

High Incidence of Thrombophilia Detected in Chinese Patients with Venous Thrombosis

H. W. Liu¹, Y. L. Kwong, C. Bourke, C. K. Lam, A. K. W. Lie, D. Wei, L. C. Chan

From the Department of Pathology and Department of Medicine, Queen Mary Hospital, University of Hong Kong, Hong Kong

Summary

Venous thromboembolism is rare in Chinese. To determine the incidence and disease profile of thrombophilia in Chinese patients with thrombosis, 52 unselected Chinese patients with documented venous thrombosis were studied for the presence of thrombophilia. Levels of antithrombin III (AT III), protein C (PC) and protein S (PS) as well as the presence of acquired lupus anticoagulant (LA) and anticardiolipin antibody (ACA) were investigated. Thirty patients were found to be abnormal. These consisted of 5 AT III deficiencies, 9 PC deficiencies, 10 PS deficiencies, 1 combined PC & PS deficiency (all in the heterozygous range), and 5 patients with LA and/or ACA. When the patients with LA and/or ACA are excluded, the incidence of hereditary thrombophilia is 25/47 i.e. 53.2% which is much higher than those reported in studies of Caucasian patients selected under strict criteria. Family studies performed in 16 cases of hereditary thrombophilia revealed involvement in 11 cases (68.7%); a total of 36 heterozygous family members were affected, most of which remain asymptomatic. Although 35 events predisposing to thrombosis (27 pregnancies, 1 oral contraceptive consumption and 7 surgical operations) were identified among these index patients, and the heterozygous family members, thrombosis was observed on only 6 occasions (17.1%). The data suggest that pregnancy and surgery do not carry the same degree of thrombotic risk in Chinese as in the Caucasian population with heterozygous AT III, PC and PS deficiency.

Introduction

Spontaneous deep-vein thrombosis (DVT) and its sequelae present in the West with an incidence that has been estimated to be around 0.05–0.1% or higher (1, 2, 3). In contrast, the Chinese population has an extremely low incidence of thromboembolism. In patients undergoing major operations and without receiving anticoagulant prophylaxis, prospective studies utilizing sensitive diagnostic methods showed that only 2.6% to 8.5% of patients developed venous thrombosis. Even in patients with concomitant malignancy, the incidence remained at 5.6–6.7% (4–6). In the majority of cases of venous thrombosis, no clear cut underlying abnormality can be demonstrated. Hereditary thrombophilia accounts for no more than 15–30% of the cases in reports on Caucasian patients (7–15). Factors such as young age, recurrent thrombosis, positive family history or thrombosis in unusual sites are not highly predictive for the presence of an underlying hereditary thrombophilia. As a result, many centres adopt recommended

guidelines for screening patients with thrombosis, since the prognosis and long term management of familial thrombophilia are different (16–18). In view of the rarity of the condition in our population, we undertook to investigate 52 consecutive Chinese patients with documented venous thrombosis who were referred to Haematology Section, QMH for the presence of AT III, PC, PS deficiency and the presence of LA/ACA to see if thrombophilia constitutes an important cause of venous thrombosis in our population. Family studies were also performed following confirmation of a positive case.

Patients, Materials and Methods

Patient Selection

Between September 1989 to August 1992, 52 consecutive Chinese patients (25 males; 27 females; age 14–88 years [mean 45.2 years]) with documented venous thrombosis were screened for thrombophilia at the Haematology Section, Department of Pathology, Queen Mary Hospital, a tertiary referral centre for haematological investigations. Twenty-two patients were from Queen Mary Hospital whilst twenty eight were referred from other hospitals in Hong Kong. There was no selection bias for the patients, the only criterion for inclusion into this study was documentation of a venous thrombosis, irrespective of age, site, previous episodes of thrombosis, family history or presence of provoking events. The site and extent of the thrombosis were confirmed by either venography (23 cases) or ultrasonography (25 cases) or both (3 cases). Pulmonary embolism was detected by ventilation-perfusion scan and visceral and cerebral thrombosis were confirmed by angiography. For patients on anticoagulant therapy, screening was carried out after cessation of anticoagulation therapy for at least 4 weeks. Detailed medical, drug and family histories were obtained, with special attention being paid to factors that might alter the AT III, PC and PS levels, e.g. liver/renal impairment, pregnancy. Venepunctures were performed after informed consent.

Blood Samples

Venous blood was collected into 3.2% trisodium citrate (9:1 ratio) and processed immediately (within 1 hour) for platelet poor plasma viz. double spun specimens at 3000 rpm × 10 min and tested on the same day or kept frozen at –70 C until further study. Sera separated from clotted blood were saved for anticardiolipin antibody study. All abnormal findings were confirmed by repeat studies 4 to 6 weeks later. Family members of patients suffering from AT III, PC or PS deficiencies either singly or combined, if available, were also studied.

Normal ranges of AT III, PC and PS were determined by studying 48 healthy adult Chinese individuals.

Coagulation Tests

Prothrombin time (PT), activated partial thromboplastin time (APTT) and thrombin time (TT) were measured by standard techniques. Fibrinogen concentration was assayed by the Clauss method.

Correspondence to: Dr. L. C. Chan, Haematology Section, Department of Pathology, University of Hong Kong, Queen Mary Hospital, Hong Kong

¹Current address: Hong Kong Red Cross Blood Transfusion Service, Kowloon, Hong Kong

Table 1 Age, site and type of thrombosis in relation to presence or absence of underlying thrombophilia

Site and type Thrombosis	Thrombophilia detected					No abnormality detected	Total
	ATIII	PC	PS	PC + PS	LA/ACA		
Mean age at thrombosis	29	33	38	37 ¹	32	49	
Site & type of thrombosis							
Calf	4	3	2	1	1	13	24
Calf (recurrent)	0	2	3	0	3	4	12
Calf + pulmonary embolism	0	3	3	0	1	2	9
Calf + visceral thrombosis	0	1	1	0	0	1	3
Pulmonary embolism	0	0	0	0	0	1	1
Visceral thrombosis	1	0	0	0	0	0	1
Cerebral thrombosis	0	0	1	0	0	1	2
Total	(5	9	10	1	5) = 30	22	52

¹) Only one proband identified.

Thrombophilia Screening

a) *AT III and protein C.* Antithrombin III heparin cofactor activity and protein C activity were measured by functional amidolytic assays (Stachrom AT III & Stachrom Protein C, Diagnostica Stago, Asnières, France) according to the manufacturer's instruction. Briefly, for AT III assay, plasma or standard was diluted in heparinized buffer and incubated with a fixed excess of thrombin. The residual thrombin was measured by its action on the chromogenic substrate CDS 34.47 as the change of O. D. at 405 nm. For protein C assay, diluted plasma or standard was activated by Protac, an extract of Agkistrodon contortrix venom, and the activated protein C was measured by its action on the chromogenic substrate CBS 65.25 as the change of O. D. at 405 nm.

b) *Protein S.* Total and free Protein S were determined by an enzyme linked immunosorbant assay (ELISA) utilizing a polyclonal rabbit antihuman protein S antibody (Dakopatts Ad, Sweden) as the capturing antibody. Polyethylene glycol 8000 was used to precipitate the C4b bound fraction for the measuring of functionally important free PS (19).

c) *Lupus anticoagulant.* Lupus anticoagulant was detected by the combination of APTT, platelet neutralization procedure, kaolin clotting time and dilute Russell's viper venom time as described previously (20–23). Anticardiolipin IgG and IgM antibodies were quantitated by ELISA (QALA, Cheshire Diagnostics Ltd., Chester, England). As chronic hepatitis B infection is endemic in Hong Kong, liver enzymes and HbsAg serology were determined.

d) *Factor assays.* In patients with prolonged PT or combined PC and PS deficiency, multiple clotting factor assays by one-stage technique were performed to rule out acquired deficiency.

Results

Controls

The normal ranges (mean \pm 2 standard deviations) established from 48 healthy Chinese adults are as follow: AT III 0.8–1.2 U/ml, protein C 0.7–1.4 U/ml, total/free protein S 0.64–1.1/0.18–0.35 U/ml for female and 0.8–1.1/0.22–0.39 U/ml for male. Deficiency is defined as less than 2 standard deviations below the mean. Normal ACA levels (IgG/IgM) are <26 GPL/MPL U/ml, as determined using sera from 20 healthy Chinese subjects.

Patients

Of the 52 index patients studied, 30 were found to be abnormal. The age, site and frequency of thrombosis showed no correlation with the

type of deficiency present (see Table 1). Thrombosis occurred most frequently in deep veins of the lower limbs. Details of the abnormal findings are shown below:

Antithrombin III deficiency. Five patients (1 male and 4 females) were identified as AT III deficient levels ranging from 0.47 to 0.60 U/ml (mean 0.54 U/ml), giving an incidence of 9.6%. The mean age at thrombosis was 29. Four developed DVT involving lower limbs; in three, these occurred spontaneously whilst in the fourth, thrombosis followed pleurodesis. The fifth patient developed multiple spontaneous visceral vein thrombosis resulting in splenomegaly and portal hypertension. Three members from 3 families were studied. Two of them who were from different families were found to be below the normal range but both remained asymptomatic.

Past history revealed a pregnancy and a surgical episode in 2 of the probands, and a pregnancy in 1 of the affected family members, but no thrombosis occurred despite the lack of any anticoagulant prophylaxis.

Protein C deficiency. Nine patients (8 males and 1 female) with protein C deficiency ranging from 0.17 to 0.65 U/ml (mean 0.45 U/ml) were found, given an incidence of 17.3%. The mean age at thrombosis was 33. One proband was taking oral contraceptive at the time of thrombosis, otherwise no provoking event could be identified. Family studies were performed in 7 cases. Among the 54 members tested, 25 heterozygotes were found in 6 families (range 0.37–0.65 U/ml), amongst whom, there were 9 pregnancies and 5 surgical procedures without anticoagulant prophylaxis. Of these, only 1 (pregnancy) was complicated by DVT. Another affected family member developed coronary arterial thrombosis at the age of 29. No apparent risk factor other than protein C deficiency was present. The incidence of thrombosis among the heterozygous family members is 8%.

Protein S deficiency. Ten patients (5 males and 5 females) with protein S deficiency were found, giving an incidence of 19.2%. In this study, no sex differences were noted with the total/free protein S levels ranging from 0.39–0.91/0.01–0.15 (mean 0.59/0.05) U/ml. Four probands (1 male and 3 females) had total protein S levels within the lower normal range, but the functionally important "free protein S" fraction was diminished in all. The mean age at thrombosis was 38. Most of the events occurred spontaneously with no apparent provoking event, except in a patient who developed DVT in the early post-partum period.

Family studies were performed in 5 cases. Among the 22 members tested, 8 members from 2 families were affected (3 males and 5

Table 2 Clinical features in probands and heterozygous family members with hereditary thrombophilia

	ATIII deficiency		PC deficiency		PS deficiency		Combined PC & PS deficiency	
	Probands	Family members ¹	Probands	Family members ¹	Probands	Family members ¹	Probands	Family members ¹
	5	2	9	25	10	8	1	2
1) History of thrombosis	5	0	9	2	10	0	1	0
Provoking events								
- None (spontaneous)	3	-	8	1	8	-	1	-
- Pregnancy	1	-	-	1	2	-	-	-
- Oral contraceptive	-	-	1	-	-	-	-	-
- Surgery	1	-	-	-	-	-	-	-
2) Asymptomatic	-	2	-	23	-	8	-	2 ²
3) Uneventful pregnancies identified in the past	1	1	-	7	9	5	-	-
4) Uneventful surgeries identified in the past	1	-	-	4	-	1	-	-

¹) Only affected family members are included in this table.

²) 1 had protein S deficiency only (see text)

females) with mean total/free protein S level of 0.60/0.14 U/ml for male and 0.54/0.13 U/ml for female. All had low free protein S and all but one had low total protein S as well. All of them remained asymptomatic at the time of analysis. There were 5 pregnancies and 1 episode of surgery in the past among these family members, none of which are complicated by thrombosis despite the lack of anticoagulant prophylaxis. History also revealed 9 uneventful pregnancies (without anticoagulant prophylaxis) in 4 probands. Therefore among the 16 provoking events (pregnancy and surgery) identified in the probands and affected family members, only 1 (6.3%) was complicated by thrombosis.

Combined protein C and protein S deficiency. One patient (1.9%) with combined PC and PS deficiency was found. The PC and total/free PS levels were 0.60 U/ml and 0.57/0.02 U/ml respectively. Liver function tests and factor VII were within normal ranges. Family study revealed that this patient's son had combined Protein C and S deficiencies whilst her sister revealed Protein S deficiency only.

Lupus anticoagulant/anticardiolipin antibody. Out of the 52 with documented thrombosis, the only abnormality in five patients (1 male and 4 females) was the presence of LA and/or ACA (ACA alone 2, LA alone 1, LA & ACA 2). Four of them had recurrent episodes of first trimester abortion, intrauterine death, either spontaneously or complicating pregnancy. All ACA detected were IgG, range 86–420 (mean 248.5) GPL u/ml. None of them had symptoms or autoimmune markers suggestive of an underlying connective tissue disease.

Family studies. Although only 4 index cases gave a family history of thrombosis, in 16 patients in which family members can be studied, 11 revealed affected relatives (68.7%).

Overall incidence of hereditary thrombophilia. When the five patients with LA and/or LCA are excluded from the 52 cases with documented DVT, the incidence of hereditary thrombophilia is $25/47 \times 100\% = 53.2\%$.

Table 2 summarises the clinical features of the patients in comparison with heterozygous family members with inherited thrombophilia. Despite 35 provoking events (27 pregnancies, 1 oral contraceptive use, and 7 surgical operations), thrombosis was observed in only 6 occasions.

Discussion

Hereditary Thrombophilia

The incidence of hereditary thrombophilia (AT III, PC and PS deficiency: 9.6%, 17.3% and 19.2% respectively) is 3–10 times that of the Caucasian series (1, 2, 4, 12, 24) with an overall incidence of 48%. This is not due to overrepresentation by any single defect, but a generalized increase in the incidence of AT III, PC and PS deficiency as compared to those of the West. Our data also show that the above events were not predictive of detecting an underlying abnormality. Provoking events were rarely present in patients as 80% of the thrombosis occurred spontaneously (Table 2). Although a positive family history was present in only 16% (4/25) of our patients, further screening of family members revealed involvement in 11/16 cases i.e. 68.7%, a figure comparable to the 80% reported in Dutch and French series (24).

The prevalence of LA/ACA is also high in our series (9.6%) compared to those reported in the West (c 2–4%) (5, 6, 15). None of our patients were suffering from connective tissue disease. Since 3 out of 5 patients possessed either LA or ACA alone, it is important to perform both assays in patients with thrombosis. In a previous study we showed that Chinese patients with LA had only moderately prolonged APTT, and more sensitive tests should therefore routinely be used for its detection (25).

Patients with recurrent thrombosis had a slightly higher incidence of detectable abnormality (8/12 = 66.7%) than those suffering from a single episode of thrombosis (22/40 = 55%). However, when results of LA/ACA was excluded, the difference disappeared. Pregnancy, use of oral contraceptive and surgery are known to provoke venous thrombosis in patients with thrombophilia. Apart from LA/ACA, our data did not reveal a high incidence of thrombosis complicating pregnancy, oral contraceptive usage or surgery in either the probands or heterozygous family members. Only 6 out of 35 provoking events (17.1%) identified were complicated by thrombosis (Table 2). In the West, the risk of thrombosis in heterozygotes not receiving prophylaxis during a pregnancy are as high as 60% for AT III, 26% for protein C and 17% for protein S deficiencies (26, 27).

Our findings of a high incidence of hereditary thrombophilia (53.2%) among Chinese patients with venous thrombosis, together with the lack of predictive risk factors, indicates that screening for thrombophilia is to be highly recommended in all Chinese patients with documented venous thrombosis. As this is a retrospective analysis, it will be important to confirm the findings in a larger prospective study.

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