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ORIGINAL ARTICLE

Lupus anticoagulants and anti-phospholipid antibodies as risk factors for a first episode of ischemic stroke

S. SAIDI,* T. MAHJOUB* and W. Y. ALMAWI†

**Research unit of Hematological and Autoimmune Diseases, Faculty of Pharmacy, University of Monastir, Monastir, Tunisia; and †College of Medicine and Medical Sciences, Arabian Gulf University, Manama, Bahrain*

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Summary. *Background:* Antiphospholipid antibodies (aPLA) and lupus anticoagulant (LAC) were shown to precipitate thromboembolic events. Their association with ischemic stroke remains to be seen. *Objectives:* We investigated the contribution of LAC, and antibodies directed against the phospholipids cardiolipin (aCL), phosphatidylserine (aPS), and the phospholipid-dependent cofactors β2-glycoprotein I and annexin V, to the risk for ischemic stroke. *Patients/methods:* LAC and antibody levels were measured in 208 stroke patients and 203 age- and gender-matched control subjects. *Results:* Positive LAC resulted in an increased risk for stroke [OR (95% CI) = 8.1 (2.4–27.5)]. Significant elevation in aPS IgG, aCL IgM and aCL IgG titers, and increased prevalence of elevated aPS IgG, aCL IgM and aCL IgG (based on P95 cutoff values of healthy individuals) were seen in patients. aPS IgG was associated with cardioembolic, whereas aCL IgG and IgM were elevated in lacunar, atherosclerotic and cardioembolic, and LAC positivity was documented only in lacunar stroke subtypes. The co-presence of LAC with a positive aCL IgM/IgG or aPS IgG did not affect the overall risk for stroke. Multivariate analysis confirmed the association of positive LAC with stroke [aOR (95% CI) = 9.7 (1.8–52.5)], and demonstrated a clear gradation of increasing risk of stroke associated with the four categories of aCL IgG and aPS IgG, and identified aCL IgM P95 as independent predictors of stroke after adjusting for potentially confounding covariates. *Conclusions:* Our study demonstrates that the presence of LAC, and elevated aCL IgG and aPS IgG antibodies are risk factors for stroke.

Keywords: antiphospholipid antibodies, ischemic stroke, lupus anticoagulant, thrombosis.

Correspondence: Wassim Y. Almawi, Department of Medical Biochemistry, College of Medicine & Medical Sciences, Arabian Gulf University, PO Box 22979, Manama, Bahrain.
Tel.: +973 39717118; fax: +973 17271090.
E-mail: wassim@agu.edu.bh

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Introduction

Ischemic stroke is a common disease in Western society, with multiple etiologies and major clinical manifestations [1,2]. The pathogenesis of ischemic stroke is complex, and several studies documented hypercoagulable states as a significant mechanism underlying stroke. This was hypothesized to be a result of specific genetic factors, including genetic background and mutations in procoagulant factors [3–5], and also to acquired risk factors [1,6,7]. The latter include protein C, protein S, or antithrombin III deficiencies, activated protein C resistance and anti-phospholipid antibodies (aPLA), including anticardiolipin (aCL) antibodies or lupus anticoagulant (LAC), which influence stroke susceptibility owing to their capacity to disturb normal hemostatic mechanisms [8–11]. aPLA are a heterogeneous family of antibodies that target negatively charged phospholipids, phospholipid-binding proteins, or both [12–14]. While aPLA are clinically associated with a state of hypercoagulation and prothrombotic disorders, the exact mechanism underlying their prothrombotic effects remains unknown [15].

aPLA are detected either functionally, owing to their ability to prolong coagulation time in a phospholipid-dependent coagulation test (LAC), or by measuring specific [anticardiolipin (aCL) and antiphosphatidylserine (aPS)] antibodies by specific immunoassays, using anionic phospholipids as antigens [16,17]. The contribution of LAC to the overall risk of both venous and arterial thrombosis [13,18], including ischemic stroke [18,19], is now well recognized. A meta analysis of five studies involving 753 patients and 234 controls concluded that LAC, more so than aCL, is a strong risk factor for thrombosis, irrespective of the site and type of thrombosis, and suggested that LAC and high-titers aCL IgG detection, rather than detection of specific aPLA antibodies (whose predictive value is unclear), can identify patients at risk for thrombosis [18].

While the contribution of aPLA (including LAC and aCL antibodies) to thrombosis is well established, their role as independent risk factors in the pathogenesis of ischemic stroke yielded apparently conflicting results. An association between different types of aPLA and stroke was suggested by several case–control studies [9–11,20,21], but not by others [22,23]. It was also suggested that the contribution of aPLA to stroke

pathogenesis is both age [20,22,24] and gender [20] dependent, and may depend on the presence of other contributory factors (older age, malignancy and cardiovascular risk factors) [25]. These conflicting results could be explained by differences in ethnic origin [26–28], inherent variation in aPLA levels [14,29] and in the failure in some studies to account for the contribution of covariates [25,30]. Here we investigated whether LAC and aPLA are independently associated with ischemic stroke in Tunisian patients with a first stroke.

Subjects and methods

Patients and control subjects

This was a retrospective case–control study involving 208 consecutive patients with a first ischemic stroke, recruited from the service of Neurology in CHU Sahloul in Sousse, Tunisia. Ischemic stroke was defined as the acute onset of clinical signs of focal disturbance of cerebral function as a result of cerebral ischemia, with symptoms lasting more than 24 h. Diagnosis of ischemic stroke was based on clinical and neurological examination, together with brain CT, magnetic resonance imaging (MRI) and cerebral angiography. In addition, 24-h electrocardiography (ECG), echocardiography, carotid ultrasound and functional evaluations (speech, occupational therapy, etc.) were performed as part of the patient workout. Brain computed tomography (CT) was employed to differentiate between ischemic and hemorrhagic stroke, with etiologic stroke subtypes classified as per the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria [2].

Exclusion criteria included non-atherosclerotic causes, rheumatic heart disease, ventricular arrhythmias, uncompensated heart failure, stroke secondary to atrial fibrillation, hematoma, brain tumors, accidental or iatrogenic stroke, arterial malformation and recent acute myocardial infarction. Information on cardiovascular and cerebrovascular risk factors was obtained at baseline. Cardiovascular disease was positive if subjects reported a history of heart disease or stroke, whereas diabetes was assessed according to fasting blood glucose and/or use of glucose-lowering drugs (including insulin). Obesity was defined as a body mass index (BMI) of 30 kg m^{-2} or higher, and hypertension was defined as blood pressure (BP) > 160/95 mmHg on two separate occasions, and/or the use of antihypertensive therapy.

A total of 203 healthy volunteers and hospital staff served as controls. Controls were frequency matched to cases with regards to age, gender and ethnic/regional origin; none of the controls reported a previous history of cerebrovascular or cardiovascular disease, or thromboembolic disorders (Table 1). Controls were matched to patients with respect to age, gender, BMI and to the prevalence of smokers. Mean systolic and diastolic BP, and number of hypertensive individuals were significantly higher in patients. Significant elevation in serum glucose, triglycerides, creatinine and lower levels of serum high-density lipoproteins (HDL) were seen in patients than controls. Complete demographic and clinical details, including family history of cerebrovascular/cardiovascular diseases and conventional stroke risk factors (hypertension, diabetes, smoking, obesity), were taken for all participants. The study was carried out in accordance with the guidelines of the Helsinki Declaration of 1975, and had the approval of the University of Monastir Ethics Committee; written informed consent was obtained from all participants.

Table 1 Characteristic of study subjects

Characteristic	Patients*	Controls*	OR (95% CI)
Age (years; mean ± SD)	62.6 ± 12.3	62.8 ± 11.8	
Male/female	104:104	114:89	
Hypertension†; number (% total)	145 (67.1)	53 (21.0)	
Anti-β2GPI			
IgM	5.2 (1.2–26.0)‡	5.0 (1.0–22.0)	1.1 (1.0–1.1)
IgG	4.5 (1.0–29.5)	4.7 (1.0–23.0)	1.0 (0.9–1.1)
Anti-annexin V			
IgM	5.0 (0.9–26.0)	6.0 (2.0–21.0)	1.0 (0.9–1.1)
IgG	6.0 (2.2–25.4)	6.0 (2.4–20.9)	1.0 (1.0–1.1)
Anti-phosphatidylserine			
IgM	6.0 (0.5–29.0)	6.0 (0.8–28.6)	1.0 (1.0–1.1)
IgG	7.5 (3.0–39.6)	6.4 (2.6–18.3)	1.1 (1.1–1.3)
Anti-cardiolipin			
IgM	4.0 (1.0–17.4)	3.2 (1.0–12.6)	1.2 (1.0–1.3)
IgG	14.0 (1.5–34.0)	7.0 (1.9–24.4)	1.1 (1.1–1.2)
LAC positivity	23 (11.1)‡	3 (1.5)	8.1 (2.4–27.5)

*Study subjects comprised 203 controls and 208 stroke patients. † Blood pressure of ≥ 140/90 mmHg, and/or use of antihypertensive medications. ‡Median (range) of IgM (in MPL) and IgG (in GPL) values.

Antibody determination

Blood samples were obtained in sodium citrate (0.129 mol L^{-1}) tubes, and platelet-poor plasma was prepared by double centrifugation at 4000 × *g* for 20 min; 100- to 200-μl aliquots were immediately stored at −70 °C pending analysis. Samples were obtained at least 2–3 months after the acute event. LAC detection was carried out on fresh plasma, whereas the detection of other antibodies was performed on either frozen or once-thawed samples.

LAC was assayed in duplicates, as detailed elsewhere [17], with normal values defined from first to 99th percentile figures established for healthy individual plasmas [29]. Individuals who tested positive in the first round were retested 6–8 weeks later. LAC positivity was scored if positive results were attained on two occasions 6–8 weeks apart. LAC was detected using dilute Russell Viper Venom Time (dRVVT), which screens for Lupus-like anticoagulants (aPLA), and DVV Confrim (ratio) confirms LAC presence in plasma as described [31]. Viper venom is snake venom which, in the presence of calcium ions, converts factor (F)X to its activated form FXa, which together with phospholipid and FV, converts prothrombin to thrombin. LAC was detected using the Bioclot LA kit, according to

manufacturer's instructions (Biopool, Umea, Sweden). For samples with prolonged time, the corresponding clotting time of a 1:1 mixture of sample and healthy pooled plasma was performed; samples with a sample/healthy ratio of more than 1.15 indicated the presence of an inhibitor activity, thereby confirming LAC-like activity.

Samples were also tested in duplicates for aCL, aPS, and anti-β2-glycoprotein I (GPI) IgM and IgG antibodies in duplicates by quantitative sandwich EIA using REAADS kits (Corgenix, Broomfield, CO, USA), and anti-annexin V antibodies by quantitative sandwich ELISA using Zymutest® Anti-Annexin V kits (HYPHEN BioMed, Neuville-sur-Oise, France), according to the manufacturers' instructions. Results were given as mean IgG antiphospholipid (GPL) or mean IgM antiphospholipid (MPL) units. Anti-GPI IgG < 16.66 GPL, anti-GPI IgM < 16.08 MPL, aCL IgG < 20.99 GPL, aCL IgM < 9.85 MPL, aPS IgG < 15.45 GPL, aPS IgM < 18.28 MPL, anti-annexin V IgG < 15.99 GPL and anti-annexin V IgM < 15.41 MPL were considered normal values.

Statistical analyses

Statistical analyses were performed on SPSS software (Statistical Package for the Social Sciences, version 14.0; SSPS Inc, Chicago, IL, USA). Quantitative data are described as medians, and upper and lower quartiles. The differences between patients and controls were determined using the Mann–Whitney comparison by ranks, as IgG and IgM antibody values showed a non-parametric distribution [29]. Antibody percentiles were calculated based on the distribution of the healthy control group. Logistic regression analysis, performed in a monoparametric then a multiparametric way, taking the healthy controls as the reference group, was done to analyze LAC and aPLA as risk factors for ischemic stroke; aCL and aPS antibodies were used as continuous, and then as categorized variables with LAC in order to determine the crude odds ratios (OR) and 95% confidence intervals (95% CI) associated with the risk of stroke.

Results

Association of LAC and antiphospholipid antibodies with stroke

Among the 208 patients included, 23 (11.1%) were positive for LAC, of whom 13 (56.5%) were men. The mean age of LAC-positive (64.6 ± 9.2 years) and LAC-negative (62.6 ± 12.7 years) patients was comparable. Only three out of the 203 control subjects (1.5%) were LAC-positive (one male, two females), resulting in a 8.1-fold increased risk for stroke for LAC-positive individuals (Table 1). Significant elevations in aPS IgG [OR (95% CI) = 1.1 (1.1–1.3)], aCL IgM [OR (95% CI) = 1.2 (1.0–1.3)] and aCL IgG [OR (95% CI) = 1.1 (1.1–1.2)] were seen in patients than in control subjects (Table 1). The median values of the other antibodies screened were comparable between patients and controls. Antibody-positive cases were then categorized based on P95 cutoff values of healthy individuals. Significantly increased prevalence of elevated aPS IgG [16.4% vs. 4.5%; OR (95% CI) = 4.2 (1.9–8.9)], aCL IgM [24.2% vs. 5.0%; OR (95% CI) = 6.1 (3.0–12.3)] and aCL IgG [36.2% vs. 4.5%; OR (95% CI) = 12.1 (5.8–24.9)] antibody cases were seen in patients than in controls.

LAC and antiphospholipid antibodies in stroke subtypes

Table 2 summarizes the association of aCL and aPS antibodies and LAC positivity with stroke subtypes. aPS IgG was associated with cardioembolic stroke, whereas elevation in aCL IgG and IgM positivity was seen in lacunar, atherosclerotic and cardioembolic stroke subtypes. Interestingly, LAC positivity was documented only in lacunar, but not other stroke subtypes.

Anti-phosphatidylserine and anti-cardiolipin antibodies

The association of aPS and aCL antibodies with stroke was confirmed by percentile analysis in which each antibody was split into four categories: percentiles 1–75 (P75), 81–90 (P90), 91–95 (P95), and > 96 (P99). For each of the aPS and aCL antibodies, the adjusted odds ratio increased as the percentile value increased. The strongest risk factors were for aCL IgG, in which the P99 was associated with a 43.5-fold higher risk than P75, and for aPS IgG where P99 was associated with a 31.3-fold increased risk than P75 (Table 3). This association of these antibodies with stroke was further analyzed by examining the proportion of antibody-positive individuals based on a pre-set cut-off level (mean + 3SD of healthy controls). An increased prevalence of individuals with elevated aPS IgG (OR = 7.7; 95% CI = 2.9–20.0), aCL IgM (OR = 7.4; 95% CI = 2.2–25.3) and aCL IgG (OR = 9.6; 95% CI = 3.4–27.7) antibodies was seen in patients than in control subjects (Table 3).

Table 2 Odds ratios (95% CI) for stroke for antiphospholipid antibodies and LAC according to stroke subtype

Antibody	Isotype	Lacunar*	Atherosclerotic*	Cardioembolic*	Others*
Anti-phosphatidylserine	IgG	2.4 (0.8–7.1)†	1.5 (0.6–3.8)	4.0 (1.5–11.0)	2.6 (0.7–9.6)
Anti-cardiolipin	IgM	4.4 (1.6–11.7)	3.1 (1.3–7.5)	3.5 (1.3–9.9)	3.4 (1.0–11.6)
	IgG	8.0 (3.1–20.3)	5.1 (2.2–11.9)	4.9 (1.8–13.2)	5.2 (1.6–16.3)
LAC positivity		8.8 (1.8–43.1)	4.0 (0.9–18.9)	4.7 (0.8–26.5)	4.3 (0.6–31.6)

*Stroke patients comprised 44 with lacunar, 109 with atherosclerotic, 37 with cardioembolic, and 18 with other stroke subtypes. †OR (95% CI).

Table 3 Influence of anti-phospholipid antibody cut-off level on the stroke risk

Cut-off	Patients	Controls	OR (95% CI)
Anti-phosphatidylserine IgG			
P75 (8.06)*	130 (62.5)†	170 (83.7)	Reference
P90 (10.64)	44 (21.2)	27 (13.3)	2.4 (1.2–4.8)
P95 (13.00)	16 (7.7)	4 (2.0)	7.6 (2.0–28.6)
P99 (18.28)	18 (8.7)	2 (1.0)	31.3 (6.2–166.7)
X ± 3SD	34 (16.3)	5 (2.5)	7.7 (2.9–20.0)
Anti-cardiolipin IgM			
P75 (4.74)	141 (67.8)	167 (82.3)	Reference
P90 (6.43)	33 (15.9)	29 (14.3)	1.6 (0.8–3.2)
P95 (8.10)	16 (7.7)	4 (2.0)	6.6 (1.7–25.0)
P99 (11.59)	18 (8.7)	2 (1.0)	8.5 (1.7–43.5)
X ± 3SD	21 (10.1)	3 (1.5)	7.4 (2.2–25.3)
Anti-cardiolipin IgG			
P75 (10.09)	120 (57.7)	189 (93.1)	Reference
P90 (13.99)	52 (25.0)	10 (4.9)	9.4 (4.1–21.7)
P95 (17.30)	12 (5.8)	3 (1.5)	9.0 (2.0–40.0)
P99 (23.96)	24 (11.5)	1 (0.5)	43.5 (5.4–333.3)
X ± 3SD	34 (16.3)	4 (2.0)	9.6 (3.4–27.7)

*Percentile (MPL/GPL antibody levels). †Number of subjects (percent of total).

Combinations of LAC with other antibodies

Of the 208 stroke patients included, 23 had a positive LAC, compared with three of the 203 control subjects. When LAC was considered in the co-presence of aCL and aPS antibodies, and taking LAC-/Ig- as reference for calculating the OR (95% CI), the majority of the 23 patients had positive LAC without aCL IgM (23/23) and IgG (19/23), as well as aPS IgG (7/23) antibodies. None of the three control subjects with a positive LAC had elevated aCL IgM and IgG, or aPS IgG antibodies (Table 4). Compared with unselected cases, the presence of LAC without antibodies to either aCL or aPS did not affect the overall risk for stroke (Table 4).

Regression analysis

The association of LAC and other antibodies with stroke was examined at the univariate and then at the multivariate levels.

Table 4 Influence of combinations LAC with other antibodies on the stroke risk

Antibody	Isotype	Status	Patients*	Controls*	OR (95% CI)
αPS	IgM	+/–†	22	3	8.0 (2.4–27.3)
		+/+†	1	0	0.7 (0.5–0.9)
	IgG	+/–†	16	3	6.4 (1.8–22.3)
		+/+†	7	0	0.8 (0.7–1.0)
αCL	IgM	+/–	23	3	8.9 (2.6–30.3)
	IgG	+/–†	19	3	7.8 (2.3–26.8)
		+/+†	4	0	0.9 (0.8–1.0)

*LAC-positive cases included 23 patients and three controls. †Indicates LAC positive (+)/antibody positive (+) or negative (–). Elevation in antibody titers was based on the following: aPS IgM > 18.28 MPL; aPS IgG > 15.45 GPL; aCL IgM > 9.85 MPL; aCL IgG > 20.99. LAC, lupus anti-coagulant; αPS, anti-phosphatidylserine; αCL, anti-cardiolipin.

Table 5 Regression analysis*

Antibody	aOR	95% CI
LAC	**9.7**	**1.8–52.5**
Anti-cardiolipin IgM		
P75	1.00	Reference
P90	1.5	0.6–3.4
P95	8.3	2.1–34.5
P99	2.9	0.4–22.6
Anti-cardiolipin IgG		
P75	1.0	Reference
P90	7.2	1.8–29.0
P95	9.8	3.1–31.3
P99	**14.8**	**1.7–128.9**
Anti-phosphatidylserine IgG		
P75	1.0	Reference
P90	2.3	1.0–5.7
P95	8.6	1.9–38.3
P99	**22.8**	**3.4–151.3**

*Adjusted for systolic and diastolic blood pressure, total cholesterol, LDL, and triglyceride levels. aOR, adjusted odds ratio.

Univariate analysis demonstrated a positive relationship between LAC ($P < 0.001$), and elevated (P99) aCL IgM ($P = 0.003$) and IgG ($P = 0.001$) antibody titers with increased stroke risk. Multivariate analysis confirmed the association of positive LAC [aOR (95% CI) = 9.7 (1.8–52.5)] with stroke, and demonstrated a clear gradation of increasing risk of stroke associated with the four categories of aCL IgG and aPS IgG, and identified aCL IgM P95 [aOR (95% CI) = 8.3 (2.1–34.5)] as independent predictors of stroke after adjusting for the potentially confounding covariates: systolic and diastolic blood pressure, total cholesterol, LDL and triglyceride levels (Table 5).

Discussion

Consistent with previous studies linking positive LAC and heightened aPLA response with increased risk of ischemic stroke, our population-based case–control study indicated that LAC, aCL IgG and aPS IgG constituted independent risk factors for the etiology of ischemic stroke, with adjusted odds ratios of 9.69 (95% CI = 1.79–52.49), 11.85 (95% CI = 1.40–100.43) and 21.44 (95% CI = 2.77–165.62), respectively. Adjustment made included age and gender, together with traditional risk factors of stroke (arterial hypertension, diabetes and hyperlipidaemia). Unlike previous population-based studies, in which only specific antibodies (notably aCL) were tested, thus neglecting the possible role of the clinically more relevant LAC [18,19], we included both functional (LAC) and antibody-based (EIA) assays in analyzing the aPLA contribution to stroke.

Our findings are reminiscent of earlier studies demonstrating an independent association between aCL [9,21,24], aPS [10], and anti-phosphatidylinositol [32] antibodies and ischemic stroke. An earlier hospital-based case–control study of 524 consecutive ischemic stroke patients (and 1020 controls) reported high positive aCL titer in 34% of cases compared

with 11% of controls, resulting in an adjusted OR of 4.0; IgG being associated with higher OR than IgM [21]. A more recent German study reported on high prevalence of aPS IgG in stroke cases (57.7%) than in healthy controls (4.8%), thereby prompting the speculation of a possible role for these antibodies in stroke etiology [33]. In contrast to our findings, the study of Kahles *et al*. [33] suggested a role for anti-β2-GPI in stroke pathogenesis, evidenced by the increased prevalence of anti-β2-GPI antibodies in stroke patients (20.8%) compared with normal controls (3.6%).

Other studies found no strong association between aPS or aCL and the risk of stroke. A Canadian study suggested that aPLA did not constitute an independent risk factor for ischemic stroke in unselected persons, evidenced by the comparable prevalence of aPLA in stroke patients (12%) and control subjects (10%), after correcting for known risk factors [23]. The Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) study demonstrated a lack of difference in the prevalence of the aCL isotypes between patients with cerebral hemorrhage and cerebral infarction and controls, after adjusting for a number of conventional stroke risk factors [30], thus prompting the conclusion that aCL do not constitute an independent risk factor for stroke, without ruling out a potential association with future stroke [30].

It was of interest to note that aCL IgG, and also IgM, were initially positively associated with ischemic stroke in our population. IgG is the major isotype of pathogenic autoantibodies, whereas increased level of IgM antibodies is rarely seen, and is typically linked with diseases associated with ongoing immunity. While IgM and IgG aCL antibodies were suggested to differ with regards to cross-reactivity with other phospholipids [12], it remains to be seen as to the functional relevance of this claimed altered restricted reactivity. In our hands, of the two aCL antibody isotypes, only IgG remains significantly associated with ischemic stroke after controlling for potential covariates, thereby minimizing or even ruling out any functional significance of elevated aCL IgM in stroke pathogenesis, as was also found elsewhere [11,30].

It was suggested that aPLA may not constitute an independent risk factors for ischemic stroke *per se*, but rather markers of [7,34]. aPLA were proposed to contribute to the pathogenesis of stroke in a select group of patients, including females [20], and young [22,25] patients. This was exemplified by the findings of Heizlef *et al*. [22], that elderly patients with elevated aCL IgG but negative anti-β2-GPI are not at an increased risk of incident ischemic stroke, in contrast to young ($<$ 40 years of age) stroke patients. In our hands, differential association of aPS (IgG) and aCL (IgG and IgM) antibodies, together with LAC positivity, was noted in specific stroke subtypes (TOAST criteria), suggesting a role for these antibodies in the pathogenesis of specific stroke types.

While the association between aPLA and stroke has been reported in several studies, it remains to be seen whether aPLA plays a causal role in the pathogenesis of stroke, or their presence is simply a consequence (or even an unrelated event) of ischemic stroke pathogenesis. Two earlier studies are in favor of a causative, more so than a consequential role, for aPLA in stroke pathogenesis [35,36], as evidenced by the increase in aPLA levels just before the onset of symptoms. While it is tempting to speculate that the increased prevalence of aCL IgG, aPS IgG, and positive LAC, play a causative role in precipitating ischemic stroke, validation of our data by a prospective study that includes repeated serum measurements of these and potentially other markers will be needed to confirm, or alternatively rule out this assumption.

We conclude that aPLA are associated with an increased risk of ischemic stroke. Our study has strengths, namely that patients included only ischemic stroke cases, and that both functional (LAC) and serologic (ELISA) assays were employed in assessing the contribution of aPLA to stroke. However, our study has some limitations, namely with regards to the relatively small sample sizes, to the inability to assign a pathogenic nature to elevated aPLA titers in stroke pathogenesis, and that it was limited to (North African) Tunisian Arabs, thereby necessitating follow-up studies in stroke cases from different ethnic groups. In addition, while we adjusted for a number of conventional stroke risk factors, we can not exclude the possible contribution of other unmeasured risk factors. Large independent prospective population-based studies with different ethnicity are needed to confirm the contribution of these and other aPLA to the risk of stroke.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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