นิพนธ์ต้นฉบับ

Two case report of protein C deficiency and venous thrombosis.

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Watananukul P, Intaragumtornchai T, Khunprayoon S. Two case report of protein C deficiency and venous thrombosis. Chula Med J 1990 Jun; 34(6) : 431-439

Investigation of 5 patients, age 32-39 years, who were admitted to Chulalongkorn Hospital with spontaneous deep vein thrombosis for congenital anticoagulant deficiency, such as protein C using a functional assays,and antithrombin III using an immunological and a functional assays. Subsequent investigations identified two patients to be protein C deficienty.

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Received for publication. March 21, 1990.

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เผือดศรี วัฒนานุกูล, ธานินทร์ อินทรกำธรชัย, ศรีนุช คุณประยูร. หลอดเลือดดำอุดตันเนื่องจากขาดโปรตีน ซี. จุฬาลงกรณ์เวชสาร 2533 มิถุนายน ; 34(6) : 431-439

ในระยะเวลาตั้งแต่ปี พ.ศ. 2520 เป็นต้นมา มีผู้ป่วยที่เป็นหลอดเลือดดำอุดตันมารับการรักษาที่โรงพยาบาลจุฬาลงกรณ์ จำนวน 504 รายด้วยกัน แต่มีผู้ป่วยที่ต้องอยู่หอผู้ป่วยในของโรงพยาบาลจุฬาลงกรณ์ เพียง 120 ราย อายุระหว่าง 15-75 ปี (เฉลี่ย 48.9) ขณะนั้นยังไม่สามารถทำการทดสอบเกี่ยวกับการหาสาเหตุได้ ตั้งแต่ต้นปี 2532 ทางหน่วยโลหิตวิทยา ภาควิชาอายุรศาสตร์ เริ่มทำการทดสอบเกี่ยวกับสาเหตุการขาด natural anticoagulant แต่กำเนิดได้โดยได้รับทุนวิจัยรัชฎาภิเษกสมโภช จึงเริ่มทำการศึกษาผู้ป่วยที่มาด้วยหลอดเลือดดำอุดตันและมีอายุต่ำกว่า 40 ปี ซึ่งมีเพียง 5 รายพร้อมกับอาสาสมัคร 50 ราย อายุระหว่าง 15-60 ปี พบว่า 2 จาก 5 รายนั้นเกิดจากการขาดโปรตีน ซี แต่กำเนิดเมื่อเทียบกับค่าปกติของอาสาสมัคร

protein c is a vitamin K - dependent serine protease zymogen.[1-2] Both bovine and human activated protein C are potent anticoagulant enzymes that destroy purified bovine Factors Va, VIIIa, Xa binding sites and prothrombinase activity of washed bovine platelets.[3,9] Purified human activated protein C selectively destroys Factors V ang VIII in human plasma,[5] and it has been suggested that combined Factor V/VIII deficiency disease is due to a deficiency of a plasma inhibitor of activated protein C.[10] Endothelial cells contain a cofactor that potendtlu stimulates the activation of protein C by thrombin,[11] and thrombomodulin as shown in Fig I. Regulation of protein C activation is also dependent on protein S, another vitamin K-dependent plasma protein. Activated protein C functions as an anticoagulant by inactivating fators Va and VIIIa and, possibly, by stimulating fibrinolysisi

Thrombotic disease is associated with protein C deficiency as the impaired inactivation of factors Va and VIIIa promotes excessive formation of fibrin. About 50% of all patients have thrombotic episodes before the ag of 30 years, often occuring without an apparent cause

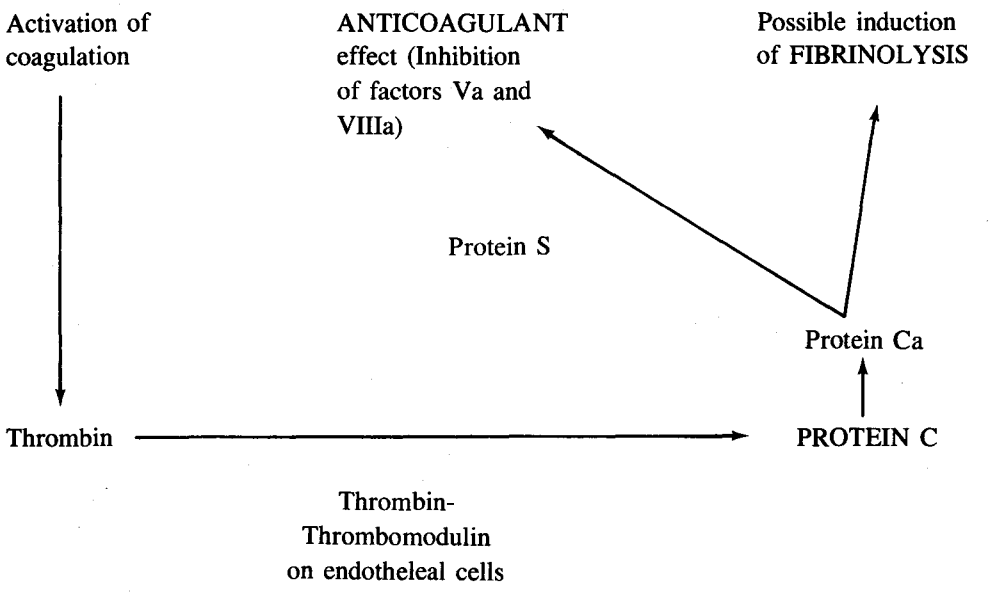

Figure 1. Role of protein C in regulating coagulation.

Recent descriptions of families with thrombotic disease and isolated protein C deficiency[12-17] have confirmed biochemical findings on the anticoagulant properties of protein C recently reveiwed by Esmon.[18] The first family with recurrent venous thromboembolism due to protein C deficiency, transmitted as an autosomal dominant trait, was reported in 1981 by Griffin.[14] In heterozygotes, protein C concentation is about 50% of normal. Hemozygosity with very low or unmeasurable levels of protein C has been described, particularly in newborns with severe visceral vein thrombosis or purpura fulminans.[19]

Cengenital protein C deficiency have been classified into two broad categories: type I with parallel decrease in activity and protein concentration, and type II, with normal protein concentration but reduced activity due to the presence of a dysfunctional protein C.

Functional assays of protein C was chosen for the screening of protein C deficiency because cases of dysfunctional protein C (type II) with normal levels of protein C antigen but low levels of protein C activity would remain undetected if only immunoassays were employed.[20-21] The functional clotting method has the theoretical advantage of exploring indirectly the action of activated protein C on factors Va and VIIIa, the natural protein substrates of the enzyme.

Patients and method

From 1977-1989 we had 120 patient admitted with deep vein thrombosis, from the beginning of 1988 we had 5 patients, age under 40 years whom we screened for protein C and antithrombin III defiency. The five patients with 3 males and 2 females; none of them had received the contraceptive pills or an anticoagulant, the age was 32-39 years (mean 32.4 years) and they had experienced one to more episodes of deep vein thrombosis or pulmonary or both. Deep vein thrombosis was confirmed by vascular scanning or venography or both and pulmonary embolism by perfusion lung scanning. None of them had a family history of deep vein thrombosis.

Protein C functional activity was evaluated according to the functional clotting method.[14,20.22.23] Antithrombin III was determinated by immunodiffusion using Nor-Partigen immunodiffusion plate, Behring, and assay of antithrombin III with chromogenic substrates.[24,25] Other variables of coagulation were done such as prothrombin time,[26-28] activated partial thromboplastin time,[29-31] thrombin time,[32] fibrinogen[33] euglobulin lysis time[34], clotting factots II[35] VII,[36] X[37-38] and plasminogen was assayed with specific amidolytic method using the substrate S-2251[39,40]

Results

Table 1 shows the clinical characteristics of the five patients with deep vein thrombosis, age 32-39. all of whom came in with leg edema. Only the first case had recurrent spontaneous deep vein thrombosis and recurrent pulmonary embolism at least 4 times in a 7 month period, the other 4 had only deep vein thrombosis.

Table 1. Clinical characteristics of 5 patients.

No.	Sex	Age (years)	Sites of vein thrombosis	Pulmonary thrombosis	Presenting Symptoms	Precipitating Condition
1	M	36	Inferior vena cava Both common iliac Both femoral Visceral vessels	complete obstruction of right lung	Chest pain, abdominal pain and both leg edema	Spontaneous
2	M	32	Inferior vena cava Both common iliac Both femoral Visceral vessels	-	Both leg edema	Spontaneous
3	M	39	Left femoral	-	Left leg edema	Spontaneous
4	F	36	Left iliofemoral	-	Left leg edema	Post-operative myomectomy
5	F	32	Left femoral	-	Left leg edema	Post partum

Case 1 :

A 36 years old welder who denied any family history of thrombotic disease, smoked 10-20 cigarette a day for 10 years, and always in good health until January 1988 when he had the first episode of spontaneous chest pain and the diagnosis of pulmonary thrombosis of the right lung, thrombosis of left femoral vein, both common iliac veins and inferior vena cava was made by vascular

scanning using 99 mTc-MAA alpha DTPA. His hemoglobin was 10.4 gm% Wbc 9.8×10^9/L, N 87%, L 13 %, Platelet 205×10^9/L, aPTT 37.8/39.6 seconds, PT 11.6/12.3 secoonds, TT 12.4/12.2 seconds fibrinogen 2.37 gm/L, factors II, V, VIII and X were within noral limits. Antithrombin III was 0.13 gm/L by immunodiffusion and was 1110% assayed with chromogenic substrates. The plasminogen was 132% assayed with specific amidolytic method using the substrate S-2251 and the protein C functional activity was only 4.6% and other detail of coagulation tests are shown in the table 2.He was then treated with a continuous infusion haparin of 30,000-35,000 units daily for 3 weeks, and maintained on coumarin until April, 1988 when he stopped taking the medicine and developed severe continuous abdominal pain and upper thighs edema. Repeated vascular scanning showed about the same results as the previous study. He was immediately heparinized on continuous infusion with little improvement of his abdominal pain; 3 days after admission he was underwent abdominal exploratory laparotomy becouse of guarding, tenderness, mucous bloody stool and a diagnosis of acute peritonitis.

Table 2. Laboratory Results in 5 Cases.

Tests	Normal Range±2SD (n=50)	Case 1	Case 2	Case 3	Case 4	Case 5
Protein C activity %	161.4+43.50 (149.83−172.97)	4.6	16.8	143.5	132.0	165.3
Antithrombin III Immunological (gm/L)	0.24±0.06 (0.232−0.248)	0.31	0.29	0.36	0.25	0.24
Functional %	115.69±19.32 (113.01−118.37)	110	126	137	129	132
Plasminogen activity %	126.50+36.22 (122.2−130.8)	132	140	110	128	132
Activated partial thromboplastin time (sec.)	34−45	53	49	37	42	37
Prothrombin time (sec.)	11.5−13.0	12.5	11.8	12.4	11.5	12.0
Thrombin time (sec.)	10.5−13.4	12.0	12.5	11.6	12.0	11.8
Fibrinogen (gm/1)	1.5−4.0	2.5	2.2	2.8	2.7	2.7
Factor II activity %	90−120	110	125	119	125	134
Factor V activity %	90−120	132	116	126	138	126
Factor VII activity %	90−120	137	126	118	112	106
Factor X activity %	90−120	130	116	120	121	130
Hematocrit %	35−45	40	36	38	35	36
White cell count × 10^9/L	5−10	6.5	7.8	5.9	7.9	5.7
Platelet count × 10^9/L	150−400	210	268	310	320	268

( ) = 95% confidence interval)

The surgeon found marked dilatation of the subcutaneous preperitoneal vein, serosangninous intraperitoneal fluid of 200 ml, dilated mesenteric vein and tributaries, swelling and congestion of small bowel; all secondary to inferior vena cava, and mesenteric venous thrombosis. The surgeon did not do anything further because of the patient's condition. He was given heparin continuously postoperatively for 4 weeks with good response in the sense that there was much less guarding, tenderness and reduction of the abdominal pain and leg edema. He was discharged on coumarin anticoagulation therapy. He was again hospitalized for a massive pulmonary embolus in July 1988 when he suddenly developed severe chest pain, shortness of breath, hemoptysis and cyanosis after he again stopping he coumarin for 2 weeks. His blood pressure was 70/0 mmHg. at the time of admission, respiration rate was 46 per/minute. He was immediately heparinized 40,000 units/24 hours continuous infusion with little inprovement. Inspite of other supportive treatment his general condition deteriorated and he expired shortly after admission.

The case described here demonstrated that case No 1 had a marked decrease in functional assay for protein C in the plasma and is associated with a history of recurrent and massive thromboses. No plasma protein C antigen is available so that study of protein C was limited to functional assay. John H Griffin[41] reported that protein C levels between 38-49% are sufficeintly low to result in thrombotic disease. It is an impression that long-term treatment with oral anticoagutant drug is benificial for the prevention of recurrence venous thrombosis. Although this is not proven it is supported by the facts that this patient had recurrences of the disease shortly after the withdrawal of the coumarin and remained asymptomatic during long-term treatment.

Case 2 :

A 32 ?years old mentally retarded was first admitted at Chulalongkorn Hospital in February 1987 because of spontaneous bilateral leg edema for many months prior to admission. The diagnosis of thrombosis of the inferior vena cava, both common iliac and both femoral veins was made by venography. He was treated with continuous heparin infusion 20,000-30,000 units per day with very good response, then he was discharged on coumarin. He was lost to follow up and did not take coumarin for 5-6 months. He was admitted for the second time in August 1988 with the same symptoms and signs and venogram. His hemoglobin was 12.2 gm%, wbc 7.4×10^9/L, N 74%, L 26%, platelet 301×10^9/L, aPTT 36.4/37.6 seconds, PT 12.1/12.8 seconds, TT 14.2/13.6 seconds, fibrinogen 2.68 gm/L, factors II, V, VII and X were within normal limits, Antithrombin III was 0.29 gm/L by immunodiffusion and was 126% assayed with chromogenic substrates, the plasminogen was 140% assayed with specific amidolytic method using the substrate S-2251 and the protein C functional activity was 16.8% and other detail of coagulation tests are shown in the table 2. He was treated with a continuous fusion of heparin 30,000 units daily for 3 weeks and anticoagulated with coumarin with good results. Because of his mental retardation and no one to take care him at home, he could not take coumarin regularly, However, he is still alive at the time of reporting and came to the hospital from time to time when ever the leg edema became worse.

Discussion

Antithrombotic treatment with vitamin K antagonist[13,15,43] is effective for the prevention of thrombosis. However, during the initial phase of oral anticoagulant treatment, protein C deficient patients are particularly susceptible to coumarin-induced hemorrhagic skin necrosis; and long-term treatment with coumarins itself carries a risk of bleeding complication, Recently, it has been demonstrated that stanozolol, an anabolic steroid can raise plasma protein C concentrations 1.5 folds in healthy volunteers.[42] Since most patients with hereditary protein C deficiency are heterozygotes[43] having protein C concentrations around 0.5 U/ml, it was hypothesized that oral administration of stanozolol may raise protein C concentration to normal level, thus cerrect the phenotypic defect in these patients.

Oral administration of stanozolol to type I protein C deficient patients will reise plaasma protein C approximately 1.6 folds to subnormal or normal levels. This is in accordance with previous observations in individual patients treated with stanozolol or danazol, another anabolic steroid.[44-46] Even after one week of treatment with stanozolol (5 mg bid), the increase in plasma protein C is already significant. Prolonged treatment for a period of 3 months will keep the protein C level within the normal range, as has been demonstrated in an Italian type I protein C deficient patient.[44] Shortly after withdrawal of stanozolol, the plasma concentration of protein C returns to pre-treament level. During the treatment period the ratio between protein C activity and protein C antigen does not change; indicating that the biological activity of protein C as assessed in our functional assay was not influenced by stanozolol the mechanism of action of stanozolol remains obscure. Whether stanozolol increases protein C synthesis in the liver or its release from the liver, or whether it reduces the catabolic rate of peotein C, is not known. However, it has been suggested that hepatocytes contain steroid receptors capable of reacting with anabolic

steriod[45], in this way increasing the synthesis rate of protein C.

The effect of stanozolol is not restricted to protein C alone, for stanozolol has also a substantial effect on other plasma proteins involved in fibrin formation and degradation. In healthy individuals stanozolol changes fibrinolytic parameters in such a way that the net effect seems to favour fibrinolysis.[46] This effect is thought to be mediated by an increase both in tPA activity which is mainly caused by a decrease in tPA inhibitor activity and in the concentration of free plasminogen, on the other hand in healthy individuals stanozolol will increase the plasma levels of the procoagulant factors II, IX and X while it has no effect an factor VII and vIII and decreases the fibrinogen cencentration.[42,46] A.M. Broekmans found that stanozolol can increase not only antithrombin III and protein C but also heparin cofactor II, a plasma inhibitor of thrombin.[47] Stanozolol has no effect on plasma proteins, the protein cofactor of activated protein C.[48]

Acknowledge

1. The Rachadapiseksompoj Fund 1989.
2. International Clinical Epidermilogy Unit

References

1. Stenflo J. A new Vitamin-K dependent protein. Purification from bovine plasma and preliminary characterization. J Biol Chem 1976 Jan 25 ; 251 (2) : 355-63
2. Esmon CT, Stenflo J, Suttie JW, Jackson CM. A new vitamin K-dependent protein; a phospholipid-binding zymogen of a serine esterase. J Biol Chem 1976 May 25 ; 251 (10) : 3052-6
3. Kisiel W, Canfield W, Ericsson LH, Davie EW. Anticoagulant properties of bovine plasma protein C following activation by thrombin. Biochemistry 1977 Dec 27 ; 16 (26) : 5824-31
4. Kisiel W. Human plasma protein C: isolation, characterization, and mechanism of activation by alpha-thrombin. J Clin Invest 1979 Sep : 64 (3) : 761-9
5. Marlar RA, Kleiss AJ, Griffin JH. Anticoagulant action of human protein C. Protides Biol Fluids Proc Collo 1980 ; 28 : 314-45
6. Canfield W, Neshien M, Kisiel W, Mann K. Proteolytic inactivation of bovin Factoe Va by bovin activated protein C. Circulation 1978 ; 210 Suppl. 2 : 57-8
7. Vehar GA, Davie EW. Preparation and properties of bovine Factor VIII (antihemophilic factor). Biochemiatry 1980 Feb 5; 19 (3) : 401-10
8. Comp PC, Esmon CT Activated protein C inhibits platelet prothrombin-converting activity. Blood 1979 Dec; 54 (6) : 1272-81
9. Dahlback B, Stenflo J. Inhibitory effect of activated protein C on activation of prothrombin by platelet bound factor Xa. Eur J Biochem 1980 Jun; 107 (2) : 331-5
10. Marlar RA, Griffin JH. Deficiency of protein C inhibitor in combined Factor V/VIII deficiency disease. J Clin Invest 1980 Nov ; 66 (5) : 1186-9
11. Esmoon CT, Owen WG. Identification of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. Proc NaltAcad Sci U.S.A. 1981 Apr ; 78 (4) : 2249-52
12. Bertina RM. Broekmans AW, Van Der Linden IK. Mertens K. Protein C deficiency in a Dutch family with thrombotic disease. Thromb Haemst 1982 Aug ; 48 (1) : 1-5
13. Broekmans AW, Veltkamp JJ. Bertina RM Congenital protein C deficiency and venous thromboembolism. study of the Dutch families. N Engl J Med 1983 Aug 11; 309 (6) : 340-4
14. Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Widemann C. Deficiency of Protein C in congenital thrombotic disease. J Clin Invest 1981 Nov; 68 (5) : 1370-3
15. Horellou MH, Conard J, Bertina RM, Samama M. Thromboses veineuses associess of un deficit constitutionnel on proteine C: trois observations. Presse Medicale 1983 ; 12 : 2259
16. Marlar RA, Endres-Brooks JL. Recurrent thromboembolic due to heterozygous protein C deficiency. Thromb Haemost 1983 ; 50 : 331
17. Pabinger-Fasching I, Bertina RM, Lechner K, Niessner H, Horninger CH. Protein C deficiency in two Austrian Families. Thromb Haemost 1983 Dec 30; 50 (4) : 810-3
18. Esmon CT. Protein-C. Biochemistry, physiology and clinical implications. Blood 1983 Dec; 63 (6) : 1155-8

19. Branson HE, Katz J, Masble R, Griffin JH. Inkented protein C defiiciency and caumarin-reaponsive chronic relapsing purpura fulminans in newborn infant. Lancet 1983 Nov 19 ; 2 (8360) : 1165-8
20. Bertin RM. Broekmans AW, Krommenhoek-van Es C, Van Wijngaarden A. The use of a functional and immunologic assay for plasma protein C in the study of the heterogeneity of congenital protein C deficiency. Thromb Haemoast 1984 Feb ; 51 (1) : 1-5
21. Esmon CT. Protein - C: biochemistry, physiology and clinical implications. Blood 1984 Dec; 63 (6) : 15-7
22. Meade TW, Stirling Y, Wilkes H, Mannucci PM. Effects of oral contraceptives and obesity on protein C antigen. Thromb Haemost 1985 Apr 22; 53 (2) : 198-9
23. Mannucci PM, Boyer C, Tripodi A, Vigano-D' Angelo S, Wolf M, Valsecci C, D'Angelo A, Mayer D, Larrieu MJ. Multicenter comparison fo five functional and two immunological assays for protein C. Thromb Haemost 1987 Feb 3; 57 (1) : 44-8
24. Mancini G, Carbonara AO, Heremans JF. Immunological quantitation of antigens by single radial immunodiffusion. Int J Immunochem 1965 Sep ; 2 : 235-54
25. Abildgard U, Lie M, Odegard OR. Antithrombin (heparin cofactor) assay with new chromogenic substrates (S-2238 and chromozym TH) Thromb Res 1977 Oct ; 11 (4) : 549-53
26. Thomson JM. Blood Coagulation and Haemostasis ; a practial Guide. 2 nd ed. Edinburgh: Churchill, Livingstone, 1980. 319
27. Ingram GIC, Hills M. Refernce method for the one-stage prothrombin time test on human blood. Int Thromb Haemost 1976 Aug 31; 36 (1) : 445-51
28. Thomson JM. Blood Coagulation and Haemotasis; a Practical Guide. 3rd ed Edinburgh: Churchill, Linvingstone, 1985. 403
29. Poller L, Thomson JM. The partial thromboplastin (cephaline) time test. J Clin Pathol 25, 1972 Dec; 25 (12) : 1038-44
30. Thomson JM. The activated partial thromboplastin time in "Blood coagulation and haemostasis : a practical guide". 3rd ed. Edinburgh: Churchill, Livingstone. 1985. 334-6
31. Stevenson KJ, Easton AC, Curry A, Thomson JM, Poller L. The reliability of activated partial thromboplastin time methods and the relationship to lipid composition and ultrastructure. Thromb Haemost 1986 Apr 30 ; 55 (2) : 250-8
32. Jim RTS. A study of the plasma thrombin time. J Lab Clin Med 50 : 1975 Jul : 50 (1) : 45-60
33. Clauss A. Gerinnungsphysiologische Schnellmethode Zur Bastimmung des Fibrinogens. Acta Haematol 1975 : 17 :237-46
34. Copley AL, Niewiarowski S, Marechel J. A micromethod of englobulin fibrinolysis in plasma of human subjects and small laboratory animals. J Lab Clin Med 1959 Mar ; 53 (3) : 468-73
35. Denson Kwe, Bonnett R, Biggs R. Br J Hematol 21 : 1971 : 291
36. Poller L, Thomson JM, Sear CHJ, Thomas W. Identification of a congenital defect of factor VIII in a colony of beagle dogs : the clinical use of the plasma. J Clin Pathol 1971 Oct ; 24 (7) : 6626-34
37. Aurell L, Fribergeer P, Kalsson G, Claeson G. A new sensitive and highly specific chromogenic peptide substrate for factor Xa. Thromb Res 11 ; 1977 Nov ; 11 (5) : 595-609
38. Aurell L, Simonsson R, Arielly S, Karlsson G, Friberger P, claeson G. New chromogenic peptide substrates for factor Xa. Haemostasis 1978 ; 7 (2) : 92-4
39. Soria J, Sroias C, Samama M. A plasminogen assay using a chromothrombolysis. In: Davidron JF, Rowan RM. Samara MM, Desnoyers J, eds. Progress in Chemical Fibrinoltsis and Thrombolysis. Vol 3 New York: Raven Press, 1978. 337-46
40. Friberger P, Knos M, Gustavsson S, Aurell L, Claeson G. Methods for determination of plasmain, antiplasmin and plasminogen by means of substrate S-2251. Haemostasis 1978 : 7 (2-3) : 138-45
41. Griffin JH, Evatt B, Zimmerman TS, Kleiss AJ, Wideman C. Deficiency of protein C in congenital thrombotic disease. J Clin Invest 1981 Nov; 68 (5) : 1370-73
42. Kluft C, Bertina RM, Preston FE, Malia RG, Blamey SL, Lowe GDO, Forbes CD. Protein C, an anticoagulant peotein, is increased in healthy volunteers and srugical patients after treatment with stanozolol. Thromb Res 1984 Feb 1 ; 33 (3) : 297-304
43. Broekmans AW. Hereditary protein C deficiency. Hemostasis 1985 : 15 (4) : 233-40
44. Mannucci Pm, Bottasso B, Sharon C, Tripodi A. A treatment with stanozolol of type I protein C deficiency in a Italian family. Da Ricerca Clin

Lab 1984 ; 14 : 673-80
45. Gonzalez R, Alberca I, Sala N, vicente V. Protein C deficiency-response to danazol and DDAVP. Thromb Haemost 1985 Jun 24 ; 53 (3) : 320-2
46. Madanes AE, Farber M. Danazol. Ann Intern Med 96 ; 1985 May ; 96 (5) : 625-30
47. Kluft C, Preston FE, Malia RG, Bertina RM, Wijngaards G, Greaves M, Verheijen JH, Dooijewaad G. Stanozolol-induced changes in fibrinolysis and coagulation in healthy adults. Thromb Haemost 1984 Apr 30 ; 51 (2) : 157-64
48. Tollefsen DM, Blank M. Detection of new heparin-dependent inhibitor of thrombin in human plasma. J Clin Invest 1981 Sep ; 68 (3) : 589-96