests. The patients diagnised with antiphospholipid syndrome have been tested for nine visceral parasitary diseases and bacterial sepsis by three ways: i) serological investigations (ELISA IgM for *Toxoplasma gondii*, *Larva migrans visceralis*, *Cysticercus* sp., *Trichinella* sp., *Giardia intestinalis*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Borrelia burgdorferi* sensu lato, and IgG for *Toxoplasma gondii*) ii) statistical analyses (Bravais-Pearson correlation coefficient R^2) and iii) evaluation of the effect of the anti-parasitary and antimicrobial treatments upon the symptoms of the antiphospholipid syndrome. A statistically significant positive linear correlation was established between the antiphospholipid syndrome and eight of the nine etiological agents, i.e. (*Giardia intestinalis*, *Borrelia burgdorferi*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *Larva migrans visceralis*, *Mycoplasma pneumoniae*, *Toxoplasma gondii*, *Cysticercus* sp.). The etiologic treatments for parasitoses and bacterial sepsis have proved to eliminate the respective parasitic and bacterial agents, and also to improve the health status and to turn specific antiphospholipid syndrome tests into negative. Coexistence of the antiphospholipid syndrome with some parasitic and bacterial infection in 96.6 % of patients, the statistically positive correlations, as well as the effect of the etiological treatment upon the antiphospholipid syndrome, can be considered strong arguments for the hypothesis that the etiologic agents of these infections can favor or even induce pathological processes that determine the antiphospholipid syndrome..

Keywords: *antiphospholipid syndrome, correlation coefficient, antiphospholipid antibodies*

1. INTRODUCTION

Antiphospholipid antibody syndrome (APLS) is regarded as an autoimmune disorder commonly called Antiphospholipid syndrome (APS), Hughes syndrome (in honor of the doctor rheumatologist

[1] Department of Parasitology and Immunology, Fundeni Clinical Institute, Bucharest
*Corresponding author e-mail address: ***teodorescubiologie@yahoo.com***
[2] Faculty of Biology, University of Bucharest
[3] Neurosurgery Clinic, Saint Pantelimon Emergency Hospital, Bucharest
[4] Hematology Clinic, Fundeni Clinical Institute, Bucharest

Graham R. V. Hughes who first described it in 1983) or "sticky blood syndrome" (because it causes the thickening of the circulating blood). Similar to other immune diseases, in which the immune system targets the own body tissues (autoimmunity), in the APS, abnormal antibodies occur, called antiphospholipid antibodies (APL), specific to anionic phospholipids on plasma membranes. These antiphospholipid antibodies are targeting cardiolipin, a normal anionic phospholipid present in the cells membrane, β_2-glycoprotein-I, a plasmatic protein attached to cardiolipin and lupus anticoagulant factors, represented by phospholipids and proteins associated with the cell membrane.

The presence of these APLs may increase the blood coagulation rate both in arteries and veins, affecting the normal functions. Thromboses occurred in the placenta vessels may cause an increased risk of pregnancy-related complications (miscarriage stillbirth, preterm delivery, severe preeclampsia). The exact cause of the APS is not known, but in all cases it is associated with thrombosis and vascular disease, affecting the cerebral vascular system (migraine, diplopia, memory loss, ataxia, cerebrovascular accidents or "multiple sclerosis-like" features), coronary arteries (heart valve disease, endocarditis, myocardial infarction), pulmonary system (pulmonary embolism), arterial or venous system in the extremities (with paraesthesia), hepatic and renal veins, ocular arteries and veins, or adrenal glands [1-28]. Some authors imply as possible causing agents the environmental factors (viral, bacterial infections) and certain medications.

Besides some conditions that favor thrombosis, important risk factors generating blood clots are surgery or pregnancy, hypertension, obesity, smoking, high levels of cholesterol, atherosclerosis, sedentary, use of contraceptive estrogens, and hormone replacement therapy.

In the laboratory, the APS is diagnosed using both liquid phase coagulation assays (lupus anticoagulant) and solid phase ELISA assays (anti-cardiolipin antibodies).

2. EXPERIMENTAL SECTION

Our study was performed on a group of 6,657 patients from the Ambulatory Neurosurgery Clinic of Saint Pantelimon Emergency Hospital, Bucharest, admitted for different vascular pathologic conditions (ocular and neurologic), between 2004 and 2009.

The APS was diagnosed in the Hematology Clinic of Fundeni Clinical Institute, Bucharest, based on the ELISA assay of three specific antiphospholipid antibodies (anti-cardiolipin, anti-lupus anticoagulant and anti-β2 glycoprotein). Additionally, to each patient the following parameters were investigated: protein C, protein S, AT III, APC-R, LDL cholesterol, triglicerides, and liver enzymes.

Parasitic and bacterial infections were diagnosed by serological investigations in the Department of Parasitology and Immunology of the Fundeni Clinical Institute, Bucharest.

The calculation of the correlation between the incidence of APS and different infections was made in the Faculty of Biology, University of Bucharest, using parametric correlations, namely Bravais-Pearson correlation coefficient (R), at a significance level of $p = 0.2$ (with values ranging in a confidence level of 80 %), using STATISTICA 9.1. software. The high significance level was considered at $p < 0.01$, while normal significance was considered at $p < 0.2$. Linear correlation is considered perfect when R^2 is equal or close to value" 1 ".

3. RESULTS SECTION

The aim of this study was to investigate the possible correlation between the presence of the APS and of simultaneous infections with nine visceral parasites and invasive bacteria, by analyzing a great number of the analyzed patients (6,657) distributed on a long period of time (six years) in an ambulatory service, allowing the proper statistical analysis of the obtained results.

The great majority of the 6,657 patients investigated in the Ambulatory of the Neurosurgery Clinic of Saint Pantelimon Emergency Hospital, Bucharest (i.e. 96.4 %) exhibited various vascular

disorders (ocular, neurological, skin and others), manifested by: skin rash named livedo reticularis (a vascular condition characterized by a purplish discoloration of the skin, usually on the legs, caused by swelling of the venules owing to obstruction of capillaries by thrombi), strong migraines, cerebral vascular accidents, pains, insensitivity, numbness, tingles in hands and foots, shank ulcerations, cardiac pains, infarct, women with premature birth or spontaneous abortions, ocular lesions detected at the back of the eye exam, performed by a neurosurgeon.

All the patients with vascular disorders were diagnosed with APS in the Hematology Clinic of Fundeni Clinical Institute, Bucharest (Table 1).

Anti-cardiolipin IgG antibodies identified by ELISA were detected in over 96 % of investigated patients, being more common than the lupus anticoagulant antibodies, which were present in about 660 patients and proved to be stronger risk factors for venous and/or arterial thrombosis than the anti-cardiolipin antibodies.

Also, the concomitant presence of both lupus anticoagulant antibodies and anti-cardiolipin antibodies in many patients was associated with a higher risk of thrombosis, comparatively with each one of the two separate antibodies.

The presence of the anti-beta-glicoprotein I in about 36 % of investigated patients could be considered significant, these antibodies being associated with thrombosis and thrombocytopenia.

Protein C, also known as autoprothrombin II A, was present in the majority of the analyzed patients (over 4.500 from all investigated patients). This is a vitamin K-dependent plasma glycoprotein with an anticoagulant role, which in its activated form is playing an important role in the common coagulation pathway, by degrading a protein that is functioning as a cofactor of the coagulation system, named the activated factor V.

Protein S is a vitamin K-dependent plasma glycoprotein synthesized in the endothelium, with a role in the anti-coagulation pathway, acting as a co-factor to Protein C (thus, anti-protein S antibodies decrease protein C efficiency).

Antithrombin III (AT III), a plasma glycoprotein that inactivates several enzymes of the coagulation system, including thrombin, was present only in 7.6 % of patients. Antithrombin III deficiency is a rare hereditary disorder in patients suffering from recurrent venous thrombosis and pulmonary embolism.

Activated protein C resistance (APC-R) can cause an increased risk of thrombosis due to a poor anticoagulant response to the activated protein C. The presence of APC-R, activated by lupus anticoagulants detected in over 470 patients represents a strong risk factor for venous thromboembolism, especially the venous one.

The lupus anticoagulant, protein S, AT III, APC-R could represent agravating factors for the occurrence of major vascular accidents in patients with APS.

Table 1: Results of the laboratory investigation of different parameters used for the diagnosis of the antiphospholipid syndrome

No. crt.	Laboratory investigations	Methods	Patients with positive values	
			Number	%
1.	Anti-cardiolipin antibodies	ELISA IgG	5187	96.6
2.	Lupus anticoagulant	DRVVT test (Dilute Russel Viper Venom Time)	659	12.7
3.	Anti-β2 glicoprotein I	ELISA IgG/IgM	1862	35.9
4.	Protein S	STA Protein C chromogen	1260	24.3
5.	Protein C	STA Protein S clotting	4539	87.5
6.	Antitrombin AT III	STA Antitrombin III	394	7.6
7.	APC-R	STA-STALCOT APC-R.(1)	472	9.1

The correlation of various parasitic and invasive bacterial infections with the antiphospholipid syndrome. The ratio between the APS incidence values (new annual number of cases with APS) and the total number of investigated patients was high, varying between 91.87 % in 2004 and 98.39 % in 2005, with values over 98 % in three years and a high mean value (96.34 %).

The possible association between antiphospholipid syndrome and nine infections, out of which five with parasitic, i.e. *Toxoplasma gondii*, *Larva migrans visceralis* (*Toxocara* sp larvae), *Cysticercus* sp., *Trichinella* sp., *Giardia intestinalis*, and four with bacterial etiology, i.e. *Chlamydia trachomatis*, *C. pneumoniae*, *Mycoplasma pneumoniae* and *Borrelia burgdorferi* sensu lato as etiologic agents was tested by serological investigations (Table 2), represented by: IgM ELISA for *Toxoplasma gondii*, *Larva migrans visceralis*, *Cysticercus* sp., *Trichinella* sp., *Giardia intestinalis*, *Chlamydia trachomatis*, C. *pneumoniae*, *Mycoplasma pneumoniae*, *Borrelia burgdorferi* and IgG ELISA for *Toxoplasma gondii* detection.

From the total number of the investigated patients, the highest number of cases exhibited concomitant infections with *Toxoplasma gondii* in 6,584 infected patients (about 99 %), *Mycoplasma pneumoniae* in >5,600 infected patients (> 84 %), and *Giardia intestinalis*, in 4,094 infected patients (61.5 %). Significant values of incidence registered also *Chlamydia pneumoniae* infection (43.5 %), *Larva migrans visceralis* (36.77 %) and *Borrellia burgdorferi* sensu lato (35.07 %). The lower number of incidence among the analyzed patients was registered for the infection with *Cysticercus* sp. and *Trichinella* sp. In all of the cases of infections with at least one of the analyzed parasites and bacteria, the APS was present. The most frequent polymicrobial associations were represented by *Mycoplasma pneumoniae*, *Giardia intestinalis* and *Chlamydia pneumoniae* (Table 2).

Statistical analysis of the obtained results. The value of R^2 is typically taken as "the percent of variation in one variable explained by the other variable". Bravais-Pearson correlation coefficient is a measure of the correlation (linear dependence) between the two variables, in our cases, APS and infection with different etiologies. A value over 0.3 was considered as positive linear relationship, with the following stratification: i) $R^2 > 0.9$ indicating a *strong significant* positive linear correlation with antiphospholipid syndrome; ii) R^2 values between 0.7 and 0.9 indicating a *significant* positive linear correlation; iii) R^2 value between 0.3 and 0.7 indicating a *moderate* positive linear correlation (Table 3). By analyzing the correlation between the number of patients with APS and the number of patients infected with the nine analyzed protozoan, helminthes and bacterial species, a statistically positive correlation was obtained in the case of seven microbial diseases, with three degrees of correlation: i) a statistically *strong significant* positive linear correlation was observed for five etiologies, out of which one protozoan (*Giardia intestinalis*), one helminthes (*Larva migrans visceralis*) and three bacteria (*Borrelia burgdorferi*, *Chlamydia trachomatis* and *Chlamydia pneumonia*), with the correlation coefficient R^2 values between 0.909 and 0.989, therefore near"1"; ii) a statistically *significant* positive linear correlation in two cases, i.e. infections with the protozoan *Toxoplasma gondii* (diagnosed by ELISA IgM) and respectively, with the bacteria *Mycoplasma pneumoniae* (R^2 values over 0.7 and under 0.9); iii) a moderate positive linear correlation, in two cases, i.e. infections with the protozoan *Toxoplasma gondii* (diagnosed by ELISA IgG) and with the *Cysticercus* helminths (R^2 values around 0.580).

No positive linear correlation between *Trichinella* and the APS was obtained (Table 3).

Linear relationships can be expressed in a graphical format where the two variables are connected *via* a straight line. The graphic analysis revealed that if the observed pattern between the two variables appeared to be linear (in case of *Giardia intestinalis* and *Borrellia burgdorferi*) (Figures 1-2), or approximately linear (in case of *Chlamydia trachomatis*, *Chlamydia pneumoniae*, *larva*

migrans visceralis) (Figures 3-5), then the correlation coefficient R^2 provideed a reliable measure of the strength of the linear relationship. In the cases of *Toxoplasma gondii* (diagnosed by ELISA IgM) and *Mycoplasma pneumoniae* infections, the pattern of the two variables appears to be less linear, proving a less strong, but however significant relationship between the two variables (Figures 6-7). In the cases of *Toxoplasma gondii* (diagnosed by ELISA IgG) and *Cysticercus* sp. the pattern appears to be nonlinear, and then the correlation was questionable or non-existent (Figures 8-9).

Table 2: The annual distribution of patients infested with visceral parasites and infected with intracellular bacteria, diagnosed by IgM and IgG ELISA (2004- 2009)

Years	Number of investigated patients	Patients with antiphospholipid syndrome	*Toxoplasma gondii* IgG+IgM	*Larva migrans visceralis* IgM	*Cystiercus* IgM	*Trichinella* IgM	*Giardia intestinalis* IgM	*Chlamydia trachomatis* IgM	*Chlamydia pneumoniae* IgM	*Mycoplasma pneumoniae* IgM	*Borrelia burgdorferi* IgM
2004	948	871 91.87%	IgG 507 (58.2%) +IgM 303 (34.78%)	223 25.6%	0	1 0.11%	494 56.7%	80 9.18%	312 35.8%	786 90.2%	107 12.2%
2005	1062	1045 98.39%	IgG 624 (59.71%) + IgM 292 (27.9%)	349 33.3%	1 0.09%	0	572 54.7%	116 11.1%	433 41.4%	804 76.9%	183 17.5%
2006	1214	1182 97.36%	IgG 543 (45.9%) +IgM 450 (38.07%)	305 25.8%	4 0.33%	0	650 54.9%	248 20.9%	467 39.5%	959 82.1%	310 26.2%
2007	1187	1150 96.88%	IgG 572 (49.7%) +IgM 554 (48.1%)	481 41.8%	2 0.17%	1 0.08%	719 62.5%	295 25.6%	545 47.3%	1021 88.7%	479 41.6%
2008	955	939 98.32%	IgG 431 (47.1%) +IgM 505 (53.7%)	501 53.3%	3 0.31%	0	821 87.4%	331 35.2%	529 56.3%	910 96.9%	552 58.7%
Total	5366	5187 96.66%	4981 96% IgG 2877 55.4% IgM 2104 40.56%	1859 25.83%	10 0.19%	2 0.03%	3256 62.7%	1070 20.62%	2286 44.07%	4480 86.3%	1631 31.44%

Table 3: Statistical correlation between the APS and infection with some visceral parasites and intracellular bacteria

Parasitic and bacterial infections in patients with APS	R^2 values
1.Statistically strong significant positive linear correlation with APS (with R^2 near "1" value)	
Infection with *Borrelia burgdorferi* sensu lato	0.9896
Infection with *Giardia intestinalis*	0.9827
Infection with *Chlamydia trachomatis*	0. 9666
Infection with *Larva migrans visceralis* (infection with *Toxocara* sp. larvae)	0.911
Infection with *Chlamydia pneumoniae*	0.9099

2.Statistically significant positive linear correlation with APS	
Infection with *Toxoplasma gondii* (diagnosed by ELISA IgM)	0.879
Infection with *Mycoplasma pneumoniae*	0.7334
3.Moderate positive linear correlation with APS	
Infection with *Toxoplasma gondii* (diagnosed by ELISA IgG)	0.5824
Infection with *Cysticercus* sp.	0.5815
4. No positive linear correlation with APS	
Infection with *Trichinella* sp.	0.1543

Influence of etiologic treatments against parasitoses and bacterial infections upon the clinical and biological parameters of the APS. All cases of infectious diseases received adequate treatments, which proved to be very beneficial to patients, determining an improvement of the health status as well as the gradual switch to negativity of the specific tests used for the diagnosis of the APS, in the 1-6 months following the confirmation of the parasitic and bacterial infections recovery (Table 4). The positive impact of the antimicrobial etiological treatment represents a strong argument to hypothesis that the physiopathological mechanisms of the APS could rely or be favored by the mechanical, chemical and physiological actions exhibited by parasites and bacteria upon patient's organisms.

Table 4: Dynamics of the number of patients exhibiting the switch to negative specific tests for the diagnosis of the antiphospholipid syndrome after the etiologic treatment of the parasitic and bacterial infection

No. crt.	Time after recovering from parasitic or bacterial infection	Patients with APS negative tests	
		Number	%
1.	I month	435	8.38
2.	II month	766	14.76
3.	III month	1582	30.59
4.	IV month	1823	35.14
5.	V month	2494	48.08
6.	VI month	582	11.22

4. CONCLUSIONS

Out of a great number of 6,657 patients with ocular and neurological vasculary disorders, investigated during a period of six years (2004-2009) in the Ambulatory Clinic of the Neurosurgery Department of Saint Pantelimon Emergency Hospital, Bucharest, the majority (6,414) were diagnosed with antiphospholipid syndrome. The same patients were tested for parasitic and bacterial infections with *Toxoplasma gondii*, *Larva migrans visceralis* (*Toxocara* sp. larvae), *Cysticercus* sp., *Trichinella* sp., *Giardia intestinalis*, *Chlamydia trachomatis*, *C. pneumoniae*, *Mycoplasma pneumoniae* and *Borrelia burgdorferi* sensu lato as etiologic agents using serological investigations with ELISA IgM and ELISA IgG tests. In all of the cases of infections with at least one of the parasites or bacteria the APS was present. A statistically positive linear correlation was obtained between seven parasitic and bacterial diseases (i.e. infections with *Borrellia burgdorferi* sensu lato, *Giardia intestinalis*, *Chlamydia trachomatis*, *Toxocara* larvae, *Chlamydia pneumoniae*, *Toxoplasma gondii*, *Mycoplasma pneumoniae)* and the APS, as indicated by the calculated Bravais-Pearson correlation coefficient. Other arguments for the correlation between the APS and some parasitic and bacterial species are represented by the high incidence of such infections (96.6 %) in patients with APS and by the positive consequences of the antimicrobial and antiparasitic treatment both upon the

patients status and the results of the laboratory investigations of the APS, suggesting that infections could favor or induce processes that determine the occurrence of the APS.

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