Detecting Anti-Prothrombin Antibodies in Young Women with Acute Ischemic Stroke

INIMIOARA MIHAELA COJOCARU1, M. COJOCARU2, R. TÂNĂSESCU1, CECILIA BURCIN3, ANDREEA CRISTINA MITU3, IULIANA ILIESCU3, LAURA DUMITRESCU3, ISABELA PAVEL3, ISABELA SILOSI4

1“Carol Davila” University of Medicine and Pharmacy, Clinic of Neurology, “Colentina” Clinical Hospital, Bucharest
2“Titu Maiorescu” University, Faculty of Medicine, Department of Physiology, Bucharest
3Clinic of Neurology, “Colentina” Clinical Hospital, Bucharest
4University of Medicine and Pharmacy, Faculty of Medicine, Department of Immunology, Craiova, Romania

Prothrombin (PT) is a target for antibodies with lupus anticoagulant (LA) activity. Anti-prothrombin antibodies (aPT) were recently identified as antibodies directed toward a phospholipid-binding protein. aPT are a new serologic marker of antiphospholipid syndrome.

The objective was to detect aPT in a group of 46 patients with acute ischemic stroke in order to correlate their presence with clinical diagnosis, laboratory and neuroradiological findings.

We tested aPT, lupus anticoagulant (LA), anticardiolipin (aCL), and anti-β2-glycoprotein I antibodies (anti-β2-GPI) in 46 young women with acute ischemic stroke aged 34–45 years and 43 patients with nonischemic neurologic diseases and 141 normal controls. Anti-prothrombin antibodies were detected by calcium-containing aPT ELISA, aCL and anti-β2-GPI by ELISA. All samples were screened using the activated partial thromboplastin time (aPTT); the dilute Russell viper venous time (dRVV) coagulation test was performed. The results were statistically analyzed.

Anti-prothrombin antibodies were found in 26 (57%) of 46 stroke patients. Out of 43 patients with nonischemic neurological disorders, 2 (4.18%) were positive for aPT. aPT were detected in one (0.70%) of the normal controls. Ten stroke patients (21%) were positive for IgG aPT only, 9 stroke patients (18.2%) for IgM aPT only, and 8 stroke patients (16.9%) for both IgG and IgM isotypes of aPT. Two nonischemic neurological disorders patients (4.18%) presented IgM isotype of aPT. Patients with ischemic stroke presented aPT much more frequently than the healthy controls (OR 182.00 [95% CI 23.382-1416.6], p<0.0001). Patients with ischemic stroke presented aPT much more frequently than the nonischemic neurological disorders patients (OR 26.650 [95% CI 5.743-123.66], p<0.0001). When IgG or IgM aPT were considered separately, they were more frequently found in patients with ischemic stroke than in healthy control group (OR 38.889 [95% CI 4.817-313.95], p<0.0001) and (OR 34.054 [95% CI 4.178-277.5], p<0.0001), respectively. Simultaneous positive titers for both isotypes of aPT (IgG and IgM) were more frequently found in patients with ischemic stroke than in healthy control group (OR 29.474 [95% CI 3.573-243.12], p<0.0001). Eleven stroke patients (43%) were negative for aCL, LA and anti-β2-GPI, but positive for aPT (OR 0.03287 [95% CI 0.001794-0.6022], p<0.001). aCL, LA and anti-β2-GPI were not found both in nonischemic neurological disorders patients and in healthy controls.

In conclusion, aPT may play a role in the pathogenesis of ischemic stroke, their presence being associated with thrombosis.

Key words: anti-prothrombin antibodies (aPT), ischemic stroke, young women.

Anti-prothrombin antibodies belong to the “family” of phospholipid antibodies (aPL). Prothrombin (PT) was the first cofactor for phospholipid antibody determination to be identified based on the observation that lupus anticoagulant activity in patient plasma specimens increased upon addition of normal plasma. A number of groups reported patients with lupus anticoagulant (LA) and acquired hypoprothrombinemia attributable to the rapid elimination of prothrombin-antiprothrombin complexes [1–3].

Prothrombin (factor II) is a vitamin K-dependent glycoprotein with a molecular mass of 72 kDa and a plasma concentration of roughly 0.1 g/l. Coagulation factors Xa and Va and Ca2+ mediate the conversion of prothrombin to thrombin [4].

It should be mentioned that two different targets are observed: a) epitopes on the PT molecule itself.
bound to oxygenated polystyrene, and b) epitope(s) formed by the phosphatidylyserine/prothrombin (PS/PT) complex. There are numerous potential links between aPL and coagulation disorders, including interaction of aPL and a cofactor, β2-glycoprotein I, which itself is involved in coagulation mechanisms [1].

Several hypotheses explain the relationship between the antiphospholipid antibody syndrome (APS) and thrombosis, but the underlying mechanism remains unclear. The mechanisms that cause the appearance of autoantibodies are not understood [5][6].

While the specific mechanism of antiphospholipid antibody-related coagulopathy is unknown, it is clear that aPL are associated with an immune-mediated prothrombotic state. Compared to normal, factors responsible for autoantibody synthesis are more complex [7–10].

Cerebral ischemia is the most common arterial thrombotic manifestation associated with the presence of aPL. Antiphospholipid antibodies are a heterogeneous group of autoantibodies with different isotypes, with varying specificities. In patients with neurologic disorders, diagnostic value of aPL can be increased by patient testing for antibodies against β2 glycoprotein I, (anti-β2-GPI), PT, C and S proteins, anexin, etc. [11–14].

The importance of aPL as a cause of ischemic stroke in young adults is well demonstrated in the previous studies [6][14][15].

The possible association between anti-prothrombin (aPT) antibodies and thrombosis is a subject of debate [1–4][15–23].

The objective of this study was to detect aPT in young patients with ischemic stroke in order to understand the underlying pathogenesis and to correlate their presence with clinical diagnosis, laboratory and neuroradiological findings.

MATERIAL AND METHODS

Fourty-six sera from young women with ischemic stroke aged 34–45 years and 43 control sera from patients with nonischemic neurological disorders and 141 healthy subjects were tested for aPT, lupus anticoagulant (LA), anticardiolipin (aCL), anti-β2-GPI.

For all eligible patients, the informed consent was given for the use of their blood in this study. The research received approval by the ethical committee of the institution.

During routine venipuncture, 2–3 ml of blood was drawn, and immediately centrifuged. Sera were then frozen and kept at −20°C, until the assays were performed.

Anti-prothrombin antibodies were detected by calcium-containing aPT ELISA. Anticardiolipin antibodies and anti-β2-GPI were detected by ELISA.

The presence of LA was tested according to the recommended criteria from the International Society on Thrombosis and Hemostasis Subcommittee on Lupus Anticoagulant-Phospholipid-dependent antibodies. All samples were screened using the activated partial thromboplastin time (aPTT); the dilute Russell viper venom time (dRVV) coagulation test was performed.

Cerebral ischemia was documented by an imaging technique, such as computerized tomography (CT scan) or magnetic resonance imaging (MRI).

Ultrasound examination of cervico-cerebral arteries and of heart was performed.

The results were statistically analyzed. Comparison between patients and controls and patients groups was expressed as odds ratio with its 95% confidence interval (OR [95%CI]), where a lower limit >1.0 was considered significant. All p values were determined by Fisher’s exact test. A value of p<0.05 was considered statistically significant.

RESULTS

It was no history of oral contraceptive drugs administration and of smoking in stroke patients.

No arterial or cardiac abnormality was observed at ultrasound examination.

Anti-prothrombin antibodies were found in 26 (57%) of 46 stroke patients.

Out of 43 patients with nonischemic neurological disorders, 2 (4.18%) were positive for aPT.

Anti-prothrombin antibodies were detected in one (0.70%) of the normal controls.

Ten stroke patients (21%) were positive for IgG aPT only, 9 stroke patients (18.2%) for IgM aPT only, and 8 stroke patients (16.9%) for both IgG and IgM isotypes of aPT.

Two nonischemic neurological disorders patients (4.18%) presented IgM isotype of aPT.

Patients with ischemic stroke presented aPT much more frequently than the healthy controls (OR 182.00 [95% CI 23.382-1416.6], p<0.0001).

Patients with ischemic stroke presented aPT much more frequently than the nonischemic neurological
disorders patients (OR 26.650 [95% CI 5.743-123.66], p<0.0001).

When IgG or IgM aPT were considered separately, they were more frequently found in patients with ischemic stroke than in healthy control group (OR 38.889 [95% CI 4.817-313.95], p<0.0001) and (OR 34.054 [95% CI 4.178-277.54], p<0.0001), respectively.

Simultaneous positive titers for both isotypes of aPT (IgG and IgM) were more frequently found in patients with ischemic stroke than in the healthy control group (OR 29.474 [95% CI 3.573-243.12], p<0.0001).

Eleven stroke patients (43%) were negative for aCL, LA and anti-β2-GPI, but positive for aPT (OR 0.03287 [95% CI 0.001794-0.6022], p<0.001).

Anticardiolipin antibodies, LA and anti-β2-GPI were not found both in nonischemic neurological disorders patients and in healthy controls.

Thrombocytopenia was also observed in some cases, but without statistical significance.

**DISCUSSION**

The association between aPT and ischemic stroke (several controversies) was analyzed in relation to the APS [14–19].

Prothrombin (PT) appears to be a common antigenic target for aPL. Prothrombin is an additional PL-binding protein that can be recognized by aPL-positive sera, and aPT have been reported to affect the coagulation process and to be responsible for LA activity.

Antibodies directed to PT have been also shown to be responsible for the LA activity. The LA activity might not be caused by aPT alone but by a combination of different types of antibodies (e.g. anti-β2-GPI). Ischemic events such as focal cerebral ischemia is the most common neurologic disorder associated with aPL; however, myelopathy, Guillain-Barré syndrome, migraine, and chorea also have been frequently reported in aPL-positive patients [18][19][22][24–27].

There is now general agreement that PT, another PL-binding protein, is also one of the target antigens of aPL. Anti-prothrombin antibodies directly inhibit PT activation. Based on these observations, the role of aPT as laboratory criteria for the APS remains to be established [20].

A recent article showed that aPL can mediate their effects by direct interaction with neuronal tissue. In particular, the authors described the functional interactions of aPL with neuronal cell membranes, showing that purified IgG from a patient with APS induced depolarization of synaptosomes [3][21].

Whether the presence of aPT further increases the risk of thrombosis carried by lupus anticoagulants and, possibly, aCL has still to be defined. Their presence did not correlate with that of aCL, anti-β2-GPI, LA and/or anti-protein S [25][28][29].

Antibody levels and frequencies of positivity of aPT were significantly different between the studied groups [23].

The addition of aPT data increased the proportion of ischemic stroke patients with at least one type of APS marker from 65 to 78% [24][27][28].

The finding that aPT are common in ischemic stroke supports the hypothesis that ischemic stroke is a form of APS.

Ischemic events have been described in aPL-positive young patients and children.

From a clinical point of view, aPT correlate with thrombotic events in many of the published studies, even if comparisons between series are difficult because of the technical heterogeneity of the assays used. Anti-prothrombin antibodies can identify almost all the LA-positive samples [17][19][20][22][25]

It was observed that 48% of the patients with aPL-related clinical features, who were negative for aCL, LA and anti-β2-GPI, had aPT, suggesting that testing for these antibodies could be of added clinical benefit in patients who are negative for the routinely used tests [23].

Anti-prothrombin antibodies can be detected in 50–90% of patients with aPL, depending on which test system is used. The PS/PT complexes are strongly associated with manifestations of APS and the occurrence of LA.

A statistically significant association between moderate to high titers of IgG aPT and seizures was found [21].

For the first time aPT in Romania were studied in this work. It is a new field of research that contributes to understanding of mechanisms of thrombosis in ischemic stroke.

Further studies are needed to evaluate the potential relevance of aPT for prediction of thrombosis in stroke.
CONCLUSIONS

1. This study demonstrates a significant frequency of aPT in young women with ischemic stroke without any identifiable risk factor.
2. Anti-prothrombin antibodies may play a role in the pathogenesis of ischemic stroke in these patients; their presence is associated with thrombosis, making these antibodies potential markers for APS.
3. Testing for aPT could be of clinical benefit in patients who are negative for the routinely used tests, suggesting an appropriate therapeutical approach and monitoring of the disease.
REFERENCES


Received August 6, 2008