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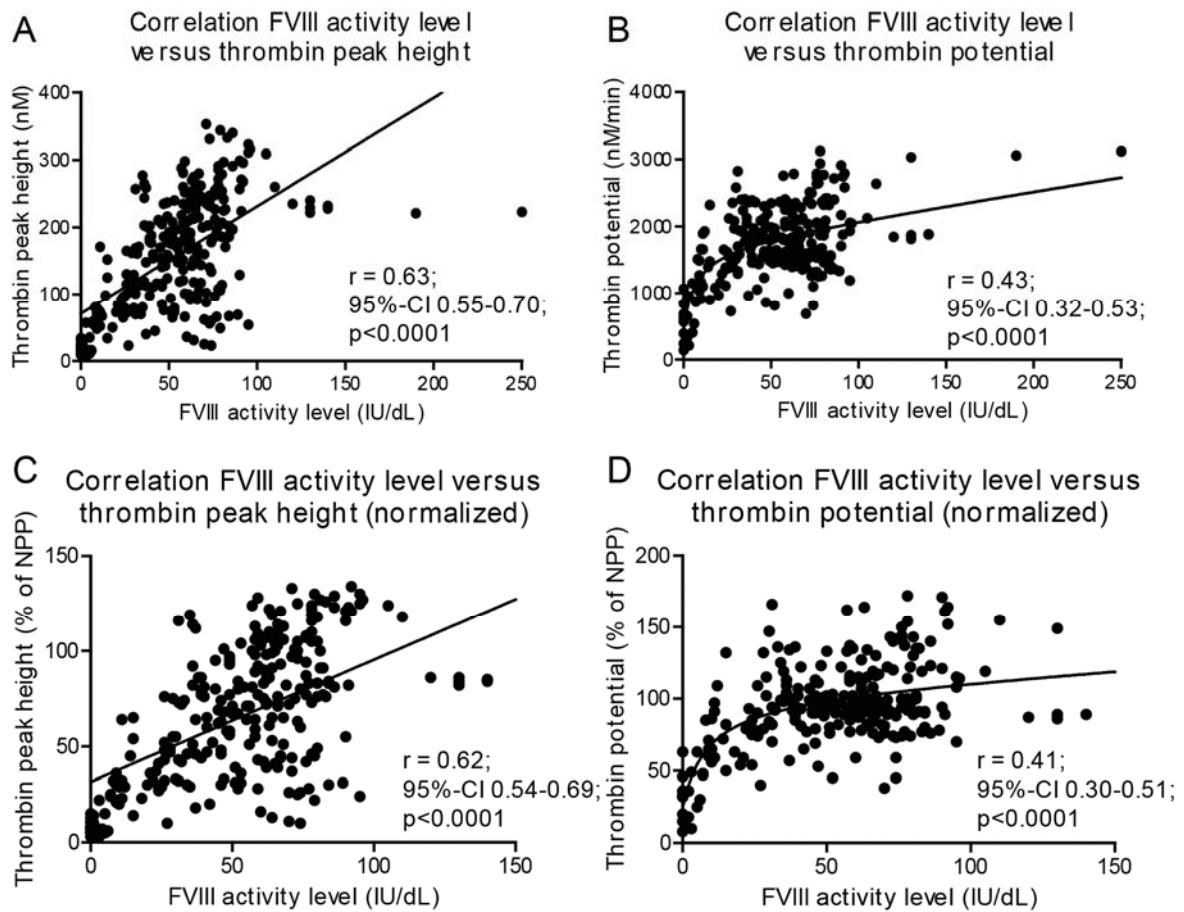
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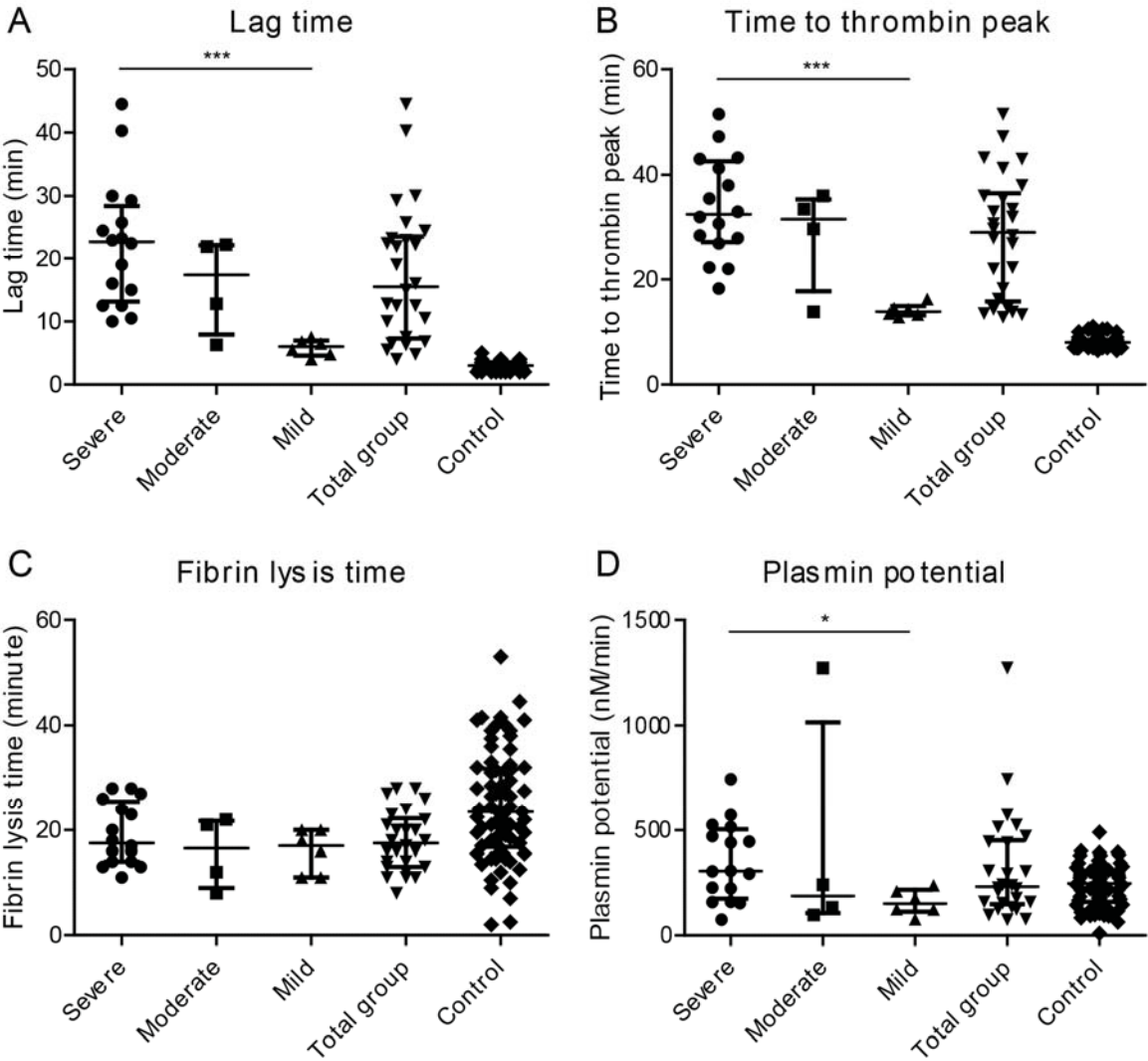
SUPPLEMENTARY FIGURES

Supplementary figure 1



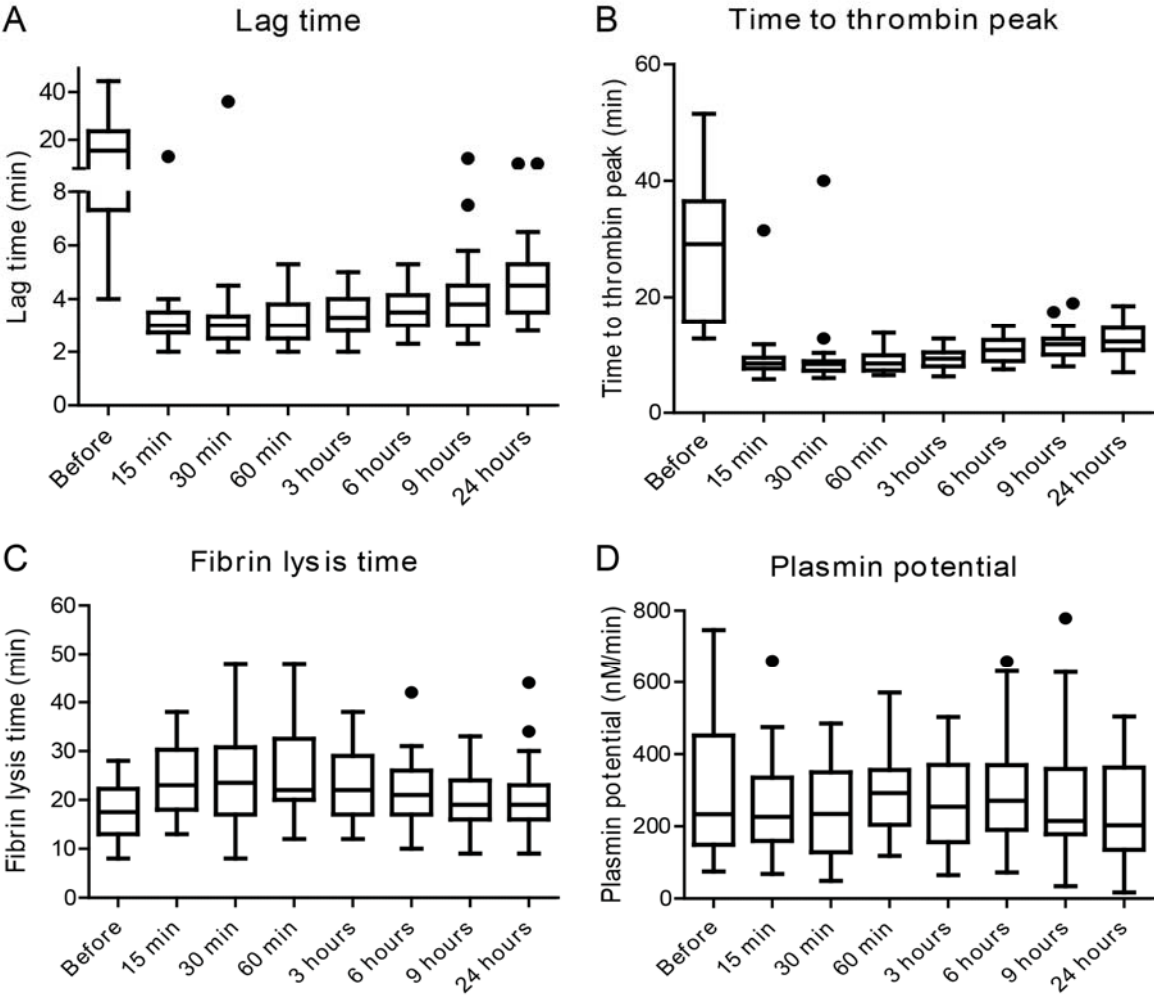
- A. Correlation between FVIII activity level (one stage) and thrombin peak height (absolute value),  $r=0.63$ ; 95%-CI 0.55-0.70;  $p<0.0001$ .
- B. Correlation between FVIII activity level (one stage) and thrombin potential (absolute value),  $r=0.43$ ; 95%-CI 0.32-0.53;  $p<0.0001$ .
- C. Correlation between FVIII activity level (one stage) and thrombin peak height (as percentage of normal pooled plasma),  $r=0.62$ ; 95%-CI 0.54-0.69;  $p<0.0001$ .
- D. Correlation between FVIII activity level (one stage) and thrombin potential (as percentage of normal pooled plasma),  $r=0.41$ ; 95%-CI 0.30-0.51;  $p<0.0001$ .

Supplementary figure 2



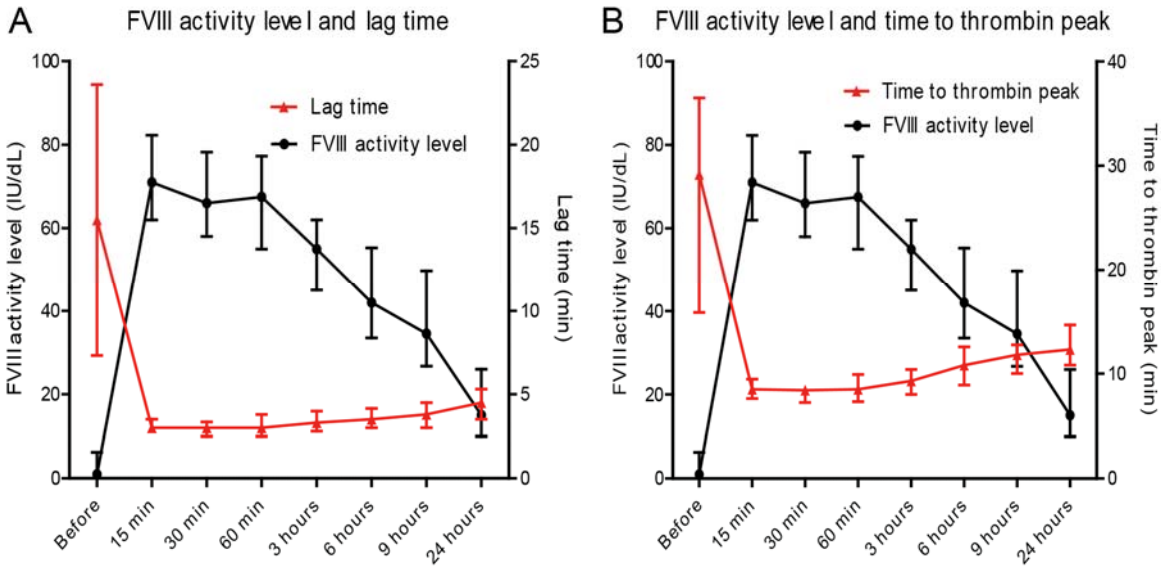
Baseline (pre-bolus) NHA parameters. Parameters given are lag time (A), time to thrombin peak (B), fibrin lysis time (C) and plasmin potential (D). Group data consist of all patients and the control represents all normal pooled plasma samples that ran as control with the NHA (n=73). Lines represent median with interquartile range in all graphs. \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

Supplementary figure 3



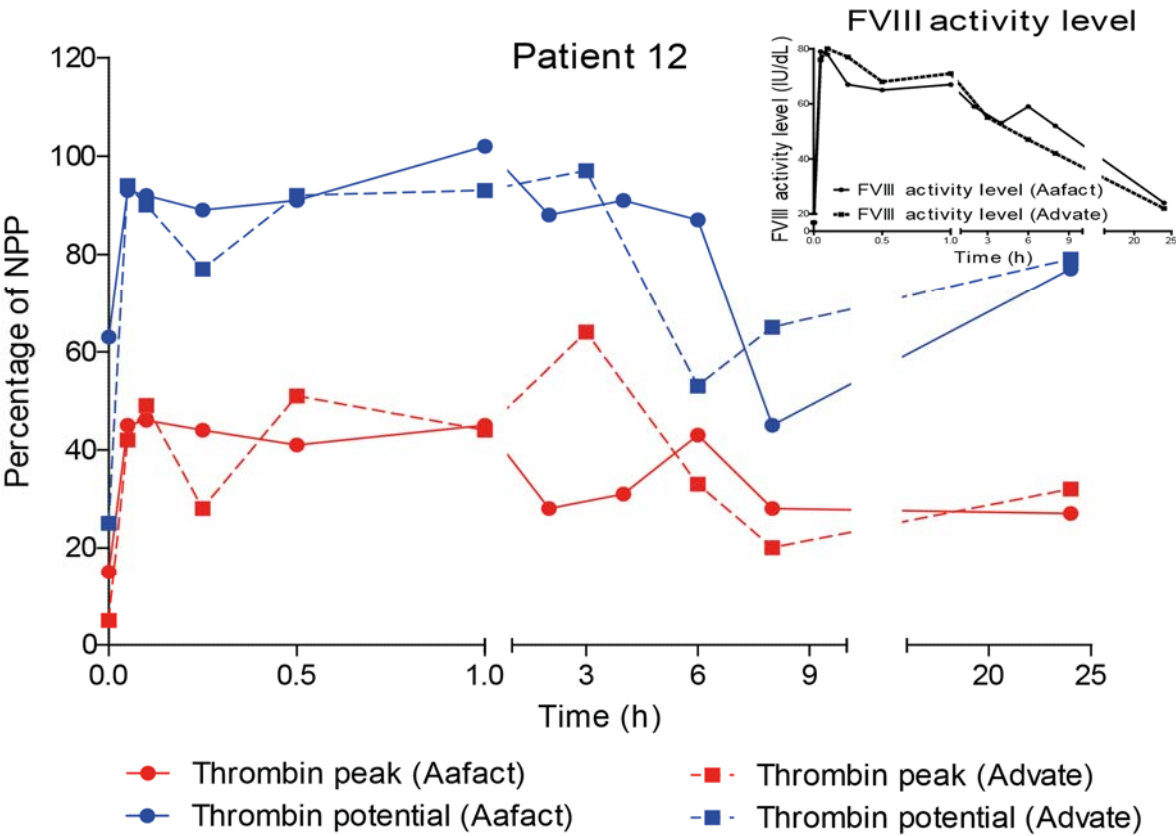
Characteristics of the NHA after a bolus of factor VIII replacement therapy. Lag time (A) and time to thrombin peak height (B). Fibrin lysis time (C) and plasmin potential (D). All values are given before and at set time points after the bolus of replacement therapy and are shown as Tukey box-and-whiskers in which box represents median with interquartile range, whiskers indicate minimum and maximum, dots represent outliers.

Supplementary figure 4



Reached FVIII activity level (in IU/dL on left y-axis; black) before and post infusion of FVIII replacement and lag time (4A; in min) and time to thrombin peak (4B, in min), both in red on right axis. Dots represent median and error indicates interquartile range.

Supplementary figure 5



The pharmacokinetic and pharmacodynamic study was performed twice in one patient. The FVIII activity level (in black, insert) before and at different time points post infusion of a bolus of FVIII replacement therapy (23 IU/kg Aafact (round symbol and continuous line) and 26 IU/kg Advate (square symbol and dotted line), and thrombin peak height (red) and thrombin potential (blue), both as percentage of NPP.

FVIII: factor VIII; NPP: normal pooled plasma.

SUPPLEMENTARY TABLES

Table 1: Clinical characteristics of individual patients.

Patient	Age (years)	Weight (kg)	FVIII one-stage pre bolus (IU/dL)	FVIII chromogenic pre bolus (IU/dL)	Factor VIII product	Total dosage of replacement therapy (IU)	Bolus of replacement therapy (IU/kg)	NBA concentration (NBU/mL)	NLTIA concentration (NLTIU/mL)
<b>Severe hemophilia</b>									
1	51	87	< 1	-	Advate	2160	25	0	-
3	33	66	2	2	Advate	2160	33	0	-
7	43	92	< 1	1	Advate	2500	27	0	0
8	2	11	11*	15*	Kogenate	1000	91	0,3	0,15
10	38	108	< 1	< 1	Advate	3000	28	0	0
11	29	68	< 1	< 1	Aafact	1800	26	0	0
13	6	19	< 1	4	Advate	750	39	0	0,1
14	7	24	< 1	< 1	Helixate	1000	42	0	0
15	35	85	1	< 1	Advate	3000	35	0	0,04
16	61	78	4	5	Aafact	2000	26	-	-
17	41	85	< 1	1	Aafact	3000	35	0	0
22	62	55	< 1	2	Advate	1500	27	0	0
24	60	89	< 1	< 1	Aafact	3000	34	0	0
27	60	71	< 1	2	Helixate	3500	49	0	0

<b>29</b>	20	134	1	3	Haemate P	4000	30	0	0
<b>33</b>	55	91	< 1	4	Advate	2500	27	0	0
<b>Moderate hemophilia</b>									
<b>12-1</b>	50	72	5	-	Aafact	1700	23	-	-
<b>12-2</b>	56	77	5	1	Advate	2000	26	0	0
<b>19</b>	70	85	6	8	Helixate	2500	29	0	0
<b>20</b>	65	60	6	9	Helixate	3000	50	0	0
<b>Mild hemophilia</b>									
<b>9</b>	77	70	11	12	Refacto	2000	29	0	0
<b>18</b>	42	105	21	10	Helixate	2500	24	0	0
<b>21</b>	68	88	7	6	Kogenate	3000	34	0	0
<b>30</b>	50	94	9	6	Advate	2500	27	0,3	0,07
<b>31</b>	52	73	7	5	Kogenate	2000	27	0	0
<b>32</b>	51	90	9	9	Advate	2500	28	0	0

\* Residual FVIII due to administration of FVIII concentrate every 24 hours because of bleeding symptoms otherwise.

- Not applicable.



Supplementary table 2: Pre- and post-infusion values of FVIII activity level and Nijmegen Hemostasis Assay parameters as absolute values for all parameters and as percentage of normal pooled plasma (NPP) for thrombin peak height and thrombin potential. The median and interquartile range (IQR) are given for each timepoint.

	Time	Reference*	Before	15 min	30 min	60 min	3 hours	6 hours	9 hours	24 hours
<b>FVIII activity level (IU/dL)</b>	Median	60-150#	1	71	66	68	55	42	35	15
	IQR		<1-6	62-82	58-78	55-77	45-62	34-55	27-50	10-26
<b>Lag time (min)</b>	Median	3.0	16.0	3.0	3.0	3.0	3.3	3.5	3.8	4.5
	IQR	2.5-3.5	7.3-23.6	2.8-3.5	2.5-3.4	2.5-3.8	2.8-4.0	3.0-4.2	3.0-4.5	3.5-5.3
<b>Time to thrombin peak (min)</b>	Median	8.0	29.2	8.5	8.4	8.5	9.3	10.8	11.8	12.3
	IQR	7.5-8.5	15.9-36.5	7.6-9.5	7.2-8.9	7.3-9.9	8.0-10.4	8.9-12.6	10.0-12.8	10.8-14.7
<b>Thrombin peak height (nM)</b>	Median	239	23	219	208	196	174	116	112	73
	IQR	25.8	15-39	149-253	166-241	142-236	151-215	101-191	79-168	56-120
<b>Thrombin potential (nM/min)</b>	Median	1846	471	1823	1808	1846	1839	1752	1640	1392
	IQR	160	undetectable-924	1538-2168	1542-2126	1527-2148	1549-2113	1495-2146	1418-2100	1054-1660
<b>Plasmin peak (nM)</b>	Median	27.7	38	29	28	31	32	34	30	26

	IQR	8.8	24-53	23-42	17-38	25-41	22-41	22-44	23-42	18-41
<b>Fibrin lysis time (min)</b>	Median	23.5	18	23	24	22	22	21	19	19
	IQR	16.8-31.8	13-22	18-30	17-31	20-33	17-29	17-26	16-24	16-23
<b>Plasmin potential (nM/min)</b>	Median	235	234	227	234	292	254	271	215	203
	IQR	101	149-452	160-335	129-350	204-356	156-371	191-370	179-359	135-363
<b>Thrombin peak height (% of NPP)</b>	Median	§	10	87	85	80	73	51	46	32
	IQR		6-17	57-108	70-105	64-96	63-87	43-78	31-75	23-50
<b>Thrombin potential (% of NPP)</b>	Median	§	28	97	94	99	96	92	90	74
	IQR		undetectable-51	86-121	89-119	90-118	89-113	82-113	79-114	59-87

\* Reference results of the Nijmegen Hemostasis Assay based on normal pooled plasma used as positive control during every run with patient samples (in total 73 runs of one batch of NNP were analyzed).

# Reference values of FVIII activity level of the one-stage assay.

§ As this is the percentage of normal pooled plasma (NPP), the reference value is always 100%.

Supplementary table 3: Individual baseline values of FVIII activity level and Nijmegen Hemostasis Assay parameters.

<b>Number</b>	<b>Baseline FVIII activity level (IU/dL; one-stage)</b>	<b>Baseline FVIII activity level (IU/dL; chromogenic)</b>	<b>Lag Time (minutes)</b>	<b>Time To Thrombin Peak (minutes)</b>	<b>Thrombin Peak Height (nM)</b>	<b>Thrombin Potential (nM/min)</b>	<b>Fibrin Lysis Time (min)</b>	<b>Plasmin Peak Height (nM)</b>	<b>Plasmin Potential (nM/min)</b>
<b>Severe hemophilia</b>									
<b>1</b>	< 1	-	30,0	41,3	24	256	23	28	573
<b>3</b>	2	2	25,8	35,5	11	332	26	28	440
<b>7</b>	< 1	1	23,3	38,0	11	237	28	46	746
<b>8</b>	11	15	40,3	51,5	9	undetectable	28	8	75
<b>10</b>	< 1	< 1	44,5	47,3	18	143	14	49	294
<b>11</b>	< 1	< 1	10,0	28,5	13	undetectable	20	55	445
<b>13</b>	< 1	4	23,0	43,3	12	undetectable	24	54	472
<b>14</b>	< 1	< 1	22,5	43,0	25	undetectable	16	42	228
<b>15</b>	1	< 1	19,0	28,0	26	undetectable	13	52	154
<b>16</b>	4	5	29,3	32,0	16	undetectable	27	45	308
<b>17</b>	< 1	1	10,5	18,3	22	569	13	40	224
<b>22</b>	< 1	2	12,5	33,0	16	628	16	56	515
<b>24</b>	< 1	< 1	24,5	30,8	34	1069	11	80	305
<b>27</b>	< 1	2	15,0	22,0	22	684	17	23	160
<b>29</b>	1	3	16,0	27,0	32	undetectable	18	27	159

<b>33</b>	< 1	4	12,5	22,3	31	841	14	63	525
<b>Moderate hemophilia</b>									
<b>12</b>	5	-	6,3	13,8	35	1149	8	21	98
<b>12</b>	5	1	22,0	29,8	11	404	12	25	134
<b>19</b>	6	8	12,8	36,0	15	undetectable	22	38	242
<b>20</b>	6	9	22,3	33,5	16	537	21	80	1273
<b>Mild hemophilia</b>									
<b>9</b>	11	12	4,8	13,5	52	1103	20	34	211
<b>18</b>	21	10	6,8	12,8	84	1159	18	15	124
<b>21</b>	7	6	6,5	14,5	59	867	11	24	125
<b>30</b>	9	6	4,0	13,3	70	1110	20	38	239
<b>31</b>	7	5	5,5	16,3	55	876	16	20	178
<b>32</b>	9	9	7,5	14,3	79	1269	11	23	78