

Hereditary Deficiencies of Antithrombin III, Protein S, and the Protein C Pathway in Jordanian Thrombosis Patients

SUHAIR S EID

Hereditary thrombophilia is caused by various inherited disorders. Most lead to a familial tendency to recurrent venous, not arterial, thrombosis, usually at a young age, and with spontaneous onset. Most of the genetic defects known today affect the function of natural anticoagulant pathways, in particular, the protein C system.

In this study, 602 (265 female, 337 male) patients with suspected thrombosis, arterial or venous, were referred to King Hussein Medical Center in Amman, Jordan. The prevalence of hereditary deficiencies of antithrombin (AT), protein S (PS), and protein C (PC) were studied over a seven-year period (1993–2000). Activated protein C (APC-R) resistance subjects were studied over four years (1996–2000). The mean age was 30 years in females and 42 years in males. A diagnosis was established in 22.4% (n = 135) of the subjects (20.3% venous, 2.1% arterial). Protein C deficiency was found in 3.8%, protein S deficiency in 2.3% and antithrombin deficiency in 1.4% of our sample group. An APC-R problem was seen in 23.0% (n = 89) of the surveyed population. Out of the APC-R patients, 75.0% had the DNA analysis of a factor V Leiden mutation present. Of the subjects found to have the mutation 87.0% were heterozygous and 13.0% were homozygous. These results confirm that APC-R, as a result of factor V Leiden mutation, is the most prevalent cause of thrombosis, and thrombophilia is related to venous, not arterial, thrombosis.

ABBREVIATIONS: APC-R = activated protein C resistance; ASPCR = allele specific polymerase chain reaction; AT = antithrombin; CVA = cerebral vascular accident; DVT = deep vein thrombosis; FVL = factor V Lieden; N-APC-R = normalized activated protein C ratio; PC = protein C; PCR = polymerase chain reaction; PE = pulmonary embolism; PS = protein S.

INDEX TERMS: coagulation; thrombosis.

Clin Lab Sci 2002;(15)4:196

Subair S Eid MSc is Chief Officer, Coagulation Laboratory, King Hussein Medical Center, Princess Eman Research Center for Laboratory Sciences, Amman, Jordan.

The peer-reviewed Clinical Practice section seeks to publish case studies, reports, and articles that are immediately useful, of practical nature, or demonstrate improvement in the quality of laboratory care. Direct all inquiries to Bernadette Rodak MS CLS(NCA), CLS Clinical Practice Editor, Clinical Laboratory Science Program, Indiana University, Fesler 409, 1120 South Avenue, Indianapolis, IN 46202-5113. brodak@iupui.edu

Address for correspondence: Subair S Eid, PO Box 143855, Amman 11844, Jordan. +962 6 5818531, +962 6 5662260 (fax). eide_8@hotmail.com

Thrombophilia is the tendency toward recurrent venous thromboembolism usually occurring in young age. In recent years, important advances have been made in understanding the complexity of laboratory abnormalities and clinical conditions associated with an increased risk of thrombosis.¹ The term inherited thrombophilia acknowledges the presence of an inherited factor that by itself predisposes towards thrombosis. However, due to the episodic nature of thrombosis, interaction with components is required before onset of the clinical disorder. The concept of inherited thrombophilia is an operational one. After the discovery of AT, genetic disorders of PC anticoagulant pathway, namely deficiencies in PC or PS, have been demonstrated to be associated with thrombophilia.² Also the discovery of the inherited activated PC resistance (APC-R), due to a single point mutation in Factor V gene (1691 G-A in exon 10 leading to 506 Arg to Glu) is associated with a significant increase in venous thrombotic risk.³⁻⁵ The presence of APC-R and anticoagulant protein deficiencies does not seem to be a significant risk factor for arterial thrombosis.^{6,7}

Progress in the molecular basis of thrombosis has enabled a more genetically based definition to be formulated: inherited thrombophilia is a genetically determined tendency toward venous thromboembolism. Dominant abnormalities, combinations, or less severe defects may be clinically apparent from early age of onset, or a frequent recurrence with a family history. Milder traits may be discovered only by further laboratory investigation. All genetic influences and their interaction are not yet understood.⁸

In previous studies, the prevalence of hereditary thrombophilia among patients with venous thrombosis was found to be greatly dependent on criteria for selection of patients. The aim of this study was to estimate the prevalence of hereditary thrombophilia, and to estimate the prevalence of arterial thrombosis in comparison to venous thrombosis (where it can be shown that thrombophilia is related to venous thrombosis and not arterial thrombosis) in 602 consecutive Jordanian patients referred for evaluation to the hematology department/coagulation section of King Hussein Medical Center.

MATERIALS & METHODS

Patient selection

Of the 602 patients (265 female, 337 male) evaluated in our study, 150 had arterial thrombosis, and 452 had venous thrombosis. These

patients were studied from 1993–2000, for inherited PC, PS, and AT deficiency. Three hundred seventy-nine patients were studied for APC-R from 1996–2000. Of the 379 patients, DNA analysis for FVL mutation was performed for 197 patients.

Patients with malignant disorders were excluded. None of the patients had received heparin or oral anticoagulant for at least three months prior to our investigation. Lupus anticoagulants were excluded. The deficient anticoagulant proteins or APC-R had to be consistently below 2SD of the normal mean on repeated samples. Three months was the period between repeats of the sample analysis.

For this study, thrombophilia was characterized as congenital and not acquired when the deficient protein was constantly below normal levels and when the same deficiency was confirmed in at least one family member. Members of the patients' families who were found deficient in one protein or had APC-R, were not included in this study.

Laboratory evaluation

Blood samples were collected in a 9:1 blood to anticoagulant ratio with 0.13 M trisodium citrate. Platelet poor plasma was prepared by centrifugation for 15 minutes at 2500g. Plasma samples were frozen and stored at –85 °C until assayed.

In all patients, antigenic AT, PC, PS were evaluated using laurel rocket immunoelectrophoresis, immunoturbidometry, and ELISA techniques (Assera-plate, Assera-chrome Liatest, Diagnostica Stago). Functional PC and AT were evaluated using colorometric methods (chromostrate AT, Organon Teknica, Stachrom protein C, Diagnostica Stago) according to manufacturer directions.

All plasmas were diluted in a ratio of 1:5 (1 part plasma: 4 parts FV deficient plasma) and APC-resistance was performed as described previously (APC deficient plasma was purchased from Diagnostica Stago).⁹

Genomic DNA was prepared from EDTA blood. Determination of the FV Leiden mutation (G to A at position 1691) which causes APC resistance, was performed using two PCR methods. The first PCR method was a restriction enzyme method.⁴ The second was a multiplex allele-specific PCR method.¹⁰

Controls

Normal plasma was obtained from 30 normal donors for the various assays. Commercial reference plasmas (Dade-Behring, Diagnostica Stago) were used to confirm the assay results. Reference ranges were established for all anticoagulant proteins and APC-R for healthy controls. The results are as follows: free, total PS, 54% to 140%; PC functional 62% to 138%; antigenic PC 60% to 145%; antigenic AT assay was 82% to 120%; functional AT was 80% to 122%; and for normalized APC ratio: ratio >0.75.

RESULTS

602 patients met the criteria of this study, 337 were males, 265 were females. Mean age in females was 30 years (2 to 60). The mean age in males was 42 years (2 to 60). The most predisposing factors in females were pregnancy and oral contraceptives. The sites of thrombosis and the number of patients are shown in Table 1.

Detection of anticoagulant protein deficiency, and/or APC-R was established in 22.4% (n = 135), 122 with venous thrombosis (20.3%), 13(2.1%) with arterial thrombosis, while no abnormality was detected in 79% (n = 476) of the cases. Deficiencies of PS, PC, or AT were found in 7.5%. Thus AT deficiency was found in 1.4% (n = 9): 1.3% were type I, 0.16 were type II; 3.8% (n = 23) had PC deficiency: 3.1% were type I, 0.7% were type II; and 2.3% (n = 14) had PS deficiency, all were type I.

Out of the 379 patients studied from 1996-2000, 23% (n = 89) were found to have APC-R; DNA analysis could only be performed for 75% of the patients. 65 patients proved to be positive for FVL mutation, (87% were heterozygous, 13% were homozygous). In two patients no FVL mutation was detected. Two patients were found to have both PC deficiency type I, and were heterozygous for FVL mutation. Both patients had recurrent DVT, one with PE, the other with a CVA.

After investigation the mean age of patients found to have PC, PS, or AT deficiency was 30.2 years; the mean age of patients found to have APC-R was 32.3 years. Three patients, one displaying a PC

Table 1. Sites of thrombosis, and type of deficiency in 135 patients found to have hereditary thrombosis

VTH	PC	PS	AT	APC—R	Total
DVT	13	11	3	65	92
PE	4	—	1	9	14
RA	1	1	—	8	10
Others	1	—	3	2	6
TOTAL	19	12	7	84	122
ATH					
MI	—	—	2	1	3
CVA	4	2	—	4	10
TOTAL	4	2	2	5	13

ATH = arterial thrombosis; CVA = cerebral vascular accident; DVT = deep vein thrombosis; MI = myocardial infarction; PE = pulmonary embolism; RA = recurrent abortion; VTH = venous thrombosis.

Others: all the rest of the clinical presentations are shown in Table 2.

deficiency, and two with heterozygous FVL mutation, had a first thromboembolic event at ages 60, 58, and 60 respectively.

Table 2 shows the number of patients and the site of thrombosis for each protein deficiency.

DISCUSSION

Congenital AT, PC, PS deficiencies and APC-R (FVL) represent a group of heterogeneous genetic disorders commonly associated with thromboembolic disease. AT is a major protein with inhibitory function on serum proteases in blood coagulation, mainly involving thrombin and factor Xa. PC is a vitamin K-dependent serine protease which together with its cofactor PS, serves as the cofactor of activated protein C (APC), which inactivates factor Va and VIIIa. With the exploration of the anticoagulant protein C system in the 1970s and 1980s, identification of protein C, protein S, and AT deficiencies provided for approximately 10% of cases of familial thrombosis. The real breakthrough in thrombotic diagnosis came in 1993 with the discovery of resistance to activated protein C (APC-R) as a risk factor for thrombosis.³ In 135 of the original 602 patients (22.4 %), a cause of thrombosis was es-

tablished as an inherited deficiency (20.3% had venous thrombosis; 2.1% had arterial thrombosis). Protein C, PS, AT deficiency and FVL mutation have not been proven to be a cause for arterial thrombosis.^{6,7} These results support the fact that thrombophilia is related to venous thrombosis and not arterial thrombosis.

The prevalence of inherited abnormalities in thrombophilic patients has been investigated in various epidemiological studies. The most important are shown in Table 3. The prevalence of PC and PS congenital deficiencies in patients with a history of thrombosis is slightly higher than the prevalence of AT as noticed in most of the protocols, including the current study. However, studies may vary greatly from one protocol to the other because of different selection criteria and laboratory assays used.

In the current study 7.5% out of 602 patients studied from 1993–2000 were found to have AT, PC, or PS deficiencies (1.4% had AT deficiency 3.8% PC, and 2.3% PS). These figures are close to the figures found in different studies done on European and American populations. If we compare this study with a previous study done in Jordan, the percentages were

higher than this study.¹⁰ However, there is no defining number in any of the studies.

Most of our patients were from a young age group. These results are very close to the study accomplished by Awidi in Jordan.¹⁰ A high proportion of our patients were younger than 35 years when the first thrombosis occurred, with mean ages of 30.2 year for PS, PC, and AT deficiencies and 32.3 years for APC-R patients. However three patients, one displaying a PC deficiency and two with heterozygous FV Leiden had a first thromboembolic event at age 60, 58, and 60 respectively. The first one, the PC deficiency patient was diagnosed with portal vein thrombosis. The two FVL mutation patients were diagnosed with DVT. Ten of the females (20%) were suffering from recurrent abortion and were found to have a protein deficiency and FVL mutation, which means that a protein deficiency and the presence of FVL may be a risk factor for recurrent abortion. Therefore all pregnant women with a history of thrombosis should be screened at least for APC-R. Two Budd-Chiari Syndrome patients were found to have a FVL mutation present.

The two most common defects found to be a risk factor for thrombosis in this study were APC-R and FVL mutation. Of the 379 patient studies from 1996–2000, 23% were found to have APC-R. It was then found that 75% of the 89 patients with APC-R that were screened for FVL mutation were found to be positive (87% were heterozygous and 13% homozygous). These results are close to the figures found by the studies in Table 3.

This study shows clearly that hereditary causes of thrombophilia are common in young Jordanian patients with a positive family history of venous thrombosis and not arterial thrombosis. APC-R, FVL accounts for most of these.

ACKNOWLEDGEMENT

I would like to acknowledge Mr David L McGlasson, MS, CLS/NCA, for his editorial support in preparing this manuscript

Table 2. Clinical presentation and numbers in patients referred for evaluation

Arterial thrombosis		Venous thrombosis	
Diagnosis	# of patients	Diagnosis	# of patients
CVA	105	Budd-Chiari syndrome	2
MI	45	Hepatic, mesenteric thrombosis	3
		Multiple thrombotic lesions	8
		Retinal venous occlusion	8
		Submandibular thrombosis	4
		Portal vein thrombosis	18
		Auxiliary thrombosis	5
		DVT	289
		PE	63
		RA	52
Total	150	Total	452
Total ATH+VTH			602

Table 3. Prevalence of hereditary thrombophilia in selected studies

	%PS	%PC	%AT III	%APC-R
Gladson ¹²	5	4.3	—	—
Griffin ¹³	—	—	—	52
Briet ¹⁴	13	8	4	—
Heijboer ¹⁵	2.2	3.2	1	—
Taberero ¹⁷	1	1	0.5	20
Pabinger ¹⁸	2.3	3.3	5	—
Current study	2.3	3.8	1.4	23
Awidi ¹¹	6.9	7.8	4.6	—

and Dr Amid Abed Alnour PhD for his support in performing some PCR tests in his laboratory.

REFERENCES

1. Mannucci PM, Tripodi A. Inherited factors in thrombosis. *Blood Rev* 1988;2(1):27-35.
2. Griffin JH, Evatt BL, Zimmerman TS, and others. Deficiency of protein C in congenital thrombotic disease. *J Clin Invest* 1981;68:1370-3.
3. Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to previously 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.
4. Bertina RM, Koeleman BP, Koster T, and others. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994;369:64-7.
5. Voorberg J, Roelse J, Koopman R, and others. Association of idiopathic venous thromboembolism with single point mutation at Arg 506 of factor V. *Lancet* 1994;343:1535-6.

6. Conard J, Samama MM. Inhibitors of coagulation, atherosclerosis, and arterial thrombosis. *Semin Thromb Hemost* 1986;12:87-90.
7. Ridker PM, Hennekens CH, Lindpaintner K, and others. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. *N Engl J Med* 1995;332:912-7.
8. Lane DA, Mannucci PM, Bauer KA, and others. Inherited thrombophilia part I. *Thromb Haemost* 1996;76:651-62.
9. Jorquera JI, Montoro JM, Fernandez MA, and others. Modified test for activated PC resistance. *Lancet* 1994;344:1162-3.
10. Hessner MJ, Luhm RA, Pearson SL, and others. Prevalence of prothrombin G 20210 A, factor V G1691A (Leiden), and methyltetrahydrofolate reductase (MTHFR) C 677 T in seven different populations determined by multiplex allele-specific PCR. *Thromb Haemost* 1999;81:733-8.
11. Awidi AS, Abu-Khalaf M, Herzallah U, and others. Hereditary thrombophilia among 217 consecutive patients with thromboembolic disease in Jordan. *Am J Hematol* 1993;44:95-100.
12. Gladson CL, Scharrer J, Hach V, and others. The frequency of type I heterozygous protein S and protein C deficiency in 141 unrelated young patients with venous thrombosis. *Thromb Haemost* 1988;59:18-22.
13. Griffin JH, Evatt B, Wideman C, Fernandez JA: Anticoagulant protein C pathway defective in majority of thrombophilic patients. *Blood* 1993;82:1989-93.
14. Briet E, Engesser L, Brommer EJP, Brockmans AW, Bertina RM. Thrombophilia: its causes and a rough estimate of its prevalence. *Thromb Haemost* 1987;58:39.
15. Heijboer H, Brandjes DP, Buller HR, and others. Deficiencies of coagulation-inhibiting and fibrinolytic proteins in outpatient with deep vein thrombosis. *N Engl J Med* 1990;323:1512-6.
16. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for Factor V Leiden (activated protein C resistance). *Blood* 1995;85:1504-8.
17. Taberero MD, Tomas JF, Alberca I, and others. Incidence and clinical characteristics of hereditary disorders associated with venous thrombosis. *Am J Hematol* 1991;36:249-54.
18. Pabinger I, Brucker S, Kyrle PA, and others. Hereditary deficiency of anti-thrombin III, protein C and protein S: prevalence in patients with a history of venous thrombosis and criteria for rational patient screening. *Blood Coagul Fibrinolysis* 1992;3:547-53.

EDUCATION AND RESEARCH FUND, INC

Grant-in-Aid funding is available to support members of ASCLS who are conducting scientific investigation in the field of clinical laboratory science. Three \$5000 grants are given annually. Also, the Allegiance Graduate Student Award for research in the amount of \$3000 is available. Applications are due June 1, 2003 and are available on the website (www.ascls.org) in the Education section.

