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Reduced Activity of Protein S in Plasma: A Risk Factor for Venous Thromboembolism in the Japanese Population

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Abstract

The quantitative assay of protein S can help in rapidly identifying carriers of abnormal protein S molecules through a simple procedure (by determining the total protein S mass, total protein S activity, and protein S-specific activity in blood), without genetic testing. To clarify the relationship between venous thromboembolism (VTE) and protein S-specific activity, and its role in the diagnosis of thrombosis in Japanese persons, the protein S-specific activity was measured and compared between patients with thrombosis and healthy individuals. The protein S-specific activity of each participant was calculated from the ratio of total protein S activity to total protein S antigen level. Plasma samples were collected from 133 healthy individuals, 57 patients with venous thrombosis, 118 patients with arterial thrombosis, and 185 non-thrombotic patients. The protein S-specific activity of one-third of the patients with VTE was below the line of $Y = 0.85X$ (-2 S.D.). Most protein S activities in the plasma of non-thrombotic patients were near the $Y = X$ line, as observed in healthy individuals. In conclusion, it was clearly shown that monitoring protein S activity and protein S-specific activity in blood is useful for predicting the onset and preventing venous thrombosis in at least the Japanese population.

Keywords

protein S, APC anticoagulants, venous thromboembolism, protein S deficiency

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Introduction

Factor V Leiden is a well-known risk factor for thrombosis in Caucasian populations.[1,2] The risk factors for thrombosis in Japanese people were unknown[3] until some studies were conducted recently; they showed that the activated protein C (APC)-protein S system is a critical risk factor.[4-6] In our previous studies on thrombophilia, most of the participants had a dysfunction of the APC anticoagulation system, mainly due to the reduced activity of protein S in Japanese and Chinese populations.[6-9] This indicates that a quantitative analysis of the protein S activity and/or mass in plasma can be a reliable diagnostic tool for diagnosing thrombophilia in at least 2 populations.[6-9]

Based on this finding, we developed novel assays for measuring the total (sum of free and bound forms) protein S activity and total protein S mass.[10] Using these assays, it was revealed that while there are gender-dependent differences in the mass and activity levels of total protein S, the mean and reference intervals of the protein S-specific activity, calculated by dividing the activity by mass, are not different between males and females.[10] Among the apparently healthy subjects in the study, 3 individuals with protein S specific activities were lower than the mean by at least 3 standard deviations (S.D.s). Hence, type II protein S deficiency was suspected in these 3 individuals, and it was confirmed using genetic testing.[10] This implies that determination of the protein S specific activity in blood may

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be a simple and rapid method for identifying pre-thrombosis and thrombosis in individuals, irrespective of sex.[10] This test can be incorporated into the list of routine medical workup for check-ups in clinics.

This study compared the protein S specific activity levels in the plasma of patients with venous thromboembolism, arterial thrombosis, and patients without thrombosis.

Methods

Samples

Whole blood samples were collected from patients with venous thromboembolism (n = 57), arterial thrombosis (n = 118), and non-thrombotic patients (n = 185; Table S1). These samples were added to tubes containing 0.1 volume of 3.13% sodium citrate. Immediately, platelet-poor plasma was prepared by centrifugation at 1500 × g for 10 min at 4°C. Samples for the analysis of protein S-specific activity were stored in small aliquots at −80°C until they were ready for analysis.[4,5] Employees of Shino-Test Co. Ltd. were considered as healthy donors (85 men aged 19-65 years; 48 women aged 23-63 years); their blood samples were collected and processed like those of the patients.

Patients

All patient samples were collected during their first visit to Kyushu University Hospital (Fukuoka, Japan) between April 2003 and March 2006. In particular, samples from patients with venous thrombosis and with arterial thrombosis were collected before the start of anticoagulant therapy. Informed consent was obtained from each patient before the start of the survey. Authorization was obtained from the Kyushu University Ethics Committee (232, 232-01, 232-02).

Patients with suspected severe liver dysfunction, disseminated intravascular coagulation, and vitamin K deficiency, and those aged below 15 years were excluded.

Measurement of Protein S Specific-Activity

The ratio of total protein S activity to total protein S antigen level was defined as the protein S-specific activity.[10] As previously reported,[10] total protein S activity was measured using colorimetry, and the total protein S antigen level was determined via latex agglutination using an automated analyzer (Hitachi 7180; Hitachi High Technologies, Tokyo, Japan). The World Health Organization International Standard (NIBSC 03/228) was used as the primary standard. The unit was converted to μg/mL using purified protein S (1.00 IU/mL, 24.46 μg /mL).

Statistical Analysis

The methodology related to the functional analysis of specimens has been described previously.[4,5] Plasma from 339 healthy individuals was used to determine the reference intervals and cut-off values for protein S-specific activity. The average protein S-specific activity was 1.01, and the cut-off was set at 0.77 (which corresponded averagely to − 3 S.D.).

Results

Figure 1 shows a scatterplot of total protein S activity versus total protein S mass for all the study participants. The protein S activity of 133 healthy individuals (Figure 1A) was in the proximity of the Y = X plot, i.e. with a protein S activity of approximately 15-35 μg/mL protein S-equivalent.

The protein S activity in the plasma of 3/133 (2.3%) healthy individuals was below the line of Y = 0.77 X (−3 S.D.) (Figure 1A). This is consistent with the ratio of occurrence of protein S-Tokushima (p. Lys196Glu) in the Japanese population.[5,7]

The protein S-specific activity of nearly one-third of the patients with deep venous thrombosis or pulmonary embolism was below the line of Y = 0.85X (−2 S.D.) or Y = 0.77X (−3 S.D.) (Figure 1B). Notably, the protein S activity of one of the patients with deep venous thrombosis was less than 5 μg/mL protein S-equivalent (Figure 1B).

In contrast, most protein S activities of the patients with arterial thrombosis were near the Y = X line, as observed in the healthy individuals, except for 5 patients who were below the Y = 0.77X (−3 S.D.) line and one who was below the Y = 0.85X (−2 S.D.) line; this patient had an activity of 10 μg/mL protein S-equivalent, which was the lowest activity in this group (Figure 1C). Further studies are needed to determine whether the reduced protein S activity in these patients plays a role in the pathogenesis of arterial thrombosis (Figure 1C).

The protein S activity of the non-thrombotic patients (*e.g.*, patients with systemic lupus erythematosus, vasculitis syndrome, antiphospholipid antibody syndrome, *etc.* Table S1) was higher than that of the patients with arterial thrombosis (Figure 1D). However, most protein S activity levels were above the line of Y = 0.85 × (Figure 1D). Further studies are needed to explain these findings, as confounding factors may have affected these findings.

Table 1 shows the protein S-specific activity of all the study participants. At the cut-off protein S-specific activity of 0.77, the specificity and sensitivity of this test in diagnosing venous thrombosis was 98% and 19%, respectively (Table 1). The multi-pathogenic nature and reduced activity of protein S can explain this low sensitivity.

Discussion

This study showed that the measurement of protein S-specific activity is a reliable method with a high specificity for diagnosing individuals with a high risk of venous thrombosis. As shown in previous studies,[5,7,8] Japanese thrombophilia is often due to APC dysfunction; about 50% of these dysfunctions are protein S deficiency. This is consistent with our study which showed that patients with venous thrombosis had a significantly lower protein S activity than those in the other groups (Table 1). Therefore, monitoring protein S activity and protein

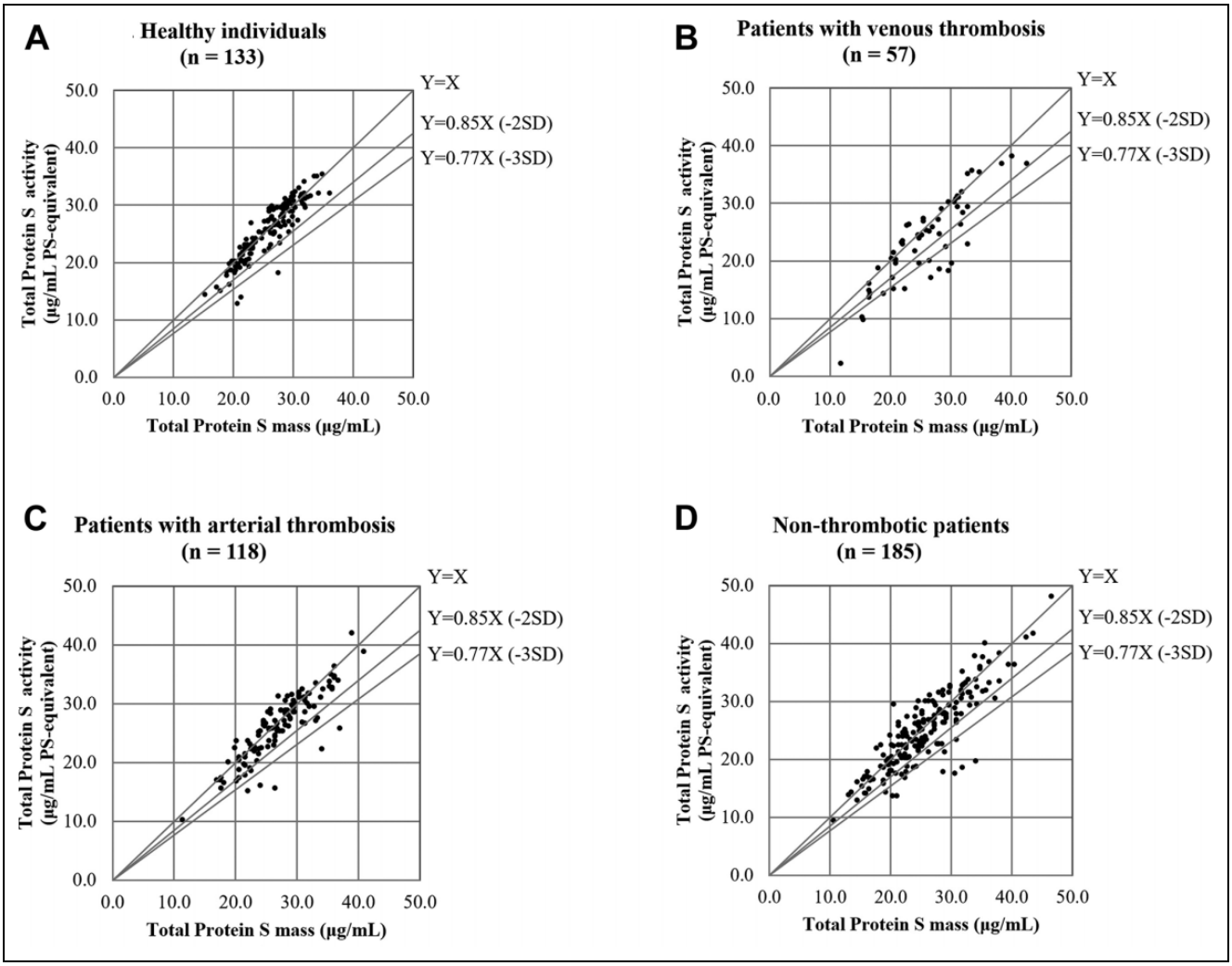

Figure 1. Quantitative analyses of protein S in plasma from healthy individuals (A), patients with venous thromboses (B), patients with arterial thromboses (C), and non-thrombotic patients (D). Protein S (PS) activity is expressed in μg/mL PS-equivalent. Each spot represents protein S specific-activity of 133 healthy individuals (A), 57 venous thrombosis patients (B), 118 arterial thrombosis patients (C), and 185 patients with non-thrombotic diseases (D). The lines Y = 0.85X and 0.77X show the boundaries of the specific-activity at [average-2 S.D.] and [average-3 S.D.], respectively. As shown in this figure, measuring the mass (x-axis) and activity (y-axis) of total (Sum of free and bound forms) protein S in plasma from individuals and calculating protein S specific-activity (Activity/mass of protein S) was shown effective to identify thrombotic condition.

Table 1. Sensitivity and Specificity of Protein S Specific-Activity.

	Protein S specific-activity				
	<0.77	≥0.77	Sensitivity	Specificity	Odds ratio (95% CI)
Healthy individuals (n = 133)	3	130		130/133 = 0.98	
Venous thrombosis (n = 57)	11	46	11/57 = 0.19		12.8 (2.8-38.8)
Arterial thrombosis (n = 118)	5	113	5/118 = 0.04		1.9 (0.4-8.2)
Nonthrombotic diseases (n = 185)	10	175	10/185 = 0.05		2.5 (0.7-9.2)

S-specific activity in blood is useful for the prevention of venous thrombosis.

By using this method, it can be seen that some healthy individuals (3/133) have low protein S activity and protein S-specific activity (Figure 1A). We predicted that these 3 individuals carried the protein S-Tokushima trait, which was confirmed by genotyping. This finding strongly suggests that the measurement of plasma protein S-specific activity can be an alternative to genotyping for diagnosing thrombosis, which is more expensive.

In conclusion, compared to genotyping, protein S specific-activity measurement is a rapid and cost-effective method to identify carriers of abnormal protein S molecules, at least in the Japanese population.

There are several risk factors of thrombosis; however, taking long flights and living in shelters after disasters favor long hours of little or no mobility are 2 major risk factors. Pregnancy and female hormone imbalance can abnormally reduce protein S activity, which leads to a higher risk of venous thrombosis. Excessive reduction of protein S activity during pregnancy retards fetal development.[6] Therefore, for individuals with a protein S-specific activity below 0.77, they can be administered aggressive prophylaxis for thrombosis. In particular, protein S-specific activity in blood should be assessed perioperatively to prevent post-operative thrombosis. On the other hand, even if patients with a protein S-specific activity of 0.77 and above, watchful waiting is required in high-risk situations of venous thrombosis such as postoperative management.

Monitoring protein S activity and protein S-specific activity can be an invaluable tool to prevent, diagnose, and select treatment not only for patients with thrombosis, but also disaster survivors, and patients with a history of recurrent miscarriage. This analysis can be used for both sexes. This approach can also facilitate personalized medical care.

Authors' Note

NH contributed to the study design. SK and NH contributed to the specimen collection. XJ, TT, HK, HY and NH contributed to the development of methods for quantitative analysis of protein S activity. XJ and TT moved to Research & Development, Shino-Test Corporation (Kanagawa, Japan). SK and NH retired from Kyushu University after completing this research. NH is now Professor Emeritus at Kyushu University School of Medicine.

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Declaration of Conflicting Interests

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Supplemental Material

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