Factor VIII half-life and clinical phenotype of severe hemophilia A

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Background and Objectives. Patients with severe hemophilia A have considerably different factor VIII half-lives. Whether this is associated with their clinical characteristics has not been reported. The aim of this study was to describe the association of factor VIII half-lives with treatment and clinical characteristics of patients with severe hemophilia A, who have been treated with individually tailored prophylaxis.

Design and Methods. Patients were selected from a single-center cohort of 214 patients with severe hemophilia, born between 1944 and 1995. To improve efficiency we measured factor VIII half-life in 42 patients selected from the extremes of the distribution of phenotypes of severe hemophilia. We assessed information on life-long joint bleeds and clotting factor consumption. Orthopedic outcome was assessed by the Pettersson score.

Results. Among these patients with severe hemophilia, factor VIII half-life ranged from 7.4-20.4 hours (median 11.8 hours). A one-hour increase in factor VIII half-life was associated with 96 (95% confidence interval (CI) 2 -190) IU less clotting factor use per kg per year. Age was an important determinant of factor VIII half-life, and explained a large part of the association between factor VIII half-life and clotting factor consumption. The median number of joint bleeds per year and arthropathy were similar for patients with different half-lives.

Interpretations and Conclusions. Among patients with severe hemophilia, treated prophylactically with clotting factor, those with a shorter factor VIII half-life required slightly more clotting factor to prevent joint bleeds and subsequent arthropathy than similar patients with a longer factor VIII half-life.

Key words: hemophilia, phenotype, half-life, factor VIII.

Haematologica 2005; 90:494-498

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Hemophilia is a rare X-linked disease characterized by the absence of clotting factor VIII or IX activity in the severe form (factor VIII/IX < 0.01 IU/mL). Without treatment patients with severe hemophilia suffer up to 96 joint bleeds and muscle bleeds per year.[1-3] Two different treatment strategies can be applied; (i) administration of factor VIII or IX when bleeding occurs (on demand), (ii) prophylactic treatment, in which factor is given at regular intervals with the aim of preventing bleeding and subsequent arthropathy.[4,5] Prophylaxis can be applied in different ways. Data from Swedish trials demonstrate that prophylactic therapy at a young age, using a high-dose regimen in which dosage is adjusted according to trough levels of factor VIII (hemophilia A 25-40 IU factor VIII on alternate days, a minimum of three times a week; hemophilia B: 25-40 IU factor IX twice weekly), prevents joint damage.[6] Results from Dutch studies have suggested that arthropathy can also be prevented using lower doses of factor concentrates for prophylaxis in an individually tailored regimen in which dosages are increased in case of spontaneous breakthrough bleeds.[7] The latter strategy allows for the study of the association between clinical characteristics, treatment and factor VIII half-life.

In pharmacokinetic studies a considerable inter-individual variation of factor VIII half-life has been observed among patients, whereas intra-individual variation of half-life is small.[8] The effect of this inter-individual variation of factor VIII half-life on treatment or clinical outcome has not been reported. The aim of this study was to describe the association between individual factor VIII half-lives,

treatment and clinical characteristics of patients with severe hemophilia A treated with individually tailored prophylactic regimens.

Design and Methods

Study design and population

Patients were selected from a previously described cohort of 214 patients with severe hemophilia, born between 1944 and 1995.[9] In short, all patients had been followed for at least 6 years, or more than 50 % of their total lifetime and visited our clinic during follow-up at least once yearly. X-rays of elbows, knees and ankles were performed every 5 years and scored according to the Pettersson score for radiological evaluation of joints as recommended by the World Federation of Hemophilia.[10] Each joint was attributed a minimum of 0 points (i.e. no arthropathy) to 13 points, resulting in a maximum total score of 78 points. Joints with arthrodeses, ankylosis or arthroplasty were assigned 13 points. All X-rays were scored by a single radiologist.

Patients who were HIV-positive, who had liver failure, low platelets, increased prothrombin time (PT), low factor V, signs of liver cirrhosis on ultrasound or who were known to have inhibitory antibodies against factor VIII were excluded. To improve efficiency we restricted measurements to 42 patients with either severe or relatively mild clinical phenotypes of severe hemophilia. Patients were selected from three different birth cohorts. A similar proportion of patients was selected from each birth cohort. The oldest patients, born between 1955 and 1965, were selected according to their last Pettersson score and the number of joints with arthropathy. Patients with the most (n=6) and with the least (n=6) affected joints were selected.

Patients born between 1965 and 1985 were selected according to a combination of three treatment characteristics.[11] For each patient we calculated a score: 2 × weekly dose (IU/kg/wk) on prophylaxis + 3 × the annual joint bleed frequency on prophylaxis – 3 × age at start of prophylaxis. Patients with the highest (n=10) and the lowest (n=10) scores were selected. The youngest patients, born between 1985 and 1995, were selected according to the age at which they experienced their first joint bleed. Patients with the youngest (n=5) and oldest (n=5) age at first joint bleed were selected. Eligible patients or parents were asked for informed consent. They were asked not to use factor VIII during the 72 hours before their visit for the assessment of factor VIII half-life. Two days before their visit to the clinic, patients were asked to complete a questionnaire to give information on their general health status and use of medication in the preceding days. Since fever has been reported to increase half-life of factor VIII, febrile patients or those feeling ill were asked to postpone their visit for factor VIII half-life measurement until they had fully recovered.[12] Since bleeding and surgery are known to decrease factor VIII half-life, patients who had had a severe bleed or surgery during the past 3 months were asked to postpone their visit for 3 months.[13,14] The Medical Ethics Committee of the University Medical Center Utrecht approved this study.

Treatment strategy

All patients were intended to receive prophylaxis, which was dosed according to individual bleeding patterns. Dosages were adjusted when breakthrough bleeds occurred.[9] Some patients, however, decided to interrupt or stop prophylaxis.[11] As prophylaxis was introduced in the 1970s, the oldest patients in this study had late access to this treatment.

Blood collection and processing

Blood samples were collected by atraumatic venipuncture without pressure into plastic tubes containing 0.109 M buffered trisodium citrate. The ratio of blood to anticoagulant was 9:1. To be sure that citrate tubes were filled without contamination from tissue fluids, the first 5 mL of blood was not used for clotting factor assays. A baseline blood sample was taken at t=0. To determine factor VIII half-life we infused 50 IU/kg of the factor VIII product regularly used by the patient. Fifteen patients used high purity plasma-derived factor VIII, 25 patients used recombinant factor VIII and 2 patients used B-domain deleted recombinant factor VIII. After infusion with factor VIII, blood samples were collected at 15, 30 minutes and 1, 3, 5, 24, 30, 48 and 60 hours.

The blood samples were centrifuged for 15 minutes at 1500 × g (2693 rpm) immediately and plasma was stored at –70°C.

Laboratory assays

A number of standard laboratory tests were performed at the Clinical Chemistry Department of our hospital. First, plasma concentrations of C-reactive protein (CRP) were determined to assess sub-clinical infection. Concentrations above 15 mg/L were considered increased. Second, hematocrit was measured and was considered normal from 20 to 55%. All hemostasis-related tests were performed at the Thrombosis and Hemostasis Diagnostic Laboratory of our institute. Factor VIII activity was determined using the one-stage method. Since inhibitory antibodies against factor VIII may influence factor VIII half-life,[15] the presence of these antibodies was assessed in pre-infusion samples using the Nijmegen-

variant of the Bethesda assay.[16,17] Inhibitor concentrations of >0.3 Bethesda units (BU)/mL were considered positive. Pre-infusion von Willebrand factor (VWF) antigen levels were determined in a previously described immunosorbent assay using polyclonal antibodies.[18] Pooled plasma from 40 healthy donors was used as a reference.

Data analysis

We used the pharmacokinetic program PK solutions 2.0 (Summit Research Services) to calculate factor VIII half-life. The last five years of follow-up were used to estimate annual clotting factor use (IU/kg/yr), number of joint bleeds per year and weekly dose on prophylaxis (IU/kg/wk). Linear regression analysis was used to assess the relation between clinical characteristics and factor VIII half-life and between factor VIII half-life and determinants of factor VIII half-life, such as age, blood group and VWF antigen. Factor VIII half-life on recombinant and plasma-derived products was compared using the independent sample T-test. A χ^2 test was used to compare the number of patients on recombinant products according to clinical phenotype. Since Pettersson scores showed a skewed distribution with many zero scores, a generalized linear model with gamma distribution and log link[19] was used to model the association between Pettersson score and factor VIII half-life. Furthermore, since Pettersson scores are highly dependent on age,[20] the findings were adjusted for age at Pettersson score.

Results

Forty-two patients with severe hemophilia A were selected. CRP and hematocrit were normal in all patients and none of the patients had inhibitory antibodies against factor VIII. The mean age was 28.8 (standard deviation (SD) 11.8) years. Factor VIII half-life ranged from 7.4 to 20.4 hours (median 11.8 hours) and was similar for plasma-derived and recombinant products, being 11.8 and 13.3 hours, respectively (p=0.09). Recombinant products were used by 67% of patients with a more severe clinical phenotype and by 52% of patients with a milder clinical phenotype (p=0.17). B-domain deleted products were only used by 2 of the patients with a more severe phenotype and its effect could therefore not be tested. Analyses including and excluding these 2 patients were similar.

Factor VIII half-life according to clinical phenotype

The median factor VIII half-life was 12.8 hours (interquartile range (IQR) 11.0-14.7) among patients with a more severe clinical phenotype and 12.8 hours (IQR 11.0-14.3) among patients with a milder clinical phenotype (p=0.78). The median age in these two groups was similar, being 27.7 and 29.8 years for patients with more severe and milder clinical phenotype, respectively (p=0.56).

Table 1. Clinical characteristics of patients with severe hemophilia A according to factor VIII half-life.

	Factor VIII half-life > 12 h		*Factor VIII half-life < 12*		*p value*
n	20		22		
Age (yr)	21.9	(15.2-28.4)	35.7	(22.7-43.8)	0.06
Weight (kg)	67.0	(55.6-79.2)	74.5	(64.2-85.3)	0.11
Treatment characteristics					
Age at start of prophylaxis (yr)	4.5	(3.0-7.4)	8.7	(5.0-18.2)	0.21
Annual clotting factor use (IU/kg/yr)	2217	(1589-2700)	1588	(816-2156)	0.03
Weekly dose on prophylaxis (IU/kg/wk)	44	(35-55)	33	(24-49)	0.14
Joint bleed frequency (per year)	2.9	(1.1-4.4)	2.6	(1.0-4.8)	0.84
Clinical manifestations					
Age at first joint bleed (yr)	2.0	(1.0-4.0)	2.1	(1.2-3.8)	0.38
Pettersson score	0	(0-11)	15	(4-32)	0.90
Plasma factors					
VWF-Ag (IU/mL)	0.98	(0.81-1.19)	1.36	(1.14-1.80)	< 0.01
Blood group 0 [n (%)]	14	(70)	8	(36)	0.03

Values are medians (IQR) or numbers (%). p: value adjusted for age at Petersson score.

Clinical characteristics and factor VIII half-life

The clinical characteristics of the study population according to strata of factor VIII half-life are presented in Table 1. Patients with a longer factor VIII half-life were older than patients with a shorter half-life. Similarly, weight tended to be higher in patients with a longer factor VIII half-life. The age at start of prophylaxis tended to be earlier in patients with shorter factor VIII half-life. The median annual factor VIII consumption per kg was higher in patients with a shorter factor VIII half-life. A one-hour increase in factor VIII half-life was associated with 96 (95%-confidence interval (CI) 2-190) IU less clotting factor use per kg per year (p<0.05) (Figure 1). Age was an important determinant of factor VIII half-life. After adjustment for age the association between factor VIII half life and clotting factor use reduced considerably; each hour increase in factor VIII half-life was associated with 36 (CI -41 to113) IU/kg/yr less clotting factor use (p=0.36). Accordingly, patients with a shorter factor VIII half-life tended to need a higher weekly dose on prophylaxis. The median annual joint bleed frequency was comparable between patients with a half-life

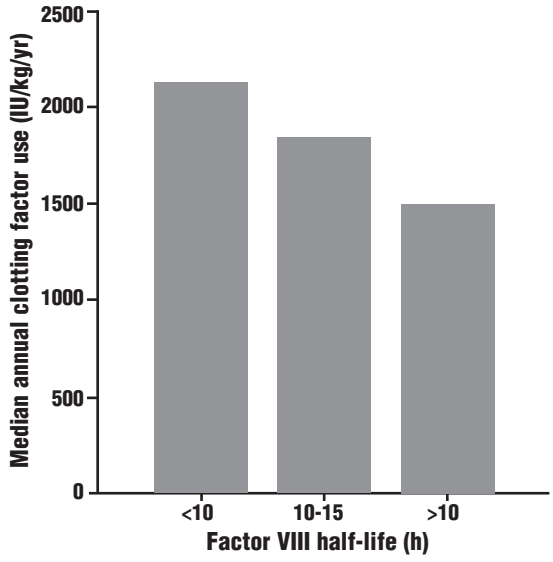

Figure 1. Half-life and annual clotting factor use.

shorter than 12 hours and patients with a factor VIII half-life longer than 12 hours. The age at the first joint bleed was similar in patients with a shorter and a longer factor VIII half-life. The median Pettersson score was 0 points (IQR 0-11) in patients with a factor VIII half-life shorter than 12 hours and 15 points (IQR 4-32) in patients with a factor VIII half-life longer than 12 hours (p=0.90). Regression analyses with factor VIII half-life as a continuous variable and adjusted for age at Pettersson score showed that factor VIII half-life was not associated with Pettersson score (*results not shown*).

Determinants of factor VIII half-life

VWF:Ag and factor VIII half-life increased with age (Table 2). An increase of ten years in age was associated with an increase of 0.16 U/mL of VWF:Ag. Patients with blood group O had lower VWF:Ag. The mean VWF:Ag level in patients with blood group O was 1.09 U/mL (SD 0.25 U/mL), compared to 1.54 U/ml (SD 0.79 U/mL) in patients with other blood groups (p=0.02). As expected, patients with a higher VWF:Ag concentration had a longer factor VIII half-life. An increase of 0.10 U/mL VWF:Ag was associated with 16.6 (95%-CI: 9-24) minutes longer factor VIII half-life (p<0.01).

Discussion

This study describes the association between factor VIII half-life and the clinical characteristics of patients with severe hemophilia. We found that shorter factor VIII half-life was associated with higher annual clotting factor use, which was largely explained by the association between age and factor VIII half-life. Among these prophylactically treated patients, joint bleed frequency and arthropathy as measured by the Pettersson score did not differ between patients with a shorter and patients with a longer factor VIII half-life. VWF and blood group were other determinants of factor VIII half-life.

Table 2. Factor VIII half-life and VWF:Ag according to age.

		Factor VIII half-life (h)	*VWF:Ag (IU/mL)*
10-20 years	(n=13)	11.2 (10.5-14.8)	1.13 (0.89-1.27)
20-30 years	(n=12)	11.5 (10.9-13.8)	1.08 (0.84-1.32)
> 30 years	(n=17)	13.7 (12.0-16.9)	1.30 (1.10-1.67)
p for trend		0.063	0.045

Values are medians (IQR).

This is the first study to describe the association between factor VIII half-life and clinical characteristics of severe hemophilia. Since it was too much effort to measure factor VIII half-life in all 214 patients, we restricted the study population to the extremes of the distribution of clinical phenotype of severe hemophilia. This selection does not influence the validity of the results, but provides a wide distribution of phenotypes and therefore a higher probability of detecting an association between clinical phenotype and factor VIII half-life. We selected patients with different ages in order to be able to study the effect of age on factor VIII half-life.

It may be argued that the potential role of non-neutralizing antibodies was not ruled out, since these were not detected by functional laboratory assays. However, none of the patients had a history of inhibitory antibodies and all patients had had more than 1000 exposures by the time of the factor VIII half-life measurement. It is therefore very unlikely that the patients in our study have non-neutralizing antibodies.[21]

We found that patients with a shorter factor VIII half-life needed more clotting factor in order to maintain a similar joint bleed frequency as that in patients with a longer factor VIII half-life. This association was for a large part explained by the influence of age on factor VIII half-life. The number of subjects in our study was too small to assess the association between factor VIII half-life and clotting factor consumption stratified for age.

Inter-individual variation of factor VIII half-life was in accordance with other reports: previously reported ranges vary between 2.6 and 30 hours.[8,13,22-25] Limited data are available on factors responsible for the intra-individual variation in factor VIII half-life. We found that age was an important determinant of FVIII half-life. In contrast, Vlot *et al.* did not find an association

between age and factor VIII half-life.[26] This may be explained by the smaller age variation in their study population. In both the present study and in studies by Fijnvandraat *et al*. and Vlot *et al*., pre-infusion VWF:Ag influenced factor VIII half-life.[8,26]

Furthermore, in both the present study and in a study by Vlot *et al*., factor VIII half-life was influenced by blood group; patients with blood group O have a shorter factor VIII half-life than do patients with group A, B or AB, which may at least partly be explained by lower VWF:Ag levels in patients with blood group O.[26] Weight, length, presence or absence of the factor VIII gene inversion and Rhesus phenotype did not influence factor VIII half-life.[26]

Our results suggest that patients with a shorter factor VIII half-life need more intensive treatment; factor VIII half-life may be useful in dosing prophylactic treatment. It would be worthwhile studying whether assessment of factor VIII half-life very early in life (e.g before the start of prophylaxis) could contribute to improvement of treatment and prevention of joint damage among patients with severe hemophilia.

In conclusion, among these patients with severe hemophilia, treated prophylactically with clotting factor, those with a shorter factor VIII half-life required slightly more clotting factor to prevent joint bleeds and subsequent arthropathy than did similar patients with a longer factor VIII half-life.

KvD, JGvdB, PJL, PGdG, EPM-B, GR, DEG, HMvdB: substantial contributions to conception and design of the study, acquisition, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, or final approval of the version to be published.

The authors would like to thank Els Haan for her help in collecting the samples and Margriet van Klaren for performing all factor VIII assays.

Manuscript received January 28, 2005. Accepted February 26, 2005.

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