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Acquired Factor X Deficiency in a Patient with Amyloidosis

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In 1956, Telfer, Denson and Wright described a patient with a previously unknown clotting defect, and in 1957 Hougie, Barrow and Graham described a similar case. The deficiency of these two patients was later shown to be identical [Denson (1958)]. The clotting factor derives its original name from these two patients — Prower and Stuart, but is now referred to as factor X. Since then some new cases have been described [Roos, van Arkel, Verloop and Jordan (1959); Rabiner and Kretchmer (1961)], and some of the cases which previously had been described as factor VII deficiency were by reinvestigation found to have factor X deficiency. In the hitherto described cases the defect has been congenital, and it appears to be inherited as a recessive, autosomal characteristic [Graham, Barrow and Hougie (1957); Roos et al. (1959); Rabiner et al. (1961)].

The purpose of this paper is to report a patient who probably has an acquired, selective deficiency of factor X. The patient has a severe bleeding tendency, and was shown to have extensive amyloidosis in the spleen and liver.

Methods

Antihaemofilic factors A, B and C were determined according to Egeberg (1960).

Bleeding time was determined by a modification of the method described by Ivy, Shapiro and Melnick (1935).

Capillary fragility was examined by the application of suction cups 20 mm in diameter to the supraspinatus region with 200 mm Hg for 1 minute.

Cephalin time was performed by the method of Waaler (1959).

Circulating anticoagulants. Normal citrated human plasma was mixed with citrated plasma from the patient in the proportions shown in Fig. 2. Quick's tissue thromboplastin time, the cephalin time, the recalcification time and the P and P test were performed on the plasma mixtures, on the normal plasma, and on the patient's plasma.

Collection of blood. Blood was collected from the antecubital vein with sharp silicone-treated needles. The first 2—3 ml of blood were discarded, and the blood was then allowed to flow freely into the test tubes. The first 5 ml portion was collected in a glass tube and

incubated at 37° C for exactly 3 hours (the serum from this portion was used in the thrombo-plastin generation test). The two following portions were collected in lusteroid tubes. In one tube, containing 0.5 ml of a 2% potassium oxalate solution, 4.5 ml of blood was collected, and in another tube containing 1 ml of a 3% trisodium citrate dihydrate solution 9 ml of blood was collected. Both tubes were centrifuged at 1700 R.C.F. for 30 minutes at + 4° C. The plasma was collected with silicone-treated pipettes into silicone-treated glass tubes. The oxalated plasma was immediately adsorbed with barium sulphate (100 mg/ml). This adsorbed plasma was used in the thromboplastin generation test. All other clotting tests were performed on the citrated plasma. Both plasmas were kept in an ice bath until used.

Factor X was determined in a one-stage system, using Mr. Stuart's plasma as substrate. This plasma was kindly sent to us by Dr. Graham, Chapel Hill, N. C. In the experiment described in Table 2, the patient's own plasma was used as substrate. The clotting mixture contained 0.2 ml of the patient's plasma, 0.2 ml tissue thromboplastin, 0.2 ml of a 10% solution of the plasma to be tested, and 0.2 ml of $CaCl_2$ 0.025 M. A correlation graph was prepared using normal, human, citrated plasma in dilutions from 10 to 0.5%. The dilutions were made as described by Hjort, Rapaport and Owren (1955).

Fibrinogen was determined by the method of Blombäck (1958).

Fibrinolysis. The euglobin lysis time [v. Kaulla (1958)] was used.

Platelets were counted by the method of Björkman (1959).

Proaccelerin (factor V) was determined by the method of Aas (1952).

Proconvertin (factor VII). The method of Aas (1952).

Prothrombin (factor II).

a) *Factor X insensitive method.* The original method of Hjort, Rapaport and Owren (1955) was made insensitive to factor X by adding a prothrombin-free eluate from normal serum to the clotting mixture [Hasselback and Hjort (1960)].

b) *Factor X sensitive method.* The method of Hjort, Rapaport and Owren (1955) was used.

Prothrombin-proconvertin (PP) was determined according to Owren and Aas (1951).

Russell's viper venom time: 0.2 ml citrated plasma + 0.2 ml Russell's viper venom (Stypven; Burrough & Welcome 1/10 000) + 0.2 ml $CaCl_2$ 0.025 M. The test was performed at + 37° C.

Tissue thromboplastin was prepared from human brain according to Owren (1947).

Tissue thromboplastin time (Quick-time) was performed as described by Quick (1935). Human tissue thromboplastin was used.

Thromboplastin generation test. The serum and the adsorbed, oxalated plasma (see above) were diluted 1/20 with physiological saline, containing 1/5 its volume of isotonic veronal buffer (pH = 7.3). In a glass test tube 1 ml of the serum dilution + 1 ml of the diluted, adsorbed, oxalated plasma + 1 ml of lipid material from human brain, 0.06 mg/ml [Bell and Alton (1955)] were mixed and incubated at 37° C for 6 minutes. Then, 1 ml of the previously warmed $CaCl_2$-solution 0.025 M was blown into the tube. After 5, 7, 10, 15 and 20 minutes aliquots of 0.2 ml were removed from this mixture and blown into 0.2 ml of normal Dowex-50 treated plasma. The clotting times were then recorded.

Whole blood clotting time was determined in silicone-treated glass capillaries [Gelin, Korsan-Bengtsen, Ygge and Zederfeldt (1961)].

Case Report

The patient is a 50 years old woman, who previously has been in good health. She has not bled abnormally after deliveries, extraction of teeth, or injuries.

In April and June 1960, the patient was admitted to the hospital for removal of varicose veins on the legs. She bled profusely during the operations, and was therefore transferred to the medical department for further investigation of the hemostatic function.

In the following year, she had repeated and severe uterine bleedings which required many blood transfusions. Ecchymoses in the skin occurred by slight pressure. The bleeding tendency gradually increased, but she was nevertheless in a remarkably good condition. Prednisolone, 15—30 mg daily, reduced the bleeding tendency considerably.

The physical examination, which was performed at several occasions showed a healthy looking, normally built woman. The only pathologic observation was an enlarged liver and spleen. X-ray of the chest showed normal lungs and heart. Biligraphy showed a normal gall bladder. Electrocardiogram was normal at several occasions.

The urine and the renal function were normal. Examination of the blood revealed normal values of haemoglobin except in periods with bleeding. The white cell count, the differential count, and the platelet count were within normal limits. The bone marrow from sternum was normal.

Coomb's test was negative. No L. E. (lupus erythematosus) cells was found. The following tests of the liver function were repeatedly normal: bilirubin in serum, thymol flocculation test, alkaline phosphatases, bromsulfalein test and paper electrophoresis. Immune-electrophoresis was normal. E.S.R. was 7 and 9 mm/hour at the time of the varix operations, but has later been 30—50 mm/hour. Temperature, pulse rate and blood pressure were normal.

In the beginning of September 1961, when the patient had just recovered from uterine bleeding, she suddenly had an intraabdominal bleeding which required urgent surgery. The bleeding came from the splenic vein and splenectomy was therefore performed. Biopsy from the liver was also made. The histologic examination of the spleen and liver showed extensive amyloidosis. No primary cause could be demonstrated. — In the postoperative period, the intraabdominal bleeding continued, and after a week reoperation was necessary. In the following month the bleeding slowly ceased. The bilirubin in plasma increased to 11 mg% during the first week after the last operation and returned slowly to normal values. The alkaline phosphatases were also increased in the postoperative period.

The course was further complicated by infection in an abdominal haematoma. At present, however, she is in a relatively good condition.

Results

General analyses of the haemostatic mechanism

From Table 1 it is evident that the coagulation screening tests were markedly abnormal. Thus, there was a prolongation of the whole blood clotting time, the cephalin time, the Russell's viper venom time and the tissue thromboplastin time. Fig. 1 shows that the thromboplastin generation test also was abnormal. These resuls indicate a defect both in the extrinsic and intrinsic clotting system. By specific tests, all factors were found to be normal except factor X. Factor X deficiency can explain the abnormal results of the screening tests.

As will be seen, the capillary fragility was increased and the bleeding time was prolonged. In congenital deficiency of factor X the bleeding time is usually normal, but it is often prolonged in patients with amyloidosis [R u k a v i n a (1956)].

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Table 1: General analyses of the haemostatic mechanism in June 1961

|	Patient	Normal values
Bleeding time	> 30 minutes	< 11 minutes
Platelets	350 000/mm³	150—350 000
Capillary fragility	25 petechiae	< 5 petechiae
Whole blood clotting time	30 minutes	< 11 minutes
Cephalin time	200 seconds	< 90 seconds
Russell's viper venom time	50 seconds	< 30 seconds
Quick's tissue thromboplastin time	62 seconds	< 18 seconds
Antihemophilic factor A	96%	60—150%
Antihemophilic factor B	66%	70—140%
Antihemophilic factor C	79%	70—140%
Proconvertin (factor VII)	100%	67—133%
Proaccelerin (factor V)	100%	
Prothrombin (Stuart insensitive method)	110%	
Prothrombin (Stuart sensitive method)	10%	
Stuart-Prower factor (factor X)	$< 1\%$	
Fibrinogen	0.42%	0.22—0.49%
Euglobulin lysis time	> 3 hours	> 2 hours
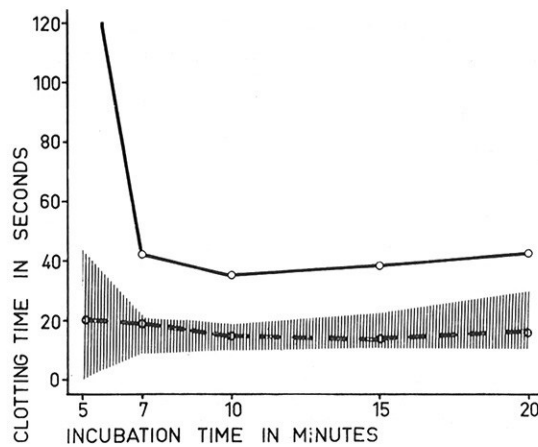
Fig. 1: *Thromboplastin generation test.* O————O Serum and adsorbed plasma from the patient. O — — — O Serum from a normal subject, adsorbed plasma from the patient. Shaded area shows the normal range with 95% confidence

Cross matching experiment

To confirm the diagnosis, a cross matching experiment involving mixtures of plasmas from the patient and from Mr. Stuart was performed. The result is presented in Table 2.

Table 2: Cross matching experiment with plasma from Mr. Stuart performed with Quick's tissue thromboplastin test

Citrated plasma from Mr. Stuart (freeze dried)	= 113 seconds
Citrated plasma from the patient	= 58 seconds
Equal parts of citrated plasma from Mr. Stuart and citrated plasma from the patient	= 63 seconds
Citrated plasma from Mr. Stuart and 1/10 its volume of normal citrated plasma	= 30 seconds
Citrated plasma from the patient and 1/10 its volume of normal citrated plasma	= 22 seconds

As will be seen, the patient's plasma was not corrected by Mr. Stuart's plasma.

Investigation of circulating anticoagulants

Fig. 2 illustrates the results of Quick's tissue thromboplastin test and the cephalin test on mixtures of normal plasma and the patient's plasma. The clotting times of the patient's plasma were corrected by addition of 10—20% of normal plasma. Identical curves were obtained after incubation of the plasma mixtures for 1 hour at 37° C. Similar experiments with recalcification of the plasma mixtures gave the same results. These experiments indicate that the patient's plasma did not contain circulating anticoagulants.
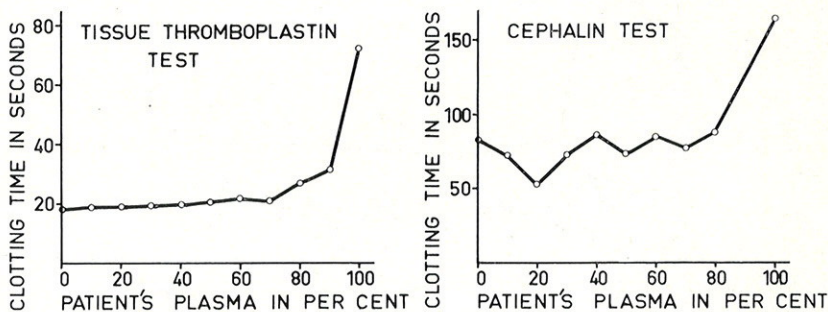

Fig. 2: Test for circulating anticoagulants. Citrated plasma from the patient was mixed with normal, citrated plasma in the proportions shown in the figure. On these plasma mixtures, Quick's tissue thromboplastin test and the cephalin test were performed

In vivo correction studies

One liter of normal, fresh, citrated plasma was given intravenously to the patient during 25 minutes. Blood samples were collected from the patient before

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and immediately after the infusion and then at intervals which are shown in Table 3.

Table 3: In vivo correction experiment

	P. P. in %	Quick time in seconds	"Specific" factor X in seconds
Before infusion	10	71	69
Immediately after infusion of 1 liter of plasma	13	58	67
20 minutes after infusion	10	67	70
40 minutes after infusion	10	69	69
75 minutes after infusion	9	70	71
The infused plasma	100	14	33 (= 100%)

The factor X activity and the PP activity did not increase significantly. The tissue thromboplastin time decreased with only a few seconds immediately after the infusion had been finished but returned to the initial values after about 20 minutes. This result indicates that the factor X activity rapidly disappeared from the patient's blood in vivo.

Analyses of clotting factors on members of the family

The patient's four children and her sister and brother were investigated. None of them had any defect of the haemostatic function.

Discussion

From our studies, we must conclude that the patient has an isolated, serious factor X deficiency. Assays of small amounts of factor X are not reliable but the patient's plasma appeared to contain less than 1% of factor X activity. Apparently, this is the first report of an acquired isolated deficiency.

The following observations indicate that the deficiency in our patient is acquired:

1. Increased bleeding tendency did not occur before the patient was 50 years old. In previously described cases of factor X deficiency of this severity, bleeding tendency has appeared in the childhood.

2. Factor X deficiency seems to be inherited as a highly penetrant, recessive, autosomal characteristic. As our patient has a severe defect, she should be a homozygote and her children heterozygotes. It is known that heterozygotes have demonstrable clotting defects [Graham et al. (1957); Roos et al. (1959)]. All the children of the patient have, however, normal clotting tests. Therefore, inheritance seems unlikely in this case.

3. The turnover of factor X has been studied in some previously reported cases. It has been shown that after infusion of normal plasma the activity has remained in the blood for several days [Crockett, Shotton, Craddock and Leavell (1949); Graham (1960); Duckert (1960)]. In the present case, infusion experiments showed that the factor X activity disappeared from the blood in a few minutes. Therefore, the deficiency may not be due to an insufficient synthesis of factor X, but rather to an increased inactivation or consumption in vivo.

As previously mentioned, our patient has extensive, apparently primary, amyloidosis. The possibility of a relationship between the clotting defect and the amyloidosis must, therefore, be taken into consideration.

Summary

A patient with extensive amyloidosis and a selective factor X deficiency is described. The following observations indicate that the factor X deficiency in this case is not inherited.

1. The first symptoms of a bleeding tendency appeared at an age of 50 years.
2. The patient's four children had no clotting defect.
3. After infusion of 1 liter of fresh plasma no increased factor X activity was observed. No anticoagulants could be demonstrated in vitro.

Résumé

Un patient souffrant d'une amyloidose généralisée et d'une déficience isolée du facteur X est décrit. Les observations suivantes indiquent que dans ce cas le défaut en facteur X n'est pas congénital.

1. Les premiers symptomes d'une tendence hémorragique ont apparu à l'âge de 50 ans.
2. Les quatre enfants du malade n'ont pas de défauts de la coagulation.
3. Après l'infusion d'un litre de plasma frais aucune augmentation de l'activité du facteur X n'est observée. La présence d'un inhibiteur ne peut pas être mise en évidence in vitro.

Zusammenfassung

Eine Patientin mit ausgedehnter Amyloidose und isoliertem Faktor-X-Mangel wird beschrieben. Die folgenden Beobachtungen zeigen, daß der Faktor-X-Mangel in diesem Fall nicht vererbt ist:

1. Die ersten Symptome der Blutungsneigung traten erst mit 50 Jahren auf.

2. Die 4 Kinder der Patientin hatten keine Gerinnungsstörung.

3. Nach Infusion von 1 Liter frischem Plasma konnte keine erhöhte Faktor-X-Aktivität gefunden werden. Es konnten keine Gerinnungshemmstoffe in vitro nachgewiesen werden.

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