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From the Institute for Thrombosis Research, University Hospital, Rikshospitalet,
(Head: P. A. Owren, M. D.), Oslo, Norway

The Effect of Plasma and Cohn's Fraction I on the Duke and Ivy Bleeding Times in von Willebrand's Disease

By

Christian F. Borchgrevink, Olav Egeberg, Hans Chr. Godal and Peter F. Hjort

Von Willebrand's disease is defined in this paper as an inherited autosomal dominant hemorrhagic diathesis characterized by a prolonged bleeding time and a deficiency of factor VIII (38). The terminology and the relationship to the capillary disorders and to the qualitative platelet deficiencies are not considered here.

In 1956 Nilsson et al. (36) observed that an intravenous infusion of a purified Cohn's plasma fraction I not only increased the concentration of factor VIII, but also *normalized the bleeding time.* This observation was extended in subsequent studies (33, 34, 35, 37, 38), leading to the conclusion that the prolonged bleeding time in von Willebrand's disease is caused by the lack of a normal plasma factor.

We were unable to confirm these results with the techniques used in our laboratory. One explanation could be a difference in the bleeding time technique: the Swedish workers used the Duke (18), while we were using the Ivy (26) technique. Therefore, we repeated our studies using both techniques. We found that infusions of plasma or plasma fraction I did shorten the Duke bleeding time, but had little or no effect on the Ivy bleeding time. These results are presented and discussed in this paper.

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Case reports

Case 1. A 13-year-old boy. The maternal grandfather was a bleeder; the mother and five of her siblings had a bleeding tendency similar to that of the patient. From the age of four, he had bruised easily and bled unusually long from minor wounds. During the last two years, he had frequent nose bleedings and was once hospitalized for this reason. He had never had petechiae.

The physical examination was normal at the time of this study.

He received 500 ml of plasma fraction I, prepared from the plasma of six donors and containing 38 % factor VIII. Two days later, 350 ml of blood was withdrawn. He then received 785 ml plasma prepared from the blood of three donors and containing 14,000 platelets/mm³. The plasma was infused over 80 min. with no reaction.

Case 2. An 18-year-old female. A maternal great-grandfather was probably a bleeder, and the patient's sister bled to death as an infant. From the age of 15 months, the patient had bled abnormally: easy bruising, prolonged bleeding from small wounds and during dentition, joint bleeding on two occasions (knee and ankle), and profuse menstrual bleeding since menarche at the age of 15 years, but she had never had petechiae. She had been hospitalized 25 times and had received many transfusions. She had constantly been taking large doses of iron, because of iron-deficiency anemia.

The physical examination gave normal results at the time of this study.

She received 500 ml of plasma fraction I, prepared from the blood of six donors and containing 61 % factor VIII. Two days later, 705 ml plasma was infused over 90 min. with no reaction. The plasma was prepared from the blood of three donors and contained 11,000 platelets/mm^3.

Case 3. A 41-year-old housewife. There was no bleeding tendency in her family. She had a severe bleeding tendency from the age of two years, with easy bruising, profuse nose bleeding and heavy menstrual bleeding, but no joint bleeding. During shorter periods, she had petechiae. She had been hospitalized as an emergency case many times and had received many transfusions. For a year, she was treated with testosterone with some success, but was finally given X-ray treatment to stop menstrual bleeding.

She had a moderate iron-deficiency anemia and a slightly enlarged spleen; otherwise the physical examination gave normal results at the time of this study.

A plasma transfusion of 760 ml was given over 120 min. She had a slight chill after 45 min. The plasma was prepared from the blood of three donors and contained 9,000 platelets/mm^3. Three days later, she was given 500 ml of plasma fraction I, prepared from four donors, containing 87 % factor VIII.

Case 4. A 44-year-old housewife. Her father, his brother, a paternal first cousin, three siblings, a nephew and her daughter had a bleeding tendency similar to that of the patient. She had bled abnormally since infancy: easy bruising, increased bleeding from small wounds and at dentition (the sockets had to be sutured), excessive menstrual bleeding and profuse bleeding after childbirth. She was finally castrated by X-ray treatment.

The physical examination was normal at the time of this study.

She received 1,135 ml plasma over 120 min. with no reaction. The plasma was prepared from five donors and contained 12,000 platelets/mm^3. One day later, she was given 500 ml plasma fraction I, prepared from four donors, containing 82 % factor VIII.

Case 5. A 19-year-old nurse. Two of her mother's siblings had an abnormal bleeding tendency, and her brother bled to death at the age of three years. She had bled abnormally since infancy: easy bruising, prolonged bleeding from small wounds and after tooth extraction. Since menarche at 16 years, she had heavy menstruations and had been hospitalized for transfusions six times. She had never had bleeding into the joints or petechiae.

Physical examination was normal at the time of this study.

A transfusion of 1,036 ml plasma, prepared from four donors and containing 31,000 platelets/mm^3, was given over 55 min. with no reaction. Six days later, she received 450 ml plasma fraction I, prepared from six donors and containing 48 % factor VIII.

Material and methods

Bleeding time (Ivy). The technique of Ivy et al. (26) was used, with one modification: instead of making punctures with a mechanical stylet, we made cuts with surgical blades (Gilette surgical blades, shape E), each blade being used for one examination only. Three cuts were made on the volar surface of the forearm, 5—6 mm long, 1 mm deep and 2 cm apart. The blood was carefully absorbed with the edge of a filter paper every 30 sec.; the wound itself was not touched.

Bleeding time (Duke). The method of Duke (18) was used: one small cut was made with the same sharp blades in the lobe of the ear, and the blood was removed as described above.

Table I. "Hemostatic tests" in the patients before transfusions

Test	Patients no.					Normal	References
	1	2	3	4	5		
Bleeding time, Ivy (min)	> 30	> 30	> 30	> 30	> 30	3—11	(11)
Bleeding time, Duke (min)	> 30	> 30	> 30	> 30	—	1—5	(18)
Tourniquet test (petechiae)	30	35—40	30	40	30—40	<5—10	(45), 90 mm Hg for 5 min
Platelets (1,000/mm³)	458	223	202	184	238	138—421	(39), as modified by (21)
Adhesive platelets in vitro (%)	37	24	38	27	35	26—68	(21)
Adhesive platelets in vivo (%)	—2	—1	5	—1	—1	24—58	(10)
Clot retraction after 3 hrs (cm)	8.0	6.0	7.0	6.8	8.3	5.8—9.3	(48)
Platelet factor 3	Normal	Normal	Normal	Normal	Normal	—	(24)
Whole blood clotting time (min)	3.5	3.5	3	3.5	4	2—5	(23)
Fibrinogen (mg%)	290	302	330	315	276	150—400	(27), as modified by (19)
Cephalin time (sec)	86.5	134	121	68	125	57—64	(19)
Quick time (sec)	15.3	14.8	14.8	15.1	14.7	13—16	Human brain thrombo-plastin
P & P test (%)	102	88	105	93	83	80—120	(41)
Factor II & X (%)	90	60	92	118	96	80—120	(22)
Factor V (%)	105	130	74	66	60	80—120	(40)
Factor VII (%)	100	110	103	100	70	80—120	(41)
Factor VIII (%)	7	2	7	45	7	60—150	(19)
Factor IX (%)	102	118	70	79	80	70—140	(19)
Factor XI (=P.T.A.) (%)	89	134	87	88	78	70—140	(19)
Fibrinolysis	No	No	No	No	No	—	(4)
Hematocrit (vol. %)	45	40	37	44	41	38—54	(49)

Collection of blood for testing. Venepuncture was performed with a siliconized needle, and the first 2—3 ml of blood were discarded. Nine volumes of blood were then collected directly into a plastic tube containing one volume of 3.13 % w/v sodium citrate dihydrate. This was mixed by gentle inversion and centrifuged at 2,500 r. p. m. (ca. 1,800 G) for 30 min. at 4° C. The plasma was removed with a siliconized pipette and stored in plastic tubes at — 20° C. until assayed.

Collection of blood from donors. Fasting, compatible blood donors were bled by means of the Fenwal equipment (Fenwal Laboratories Inc., Framingham, Mass.): 500 ml of blood was collected through a siliconized needle and plastic tubing into a plastic bag containing 75 ml acid-citrate-dextrose solution, U. S. P. formula A. The blood was discarded if there were any difficulties during collection.

Preparation of plasma for infusion. Immediately after collection, the blood bags were centrifuged at 2,500 r. p. m. (ca. 1,400 G) for 30 min. at 4° C. The clear, platelet-poor plasma was then squeezed into a plastic bag

Table II. The effect of plasma and plasma fraction I on the Duke bleeding time. The bleeding time was measured before the infusion, and 15 min., 4 hours and 20—24 hours after completion of the infusion of plasma or plasma fraction I

Patient no.	Duke bleeding time (min)							
	Plasma				Plasma fraction I			
	Before	15 min	4 hrs	20–24 hrs	Before	15 min	4 hrs	20–24 hrs
1	> 30	8	11	28	> 30	5	5.5	> 30
2	> 30	23.5	14	> 30	> 30	25	18	20
3	> 30	26	30	> 30	> 30	6.5	14	> 30
4	> 30	27	12	16	17	8	14	17

Table III. The effect of plasma and plasma fraction I on the Ivy bleeding time. The bleeding time was measured before the infusion, and 15 min., 4 hours and 20—24 hours after completion of the infusion of plasma or plasma fraction I

Patient no.	Ivy bleeding time (min)							
	Plasma				Plasma fraction I			
	Before	15 min	4 hrs	20–24 hrs	Before	15 min	4 hrs	20–24 hrs
1	> 30	30	> 30	> 30	> 30	17.5	24.5	> 30
2	> 30	> 30	> 30	> 30	> 30	> 30	> 30	> 30
3	> 30	> 30	> 30	> 30	> 30	17	> 30	> 30
4	> 30	26, 27, 30	> 20	> 30	> 30	13,20,>30	18,30,>30	> 30
5	> 30	21,27,>30	> 30	> 30	> 30	> 30	> 30	> 30

and infused into the patient. With this technique, the blood was rapidly chilled and exposed only to non-wettable surfaces. The infusion started within one hour after collection of the blood.

Preparation of Cohn's plasma fraction I. Plasma, prepared as for infusion, was immediately cooled and precipitated with 8 % ethanol, according to method VI of Cohn et al. (14), using siliconized equipment. After centrifugation, the precipitate was dissolved in 500 ml of a solution containing 0.9 % NaCl and 0.6 % sodium citrate, and immediately infused into the patient. The infusion always started within 4 hours of collection of the blood. No side effects were observed.

"*Hemostatic tests*" were carried out by the techniques referred to in table I.

Results

The hematocrit values decreased in proportion to the volumes infused. The other results are presented in tables II—V. Since the number of patients is small, we have not calculated mean values.

Duke bleeding time. In all experiments there was a clear-cut decrease in the Duke bleeding time, but in only one patient did we obtain a bleeding time barely within the normal range (table II). The effect was marked during the first hours after infusion, and could hardly be demonstrated after 20—24 hours. Fresh plasma and plasma fraction I had similar effects, but the response to

Table IV. The effect of plasma and plasma fraction I on the concentration of factor VIII. Factor VIII was assayed before the infusion, and 15 min., 4 hours and 20—24 hours after completion of the infusion of plasma or plasma fraction I

Patient no.	Concentration of factor VIII (%)							
	Plasma				Plasma fraction I			
	Before	15 min	4 hrs	20–24 hrs	Before	15 min	4 hrs	20–24 hrs
1	19	56	53	32	7	30	53	44
2	5	55	32	41	2	20	17	14
3	7	54	62	50	50	71	73	59
4	45	86	77	64	50	61	86	63
5	7	21	34	23	12	19	38	16

Table V. The effect of plasma and plasma fraction I on the platelet count. The platelets were counted before the infusion, and 15 min., 4 hours and 20—24 hours after completion of the infusion of plasma or plasma fraction I

Patient no.	Platelet counts (1,000/mm^3)							
	Plasma				Plasma fraction I			
	Before	15 min	4 hrs	20–24 hrs	Before	15 min	4 hrs	20–24 hrs
1	439	418	429	463	458	456	444	461
2	204	198	231	215	223	211	234	217
3	202	175	210	190	190	204	226	217
4	184	134	148	137	130	128	123	118
5	238	186	197	204	243	237	229	222

fraction I was somewhat greater. There was no correlation between the shortening of the bleeding time and the amount of plasma infused or the concentration of factor VIII in the fraction I administered.

Ivy bleeding time. The results are collected in table III. The pattern of response was similar. However, the effect was very small; indeed, in three patients out of five, 705—1,135 ml of fresh plasma had no effect at all. In no case did the bleeding time become normal.

Factor VIII. The results are given in table IV. In all patients the concentration of factor VIII increased markedly, and in some patients the increase was greater than expected on the basis of the amount infused. The activity disappeared more slowly than in transfused hemophiliacs. There was no parallel between the bleeding times and the factor VIII concentrations achieved, and the effect on the bleeding time disappeared more quickly than the effect on factor VIII.

Platelets. The platelet count decreased slightly, probably due to hemodilution (table V). The decrease was too small to influence the bleeding time.

Discussion

1. *Therapeutic effect*

Many authors have reported a therapeutic effect of blood, plasma and plasma fractions on bleeding in patients with von Willebrand's disease (1, 5, 15, 17, 31, 33, 34, 37). Such observations are impressive at the bedside, but difficult to evaluate scientifically. There are also reports of little or no effect of transfusions (7, 12), and Biggs and Macfarlane (8) and Valberg and Brown (47) maintained that these patients often do not bleed profusely at operations; they bleed primarily from skin and mucous surfaces. It is possible that therapeutic failures could be explained on the basis of insufficient amounts transfused; nevertheless, the clinical evidence appears to be suggestive only.

To illustrate the difficulties involved in the clinical observation of bleeding, reference can be made to the discussion on the effect of non-viable platelets in thrombocytopenia. Clinical experience strongly suggested a hemostatic effect of such platelets (29), but no effect was found in quantitative animal experiments (20).

2. *The effect on the bleeding time*

Nilsson et al. (33, 34, 35, 36, 37, 38) have shown that normal plasma and plasma fraction I—O normalize the prolonged Duke bleeding time in patients with von Willebrand's disease. A clear-cut dose-response relationship has not been reported. An ordinary blood transfusion had no effect; "half a dose" of fraction I—O (prepared from 500—700 ml fresh plasma) did not always correct the bleeding time, but "one dose" of fraction I—O or 400—800 ml fresh plasma normalized the bleeding time. The effect lasted for 24—48 hours. It was not related to platelets, fibrinogen or factor VIII. Fraction I—O prepared from hemophilic plasma was effective (two experiments), but the same fraction prepared from the plasma of patients with von Willebrand's disease had no effect (one experiment). Based on this evidence, they postulated that normal plasma contains a "bleeding time factor" which is lacking in von Willebrand's disease.

This effect of normal plasma on the bleeding time has been observed by many others (1, 2, 6, 15, 16, 30, 31, 42, 46, 47). There are also reports of no effect, but, except for McMillan's (32) study, they are less extensive than the positive reports (3, 7, 25, 28, 43, 44). Nearly all of these authors used the Duke method; two used the Ivy method (7, 25), and one used single stabs on the forearm (32). A few authors did not specify their method (3, 28, 42).

It could be argued that the effect might be unspecific, and this argument appears to be supported by the observation that fraction I stops bleeding and normalizes the bleeding time in thrombocytopenia (13, 50). However, Nilsson et al. (35) found no effect of fraction I—O in three patients with thrombocytopenia and in one patient with macroglobulinemia of Waldenström. Further, they found no effect of fraction I—O prepared from patient with von Willebrand's disease.

We found a definite effect on the Duke bleeding time of both fresh plasma and fraction I, but the effect was much smaller than that reported by the Swedish workers. Both plasma and fraction I were prepared as carefully and rapidly as possible. The effect on the Ivy bleeding time was considerably smaller: the infusion of about one liter of normal plasma pro-

duced either no or only a questionable effect.

These results raise three questions:

a) What is the reason for the difference between the results obtained with the Ivy and Duke techniques? The Duke wound is smaller and deeper than the Ivy wound, but this can hardly explain the difference. Nor can the difference be due to the pressure used in the Ivy method, since parallel tests with no pressure gave similar results. Thus, we do not know the reason for the difference, but our observations may explain some of the discrepancies in the literature.

b) Which method more truely reflects the hemostatic efficiency in a patient? This question cannot be answered at present, but the fact that patients have been safely operated upon after infusions of fraction I—O may suggest that the Duke bleeding time is a better practical guide than the Ivy bleeding time. We have also observed a family with prolonged Ivy bleeding time but no bleeding tendency (unpubl. observation). A comparison of the two techniques in patients with different bleeding disorders may throw further light on this problem.

c) Are our result compatible with the postulated "bleeding time factor"? The results with the Duke bleeding time do support the existence of such a factor. The negative results with the Ivy bleeding time could be due to qualitative or quantitative differences in the effect of the "bleeding time factor" on the two methods. It appears unlikely that the hemostasis of the two types of wounds should be qualitatively different. It is also difficult to accept the explanation that too little plasma was given: about one liter of fresh normal plasma ought to have had some effect. At present, therefore, both of these explanations appear unsatisfactory. Although the positive results with the Duke bleeding time carry more weight than the negative with the Ivy method, this discrepancy must be elucidated before the "bleeding time factor" can be finally accepted.

3. *The effect on factor VIII*

Nilsson et al. (33, 35) and Cornu et al. (15) have observed that factor VIII increased more than expected and disappeared more slowly than in transfused hemophiliacs. Our results support these observations.

Summary

Five patients with von Willebrand's disease (prolonged bleeding time, reduced concentration of factor VIII and bleeding manifestations) were transfused with 705—1,135 ml of fresh citrated plasma collected without exposure to wettable surfaces, and also with 500 ml of Cohn's fraction I.

The Duke bleeding time became shorter in all experiments, but in only one did it became normal. The Ivy bleeding time showed little or no response. The significance of these observations is discussed.

Factor VIII increased markedly, and decreased more slowly than in transfused hemophiliacs.

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