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The Frequency of Heterozygous Protein C Deficiency and Heterozygous Protein S Deficiency in Both Normal and Cerebral Thrombotic Conditions

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Summary. **Protein C and protein S activities were assayed in 508 healthy subjects and in 121 patients with cerebral thrombosis, and the frequencies of the congenital deficiencies of these physiological anticoagulant proteins were examined. In the healthy subjects, one case of heterozygous protein C deficiency (type I) and two cases of heterozygous protein S deficiency (type I and type II) were found. The frequencies of heterozygous protein C deficiency and heterozygous protein S deficiency in healthy subjects were 0.19% and 0.38%, respectively. In patients with cerebral thrombosis, one case of heterozygous protein C deficiency (type I) and 8 cases of heterozygous protein S deficiency (type I, 3; type II, 3; type III, 2) were found. The frequencies of heterozygous protein C deficiency and heterozygous protein S deficiency in cerebral thrombosis were 0.8% and 6.4%, respectively. In cerebral thrombosis the frequency of heterozygous protein S deficiency was higher than that of heterozygous protein C deficiency; heterozygous protein S deficiency seems to be a risk factor for the later onset of cerebral thrombosis.**

INTRODUCTION

The physiological plasma coagulant inhibitors protein C and protein S function to inhibit intravascular coagulation. Hereditary deficiencies and abnormalities among these inhibitors have been linked to recurrent thrombosis.[1] Protein C and protein S are vitamin K dependent plasma proteins which are synthesized in liver cells[2-4]; protein S functions to inhibit the coagulant co-factors Va and VIIIa by serving as a cofactor for activated protein C.[5-7] Therefore, protein C or protein S deficiency plays a pivotal role in hereditary thrombophilla.[8,9] In protein C deficiency, two types are recognized: in type I, the patient has reduced protein C functional activity and antigen; in type II, the patient has reduced functional activity but has significant quantities of the antigens.[10] In protein S deficiency, there are also two types,[11] but a precise examination has not yet been performed, because a generally accepted simple and specific assay method for protein S functional activity remains to be established. We have already reported a specific and simple assay method for protein S functional activity using protein C activated by a venom activator.[12] Protein C or protein S deficiency is mostly associated with venous thrombosis.[8,9,13] It should be noted that several cases associated with cerebral thrombosis in protein C or protein S deficiency have also been reported,[10,14-16] and that aging is an important factor for thromboembolic events in these patients.[9] We examined the frequencies of protein C and protein S deficiency in normal healthy Japanese subjects and in patients with cerebral thrombosis using our screening assay method and discussed the influence of aging on thrombotic episodes of heterozygous protein C and protein S deficiencies.

PATIENTS AND METHODS

One hundred and twenty-one patients with chronic stages cerebral thrombosis who were diagnosed by computerized tomography of the brain, were not taking any oral anticoagulants nor any oral contra-

ceptives, and who were without liver disease, disseminated intravascular coagulation or other massive thromboembolic diseases, were studied. The liver function of macromolecular synthesis was measured by the serum level of nonspecific cholinesterase (ChE) activity, and the influence of oral anticoagulants was excluded by thrombotest assay. Five hundred and eight clinically healthy subjects were also studied.

Blood samples were collected into 38 g/dl of trisodium citrate solution at a blood: anticoagulant ratio of 9 : 1. Platelet poor plasma was prepared by centrifugation at 4°C (2000 Xg for 20 min), then kept frozen at −70°C until used. Pooled normal plasma from 30 clinically healthy normal subjects who had not been taking any drugs was used as the standard plasma. Protein C and protein S activities in normal plasma are stable for a month at −70°C and the coefficient of variation (CV) for protein C and protein S activities under such conditions is less than 5%. Protein C activity was measured by selective spectrophotometry in a centrifugal analyzer using a chromogenic substrate[17] and by anticoagulant activity using a commercialized kit (protein C Reagent, Coagulometric, Behringwerke AG, Marburg, F.R.G.) and a KC 10 coagulometer (Heinrich Amelung GmbH, Lieme, F.R.G.). Protein S activity was measured by anticoagulant activity using protein C activated by venom activator and by a KC 10 coagulometer.[12] Protein C antigen was measured by the Laurell rocket technique[18] and by an enzyme linked immunosorbent assay (ELISA) kit (Boehringer Mannheim/Yamanouchi Co., Ltd. Mannheim, F.R.G.).

Protein S complexed to C4b-binding protein (C4bp) was precipitated with 3.75% polyethylene glycol 8,000 according to a report by Comp,[11] and total protein S antigen in plasma and free protein S antigen in a supernatant were assayed by ELISA kit (Asserachrom protein S; Diagnostica Stago, Francoville, France) and the Laurell rocket technique.[11] The qualitative analysis of protein S was performed by two-dimensional immunoelectrophoresis according to the report by Comp.[11] C4bp was measured by Laurell rocket technique using an antibody to C4bp (Assera C4bp, Diagnostica Stago, Francoville, France). Antithrombin III activity was measured using a chromogenic substrate (Testozym Test, ZYM, Kabivitrum Stockholm, Sweden). ChE activity was measured according to the method by Michel[19] and Thrombotest assay was done by the method of Owren.[20]

The classification of protein C deficiency was based on the proposal by Bertina et al.,[10] and protein S deficiency was classified into three types. All types had reduced activity, but the antigen level varied. Type I had a reduced total and free antigens to the same level of activity, type II had normal levels of total and free antigens, and type III had reduced free antigens but normal levels of total antigens.

Mean±SD and range of assay system of activity and antigen in protein C from 40 normal healthy subjects (divided into two groups, less than and more than 50 years of age, as shown in Table 1). In the group aged over/50, protein C activity (amidolytic and anticoagulant) and antigen showed a sex difference. The protein C activity and antigen was higher in females than in males, as reported by Tahara et al.[21] previously. The mean value and range of individual values of other assay systems in normal healthy subjects were found to be independent of age (at more than 20 years old) and sex. Cardroy et al.[22] already reported that free protein S antigen was higher in normal subjects more than 40 years old than in those less than 40 years old, and was higher in males than in females. We, however, could not find any differences in protein S activity by sex or age. Protein C, protein S and antithrombin III dificiencies were diagnosed as follows: protein C, protein S and antithrombin III activities were below the lower limit of the normal range of each assay system.[18] The

Table 1. Protein C Activity and Antigen in Two Groups of Healthy Normal Subjects, Either Less Than or More Than 50 Years of Age.

	50 years old>		50 years old≦			
			Male		Female	
Protein C activity	mean±SD (%)	range (%)	mean±SD (%)	range (%)	mean±SD (%)	range (%)
Amidolytic	109±18	70-158	86±12	70-110	104±18	78-158
Anticoagulant	104±29	63-160	74±12	63-100	102±29	68-160
Antigen	92±27	63-160	100±12	74-119	117±13	95-144

mean value (mean±SD) and range was as follows: protein S activity, 102±21%, 68-143%; protein S total antigen, 106±25%, 60-115%; protein S free antigen, 106±20%, 74-145%; antithrombin III activity, 100±15%, 70-134%; ChE activity, 1.00±0.22 ΔPH, 0.6-1.45 ΔPH; thrombotest; 78.5±15.9%, 40-120% and C4bp, 93±16%, 65-140%.

RESULTS

Only one case of protein C deficiency was found among the 508 healthy subjects examined (Table 2). Protein C activity (amidolytic and anticoagulant) and antigen were reduced to the same degree. Therefore, this was a case of type I deficiency. In fact, the subject had never had a history of thromboembolic disease even at the time of study. (Her family study is shown in Table 3). Protein C activity and antigen were reduced slightly in the father, to the same degree as the patient, this being a case of heterozygous protein C deficiency. Her father had had no episode of thromboembolic disease before the study.

Two cases of protein S deficiency were found among the 508 healthy subjects (Table 4). In the first case, protein S activity and antigen were reduced to the same degree, exibiting type I deficiency. In the second, protein S activity was moderately reduced, but total and free antigens were normal. This case constituted a type II deficiency. (A study of these two families is shown in Table 5). In the family labeled M. I., the mother's protein S activity and antigen were low to the same degree as the patient. In the family K.A., protein S activity was low in the mother and sister to the same degree as the patient, but total and free protein S antigens were normal. These two cases were heterozygous protein S deficiencies.

In the 121 patients with cerebral thrombosis, one case of protein C deficiency was found. Both amidolytic and anticoagulant protein C activities

Table 2. One Case of Protein C Deficiency among 508 Healthy Normal Subjects.
AT III=antithrombin III

Case					Protein C			Protein S activity	AT III activity
					Activity (%)		Antigen (%)		
No.	Name	Sex	Age	Type	amidolytic	anticoagulant	ELISA	(%)	(%)
1	YN	F	24	I	48	41	47	86	103

Table 3. Family Study of Protein C Deficiency in Healthy Normal Subjects.

		Protein C		
		Activity (%)		Antigen (%)
Family Y. N.	Age	anticoagulant	amidolytic	ELISA
Father	58	65	68	61
Mother	54	134	114	105
Brother	23	112	98	89

Table 4. Protein S Deficiency in 508 Healthy Normal Subjects.
AT III=Antithrombin III, C4bp=C4b Binding Protein

Case					Protein S					C 4 bp antigen	Protein C activity	AT III activity
					Activity	Antigen (%) Free		Total				
No.	Name	Sex	Age	Type	(%)	ELISA	Laurell	ELISA	Laurell	(%)	(%)	(%)
1	MI	F	30	I	49	35	41	63	44	70	145	112
2	KA	M	24	II	37	130	135	95	100	120	102	97

Table 5. Family Study of Protein S Deficiency in Healthy Normal Subjects.

		Protein S				
			Antigen (%)			
		Activity	Free		Total	
	Age	(%)	ELISA	Laurell	ELISA	Laurell
Family M. I.						
Mother	55	9	21	24	57	68
Father	57	104	62	54	63	70
Family K. A.						
Mother	59	35	115	180	110	115
Sister	29	45	96	100	75	78
Father	59	138				

Table 6. Protein C Deficiency in 121 Patients with Cerebral Thrombosis.
AT III = Antithrombin III, ChE = Non Specific Cholinesterase, TT = Thrombotest

					Protein C			Protein S	AT III	Ch-E	TT
Case			Age at		Activity (%)		Antigen (%)	activity	activity		
No.	Name	Sex	onset	Type	amidolytic	anticoagulant	Laurell	(%)	(%)	(ΔpH)	(%)
1	SY	F	87	I	56	45	35	77	76	0.80	77

Table 7. Protein S Deficiency in 121 Patients with Cerebral Thrombosis.
AT = Antithrombin III, ChE = Non Specific Cholinesterase, TT = Thrombotest

					Protein S					C 4 bp	Protein C	AT III	Ch-E	TT
					Activity	Antigen (%)								
Case			Age at			Free		Total		antigen	antigen	activity		
No.	Name	Sex	onset	Type	(%)	ELISA	Laurell	ELISA	Laurell	(%)	(%)	(%)	(ΔpH)	(%)
1	TO	M	55	I	34	41	31	50	60	69	78	69	0.84	66
2	SH	F	71	I	49	42	23	50	64	81	72	76	1.01	80
3	YY	F	75	I	50	62	54	63	70	120	99	82	0.65	67
4	TY	F	70	II	51	80	100	98	120	120	124	100	0.79	122
5	WK	F	64	II	44	101	210	98	110	110	99	88	0.66	65
6	KY	F	70	II	15	80	80	114	100	81	73	87	0.92	92
7	MN	M	76	III	57	56	41	91	88	80	82	70	0.80	66
8	TK	F	63	III	47	53	27	98	90	105	93	100	0.82	96

were reduced to the same degree (Table 6). Protein S, antithrombin III, other coagulation factors, ChE activity and the thrombotest were normal. This case was type I deficiency. The female patient had had no episode of thromboembolic disease until 87 years of age. In the 121 patients with cerebral thrombosis, 8 cases of protein S deficiencies were found (Table 7). In case numbers 1, 2 and 3, protein S activity decreased moderately and antigen levels measured by ELISA and Laurell rocket technique were also reduced to the same degree. These three cases were type I deficiencies. In case number 4, 5 and 6, protein S activity decreased moderately, but total and free antigens were normal. These three cases were type II deficiency. In case numbers 7 and 8, protein S activity decreased moderately and the free antigen was reduced to the same degree, but the total antigen was normal. These two cases were type III deficiency.

The mean age at the onset of cerebral thrombosis was 68 years, and the mean activity of protein S was 43% in these cases. C4bp, protein C, antithrombin III, other coagulation factors, ChE activity and thrombotest were normal in these cases.

The qualitative analysis of protein S is shown in Fig. 1. In two-dimensional immunoelectrophoresis, the amounts of C4bp complexed and free protein S antigens for type I (case SH) were reduced, compared with normal subjects. In type II (case WK), the amount of C4bp complexed and free protein S antigens were somewhat increased, and in type III (case MN), the amount of free protein S antigen was reduced but C4bp complexed protein S antigen was increased. In general, however, no big differences in distribution pattern in C4bp comlexed and free protein S antigens between those cases and healthy normal subjects were recognized.

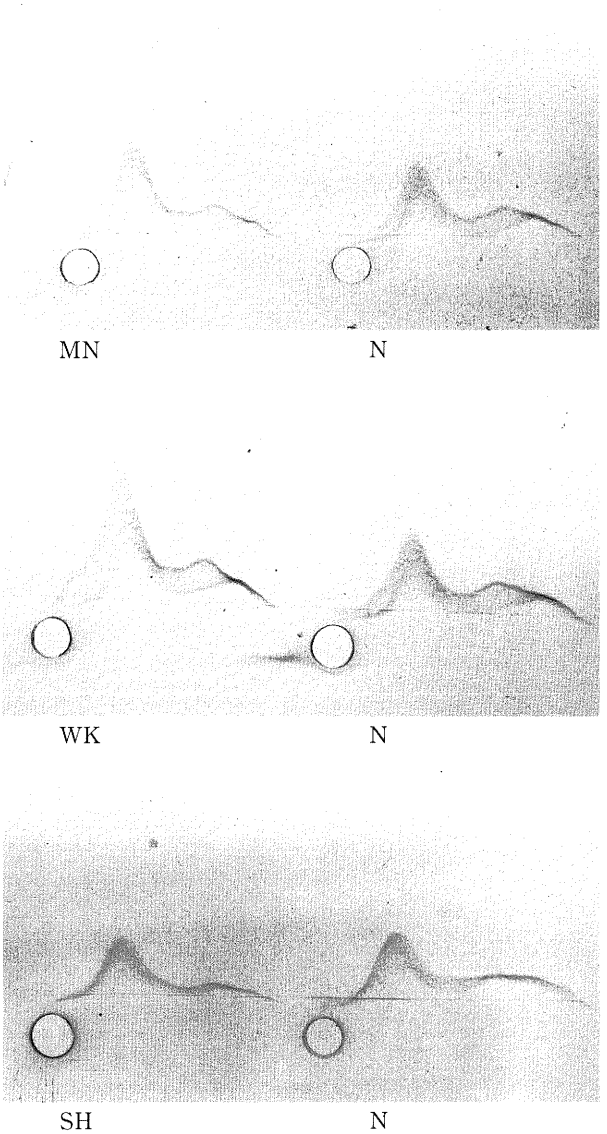

Fig. 1. Qualitative protein S analysis of protein S deficiency in cerebral thrombosis (type I: case SH, type II: case WK, type III: case MN) using two-dimensional immunoelectrophoresis. N=normal plasma

DISCUSSION

The frequencies of heterozygous protein C deficiency and heterozygous protein S dificiency in healthy subjects were 0.19% and 0.38%, respectively. These frequencies are almost the same as those reported by Bertina et al.[23] For the family Y.N., the father had protein C deficiency and the mother was normal. This case was a heterozygous protein C type I deficiency. In the study on family M.I., the mother's protein S activity and antigen decreased to the same degree as the patient. For the family K.A., protein S activity decreased to the same degree among the patient, the mother and the father, but the antigen levels were normal. Therefore, cases K.A. and M.I. were heterozygous protein S deficiencies of type I and type II respectively.

In the 121 patients with cerebral thrombosis, we found one case of protein C deficiency and eight cases of protein S deficiencies. The frequency of heterozygous protein C deficiency in cerebral thrombosis was 0.8%. Our data shows that protein C deficiency associated with cerebral thrombosis is rare, as previously reported.[10] The frequency of heterozygous protein S deficiency in cerebral thrombosis was higher than that of heterozygous protein C deficiency. We classified protein S deficiency into three types. Type I is a quantitative reduction of the total and free protein S antigen, and type II is an abnormal protein S with reduced functional activity and normal levels of the free and total protein S antigen. Type III has reduced free but normal levels of the total protein S antigen. Recently, Comp also proposed to classify the congenital protein S deficiency in to three types as I, IIa, and IIb based on total protein S levels.[24] However, the reduction of the free protein S in type III or type IIa by Comp may be due to an abnormal distribution of protein S between the free and C4bp-protein S complex. This type is not a true protein S deficiency. Therefore, type III protein S deficiency must be distinguished from type I and type II. The frequencies of type I and type II were 2.

4% each, with type III at 1.6%.

In the past, functional protein C activity was assayed for determining protein C deficiency. However, most protein S deficiency was determined by assaying the total protein S antigen.[9,25,26] The frequency of protein S deficiency may be low because type II and type III protein S deficiency are often overlooked. From our data, five out of eight cases of protein S deficiencies in cerebral thrombosis were type II or type III. Therefore, the screening assay of functional protein S activity in thromboembolic disease is quite important. In two-dimensional immunoelectrophoresis, no big difference in distribution pattern was recognized between type II protein S deficient and healthy normal subjects as has already been reported by Mannucci et al.[27] Other techniques are required to analyze the abnormal protein S in type II.

Homozygous protein C or protein S deficiency is associated with severe thrombosis and purpura fulminans in childhood,[28-31] but heterozygous deficiency of protein C or protein S is rarely symptomatic in childhood. In heterozygous protein C deficiency, the average age of onset of thrombosis was in the twenties,[32] with 50% of 48 cases of hereditary protein C deficiencies having episodes of thrombosis after 30 years old.[10] In heterozygous protein S deficiency, the average age of onset of thrombosis was also in the twenties, but range of onset age was from 15 to 68 years old; even at 35 years of age, no symptom was observed in 32% of the 71 cases.[9] Miletich et al.[33] reported the absence of thrombosis in heterozygous protein C deficiency but also suggested that other factors, ex. aging, might be needed to induce the thromboembolic complication. The age of onset of cerebral thrombosis in these cases was from 55 to 77 years (mean age: 68 years). Therefore, heterozygous protein S deficiency seems to be a risk factor for the later onset of cerebral thrombosis and the frequency of heterozygous protein S deficiency associated with cerebral thrombosis seems to be higher in females than in males.

The most commomn sites of the thromboembolic disease are in the superficial veins of the extremities, deep vein thrombosis of the leg, pulmonary emboli, mesenteric vein and cerebral vein thrombosis.[8,9,15,25,34] Until now, however, several cases of protein C or protein S deficiencies associated with cerebral thrombosis have been reported.[10,14-16] In addition, Thommen et al.[35] also reported that seven out of fourteen cases of protein S deficiencies were associated with cerebral vascular disease and that there was the possibility of a high frequency of cerebral thrombosis in protein S deficiency.

Protein C shows an anticoagulant action which arises solely from the cleavage of specific peptide bonds in factors Va and VIIIa.[36] The inactivation processes of Va and VIIIa by activated protein C require protein S[37] and platelets or the endothelial cell surface.[38-40] Activated protein C can interact with the surface of activated platelets with the presence of protein S, but can not interact with intact platelet surfaces or endothelial cell surfaces. Protein S functions as a protein C cofactor. Protein C is activated by thrombin-thrombomodulin complexes.[41] Thrombomodulin is detected on the surfaces of endothelial cells of arteries, veins and capillaries in various organs except the brain.[42] Therefore, it is probable that cerebral thrombosis has a tendency to be associated with heterozygous protein C and protein S deficiency. The reason for the high incidence of protein S deficiency in cerebral thrombosis is still unclear.

Congenital protein C and protein S deficiencies constitute individuals with true prethrombotic states because of the excessive production of factor Xa enzymatic activity.[43] It is reasonable that the initial manifestations of states of hypercoagulation occur frequently in deep veins of the lower extremities since the blood flow is slow in these regions. However, it is still undetermined whether dramatic increments of arterial thrombosis are less rare. To analyze the role of protein C and protein S in the pathogenesis of thrombus formation in later onset, screening studies of activities of these proteins in large populations across a wide variety of ages are needed.

REFERENCES

1) High KA: Antithrombin III, Protein C, and Protein S. *Arch Pathol Lab Med* **112**: 28-36, 1988.
2) Fair DS, Marlar RA: Biosynthesis and secretion of factor VII, protein C, protein S and the protein C inhibitor from a human hepatoma cell line. *Blood* **67**: 64-70, 1986.
3) Stenflo J: A new vitamin K-dependent protein: purification from bovine plasma and preliminary characterization. *J Biol Chem* **251**: 355-363, 1976.
4) Di Scipio RG, Davie EW: Characterization of protein S, a r-carboxy glutamic acid containing protein from bovine and human plasma. *Biochem* **18**: 899-904, 1979.
5) Walker FJ: Regulation of activated protein C by a new protein. *J Biol Chem* **255**: 5521-5524, 1981.
6) Walker FJ: Regulation of activated protein C by protein S, the role of phospholipid in factor Va inactivation. *J Biol Chem* **256**: 11128-11131, 1981.

7) Suzuki K, Nishioka J, Hashimoto S: Regulation of activated protein C by thrombin modified protein S. *J Biochem (Tokyo)* **94**: 699-705, 1983.
8) Gladson CL, Scharrer I, Hach V, Beck KH, Griffin JH: The frequency of type I heterozygous protein S and protein C deficiency in 141 unrelated young patients with venous thrombosis. *Thromb Haemost* **59**: 18-22, 1988.
9) Engesser L, Broekmans AW, Briet E, Brommer EJP, Bertina RM: Hereditary protein S deficiency: clinical manifestations. *Ann Int Med* **106**: 677-682, 1987.
10) Broekmans AW, Wintzen AR, Bertina RM: Clinical manifestations of hereditary protein C deficiency. *Circulation* **70 (suppl II)**: 204, 1984.
11) Comp PC, Doray D, Patton D, Esmon CT: An abnormal plasma distribution protein S occurs in functional protein S deficiency. *Blood* **67**: 504-508, 1986.
12) Kobayashi I, Amemiya N, Endo T, Okuyama K, Tamura K, Kume S: Functional activity of protein S determined with use of protein C activated by venom activator. *Clin Chem* **35**: 1644-1648, 1989.
13) Comp PC, Esmon CT: Recurrent venous thromboembolism in patients with a partial deficiency of protein S. *New Eng J Med* **311**: 1525-1528, 1984.
14) Girolami A, Simioni P, Lazzaro AR, Cordiano I: Severe arterial cerebral thrombosis in a patient with protein S deficiency (moderately reduced total and markedly reduced free protein S): A family study. *Thromb Haemost* **61**: 144-147, 1989.
15) Shen MC, Tsai W, Hsu PL: A study of hereditary thrombophilia in Taiwan, ROC. *Thromb Haemost* **62**: 442, 1989. (abstract)
16) Akiyama M, Ogawa J, Kuchiba K, Maeda T, Ikemoto S, Yokose T, Isogai Y: Arterial thrombosis associated with protein C deficiency: Report of a case. *Thromb Haemost* **62**: 383, 1989. (abstract)
17) Kobayashi I, Amemiya I, Endo T, Motegi J, Kuriharа A, Hamaoka S, Tamura K, Kume S: Amidolytic kinetic assay of protein C by selective spectrophotometry in a centrifugal analyzer. *Clin Chem* **34**: 2260-2263, 1988.
18) Bertina RM, Broekmans AW, Krommenhoek-Van ESC, 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 Haemost* **55**: 1-5, 1984.
19) Michel HO: An electric method for the determination of red blood cell and plasma cholinesterase activity. *J Lab Clin Med* **35**: 1564-1568, 1949.
20) Brozovic M: Laboratory control of anticoagulant thrombolytic and antiplatelet therapy. In: Dacie JV, Lewis SM (eds) Practical Haematology. 7th ed. Churchill Livingstone, Edinburgh-London-Melbourne-New York 1991; 335-349.
21) Tahara C, Kazama M, Miyajima Y. Matsumoto K, Abe T: Reference values of hemostasis related factors of healthy Japanese adults. II. protein C. *Thromb Res* **62**: 345-351, 1991.
22) Cadroy Y, Daviaud P, Sie P, Boneu B: Distribution of 16 hemostatic laboratory variables assayed in 100 blood donors. *Nouv Rev Fr Hematol* **32**: 259-264, 1990.
23) Bertina RM: Hereditary thrombophilia. *Thromb Haemost* **62**: 147, 1989. (abstract)
24) Comp PC: Laboratory evaluation of protein S status. *Sem Thromb Hemost* **16**: 177-181, 1990.
25) Bertina RM: Hereditary protein S deficiency. *Haemostasis* **15**: 241-246, 1985.
26) Broekmans AW, Bertina RM, Reinalde P, Engesser L, Müller HP, Leeuw JA, Michiels JJ, Brommer EJP, Briet E: Hereditary protein S deficiency and venous thromboembolism. A study in three Dutch families. *Thromb Haemost* **3**: 273-277, 1985.
27) Mannucci PM, Valsecchi C, Krachmalnicoff A, Faioni EM, Tripodi A: Familial dysfunction of protein S. *Thromb Haemost* **62**: 441, 1989. (abstract)
28) Malar RA, Montgomery R, Broekmans AW: Diagnosis and treatment of homozygous protein C-deficient children. *J Pediat* **114**: 528-535, 1989.
29) Marciniak E, Wilson HD, Marlar RA: Neonatal purpura fulminans: A genetic disorder related to the absence of protein C in blood. *Blood* **65**: 15-20, 1985.
30) Clause HL, Comp PC: The regulation of hemostasis: the protein C system. *New Eng J Med* **314**: 1298-1304, 1986.
31) Mahasandana C, Suvatte V, Marlar R, Manco-Johnson M, Jacobson L, Hathaway WE: Neonatal purpura fulminans associated with homozygous protein S deficiency. *Thromb Haemost* **62**: 301, 1989. (abstract)
32) Horellou MH, Conard J, Bertina RM, Samama M: Congenital protein C deficiency and thrombotic disease in nine French families. *Brit Med J* **289**: 1285-1287, 1984.
33) Miletich J, Sherman L, Broze G: Absence of thrombosis in subjects with heterozygous protein C deficiency. *New Eng J Med* **317**: 991-996, 1987.
34) Pabinger FI, Bertina RM, Lechner K, Niessner H, Korninger CH: Protein C deficiency in two Austrian families. *Thromb Haemost* **50**: 810-813, 1983.
35) Thommen D, Buhrfeind E, Felix R, Sulzer I, Furlan M, Lammle B: Hemostasis parameters in 55 patients with venous and/or arterial thromboembolism. *Thromb Haemost* **62**: 480, 1989. (abstract)
36) Kisiel W, Canfield WM, Ericsson LH, Davie EW: Anticoagulant properties of bovine plasma protein C following activation by thrombin. *Biochem* **16**: 5824, 1977.
37) Walker FJ: Regulation of activated protein C by a new protein: a possible function for bovine protein S. *J Biol Chem* **255**: 5521-5524, 1980.
38) Harris KW, Esmon CT: Protein S is required for bovine platelets to support activated protein C binding and activity. *J Biol Chem* **260**: 2007-2010, 1980.
39) Suzuki K, Nishioka J, Kusumoto H: Interaction of protein S with activated protein C in the formation

of the factor Va inactivation complex. *Thromb Haemost* **54**: 56, 1985. (abstract)
40) Stern DM, Nawroth PP, Harris K, Esmon CT: Cultured bovine aortic endothelial cells promote activated protein C-protein S-mediated inactivation of factor V. *J Biol Chem* **261**: 713-718, 1986.
41) Suzuki K, Kusumoto H, Hashimoto S: Isolation and characterization of thrombo modulin from bovine lung. *Biochem Biophys Acta* **882**: 343-352, 1986.
42) Maruyama I, Bell CE, Majerus PW: Thrombomodulin is found on endothelium of arteries, veins, capillaries and on syncytiotrophoblast of human placenta. *J Cell Biol* **101**: 363-371, 1985.
43) Rosenberg RD, Bauer KA: Thrombosis in inherited deficiencies of antithrombin, protein C, and protein S. *Human Pathol* **18**: 253-262, 1987.