European Review for Medical and Pharmacological Sciences 2021; 25: 7369-7374

Thrombotic microangiopathy in primary antiphospholipid syndrome is linked to stroke and less deep venous thrombosis

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Abstract. – OBJECTIVE: To compare clinical and laboratory data obtained from patients with primary Antiphospholipid Syndrome (pAPS) with and without Thrombotic Microangiopathy (TMA).

PATIENTS AND METHODS: A cross-sectional study with 66 (83.3% female) pAPS patients was performed. Demographic, clinical, drug use, antiphospholipid antibodies data were evaluated. Patients were subdivided into one of two groups: pAPS with TMA and pAPS without TMA and were compared.

RESULTS: In this sample, 5/66 (7.6%) of patients had TMA. Primary APS with TMA group exhibited a higher frequency of arterial events (100% *vs.* 54.1%, *p*=0.02), stroke (100% *vs.* 32.8%, *p*=0.001) and a lower frequency of deep venous thrombosis (0 vs. 68.9%, *p*=0.0009) compared to the patients without TMA. Analysis of therapy used in these patients showed a higher frequency of current (40% *vs.* 6.6%, *p*=0.0006) and previous glucocorticoid use (80% *vs.* 36%, *p*=0.0007) and statin use (50% *vs.* 22.9%, *p*=0.037) in the first group. The two groups exhibited no differences in the frequency of positive autoantibodies, except for higher IgG anticardiolipin titers (86 ± 52 *vs.* 34.5 ± 39 GPL, *p*=0.003).

CONCLUSIONS: Patients with pAPS and TMA have distinct clinical and laboratory spectra from those without TMA, that is characterized by an increased frequency of arterial events, stroke, and higher titers of IgG anticardiolipin; they have deep venous thrombosis less frequently.

Key Words:
Thrombotic microangiopathy, Antiphospholipid syndrome, Thrombosis, Thrombophylia, Stroke, Deep venous thrombosis.

Introduction

Antiphospholipid syndrome (APS) is a thrombophilic autoimmune disease characterized by recurrent thrombotic events and/or pregnancy morbidity associated with the presence of antiphospholipid antibodies[1].

APS is a heterogeneous condition with diverse clinical manifestations and autoantibodies profile and, unlike other thrombophilias, involves both venous and arterial vessels. Deep vein thrombosis is one of the most common clinical presentations; the arterial bed involvement may manifest as stroke, coronary artery disease, peripheral artery disease, and renal involvement[2,3].

Renal involvement in APS primarily results from renovascular thromboses involving renal arteries, intraparenchymatous arteries, glomerular capillaries, or renal veins[4]. The involvement of microcirculation in the kidney leading to APS nephropathy is called thrombotic microangiopathy (TMA). Its diagnosis requires the presence of one or more acute or chronic typical intrarenal lesions on histology[5] after ruling out other causes of renal TMA[4]. No study comparing APS patients with and without TMA has been reported previously.

Herein we studied a sample of primary APS patients to know if those with TMA are clinically and/or serologically different from those without TMA.

Patients and Methods

This is a cross-sectional study that included sixty-six primary APS patients regularly followed at a private clinic. All subjects completed the Sidney criteria for primary APS[6]. Patients of both genders with ages equal to or greater than 18 years were included. The authors fulfilled the Helsinki World declaration; the study was approved by Federal University of Bahia Ethical Committee and all participants gave informed consent.

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Stroke and vascular events were confirmed by computed tomography (CT), magnetic resonance imaging (MRI), computed tomography angiography (CTA), and/or magnetic resonance angiography (MRA). Other vascular events, arterial (including limb ischemia) or venous, were diagnosed by the clinical presentation followed by imaging confirmation by CT, MRI, CTA, MRA, Doppler sonography, ventilation/perfusion scintigraphy, and/or arteriography. Thrombocytopenia was defined as a platelet count less than 100,000/mm^3 on at least two consecutive occasions. Livedo reticularis diagnosis was based on typical *livedo reticularis* on the physical examination. In the case of clinical doubt, a skin biopsy was performed. Sneddon's syndrome was considered as the presence of livedo reticularis and stroke[7]. Data on the presence of comorbidities such as smoking, arterial hypertension (defined by blood pressure > 140/90 mmHg), and diabetes (defined as fasting glucose above 126 mg/dL) were collected. Moreover, dyslipidemia (defined by the National Cholesterol Education Program[8] as well as the use of medications (corticosteroid, warfarin, antimalarial, statins, acetylsalicylic acid), were also recorded.

Thyroid dysfunction was defined as hypothyroidism (low free T4 or high TSH levels) and hyperthyroidism (high free T4 or low TSH levels) in sera independently of thyroid symptoms.

TMA was defined by renal dysfunctions such as increased creatinine level, alterations of urinary sediment, proteinuria, and/or hypertension associated with positive renal histopathology. All patients with TMA had kidney biopsy confirming the diagnosis.

Statistical Analysis

The central tendency was expressed as mean ± standard deviation (SD). Statistical analysis was carried out using JASP 0.12.2 version Software (Amsterdam, The Netherlands). Student's *t*-test was used to compare means, and comparisons between proportions were calculated using x^2 test. *p*-values below 0.05 were considered statistically significant.

Results

Description of the Studied Sample

In the sample of 66 APS, 55/66 (83.3%) were females, and 11/66 (16.6%) were males. The main epidemiological, clinical, and treatment characteristics of this sample are in Table I.

In this sample, 5/66 (7.6%) of patients had TMA. All patients had endothelial proliferation with intimal hyperplasia of the renal arteries, redundant basal membrane, and, in some cases, basal membrane was doubled. Other causes of TMA were excluded.

All patients had systemic artery hypertension; the mean creatinine was 1.7 mg/dL. Regarding urine sediment, no one had hematuria, and cylindruria. All had proteinuria, which varied from 0.5 to 2.1 g in 24 hours. Schistocytes was verified in all 5 patients with MAT, but in small percentage varying from 0.5% to 1% (normal range: < 0.2%).

Comparison of APS Patients with and Without TMA

The comparison of clinical and epidemiological data between patients with and without limb ischemia is in Table II. In this table, it is possible to observe that the pAPS with TMA group exhibited a higher frequency of arterial events (100% *vs.* 54.1%, p=0.02), stroke (100% *vs.* 32.8%, p=0.001 and a lower frequency of deep venous thrombosis (0 *vs.* 68.9%, p=0.0009) compared to the patients without TMA. The other APS manifestations and comorbidities did not differ significantly.

Analysis of therapy used in these patients showed a higher frequency of current (40% *vs.* 6.6%, p=0.0006) and previous glucocorticoid use (80% *vs.* 36%, p=0.0007) and statin use (50% *vs.* 22.9%, p=0.037) in the TMA group in comparison to the group without TMA. There was no difference in warfarin and antimalarial use (p >0.05) between patients from the two groups (Table III).

The study of autoantibodies profile of patients in the PAPS with TMA and PAPS without TMA groups is shown on Table IV. The two groups exhibited no differences in the frequency of positive autoantibodies, except for higher IgG anticardiolipin titers (86 ± 52 *vs.* 34.5 ± 39 GPL, p=0.003) in those with TMA.

Discussion

Our results have shown that about 8% of this sample with pAPS had TMA proved by renal biopsy that was more common in those with arterial events and in those with stroke; venous events were less common in this group of patients.

TMA is a poorly studied entity in pAPS. A study by Nochy et al[5] identify 5 cases in 16 patients (31%) of TMA in patients with pAPS and renal dysfunction. So, when pAPS patients pres-

Table I. Description of studied sample (66) patients with primary antiphospholipid antibody syndrome).

Epidemiological aspects	
Mean age (SD)- years	39.1 ± 10.9
Mean disease duration (SD) (months)	74.1 ± 61.4
Ethnic background (n)	Caucasians – 50/66-75.7% Afro descendants – 16/66-24.3%
Tobacco exposure (n)	Current smokers – 7/66 Ex-smokers – 26/66
Mean body mass index (SD)- kg/m^2	28.1 ± 6.7
Clinical manifestations	
Arterial events (n)	38/66-57.7%
Venous events (n)	43/66-65.2%
Obstetrical events (n)	21/66-31.8%
Deep venous thrombosis (n)	42/66-63.6%
Livedo reticularis (n)	19/66-28.7%
Sneddon's syndrome (n)	13/66-19.6%
Stroke (n)	25/66-37.8%
Convulsions (n)	7/66-10.6%
Myocardial infarction (n)	4/66-6.0%
Angina (n)	3/66-4.5%
Thrombocytopenia (n)	7/66-10.6%
Pulmonary embolism (n)	15/66-22.7%
Thrombotic microangiopathy (n)	5/66-7.5%
Laboratory data	
Anticardiolipin IgM (n)	37/66-56.0%
Anticardiolipin IgG (n)	34/66-51.5%
Lupus anticoagulant (n)	56/66-84.8%
Triple positive (n)	26/66-39.4%
Comorbidities	
Arterial hypertension (n)	25/66-37.8%
Diabetes mellitus (n)	3/66-4.5%
Dyslipidemia (n)	20/66-30.3%
Thyroid disorder (n)	9/66-13.6%
Protein C deficiency (n)	6/66-9.0%
Treatment data	
Acetylsalicylic acid (n)	21/66-31.8%
Warfarin (n)	53/66-80.3%
Antimalarial (n)	11/66-16.6%
Statins (n)	17/66-25.7%
Glucocorticoid (n)	Current – 6/66 Previous – 26/66

ent with renal alterations, the clinician should try to clarify two points. The first is to distinguish the renal involvement of pAPS without TMA from those of pAPS associated with TMA. The second is to clarify if the TMA does not have any other cause than pAPS.

Renal involvement in pAPS without TMA may be due to renal artery thrombosis or stenosis, renal vein thrombosis, injury to the intra renal vasculature and glomerular lesions such as membranous nephropathy, minimal change disease/focal segmental glomerulosclerosis, mesangial C3 nephropathy, and pauci-immune crescentic glomerulonephritis[9,10]. Clinical manifestations are unspecific, ranging from the indolent presentation with hypertension, various degrees of proteinuria (less than 2 g in most patients), presence of active urine sediment (that can include red cell casts) in some patients, and reduced glomerular filtration rate, to acute renal failure[11].

In TMA, small vessel platelet microthrombi occur in several vascular beds, causing thrombocytopenia, microangiopathic hemolytic anemia, and organ injury that may be life-threatening[12].

Table II. Comparison of clinical and epidemiological data of primary antiphospholipid antibody syndrome (pAPS) patients with and without thrombotic microangiopathy (TMA).

	pAPS with TMA n=5	pAPS without TMA n=61	*p*
Mean age (SD) (years)	44.8 ± 4.1	38.6 ± 11.3	0.11
Female sex, n (%)	4 (80)	51 (83.6)	0.42
White race, n (%)	4 (80)	46 (75.4)	0.41
Current smoking, n (%)	1 (20)	6 (9.8)	0.19
Previous smoking, n(%)	2 (40)	24 (39.3)	0.39
Mean body mass index (SD)(kg/m2)	26.5 ± 4.1	28.2 ± 6.9	0.30
Weight (kg)	70.3 ± 12.9	73.7 ± 19.8	0.35
Height (cm)	160.4 ± 5.9	161.3 ± 8.4	0.41
Arterial events, n (%)	5 (100)	33 (54.1)	**0.02**
Venous events, n (%)	2 (40)	42 (68.9)	0.08
Obstetric events, n (%)	2 (40)	19 (31.1)	0.37
Stroke, n (%)	5 (100)	20 (32.8)	**0.001**
Sneddon's syndrome, n (%)	1 (20)	12 (16.4)	0.48
Livedo reticularis n (%)	2 (40)	17 (27.9)	0.69
Acute myocardial infarction, n (%)	0	4 (6.6)	0.28
Angina, n (%)	0	3 (4.9)	0.31
Deep venous thrombosis, n (%)	0	42 (68.9)	**0.0009**
Pulmonary thromboembolism, n (%)	0	15 (24.6)	0.11
Thrombocytopenia, n (%)	1 (20)	6 (9.8)	0.33
Limb ischemia, n (%)	1 (20)	9 (14.7)	0.38
Seizures, n (%)	1 (20)	6 (9.8)	0.25
Arterial systemic hypertension, n (%)	1 (20)	22 (36.1)	0.21
Dyslipidemia, n (%)	1 (20)	18 (29.5)	039
Diabetes mellitus, n (%)	0	3 (4.9)	0.31
Thyroidopathy, n (%)	0 920)	9 (14.7)	0.18

Table III. Comparison of used treatment in pAPS (primary antiphospholipid antibody syndrome) with and without TMA (thrombotic microangiopathy).

	pAPS with TMA n=5	pAPS without TMA n=61	*p*
Current glucocorticosteroid use, n (%)	2 (40)	4 (6.6)	**0.0006**
Previous glucocorticosteroid use, n (%)	4 (80)	22 (36)	**0.0007**
Warfarin use, n (%)	3 (60)	50 (81.9)	0.082
Antimalarial use, n (%)	0	14 (22.9)	0.17
Statins use, n (%)	3 (50)	14 (22.9)	**0.037**
Acetylsalicylic acid use, n (%)	1 (40)	20 (32.8)	0.55

Laboratory findings such as severe thrombocytopenia, schistocytes in peripheral blood smear, microangiopathic anemia, are important clues to determine the kidney injury etiology, but TMA requires a tissue diagnosis made usually by kidney biopsy. Nevertheless, the renal histopathology seen in pAPS with TMA is similar to those seen in other diseases such as hemolytic-uremic syndrome, and thrombotic thrombocytopenic purpura. HELLP syndrome, malignant hypertension, systemic sclerosis, preeclampsia or eclampsia, and medications (cyclosporine, chemotherapy)[9]. These different conditions are impossible to distinguish based solely on the pathologic findings, necessitating correlation with clinical and laboratory data[13,14]. So careful history taking, and clinical examination are necessary. The search for antiphospholipid antibodies can help to identify APS nephropathy-related TMA from the other causes[14,15]. Histological findings in this context include fibrin thrombi within glomeruli and intrarenal vascular tree, in the absence of inflamma-

Table IV. Comparison of antiphospholipid and antinuclear antibodies in patients with primary antiphospholipid syndrome (pAPS) with and without thrombotic microangiopathy (TMA).

	pAPS with TMA n=5	pAPS without TMA n=61	*p*
Lupus anticoagulant, n (%)	5 (100)	51 (83.6)	0.17
Mean IgG aCL, GPL titers (±SD)	86 ± 52	34.5 ± 39	**0.003**
Mean IgM aCL, MPL titers (±SD)	20.2 ± 13	33.2 ± 41.2	0.24
Antinuclear antibodies, n (%)	1 (20)	19 (31.1)	0.33

tory cells or immune vascular deposits that may be associated with fibrous intimal hyperplasia characterized by intense myofibroblast production leading to intimal thickening and interlobular arterial tortuosity. Focal cortical atrophy appears in areas on the surface of the subcapsular cortex, with small and sclerotic or pseudocystic glomeruli (usually in clusters) accompanying with tubular atrophy may also be seen[5].

In the present study, a positive association of TMA with previous arterial events was found. Although stroke is considered one of the most common expression of arterial events in pAPS,[2] the TMA association with stroke is a novel observation. Unlike catastrophic APS[16], TMA typically does not associate to large vessel thromboses. Even though a study by Camous et al[17] identified 7 cases of cerebral artery thrombosis in 55 patients with TMA admitted to a teaching-hospital ICU, but only one of these 7 individuals with stroke had corresponding clinical signs. Nevertheless, none of them had TMA associated to pAPS. There is also a case report, of a patient who developed severe hypertension after gemcitabine use, followed by TMA, and then stroke[18]. Another case description brings to attention a polyarteritis nodosa patient, with stroke diagnosed after a TMA development[19]. A third one describes a patient with human herpesvirus type 6 infection after melphalan use for autologous peripheral stem cell transplantation that had TMA and stroke[20]. Again, no case of pAPS was reported with TMA and stroke.

An interesting observation was the inverse relationship of deep venous thrombosis occurrence with TMA herein observed. No studies that showed this unique negative association were found for comparison. The given explanation was that the mechanism for the arterial thrombosis that predominates in the clinical picture in present context is distinct from those observed in the venous events[21,22].

In this study TMA was linked to IgG anticardiolipin titers, but not with lupus anticoagulant. Some studies have demonstrated the association of IgG anticardiolipin with arterial events in APS such as those of Matyja-Bednarczyk et al[23] that studied retrospectively 163 APS patients and found IgG anticardiolipin antibodies to be associated with arterial events independent of the titers.

This study has limitations such as its transversal design, the low number of participants and the unicentric observation. Multicentric with multiethnic participation to confirm the present data are desirable.

Conclusions

This study used a systematic design to show that individuals with pAPS associated with TMA are linked to stroke and less prone to deep venous thrombosis and have higher titers of IgG anticardiolipin antibodies.

Conflict of interests

The authors declare that they have no conflict of interests.

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