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Activated protein C resistance associated with lupus anticoagulants is a high risk in acute mesenteric venous thrombosis

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Background: **Acute mesenteric venous thrombosis (AMVT) is one of those diseases that cannot be diagnosed by specific symptoms and signs. A high misdiagnosis rate makes AMVT a final diagnosis established by exploratory laparotomy or forensic examinations earlier, during the period when computed tomography was not as efficient as it is now. The main motivation of our research was to improve the diagnosis and treatment by finding the relationship among activated protein C (APC) resistance, antiphospholipid antibodies, and AMVT in the Chinese Han population.**

Methods: **APC resistance was tested by activated partial thromboplastin time method in 70 AMVT patients and 75 healthy adult volunteers that excluded hypercoagulable states. Factor V Leiden mutation was analyzed by polymerase chain reaction with restriction fragment length polymorphism. Anticardiolipin antibodies (aCLs) were tested by enzyme-linked immunosorbent assay. A sensitive activated partial thromboplastin time-lupus anticoagulant (LA) test was used according to the guidelines.**

Results: **Only two samples had factor V Leiden mutation and were excluded. Twenty-one (30.9%) of the 68 AMVT patients had APC resistance. The rate of aCLs positive in AMVT group (13.2%) was significantly increased compared with control group (1.33%; $P = .014$). The LA-positive rate is significantly different between the AMVT and control group. Among LA-positive patients, the number of APC resistance was much higher than LA-negative patients ($P = .000$), but aCLs do not have an increased predisposition to APC resistance ($P = .85$).**

Conclusions: **APC resistance associated with LAs is a high risk in AMVT. The way aCL may affect the process of AMVT is not the same as with LA. (J Vasc Surg: Venous and Lym Dis 2014;2:155-9.)**

Activated protein C (APC) resistance is the most common risk factor for venous thrombosis, describing a lifelong increased risk of thromboembolism.[1-4] APC resistance is rather common in China, but the specific genetic defect responsible for the APC resistance, a single well-conserved G to A missense mutation at nucleotide 1691 of the factor V (FV) gene, known as factor V Leiden (FV_{Leiden}), is rare in the Asian population.[5-7] Remarkably, not only FV_{Leiden}-dependent APC resistance, but also APC resistance due to several other acquired conditions is an important risk factor for venous thrombosis.[8,9] Antiphospholipid antibodies (aPLs), mainly including anticardiolipin antibodies (aCLs) and lupus anticoagulants (LAs), are a heterogeneous group of circulating autoantibodies primarily directed against negatively charged phospholipid compounds. There are data suggesting that aPLs induce thrombosis through any one or more of several mechanisms: (1) aPL interference with endogenous anticoagulant mechanisms; (2) binding and activation of platelets; (3) interacting with endothelial cells and inducing expression of adhesion molecules and tissue factor; and (4) activation of the complement cascade.[10] Until now, studies about the relationship between aPL and the pathophysiological causes of thrombosis mostly focused on systemic lupus erythematosus and deep vein thrombosis.[3,11,12] The correlation research about the connection between aPL and acute mesenteric venous thrombosis (AMVT) is limited. We examined FV_{Leiden} gene mutation, APC resistance, aCL, and LA of 70 AMVT Chinese Han population patients in Northeast China, and analyzed the relationship between AMVT and the above factors.

METHODS

AMVT patients and controls. In this case-control study, 70 cases comprising 53 males and 17 females with an average age of 47.8 ± 20.6 years were consecutive patients diagnosed with AMVT at the First Affiliated Hospital, China Medical University, during the period from December 2006 to December 2011. Sixty cases were diagnosed by enhanced computed tomography or computed tomography veinography, and 32 cases were further treated by operation. Enhanced computed tomography or computed tomography veinography showed a filling defect in the mesenteric vein together with small bowel wall thickening, with or without

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Table I. Comparison between acute mesenteric venous thrombosis (*AMVT*) and control groups

	No.	*Mean age ± SD*	*Gender*		*Cigarette*	*Alcohol*	*Diabetes*	*APCR, No. (%)*[a]
			Male	*Female*				
AMVT	70	47.8 ± 20.6	53	17	18	9	1	23 (32.9)
AMVT[a]	68	48.5 ± 19.3	51					21 (30.9)
Control	75	44.2 ± 18.4	56	19	23	11	2	3 (4)
P value		.27	.88	-	.51	.75	.95	.000
P value[a]		.17	.96					.000

APCR, Activated protein C resistance; *FV_{Leiden},* factor V Leiden; *SD,* standard deviation.
Only the results of APC resistance show significantly different in AMVT group compared with these in the control group.
[a]Data changed after excluding two FV_{Leiden} mutation samples.

peritoneal effusion. The other 10 cases were definitely diagnosed by exploratory laparotomy. None of the 70 cases suffered from thrombotic disease before, nor did they receive anticoagulant therapy. Fifty-six male and 19 female patients recruited into the control group were apparently healthy adult volunteer blood donors from Liaoning Blood Center with an average age of 44.2 ± 18.4 years. Cases in both groups did not have a diagnosis of active cancer, were not pregnant, did not have a family history of venous thromboembolism, and did not have a biological relationship. Hypercoagulable states such as cirrhotic portal hypertension, postsplenectomy, nephrotic syndrome, and polycythemia were excluded.[1,4,6,13-16] Two weeks before blood collection, any drugs that might affect coagulation, including oral contraceptives, were forbidden (Table I). The research was approved by Ethics Committee of the First Affiliated Hospital, China Medical University.

Specimen collection. After informed consent, 5 mL of blood samples from fasting patients were collected by venipuncture into vacuum tubes containing 119 mmol/L trisodium citrate (3.8%) as soon as the diagnosis was made before any anticoagulant therapy. Samples were centrifugalized at 3000 r/minute for 20 minutes at 4°C to prepare platelet-poor plasma and serum before storage at −80°C.

Laboratory measurements. The APC resistance assay relies on the ability of APC to prolong the activated partial thromboplastin time (aPTT). The aPTT-based kits (Diagnostica Stago, Asnieres, France) together with STA-R analyzer (Diagnostica Stago) were used. Clotting was initiated by the addition of $CaCl_2$. The result is expressed as the sensitivity ratio of APC (APCsr), the ratio of the aPTT determined by the clotting time obtained in the presence of added APC (200 μg/mL) divided by the absence of APC (APCsr = [aPTT with APC]/[aPTT without APC]). Even after adding exogenous APC, the aPTT of APC resistance blood sample is not efficiently prolonged. Consequently, the decreased APCsr was considered reflective of inhibition of the effect of APC. The cutoff for APC resistance was determined in our laboratory. The value was defined as 2.0 as derived from the fifth percentile of values measured in local healthy control subjects. Samples with an APCsr ratio below 2.0 were regarded as positive, which suggested having APC resistance.

The FV_{Leiden} mutation was analyzed by polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) in all the APC-resistant test positive samples. Genomic DNA was isolated by the phenol-chloroform method, dissolved in nuclease-free water, and stored at 4°C. The forward primer 5′-GGAAACC TATACTTATAAGT-3′ and reverse primer 5′-TGTTAT-CACACTGGTGCTAA-3′were used to amplify a 323 bp fragment of the factor V gene. The PCR condition, in a 25-μg volume, comprised 1 μg of genomic template, 20 pmol of each primer, 200 mol/L of each dNTP, 2.5 mmol/L $MgCl_2$, 2.5 μL 10 × PCR buffer, and 1 unit Taq polymerase. Predegeneration was at 94°C for 7 minutes, then 94°C for 1 minute, 50°C for 30 seconds, and 72°C for 1 minute, for 35 cycles before extension at 72°C for 5 minutes. The PCR reaction was then analyzed on a 2% agarose gel and visualized by ethidium bromide staining. The products were then digested by restriction enzymes at 37°C for 12 hours and were analyzed by polyacrylamide gel electrophoresis (12%) at 50 mA constant current for 3 hours with visualization by silver ($AgNO_3$) staining. For the result of PCR, samples homozygous for FV_{Leiden} were characterized by a band at 200 bp, wild-type samples by a band at 163 bp, and heterozygous samples by bands at both 200 and 163 bp.

We detected anticardiolipin IgG, IgM, or IgA antibodies by the use of commercially available enzyme-linked immunosorbent assay (APhL ELISA Kit; Louisville APL Diagnostics, Inc, Doraville, Ga) in strict accordance with the manufacturer's instruction. Each result is expressed with binding index (BI; BI = [Optical Absorbance (OA) of sample − OA of blank]/[OA of normal pool − OA of blank]). The reference normal pool contains aCL-negative serum from 75 healthy donors. A positive value is defined as the resulting BI two standard deviations above the mean normal value.

According to the guidelines on LA testing proposed by Brandt et al in 1995,[17] as well as the update of the guidelines proposed by Pengo et al in 2009,[18] a sensitive aPTT-LA test was selected. If a sample's clotting time was longer than the cut-off value in the screening test, not affected by mixing with the pooled normal plasma from healthy donors during the mixing test, and shortened after increasing the

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