consideration of those factors that may interfere with the results should be of the utmost importance when studying APCR prevalence in venous thromboembolic patients and its association with other variables.

Also, we screened 15 families of positive APCR patients; 26.6% of the relatives revealed positive APCR-test and none of them presented VT. All the affected family members were first degree relatives to the propositus. As reported by Svensson[9] our results suggest the existence of an autosomal dominant mode of inheritance.

VT appears as a consequence of a series of environmental and genetic factors.[10] APCR is one of the factors associated to high risk of thrombosis and it is widely accepted that its frequency is higher than other genetic disorders involved in VT.

Key words

Venous thromboembolism, PCR

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References

1. Dahlbäck B, Carlsson M, Svensson P. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C. Proc Natl Acad Sci USA 1993; 90: 1004-8.
2. Bertina RM, Koelemen BPC, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369:64-7.
3. Dahlbäck B. Factor V and protein S as cofactors to activated protein C. Haematologica 1997; 82:91-5.
4. Dahlbäck B. Inherited thrombophilia: resistance to activated protein C as a pathogenic factor of venous thromboembolism. Blood 1995; 3:607-14.
5. Legnani C, Palareti G, Biagi R, Coccheri S. Activated protein C resistance in deep-vein thrombosis [letter]. Lancet 1994; 343:541-2.
6. Griffin JH, Evatt B, Wideman C, Fernández JA. Anticoagulant protein C pathway defective in majority of thrombophilic patients. Blood 1993; 82:1989-93.
7. Koster T, Rosendaal FR, de Ronde H, Briët E, Vanderbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated proteic C: Leiden thrombophilic study. Lancet 1993; 342: 1503-6.
8. Dahlbäck B. Inherited resistance to activated protein C, a major cause of venous thrombosis is due to a mutation in the factor V gene. Haemostasis 1994; 24:139-51.
9. Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994; 330:517-22.
10. D'Angelo A, Mazzola G, Crippa L, Fermo I, Viganò D'Angelo S. Hyperhomocysteinemia and venous thromboembolic disease. Haematologica 1997; 82: 211-9.

Incidence and clinical manifestations of activated protein C resistance and factor V Leiden in young patients with venous thromboembolic disease in Spain

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In order to evaluate the actual Incidence and clinical repercussion of activated protein C resistance (APCR) in our area, we performed a coagulation and thrombophilic study on 65 young patients diagnosed with deep vein thrombosis and 53 controls. Family and genetic study was carried out in APC-resistant patients. We found APCR in 26.15% of patients and the 77.7% of these and their relatives were heterozygous for factor V Leiden. There's a clear relationship between phenotype APCR and thrombosis, and also between factor V Leiden and thrombosis.

In 1993 Dahlbäck[1] described a new cause of inherited thrombophilia. The activated protein C resistance (APCR) was identified as the main risk factor of venous thromboembolic disease (VTED) because it was found in 20-60% of the patients with venous thrombosis and in 2-10% of normal population.[2,3] The molecular background for the APCR is a single point mutation in the factor V gene, which predicts substitution of an arginine at position 506 by a glutamine.[4] Mutated factor V (also called factor V Leiden) is activated by thrombin or factor Xa in the normal way, but impaired inactivation of mutated factor Va by activated protein C results in a life-long, hypercoagulability.[2] Nevertheless, the role of factor V as a cofactor of activated protein C is still far from clear.[5]

The incidence and clinical manifestations of APCR are very heterogeneous,[6-9] and the clotting assays used as screening tests are subjected to large variability.[9,10] For this reason, we wanted to evaluate the actual incidence and clinical repercussion of APCR in our area in order to establish our own normal range and to determine in which cases it's necessary to complete the study with the analysis of factor V Leiden.

We studied 65 young patients, 27 females and 38 males, aged between 18 and 50 years old, diagnosed of VTED in the Zaragoza University Hospital, Spain between January, 1993 and December, 1995. Patients taking oral anticoagulants were excluded from this study and all the plasma samples were taken at least one month after suppressing coumarins. The control group was comprised of 53 healthy subjects, with a sex and age distribution similar to the patients' group.

The coagulation and thrombophilia study in patients and controls was performed as follows:

functional antithrombin III by chromogenic assay (AT III, Isaza), antigenic protein C by ELISA (Asserachrom P.C., Boheringer Mannheim), functional protein C by clotting assay (Proclot, Isaza), functional protein C by chromogenic assay (Stachrom, Boheringer Mannheim), antigenic free and total protein S by ELISA (Stachrom, Boheringer Mannheim), plasminogen by chromogenic assay (Plasminogen, Boheringer Mannheim), Exner test or clotting test with kaolin (lupus anticoagulant assay) results were expressed as *APC-ratio*.

Table 1. Thrombosis risk for strata of APC-ratios.

APC-ratio	Odds ratio	95% confidence interval
< 2.1	13.00	1.64-103.02
< 2.2	7.65	1.66-35.19
< 2.4	2.51	0.96-6.59
< 2.6	1.82	078-4.23
< 3.0	1.44	0.69-3.00

Family and genetic study was carried out in APC-resistant patients. The genomic fragment containing 1691 G/A was amplified by PCR using the primers described by Bertina[4] (primer 5'- TGCCCAGTGCT-TAACAAGACCA-3', primer Y: 5'-TGTTATCACACTG-GTGCTAA-3'). The 267-bp fragment was digested with 2 U of the restriction enzyme Mnl I (Biolabs) to establish whether the allele was normal or mutated.

According to the anticoagulant response to APC in controls and patients, we defined three categories: normal (APC-ratio >2-4), borderline (2.04-2.4) and resistant (< 2.04) subjects.

Some inherited known causes of thrombophilia in 40% of patients was detected. The most frequent was APCR (26.15%, with 12.3% resistants and 13.8% borderlines), followed by PC (7.69%) and PS (4.61%) deficiency and, finally, ATIII deficiency (1.53%). Although APCR is the most prevalent risk factor for venous thrombosis, the clinical manifestations are less severe than antithrombin III and protein C deficiency.

The relative risk of venous thrombosis for APC-ratio, estimated by the odds ratio, is very variable, as summarized in Table 1, where confidence intervals of 95% are shown. When the APC ratio is lower than 2 1, the risk of thrombosis is increased 13-fold; lower than 2.2 it's increased almost 8-fold, and when ratio is lower than 2.4, the risk doubles. This means that there's a clear relationship between phenotype and thrombosis, because the lower the anticoagulant response to APC, the higher thrombotic risk is.

The 77.7% of resistant and 40% of borderline patients and relatives are heterozygous for factor V Leiden. So, we think it's justified to perform a genetic study of the patients with APC ratios in the lower limits of normal. The risk of deep vein thrombosis, calculated with the odds ratio (10.8) and its confidence interval of 95% (1.64-70.9) in the contingency table (thrombosis/G1691A mutation), is increased 10-fold in those heterozygous for factor V Leiden. That is, there's a relationship between genotype and thrombosis, although additional genetic factors may play a role.[12]

Acknowledgments

The authors wish to thank José Antonio Cristóbal, Professor of statistics at Zaragoza University, for his help with the statistical evaluation.

Key words

APC resistance, thrombophilia, factor V Leiden.

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References

1. Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previous unrecognized mechanism characterized by poor anticoagulant response to activated protein C prediction of a cofactor to activated protein C. Proc Natl Acad Sci USA 1993: 90:1004-8.
2. Hillarp A, Dahlbäck B, Zöller B. Activated protein C resistance, from Phenotype to genotype and clinical practice. Blood Rev 1995; 9:201-12.
3. Svensson PJ, Dahlbäck B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994; 330:517-22.
4. Bertina RM, Koeleman BPC, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369:64-7.
5. Dahlbäck B. Factor V and protein S as cofactors to activated protein C. Haematologica 1997; 82:91-5.
6. Koster T, Rosendaal FR, de Ronde H, Briët E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilla Study. Lancet 1993; 342:512-22.
7. Griffin JH, Evatt B, Wideman C, Fernandez JA, Anticoagulant protein C pathway defective in majority of thrombophilic patients. Blood 1993; 81:1989-93.
8. Rosendaal FR, Koster T, Vandenbroucke JP, Reltsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood 1995; 85:1504-8.
9. Greengard JS, Eichinger S, Griffin JH, Bauer KA. Brief report – variability of thrombosis among homozygous siblings with resistance to activated protein C due to an Arg-->Gln mutation in the gene for factor V. N Engl J Med 1994; 33 1:1559-62.
10. de Ronde H, Bertina RM. Laboratory diagnosis of APC-resistance: a critical evaluation of the test and the development of diagnostic criteria. Thromb Haemost 1994; 72:880-6.

11. Freyburger G, Bilhou-Nabera C, Dief S, et al. Technical and biological conditions influencing the functional APC resistance test. Thromb Haemost 1996; 75:460-5.
12. D'Angelo A, Mazzola G, Crippa L, Fermo I, Viganò D'Angelo S. Hyperhomocystenemia and venous thromboembolic disease. Haematologica 1997; 82: 211-9.

Atypical microangiopathy in a patient treated with ticlopidine

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Microangiopathies are rare complications during treatments with ticlopidine. We describe an atypical microangiopathy, affecting almost exclusively myocardium, and thrombocytopenia, shortly after onset of ticlopidine. The patient died a few days after. Autopsy showed no bleedings or large thrombi in most organs, but were compatible with microangiopathy in myocardial small vessels.

Ticlopidine is an inhibitor of platelet aggregation frequently being used in stroke prevention and other thromboembolic events. Its probable association with the appearance of microangiopathies, especially thrombotic thrombocytopenic purpura (TTP) has been described by several authors.[1-5] We present the case of a patient that developed atypical characteristics and deadly evolution microangiopathy in the course of treatment with ticlopidine.

The patient was a male of 46 years, without personal antecedent of interest. Three weeks before admission, he was diagnosed with left eye acute anterior ischemic neuropathy, beginning treatment with ticlopidine at usual doses (250 mg a day). At this time, blood cell count, coagulation tests and routine study of hypercoagulability were normal.

Seven days after beginning treatment, the patient presented skin rash, with pruritus and febricula, being treated with antihistaminic drugs, which improved the symptoms. In the following days, and in a progressive way, the patient complained of general discomfort, nausea and vomiting. Analytical study showed intensive thrombocytopenia (6×10^9 platelets/L) and moderate anemia, so he was refered to our Hospital.

On admission, the patient appeared ill, showing dry mucosae and injuries from scratching. No petechial purpura, evident hemorrhages or neurological abnormalities were found. Spleen and liver were not palpable. No thoracic abnormal murmurs were detected. Laboratory tests confirmed the existence of intensive thrombopenia and anemia (hemoglobin of 8.8 g/dL); leukocytes and formula were normal; schistocytes were not found in blood smears. Biochemical study showed high levels of lactodehydrogenase (LDH) (3.873 U/L) and creatinine (2 mg/dL). Bilirubin, transaminases, proteinogram, ions, glycemia and coagulation tests were normal. D-Dimers were in low levels (0,5-1 mg/L). Urine was of normal aspect; urinary sediment showed microhematuria (200 erythrocytes/µL). Coombs test was negative. Bone marrow aspirate showed megakaryocytic hyperplasia and mild eosinophilia.

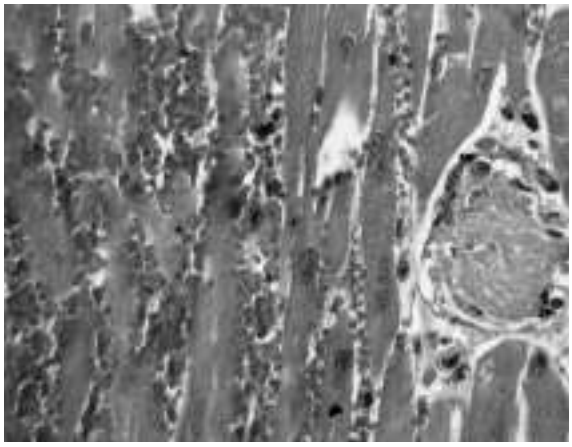

Figure 1. Thrombi and hemorrhages in myocardium.

After fluid replacement and discontinuation of ticlopidine, treatment with prednisone was administered (1 mg/kg/d). On the first day after admission the general condition improved and the urine was clear. On the second day, LDH and creatinine values were slightly lower (2.454 U/L and 1.2 mg/dL). Anemia and great thrombocytopenia persisted. On the third day, the patient entered into a sudden confusioned status; urgent cranial scanner was reported as absence of hemorrhage. Few hours later, he developed intense agitation, and died by cardiorespiratory arrest. Necropsy was performed. Hemorrhagic effusions were found only in myocardium. Macroscopic findings were irrelevant, except the presence of hemorrhages in a stripped pattern in myocardium. The microscopic examination showed the existence of multiple fibrinous thrombi in myocardial small vessels (Figure 1), and in a reduced number of small vessels in adrenal glands, liver, kidneys and pancreas.

The temporal relationship suggests that this microangiopathy was due to ticlopidine treatment.

Acknowledgments

The authors wish to thank Dr. J. Galiana and Dr. M. Saldaña for critical reading of the manuscript.

Key words

Ticlopidine, microangiopathy, Moskowitz, purpura, thrombosis.