Are the cutaneous manifestations in patients with primary antiphospholipid syndrome a marker for predicting lung manifestations?

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Abstract

Objective

The aim of this study was to investigate association between pulmonary and skin manifestations in a large group of patients with primary antiphospholipid syndrome (PAPS) as well as their connection with antiphospholipid antibodies.

Methods

Our prospective study comprises of 390 patients with primary APS. Antiphospholipid antibody (aPL) analysis included detection of aCL (IgG/IgM), β_2GPI (IgG/IgM) and LA. Distinct pulmonary and skin associations were determined, as well as their associations with aPL.

Results

In PAPS patients the presence of LA was more common in PTE (p=0.005) and in pulmonary microthrombosis (p=0.003). We revealed statistical significance considering the presence of aCL IgM and pulmonary microthrombosis (p=0.05). Skin ulcerations correlated with positive titres aCL IgM and ß2 GPI IgM (p=0.03 and 0.04, respectively), while pseudovasculitis correlated with positive titres ß2 GPI IgM (p=0.02).

PAPS patients were more more likely to develop pulmonary thromboembolisam if they had livedo reticularis (p=0.005), skin ulcerations (p=0.007), pseudovasculitic lesions (p=0.01), superficial cutaneous necrosis (p=0.005), and digital gangrene (p=0.02). Patients were also more prone to pulmonary microthrombosis if they already had livedo reticularis (p=0.03), skin ulcerations (p=0.007), pseudovasculitic lesions (p=0.05), superficial cutaneous necrosis (p=0.006), and digital gangrene (p=0.02).

Conclusion

There is strong link between some pulmonary and skin manifestations in PAPS patients, suggesting complexity and evolutionary nature of APS. The presence of skin manifestations may be a high risk factor for several types of serious pulmonary manifestations in PAPS. Certain aPL types are associated with distinct pulmonary and skin manifestation, suggesting their predictive role.

Key words

antiphospholipid antibodies; primary antiphospholipid syndrome; pulmonary and skin manifestations

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Introduction

Antiphospholipid syndrome (APS) or Hughes Syndrome represents a systemic autoimmune disorder characterised by arterial and/or venous thrombosis, multiple and recurrent fetal losses, often accompanied by a thrombocytopenia and elevated levels of antiphospholipid antibodies (aPL), such as lupus anticoagulant (LA), anticardiolipin antibodies (aCL) and anti- β_2GPI antibodies (1-3). This syndrome is considered primary (PAPS) if unassociated with any other connective tissue disease, or secondary (SAPS) if it appears in association with other autoimmune disorders, mainly systemic lupus erythematosus (SLE) (4-13). Antiphospholipid antibodies (aPL) are heterogenous and distinct group of antibodies that appears in variety of autoimmune diseases, particularly in systemic lupus erythematosus (SLE) (1). In the absence of an underlying connective tissue disorder, persistent aPL positivity is strongly associated with antiphospholipid syndrome (APS).

Pulmonary manifestations, including embolism and infarction, pulmonary hypertension, adult respiratory distress syndrome (ARDS), fibrosing alveolitis, intra-alveolar haemorrhage, primary thrombosis of lung vessels, as well as pulmonary capillaritis, are often associated with APS (14, 15). Furthermore, a postpartum syndrome and fibrosing alveolitis could be also associated with APS, although less commonly (14, 15). This spectrum of pulmonary disorders in patients with antiphospholipid antibodies is often referred as the "antiphospholipid lung syndrome".

On the other hand, skin is also an important target organ and many patients with APS may present with skin manifestations, like livedo reticularis, necrotising vasculitis, thrombophlebitis, ulcerations, livedo racemosa, digital gangrene, subungual splinter hemorrhages, superficial venous thrombosis, thrombocytopenic purpura, pseudovasculitic manifestations, extensive cutaneous necrosis, or primary anetoderma. Many of these manifestations are interrelated and may occur simultaneously in the same patient. These skin manifestations are very diverse, from minor signs to life-threatening conditions such as widespread cutaneous necrosis (16). The commonest skin manifestation in patients with antiphospholipid syndrome (APS) is livedo reticularis, which is strongly associated with the arterial and microangiopathic subtypes of APS (16). Livedo reticularis (LR) refers to the purple reticular mottling of the skin mostly involving the upper and lower limbs, resulting from a narrowing of the small and medium arteries at the dermis-subcutis border (1, 22). LR appears in areas of anastomoses between two cones where reduced blood flow is associated with the dilatation of venules and capillaries, and the development of blood inflow/outflow obstruction and hyperviscosity.

Skin manifestations are varied and although not included in the diagnostic criteria, may be the presenting feature of this syndrome. Therefore all dermatologists should investigate the possibility of APS when cutaneous findings are related to venous or arterial thrombosis. The risk of thrombosis cannot be predicted, and therefore treatment is not initiated until a thrombotic event occurs. Indefinite anticoagulation is prescribed once a thrombotic event occurs. Prognosis depends on the severity of the clinical manifestations and so, knowledge of the presentation of this disease is important for early detection and prompt treatment to prevent life-threatening consequences of this catastrophic disease process.

Skin involvement may be the first manifestation of APS in 40% of patients. One-third of these patients develop multisystem thrombotic events during the course of the disease, pointing to the importance of detecting aPL in all subjects (17).

The association between pulmonary and skin manifestations in PAPS patients, and the detection of antiphospholipid antibodies (aPL) in some of these patients pointed to the possible association between these manifestations, aPL and thrombosis in APS. In this regard, aim of our study was to observe and investigate association between pulmonary and skin manifestations, as well as with antiphospholipid antibodies in our prospective study of large cohort of PAPS patients.

Materials and methods

We recruited a total of 390 primary antiphospholipid syndrome patients (310 female and 80 male, mean age 47.4±12.1 years). The study was prospective and patients were included consecutively from 2000 until the end of 2016. We investigated association between pulmonary and skin manifestations as well as with aPL type. We also followed other patients' pathological features – neurological, cardiac, haematology, thrombotic, non-thrombotic and other – and we plan to report some of these results soon.

All patients included in the study met the 1997 revised Sapporo criteria for APS (20). The study fulfills the ethical guidelines of the most recent declaration of Helsinki (Edinburgh, 2000) and has received approval from the local ethics committee. All the patients were examined by a group made up of a rheumatologist, pulmonologist, dermatologist, neurologist, cardiologist, radiologist and haematologist.

Diagnosis of pulmonary manifestations was established by clinical findings (chest pain, dyspnea, tachypnea, cough, haemoptysis and other), followed by chest radiography, echocardiography, radionuclide lung scanning, laboratory analysis (D-dimer test), multislice computed tomography, ventilation perfusion scintigraphy and MDCT pulmonary angiography. All these tests were performed in patients with suspicious pulmonary thrombosis or embolism**.** All patients were treated according to international protocols.

Laboratory tests

Patients were evaluated for the presence of antiphospholipid antibodies with routine biochemistry and complete blood cell counts. Lupus anticoagulant (LA) was based on the initial use of phospholipid-depleted or platelet-depleted coagulation tests, such as kaolin clotting time (KCT), dilute Russell's venom viper time (DRVVT), the tissue thromboplastin inhibition test and diluted activated partial thromboplastin time. The LA tests were not performed while the patients were receiving anticoagulant therapy. Anti-cardiolipin (aCL: IgG/IgM) and anti-$\beta_2$glycoprotein I (β_2GPI: IgG/IgM) antibodies were measured by an enzyme-linked immunosorbent assay (ELISA, Binding Site) and expressed in GPL or phospholipids (MPL) units (GPL-U and MPL-U).

Also, we followed revised the laboratory criteria for antiphospholipid syndrome (20):

- Lupus anticoagulant present in plasma, on two or more occasions at least 12 weeks apart, detected according to the guidelines of the International Society on Thrombosis and Hemostasis (Scientific Subcommittee on lupus anticoagulant/phospholipid-dependent antibodies).
- Anticardiolipin antibody of IgG or IgM isotype, or both, in serum or plasma, present in medium or high titres (*i.e.* >40 GPL or MPL, or greater than the 99th percentile) on two or more occasions, at least 12 weeks apart, measured by a standardised ELISA
- Anti-β2-gycoprotein 1 antibody of IgG or IgM isotype, or both, in serum or plasma (in titres greater than the 99th percentile), present on two or more occasions, at least 12 weeks apart, measured by a standardised ELISA.

Antinuclear antibodies (ANA) were determined by indirect immunofluorescence on mouse liver and HEp-2 cell substrate. Anti-double-stranded DNA (anti-dsDNA) antibodies were determined by ELISA, Binding Site.

Statistics

Results are presented as the mean SD, except for frequencies, which are expressed as percentages. Comparisons between groups were made by means of Mann-Whitney U-tests for continuous variables, and by chi-square analysis for categorical variables. For 2 by 2 tables Yates Correction for Continuity was used if necessary. Two sided probability (p) values <0.05 (2-tailed) were considered significant. All statistics analysis was performed with using SPSS v. 20.0 statistical package.

Results

Distribution of aPL in the patients with PAPS is presented in Table I. Among 390 PAPS patients 120 of them (31%) had positive aCL IgG, 187 (48%) had positive aCL IgM, 117 (30%) had positive β_2GPI IgG, 133 (34.8%) had positive β_2GPI IgM, and 241 patients (62.4%) had positive LA.

Table I. Distribution of aPL in the patients with primary APS

aPL	PAPS n=390 n (%)
aCL IgG	120 (31%)
aCL IgM	187 (48%)
β_2GPI IgG	117 (30%)
β_2GPI IgM	133 (34.8%)
LA	241 (62.4%)

PAPS: primary antiphospholipid syndrome; aCL: anticardiolipin antibodies; β2GP: anti- β2 glycoprotein I antibodies; LA: lupus anticoagulant; aPL: antiphospholipid antibodies.

Frequency of pulmonary and skin manifestations in patients with primary APS is presented in Table II. The most common lung manifestation was pulmonary embolism and infarction (44 patients or 11.3%). Beside this, PAPS patients had primary pulmonary hypertension (5 patients or 1.3%), secondary pulmonary hypertension (14 patients or 3.6%), major pulmonary arterial thrombosis (7 patients or 1.8%), pulmonary microthrombosis (48 patients or 12.3%), acute respiratory distress syndrome (6 patients or 1.5%), pulmonary intra-alveolar haemorrhage (1 patients or 0.3%), and fibrosis alveolitis (1 patients or 0.3%).

Among skin manifestations, the most common was livedo reticularis (54 patients or 13.8%). Besides this, 31 patients (or 7.9%) had skin ulcerations, 36 patients (or 9.2%) had pseudovasculitic lesions, 9 (2.3%) had superficial cutaneous necrosis, and 4 (or 1.02%) had digital gangrena.

We performed analysis of pulmonary and skin manifestations according to different antiphospholipid antibodies (aPL) types (Table III). The presence of LA was more common in patients with PTE (p=0.005) and pulmonary microthrombosis (p=0.003). We also revealed statistical significance considering the presence of aCL IgM and pulmonary microthrombosis (p=0.05).

In PAPS patients skin ulcerations correlated with positive titres aCL IgM and β2 GPI IgM (p=0.03 and 0.04,

Table II. Demographic and clinical characteristics of the patient group.

Variable		Frequency n (%) n=390
Age	<45	79 (20.26)
	45-50	210 (53,85)
	50-55	61 (15,64)
	>55	40 (10.26)
Sex	Female	310 (79.48)
	Male	80 (20.52)
Pulmonary manifestations	Pulmonary embolism and infarction	44 (11.3%
	Primary pulmonary hypertension	5 (1.3%
	Secondary pulmonary hypertension	14 (3.6%)
	Major pulmonary arterial thrombosis	7 (1.8%)
	Pulmonary microthrombosis	48 (12.3%)
	Acute respiratory distress syndrome	6 (1.5%)
	Pulmonary intra-alveolar haemorrhage	1 (0.3%)
	Fibrosis alveolitis	1 (0.3%)
Skin manifestations	Livedo reticularis	54 (13.8%)
	Skin ulcarations	31 (7.9%)
	Pseudovasculitic lesions	36 (9.2%)
	Superficial cutaneous necrosis	9 (2.3%)
	Digital gangrena	4 (1.02%)

Table III. Associations between pulmonary and skin manifestations and aPL in PAPS patients.

Pulmonary and skin manifestations	PAPS patients, n (%)		
	positive	negative	*p*-value
	LA		
Pulmonary embolism and infarction	34 (77.3%)	10 (22.7%)	*p*=**0.005**
Pulmonary microthrombosis	37 (77.1%)	11 (22.9%)	*p*=**0.003**
	aCL-IgM		
Pulmonary microthrombosis	39 (81.2%)	9 (18.8%)	*p*=**0.05**
Skin ulcerations	25 (83.4%)	5 (16.6%)	*p*=**0.03**
	β2 GPI IgM		
Pseudovasculitis	22 (62.9%)	13 (37.1%)	*p*=**0.02**
Skin ulcerations	19 (61.3%)	12 (38.7%)	*p*=**0.04**

respectively), while pseudovasculitis correlated with positive titres β2 GPI IgM (*p*=0.02). There were no significant associations between the positive antibodies and other pulmonary or skin manifestations.

The next step in our study was to test possible associations between pulmonary and skin manifestations in our large group of PAPS patients (Table IV). In our group patients were more likely to develop pulmonary thromboembolism if they had livedo reticularis (*p*=0.005), skin ulcerations (*p*=0.007), pseudovasculitis lesions (*p*=0.01), superficial cutaneous necrosis (p=0.005), and digital gangrene (*p*=0.02). PAPS patients were more prone to pulmonary microthrombosis if they already had livedo reticularis (*p*=0.03), skin ulcerations (*p*=0.007), pseudovasculitis lesions (*p*=0.05), superficial cutaneous necrosis (*p*=0.006), and digital gangrene (*p*=0.02).

Finally, our study showed no correlation between age of patients, smoking habits, gender, lipid profile or disease duration and pulmonary and skin manifestations in PAPS.

Discussion

We present the results from our prospective study with PAPS patients, which started in 2000. It was performed in University Medical Center, "Bezanijska Kosa", Belgrade, in collaboration with other Clinical Centers in Serbia. Beside frequent clinical manifestations of antiphospholipid syndrome, we followed less common, unusual and rare manifestations, according to Ruiz-Irastorza *et al*. (21).

This is our first, national study that investigates influence of antiphospholipid antibody type on pulmonary and skin manifestations as well as association between pulmonary and skin manifestations in PAPS patients. We observed many lung and skin manifestations in primary antiphospholipid syndrome patients. Some of them occurred significantly more often than others: pulmonary embolism and infarction and pulmonary microthrombosis were the most common pulmonary manifestations, with total prevalence of 11.3% and 12.3% patients, respectively. The frequency of the most often lung manifestations in our study group was comparable to data reported in previous studies (14, 18, 19). Pulmonary emboli were not always consequence of deep venous thrombosis.

The most common skin manifestations in our group were livedo reticularis (13.8%), pseudovasculitic lesions (9.2%) and skin ulcerations (7.9%). Toubi *et al*. reported that livedo reticularis is a frequent cutaneous manifestation in patients with APS and in his study group the prevalence was 16%, but they analysed PAPS and SAPS group together (22).

Our study also indicates that presence of certain types of aPL are associated with increased probability of some lung and skin APS manifestations. Many previous studies showed that lupus anticoagulant is the strongest risk factor for thrombosis (23-25). It is considered that detection of LA is very useful for establishing thrombotic risk in patients with APS, since odds ratio for thrombosis is much higher than controls. This study indicates that presence of certain types and levels of aPL are associated with increased probability of some pulmonary manifestations. We revealed statistical significance considering the presence of LA and pulmonary embolism and infarction, and between presence of LA and pulmonary microthrombosis in PAPS group.

According to levels of specific aPL we revealed that presence of positive aCL IgM were in correlation with the appearance pulmonary microthrombosis

Table IV. Association between pulmonary and skin manifestations in PAPS patients.

PAPS patients N=390		Pulmonary thromboembolism n (%)			Pulmonary microthrombosis n (%)		
		not present	present	p	not present	present	p
Livedo reticularis	not present	300 (76.9%)	36 (9.2%)	0.005	298 (76.4%)	38 (9.7%)	0.03
	present	46 (11.8%)	8 (2%)		44 (11.3%)	10 (2.6%)	
Skin ulcerations	not present	319 (81.8%)	40 (10.2%)	0.007	315 (80.8%)	44 (11.3%)	0.007
	present	27 (6.9%)	4 (1%)		27 (6.9%)	4 (1%)	
Pseudovasculitic lesions	not present	319 (81.8%)	36 (9.2%)	0.01	313 (80.2%)	42 (10.8%)	0.05
	present	27 (6.9%)	8 (2%)		29 (7.4%)	6 (%)	
Superficial cutaneous necrosis	not present	339 (87%)	42 (10.8%)	0.005	335 (85.9%)	46 (11.8%)	0.006
	present	7 (1.8%)	2 (0.5%)		7 (1.8%)	2 (0.5%)	
Digital gangrene	not present	342 (87.7%)	44 (11.3%)	0.02	338 (86.7%)	48 (12.3%)	0.02
	present	3 (0.8%)	1 (0.2%)		3 (0.8%)	1 (0.2%)	

PAPS: primary antiphospholipid syndrome; n: number of patients.

and skin ulcerations in the PAPS patients. Nevile *et al*. found that an increase in aCL IgG level is associated with an increased risk of arterials and venous thrombosis (26). On the other hand, Marai *et al*. did not find any correlation between the aCL antibody levels and clinical manifestations of APS (27). However, our study revealed significant correlation between positive aCL IgM levels and pulmonary microthrombosis and skin ulcerations. Possible explanation for differences among these studies can be that samples were not big enough, differences in study design or different definitions of aCL positivity, diversity of antiphospholipid antibodies in terms of types, isotypes, cut-offs and laboratory methods performed for their detection (14).

Some previous studies have found a close association between clinical manifestation of APS and beta$_2$GPI antibodies (28-32). In our study skin ulcerations correlated with positive titres aCL IgM and β2 GPI IgM, while pseudovasculitis correlated with positive titres β2 GPI IgM.

Our results suggest the predictive role of positive aCL IgM and LA in severe pulmonary manifestations - pulmonary embolism and infarction and pulmonary microthrombosis in PAPS patients, and predictive role of positive aCL IgM and β2 GPI IgM in pseudovasculitis and skin ulcerations.

Toubi *et al*. reported that livedo reticularis (LR) had high association with cardiac and CNS thrombosis, which may suggest that LR-APS patients compose a subset at higher risk for thrombosis, and thus may require a closer follow-up and a more aggressive anticoagulation (22). Our study showed that PAPS patients more likely developed pulmonary thromboembolism if they had livedo reticularis, but also skin ulcerations, pseudovasculitis lesions, superficial cutaneous necrosis, and digital gangrene. Furthermore, PAPS patients were more prone to pulmonary microthrombosis if they had livedo reticularis, skin ulcerations, pseudovasculitis lesions, superficial cutaneous necrosis, and digital gangrene. Our findings confirmed that livedo reticularis, as well as some other skin APS manifestations, represent a high risk for some serious thrombosis in lung. Awareness of these associations might give us better understanding of the disease.

Conclusions

There is strong link between some pulmonary and skin manifestations in PAPS patients, suggesting complexity and evolutionary nature of APS. The presence of skin manifestations may be a high risk factor for several serious pulmonary manifestations in PAPS. Certain aPL types are associated with distinct pulmonary and skin manifestation, suggesting their predictive role.

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