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Langzeitbeobachtung der Therapie von Hämophilie A-Patienten mit einem humanen Faktor VIII-Konzentrat

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Summary in English

This doctoral thesis entitled “Long-term surveillance of the therapy of haemophilia A patients with a human plasma-derived factor VIII concentrate” was performed to assess the influence of the chronic long-term therapy with a human plasma-derived factor VIII concentrate in daily clinical practice on the health of haemophilia A patients.

Haemophilia A is a chronic disease, caused by a congenital deficiency of coagulation factor VIII, which requires life-long haemostatic treatment. The severity of bleedings, as the main clinical feature of haemophilia A, is generally correlated with the residual activity of coagulation factor VIII.

Until recently, factor VIII preparations, used to replace the deficient factor VIII, were the only treatment option for haemophilia A. Development of inhibitory antibodies against factor VIII is the most serious complication associated with the use of factor VIII products, rendering the administered factor VIII ineffective.

To date, all novel treatments still rely on some factor VIII replacement therapy. At least in the near future and probably for longer, (concomitant) therapy with factor VIII concentrates will continue to be necessary for treatment of haemophilia A, emphasising the continuous need for efficacy and safety data in terms of pharmacovigilance on factor VIII replacement therapy.

Medicines to treat haemophilia A, are authorised for use, when evidence of its efficacy and safety is limited to data of a small number of investigated patients during short-term observation periods of about six months, and thus have not been systematically assessed in all patient groups until marketing authorisation. Long-term efficacy and safety data from post-marketing surveillance are important to prove that a chronic treatment is efficacious and safe in the real-life setting by monitoring “real-life” patients of all age groups, rather than a carefully selected patient population. Medical and scientific analyses of such long-term data are crucial to detect, understand, and potentially prevent the harm resulting from (new) adverse drug reactions, including those, which only rarely occur and therefore are difficult to detect.

Therefore, data from two prospective surveillance studies investigating real-life therapies with the same human plasma-derived factor VIII concentrate were combined and analysed retrospectively. It was hypothesised that the

chronic long-term therapy with a human plasma-derived factor VIII concentrate in daily clinical practice is effective, safe, and well tolerated with no unexpected adverse effect on the health of haemophilia A patients. It was the aim of this analysis to investigate the influence of the chronic long-term treatment with the factor VIII concentrate on the health of patients with severe as well as non-severe haemophilia A including all age groups in a real-life setting. In addition, the influence of prophylactic factor VIII treatment or the switch to this regimen on the annual bleeding rate of all haemophilia A patients, and the long-term effects of this regimen on the patients' annual bleeding rates were investigated.

Starting in 1998 until 2015, data of 1418 patient-years from 198 haemophilia A patients representing all age groups and haemophilia A severities were analysed. This study covered 18 years of documentation time with a mean observation period of more than seven years per patient. It is the longest study of a single factor VIII concentrate conducted so far, investigating the therapy of haemophilia A. The only observed side effects involved low incident factor VIII inhibitor formation in patients at risk (13 % of previously untreated patients, compared with usually about 30 %). Factor VIII inhibitor development was mainly transient, with low titers, and without clinical relevance. Any, even low frequent prophylaxis was found to be significantly better than on demand and had the greatest effect on the annual bleeding rate of patients, irrespective of their age or haemophilia A severity. Patients suffered during continuous prophylaxis from a very low bleeding rate (median 1.3 compared with 31.4 under on demand), down to no bleeding per year. Patients whose regimen changed to continuous prophylaxis benefitted most (median annual bleeding rate 1.1), irrespective of age or haemophilia A severity.

This analysis demonstrates that the chronic long-term therapy with the plasma-derived factor VIII concentrate in daily clinical practice is effective, safe, and well tolerated. Thus, data on efficacy and safety obtained during chronic long-term therapy with the human plasma-derived factor VIII concentrate reaffirm that there is no unexpected adverse effect on the health of haemophilia A patients.

These results support the therapeutic concept of a life-long prophylaxis of haemophilia A patients with a human plasma-derived factor VIII concentrate.

Summary in German

Das Ziel dieser Doktorarbeit, mit dem Titel „Langzeitbeobachtung der Therapie von Hämophilie A-Patienten mit einem humanen Faktor VIII-Konzentrat", war es, den Einfluss der chronischen Langzeittherapie mit einem plasmatischen Faktor VIII-Konzentrat im klinischen Alltag auf den Gesundheitszustand von Hämophilie A-Patienten zu bewerten.

Hämophilie A ist eine chronische Erkrankung, welche durch einen angeborenen Mangel des Gerinnungsfaktors VIII verursacht wird und eine lebenslange hämostatische Therapie benötigt. Der Schweregrad der Blutungen, welche das Leitsymptom der Hämophilie A sind, korreliert in der Regel mit der Restaktivität des Gerinnungsfaktors VIII.

Bis vor kurzem waren Faktor VIII-haltige Produkte, die angewendet werden um den fehlenden Faktor VIII zu ersetzen, die ausschließliche Therapieoption der Hämophilie A. Die Bildung von inhibitorischen Antikörpern gegen den infundierten Faktor VIII ist die schwerwiegendste Nebenwirkung im Zusammenhang mit der Anwendung von Faktor VIII-Produkten, da diese den infundierten Faktor VIII wirkungslos machen. Bis heute, und auch in naher Zukunft, kann bei neuen Therapieoptionen nicht auf die (begleitende) Faktor VIII-Substitutionstherapie verzichtet werden. Deshalb werden nach wie vor kontinuierlich Wirksamkeits- und Sicherheitsdaten im Rahmen der Pharmakovigilanz zu der Therapie mit Faktor VIII-Konzentraten benötigt.

Arzneimittel zur Therapie der Hämophilie A erlangen mit begrenzten Wirksamkeits- und Sicherheitsdaten wenig behandelter Patienten und kurzen Beobachtungszeiten von etwa sechs Monaten die Marktzulassung. Somit werden bis zur Marktzulassung Wirksamkeits- und Sicherheitsdaten noch nicht systematisch für alle Patientengruppen bewertet. Langzeitbeobachtungen der Wirksamkeit und Sicherheit sind notwendig um sicherzustellen, dass die Therapie auch im realen Alltag in nicht vorselektierten Patienten-(gruppen) wirksam und sicher ist. Medizinisch wissenschaftliche Analysen von solchen Langzeitdaten sind unerlässlich, um potentiell (neue) unerwünschte Arzneimittelreaktionen, und insbesondere auch solche, die selten auftreten, zu identifizieren, zu verstehen und um diese und den daraus resultierenden Gesundheitsschaden verhindern zu können.

Deshalb wurden Daten aus zwei prospektiven Beobachtungsstudien, in denen die Therapie mit dem gleichen plasmatischen Faktor VIII-Konzentrat im klinischen Alltag untersucht wurde, zusammen retrospektiv analysiert. Es wurde die Hypothese aufgestellt, dass die chronische Langzeittherapie mit einem humanen Faktor VIII-Konzentrat im klinischen Alltag wirksam, verträglich und ohne unerwünschten Einfluss auf den Gesundheitszustand der Hämophilie A-Patienten ist. Das Ziel der Analyse war es, den Einfluss der chronischen Langzeittherapie mit dem Faktor VIII-Konzentrat im klinischen Alltag auf den Gesundheitszustand der Patienten aller Altersgruppen mit schwerer sowie nicht schwerer Hämophilie A zu untersuchen. Zusätzlich wurde der Einfluss der Prophylaxe und des Regimewechsels auf Prophylaxe auf die jährliche Blutungsrate aller Patientengruppen untersucht.

Von 1998 bis 2015 wurden Daten von 198 Patienten aller Altersgruppen und Schweregrade der Hämophilie A aus 1418 Patientenjahren analysiert. Der Beobachtungszeitraum umfasste insgesamt 18 Jahre und durchschnittlich mehr als sieben Jahre pro Patient. Es handelt sich um die bisher längste Studie eines einzelnen Faktor VIII-Konzentrats in der Hämophilie A-Therapie. Die einzigen beobachteten Nebenwirkungen waren wenige Faktor VIII-Inhibitorbildungen bei Risikopatienten (13 % der unvorbehandelten Patienten, verglichen mit üblicherweise etwa 30 %). Diese waren hauptsächlich transient, niedrig-titrig und ohne klinische Relevanz. Prophylaxe allgemein, sogar selten verabreicht, erwies sich als signifikant besser als die Bedarfstherapie und hatte den größten Einfluss auf die jährliche Blutungsrate. Patienten litten während einer kontinuierlichen Prophylaxe an sehr wenigen (Median 1,3 verglichen mit 31,4) oder keinen Blutungen pro Jahr. Patienten, deren Regime auf eine kontinuierliche Prophylaxe gewechselt wurde, profitierten am meisten (Median 1,1).

Diese Analyse bestätigt, dass die Langzeittherapie mit dem plasmatischen Faktor VIII-Konzentrat im Praxisalltag wirksam, sicher und verträglich ist. Zudem bestätigen diese Wirksamkeits- und Sicherheitsdaten, dass die chronische Langzeittherapie mit dem plasmatischen Faktor VIII-Konzentrat keinen unerwünschten Einfluss auf den Gesundheitszustand der Patienten hat.

Das Therapiekonzept einer lebenslangen Prophylaxe von Hämophilie A-Patienten mit einem plasmatischen Faktor VIII-Konzentrat wird durch diese Ergebnisse unterstützt.

List of abbreviations

IU	International Unit
PUP1-3	Previously untreated patient number 1-3
PTP1-4	Previously treated patient number 1-4
BU	Bethesda Unit

1. Comprehensive summary

1.1. Introduction

This doctoral thesis entitled "Long-term surveillance of the therapy of haemophilia A patients with a human plasma-derived factor VIII concentrate" was performed to assess the influence of the chronic long-term therapy with a human plasma-derived factor VIII concentrate in daily clinical practice on the health of haemophilia A patients.

Haemophilia A is a chronic disease, caused by a congenital deficiency of coagulation factor VIII, which requires life-long haemostatic treatment. It is a rare coagulation disorder with a prevalence, per 100,000 male births, of approximately 17 for all severities of haemophilia A and six for severe haemophilia A (< 1 % residual factor VIII activity).[1] The severity of bleedings, as the main clinical feature of haemophilia A, is generally correlated with the residual activity of coagulation factor VIII. Intracranial and gastrointestinal bleedings can be life-threatening in patients with haemophilia A. To prevent and treat bleedings and related complications and morbidities is the primary aim of haemophilia A care.[2,3] The superiority of prophylactic factor VIII replacement over on-demand treatment has been demonstrated for children with severe haemophilia A,[3-12] but it is not generally accepted for adolescent and adult patients and those with non-severe haemophilia A.[7] However, although follow-up documentation is limited, especially from real-life settings, opinion is shifting toward recommending life-long prophylaxis.[3-5,13-16]

Until recently, factor VIII preparations, used to replace the deficient factor VIII, were the only treatment option for haemophilia A. Development of inhibitory antibodies against factor VIII is the most serious complication associated with the use of factor VIII concentrates; the antibodies neutralise the infused factor, rendering patients resistant to conventional replacement treatment.[17] Factor VIII inhibitors occur more frequently in patients with severe haemophilia A than in those with non-severe haemophilia A (≥ 1 % residual factor VIII activity). The risk of inhibitor development in patients with severe haemophilia A ranges from approximately 27 % to 45 %.[18] Notably, in patients with severe haemophilia A, inhibitors develop almost exclusively within the first 50 exposure days, whereas for patients with non-severe disease the risk is life-long.[18-21]

New therapies including non-factor therapeutic agents and gene therapy have been, and are becoming increasingly available for treatment of haemophilia A. But at least in the near future and probably for longer, (concomitant) therapy with factor VIII concentrates will continue to be necessary for treatment of haemophilia A,[22,23] emphasising the continuous need for efficacy and safety data in terms of pharmacovigilance on factor VIII replacement therapy.

Pharmacovigilance is an umbrella term and is defined as the science including all activities related to monitor, detect, assess, understand, evaluate and prevent adverse effects or any other drug-related problem with the main aim of maximising patients` safety in relation to the use of medicines (benefit risk balance).[24-26]

Medicines to treat haemophilia A, are authorised for use, when evidence of its efficacy and safety is limited to data of a small number of investigated patients during short-term observation periods of about six months, and thus have not been systematically assessed in all patient groups until marketing authorisation. Further information are necessary from post-marketing surveillance to acquire systematically long-term treatment data from everyday clinical practice and to ensure consistency between the outcomes of treatment in clinical studies and in routine use. These long-term data are important to prove that a chronic treatment is efficacious and safe in the real-life setting monitoring “real-life” patients of all age groups, rather than a carefully selected patient population. Medical and scientific analyses of these long-term data are crucial to detect, understand, and potentially prevent the harm resulting from (new) adverse drug reactions, including those, which only rarely occur and therefore are difficult to detect.[24-26]

Therefore, data of two prospective observational/ surveillance studies investigating real-life efficacy and safety data in terms of pharmacovigilance on the chronic long-term treatment with the same human plasma-derived factor VIII concentrate were combined and analysed retrospectively. It was hypothesised that the chronic long-term therapy with the human plasma-derived factor VIII concentrate in daily clinical practice is effective, safe, and well tolerated with no unexpected adverse effect on the health of haemophilia A patients. Data were to be taken from clinical practice and routine daily treatment, with the aim to

investigate the influence of the chronic long-term treatment with the human plasma-derived factor VIII concentrate on the health of haemophilia A patients including all age groups and severities of haemophilia A. In addition, the influence of prophylactic factor VIII treatment or the switch to this regimen on the annual bleeding rate of haemophilia A patients of all age groups and haemophilia A severities, and the long-term effects of this regimen on the patients' annual bleeding rates were assessed.

1.2. Materials and methods

1.2.1. Study setting and design

The two studies, including 163 and 35 patients, covered nearly the same observation period (May 1998 to December 2015 and January 1998 to January 2014). Their observation plans and case report forms were highly comparable. As a result, to have a lager dataset and to increase the validity, data from both studies were combined for the main analysis on the influence of the chronic long-term treatment on the patients` health. A merge was not possible of the data needed for the additional analyses on the development of the annual bleeding rates, because these data were available in different databases with different electronic formats.

The combined prospective, multicentre long-term non-interventional study was conducted at German and Hungarian haemophilia centres.

1.2.2. Study treatment

The factor VIII concentrate administered in this study is produced from human plasma provided as a powder along with solvent for dissolution for injection and contains von Willebrand factor.

1.2.3. Participants

Patients with severe or non-severe haemophilia A were included and classified as previously treated or previously untreated. Previously untreated patients were defined as those patients who had never been treated with clotting factor concentrates. There were no specific exclusion criteria, to avoid any selection.

1.2.4. Treatment regimen, variables, and data sources

Study drug was administered at the physician's discretion. Its dosage and frequency of administration were based on the summary of product characteristics of study drug. Reasons for treatment with study drug were classified by the physician as “prophylaxis”, “bleeding” or “follow-on treatment” (on demand), or “surgery”). In addition, the doses of study drug administered, bleedings and reasons for administration were recorded in patient diaries by the patients. Patients were required to attend haemophilia treatment centres for at least one annual visit. Most of the patients had three or four routine visits per year.

Patients were divided into the following three groups depending on their treatment regimen(s) throughout their observation period during the study: (i) patients with only an on-demand or (ii) prophylactic treatment regimen and (iii) those whose treatment regimen changed during their observation time (=”switchers”). Patients whose treatment regimen changed continuously from on-demand treatment to a continuous prophylaxis during the observation period were further investigated, because these switches allowed within-patient comparison regarding the development of annual bleeding rates. Continuous prophylaxis was defined following the World Federation of Hemophilia guideline as prophylactic administration for at least 45 consecutive weeks.[3] Additionally, the frequency and dosing of the patients treated prophylactically were assessed.[27]

Variables as described below were documented by the physicians in paper case report forms, and are summarised more detailed and completely in Supplemental Table 1 (page 32).

1.2.5. Efficacy assessment

Efficacy was primarily analysed on the basis of annual bleeding rates, their development, and by assessing the expected therapeutic effect. Documented days with bleedings by patients in their patient diaries were used for calculations of the annual bleeding rates, which included traumatic and spontaneous bleedings. “Bleeding” was not predefined.

1.2.6. Safety and tolerability assessment

Adverse events – including any bleedings due to suspected lack of efficacy of treatment with study drug, aggravation of underlying or newly diagnosed diseases, and laboratory assessments – were documented. They were assessed (and, if clinically relevant, commented on) by the physician; any deviation from normal range, clinical relevance, severity, seriousness and study drug-relationship were taken into account.

1.2.7. Statistical analyses

Descriptive statistical methods were applied for the analyses. The effect of prophylaxis on the annual bleeding rate was analysed by Poisson regression with annual bleeding rate as the dependent variable, and the influence of age and/ or the haemophilia severity was investigated.

Median differences of annual bleeding rates between treatment regimens were evaluated by using the non-parametric Wilcoxon signed-rank test with each test performed at a 5 % alpha level and Bonferroni adjusted for multiple testing, with p-value < 0.05 considered statistically significant. The comparisons between treatment regimens are presented in section “4.3.6. Statistical analyses” (page 38).

1.3. Results

1.3.1. Demographic and treatment data

Long-term data from 198 enrolled male patients during the period January 1998 until end of December 2015 were analysed. Fifty-two patients were treated at Hungarian and 146 at German centres. Thirty patients were enrolled as previously untreated patients and 168 as previously treated patients. A subgroup of 160 patients had severe haemophilia A (factor VIII residual activity ≤ 1 %); 137 patients with severe haemophilia A were enrolled as previously treated and 23 patients as previously untreated patients (Table 1, page 27).

All patients were male and almost all were Caucasian, with four exceptions: two were black, one was Asian and one was Arabian. At the time of inclusion in the study, patients had a mean (± standard deviation) age of

25.1 ± 18.7 years, with ages ranging from zero to 80 years. The oldest previously treated patient turned 88 years old during the study (Figures 4 and 8, pages 53 and 85).

During the 18-year observation period, a total of 1418 patient-years were documented. The mean documentation time for all patients was 7.3 ± 5.1 years; for patients with severe haemophilia A it was 7.8 ± 5.0 years. Twenty-four patients were followed up for more than 15 years.

A total of 213,471,262 international units (IU) of study drug were administered in 130,595 treatments during the study period; of these 70.5 % were given as prophylactic treatment and 29.1 % as on-demand treatment. The mean number of infusions for all patients was 665.9 ± 580.8. On average, 31.6 ± 15.2 IU/kg per body weight were administered per exposure. Patients received on average 8.3 ± 5.1 treatments per month (Table 2, page 28).

1.3.2. Efficacy

Investigators assessed the therapeutic effect of treatment with study drug as "successful" for nearly all (99.4 %) treatments.

The mean dose of prophylaxis (31.5 ± 15.4 IU/kg; median 28.9 IU/kg; range 9.1 – 120.4 IU/kg) and the mean frequency per month (6.4 ± 5.9; median 5.7; range 0.0 – 30.0; 156 patients) of prophylactic treatments varied substantially between the patients.

Overall, considering all patients, the annual bleeding rate decreased over time, from a median annual bleeding rate of 20.7 (mean 23.7 ± 23.6; 90 patients) in the period 1998 to 2002 to finally 2.6 (mean 7.2 ± 10.4; 70 patients) in the period 2013 to 2015. In parallel, the proportion of patients receiving prophylactic treatment increased during the long-term treatment with study drug from 41.1 % (37 of 90 patients) in 2003 to finally 65.7 % (44 of 67 patients) in 2015.

Among the 163 analysed patients, a strong relationship was found between prophylactic treatment and mean annual bleeding rate (Poisson regression, $\beta = -0.02$, $p < 0.001$). That means, when administering X prophylactic administrations more per year, the annual bleeding rate decreases by 2X %. Thus, the more prophylactic administrations a patient received, the lower his annual bleeding rate. No differences in this relationship were detected

between patients with severe and non-severe haemophilia A or between age groups.

Overall, the median annual bleeding rate of the patients receiving study drug on demand was considerably higher than the median annual bleeding rate of patients treated prophylactically with study drug during the observation period of this study (Tables 4, 5, pages 48, 49, and Figure 2, page 51). Considering all patients with severe or non-severe haemophilia A, the differences in annual bleeding rates were statistically significant ($p< 0.05$) for patients treated on demand compared with those treated continuously prophylactic with study drug (all comparisons 1-4, and comparison 4 for patients with non-severe haemophilia A, described in section “4.3.6. Statistical analyses”, page 38). A remarkable drop and an even great and statistically significant ($p< 0.05$) difference/ benefit was observed for patients whose on-demand regimen changed to continuous prophylaxis (Figure 2, page 51).

Twenty-five (49 %) of the 51 patients whose treatment regimen changed from on demand to a continuous prophylaxis suffered on average from less than one bleeding per year after their switch, and of these 13 (26 %) did not suffer from any bleeding. Further five switchers suffered from less than one bleeding per year. Of the 71 patients treated continuously prophylactic doses of study drug, 25 patients (35 %) on average suffered from less than 1.0 bleedings per year, thereof eleven patients (16 %) suffered from no bleeding at all. Of these 71 patients, four additional had a mean annual bleeding rate of 1.0. Notably, none of the patients treated under on-demand regimen suffered from ≤ 1.0 bleedings per year; all of these patients had a higher mean annual bleeding rate of 1.0 (minimum= 2.9 bleedings per year).

1.3.3. Safety and tolerability

During the study period, within 1418 patient-years, ten adverse events in seven patients were considered study drug-related, resulting in an adverse drug reaction rate of < 0.1 per patient, 7.1 per 1000 patient-years, and 0.1 per 1000 study drug administrations. All adverse drug reactions involved factor VIII inhibitor formation and were documented in seven patients with severe haemophilia A (three previously untreated and four previously treated patients: “PUP1-3” and “PTP1-4”; details are presented in Supplemental Table 2, page

33, and Tables 6 and 7, pages 76 and 78). All seven patients recovered. In two patients (PUP3, PTP4), the inhibitor formation was recorded twice and in PUP3 an increased bleeding tendency was additionally documented.

The overall incidence of factor VIII inhibitor formation in previously untreated patients with severe haemophilia A was 13 % (3/23), and 4 % (1/23) for high-titre factor VIII inhibitors.[17,28] One previously untreated patient (PUP2) of African ethnicity[29,30] developed a high-titre (≥ 5 Bethesda Unit (BU)/ml) and persistent inhibitor with a peak titre of 320 BU/ml at exposure day 6.[28] After eleven months of immune tolerance induction (Bonn Protocol) with study drug, inhibitor test results remained negative. The other two previously untreated patients showed transient and low-titre inhibitors (peak titres of 0.9 and 1.6 BU/ml; exposure days 16 and 27).[17,28] PUP3 was first exposed to study drug at an early age (six months) and suffered form an intron-22 inversion.[31-33] Inhibitor results remained negative in PUP1/ PUP3 after a few days/ months of three times weekly doses of approximately 50 IU/kg study drug.

During the study period, overall four previously treated patients with severe haemophilia A developed inhibitors, thereof three high-titre inhibitors, resulting in frequencies of 3.3 inhibitors and 2.5 high-titre inhibitors in 1000 patient-years with severe haemophilia A.[17,28] Three previously treated patients developed transient factor VIII inhibitors without any change in treatment. All three previously treated patients had single high-titre inhibitor results (5 to 8 BU/ml) that were not confirmed. Consecutive low-titre inhibitor results were documented for only two of these three previously treated patients. A fourth previously treated patient (PTP3) developed a low-titre and persistent inhibitor.[28] During follow-up observation after the study period, inhibitor titre did not increase but a high-dose immune tolerance induction with study drug was initiated. After one month and an initial booster to 30 BU/ml inhibitor results remained negative.

For five patients with factor VIII inhibitor development (PUP1, PUP2, PTP2, PTP3, and PTP4), severe bleedings with coexisting inflammation, requiring ≥ 3 up to ≥ 10 consecutive days of study drug treatment, were identified as a risk factor for inhibitor formation. Two of these patients (PUP1, PUP2) needed treatment with packed erythrocytes concomitantly with study

drug. In addition, two previously treated patients with inhibitor formation were treated on demand or had been treated on demand in the past.[30-32,34-40]

1.4. Discussion

This study covered 18 years of observation and documented 1418 patient-years. It is the longest non-interventional study of a single factor VIII concentrate conducted so far, investigating real-life efficacy and safety in daily clinical routine of prophylactic or on-demand treatment of haemophilia A and its influence on patient health.[20,41-44] A broad range of patients with severe as well as non-severe haemophilia A, covering all age groups (postnatal up to 88 years), were investigated. Notably, in this study “real-life patients”, including high-risk patients, instead of carefully selected patients were observed.

The plasma-derived factor VIII concentrate was effective and well tolerated, and no pharmacological safety concern was observed. In addition to the severe form of haemophilia A, all patients with inhibitor formation showed various risk factors for inhibitor formation.[30,37] Several risk factors are known or currently under discussion: these include the regimen and intensity of factor VIII replacement, age at start of factor VIII treatment, coexisting inflammation, type of factor VIII product, factor VIII mutation type, ethnicity, family history of inhibitors, and severity of haemophilia A.[19,29-32,35,38-40,45-54] Inflammatory danger signals, such as recurrent joint bleedings and severe bleeding episodes (including surgical procedures) requiring peak treatment moments, seem to increase the risk of developing mainly transient and low-titre inhibitors without clinical relevance in both previously untreated and previously treated patients treated on demand with study drug.[17,30-32,34-40,46,53] Overall, considering applied definitions of inhibitors,[28] rates of inhibitor formation in previously untreated and previously treated patients were low, and lower than (13 % of previously untreated patients, compared with usually approximately 30 %) or similar to those reported for other plasma-derived factor VIII concentrates, including the comparisons between plasma-derived and recombinant factor VIII products.[19,20,31,32,52,55-59]

Any, even low frequent prophylaxis was found to be significantly better than no prophylaxis and had the greatest effect on the annual bleeding rate of

patients, irrespective of their age or haemophilia A severity. Patients suffered during continuous prophylaxis from a very low bleeding rate (median 1.3 compared with 31.4 under on demand), down to no bleeding per year. Patients whose regimen changed to continuous prophylaxis benefitted most (median annual bleeding rate 1.1), irrespective of age or haemophilia severity.

There were limitations in data acquisition mainly due to the long observational period, especially at the beginning of the study, mostly on account of items documented at the start of the non-interventional study two decades earlier. Therefore, underreporting of adverse events, such as those related to bleeding (for example pain or causes of (traumatic) bleeding), elective procedures and underlying haemophilia A, related comorbidities (for example arthropathy) and their aggravation cannot be ruled out. However, the documentation and reporting of clinically relevant events, for example factor VIII inhibitor formation or thromboembolic events, are assumed to have been documented completely. Annual bleeding rates were calculated on the basis of bleedings (for which no definition was prespecified) and were documented in patient diaries, without the requirement that they be medically confirmed.

1.5. Conclusions

This analysis demonstrates that the chronic long-term therapy with the human plasma-derived factor VIII concentrate in daily clinical practice is effective, safe, and well tolerated.

To date, all novel treatments still rely on some factor VIII replacement therapy, and (concomitant) therapy with factor VIII concentrates will continue to be necessary for treatment of haemophilia A. Thus, data on efficacy and safety obtained during chronic long-term therapy with the human plasma-derived factor VIII concentrate reaffirm that there is no unexpected adverse effect on the health of haemophilia A patients.

These results support the therapeutic concept of a life-long prophylaxis of haemophilia A patients with a human plasma-derived factor VIII concentrate.

2. Overview of the manuscripts

2.1. Published manuscript (*Hämostaseologie* 2019;39:360–367)
“Long-term safety and efficacy data of a plasma-derived factor VIII concentrate with von Willebrand factor for treatment of patients with hemophilia A covering 18 years” (pages 24–31)

2.2.1. Supplement of the published manuscript (pages 32–33)

2.2. Submitted manuscript (to *Haemophilia*)
“Long-term analysis of the benefit of prophylaxis for adult patients with severe and moderate haemophilia A” (pages 34–60*)*

Long-Term Safety and Efficacy Data of a Plasma-Derived Factor VIII Concentrate with von Willebrand Factor for Treatment of Patients with Hemophilia A Covering 18 Years

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Hämostaseologie 2019;39:360–367.

Abstract

We describe the results of the (to our knowledge) longest long-term noninterventional study so far performed to obtain real-life data on the treatment of hemophilia A patients with a single plasma-derived FVIII concentrate containing von Willebrand factor (pdFVIII; Haemoctin/Faktor VIII SDH Intersero). A total of 198 patients (146 in Germany and 52 in Hungary), of whom 160 had severe and 38 nonsevere hemophilia A, representing all age groups (0–88 years; mean ~25 years at inclusion) were analyzed during prophylactic or on-demand treatment over 18 years (overall 1,418 patient-years; mean >7 years). pdFVIII was very effective and well tolerated. The mean annual bleeding rate, including spontaneous and traumatic bleeds, was considerably lower for patients treated prophylactically (mean 5.4; median 3.1) than for patients treated on demand (mean 26.1; median 21.9). Inhibitors were found in 13% (3/23) and high-titer inhibitors in 4% (1/23) of previously untreated patients with severe hemophilia A. Four previously treated patients with severe hemophilia A developed inhibitors, thereof three high-titer inhibitors (3.3 and 2.5 high-titer inhibitors in 1,000 patient-years). No unexpected adverse effect on the health of the patients, no pdFVIII-related thrombosis, thromboembolic event, or hypersensitivity reaction, and no suspected viral transmission related to pdFVIII were documented.

Keywords

- hemophilia A
- plasma-derived factor VIII
- FVIII inhibitor
- noninterventional study

Zusammenfassung

Wir berichten über die Ergebnisse der unseres Wissens längsten nicht-interventionellen Langzeitstudie mit einem von-Willebrand-Faktor-haltigem humanen FVIII-Konzentrat (pdFVIII, Haemoctin®/Faktor VIII SDH Intersero®) zur Erfassung praxisnaher Daten aus der alltäglichen Therapie von Patienten mit einer Hämophilie A. 198 Patienten (146 in Deutschland und 52 in Ungarn) aller Altersgruppen (0–88 Jahre; durchschnittlich ~25

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Schlüsselwörter
- Hämophilie A
- plasmatischer Faktor VIII
- FVIII Inhibitor
- nicht-interventionelle Studie

Jahre alt beim Einschluss), 160 davon mit schwerer und 38 mit nicht schwerer Hämophilie A, wurden im Rahmen ihrer prophylaktischen oder Bedarfstherapie über 18 Jahre analysiert (insgesamt 1418 Patientenjahre; durchschnittlich >7 Jahre). Der pdFVIII erwies sich als sehr wirksam und verträglich. Die durchschnittliche jährliche Blutungsrate (spontane und traumatische Blutungen) war deutlich geringer in Patienten, die prophylaktisch (Mittelwert 5,4; Median 3,1) anstatt bei Bedarf (Mittelwert 26,1; Median 21,9) mit pdFVIII behandelt wurden. Inhibitoren wurden in 13% (3/23) und hochtitrige in 4% (1/23) der zuvor unbehandelten Patienten mit schwerer Hämophilie A dokumentiert. Vier der vorbehandelten Patienten mit schwerer Hämophilie A entwickelten Inhibitoren, 3 davon hochtitrige (3.3 Inhibitoren und 2.5 hochtitrige pro 1000 Patientenjahren). Während der Langzeittherapie mit pdFVIII wurde kein unerwünschter Einfluss auf den allgemeinen Gesundheitszustand der Patienten festgestellt. Es trat keine thromboembolische Reaktion, keine Überempfindlichkeitsreaktion, und keine Virusübertragung im kausalen Zusammenhang mit pdFVIII auf.

Introduction

Hemophilia A is a chronic disease caused by a congenital deficiency of coagulation factor VIII (FVIII) and requires lifelong hemostatic treatment. Long-term pharmacovigilance data are important to prove that a treatment is safe and efficacious in the real-life setting during long-term up to life-long therapy. Until recently, FVIII preparations were the only treatment option. New therapies including nonfactor therapeutic agents and gene therapy have been, and are becoming increasingly, available for treatment of hemophilia A. However, at least in the near future and probably for longer, (concomitant) therapy with FVIII concentrates will continue to be necessary for treatment of hemophilia A,[1,2] emphasizing the continuous need for data on FVIII replacement therapy.

Development of inhibitory antibodies against FVIII is the most serious complication associated with the use of FVIII concentrates; the antibodies neutralize the infused factor, rendering patients resistant to conventional replacement treatment.[3]

FVIII antibodies occur more frequently in patients with severe hemophilia A than in those with moderate or mild hemophilia A. The risk of inhibitor development in patients with severe hemophilia A ranges from approximately 27 to 45%.[4] Notably, in patients with severe hemophilia A, inhibitors develop almost exclusively within the first 50 exposure days (EDs), whereas for patients with nonsevere disease the risk is life-long.[4–7]

To acquire more clinical data and to ensure consistency between the outcomes of treatment in clinical studies and in routine use, long-term postmarketing noninterventional studies (NISs) are necessary.[8]

This prospective observational study was initiated to collect real-life data on long-term treatment with a plasma-derived FVIII concentrate (pdFVIII; Haemoctin/Faktor VIII SDH Intersero) stabilized with von Willebrand factor (vWF). Data were taken from clinical practice and routine daily treatment, and the aim of the study was to investigate the influence of the treatment on the health of hemophilia A patients including all age groups. Safety and effectiveness data were collected while pdFVIII was being used in the prophylactic or on-demand setting. Analysis of interim data with 109 patients was presented in 2012.[9]

Materials and Methods

Study Treatment

The pdFVIII concentrate administered in this study was manufactured by Biotest Pharma GmbH (Dreieich, Germany). pdFVIII is produced from human plasma in a manner that complies with the relevant European Pharmacopoeia monograph; it is provided as a powder along with a solvent for dissolution for injection. The pdFVIII molecule is present in a physiological complex with vWF, without added artificial stabilizers. The ratio of the quantity of vWF to FVIII activity (vWF:Ag/FVIII:C) in pdFVIII is approximately 0.4, which means a significant excess of binding sites in vWF for FVIII. The manufacturing process of pdFVIII includes two virus inactivation steps using solvent/detergent (Polysorbate 80/TNBP) and a heat treatment at 100°C. These two steps ensure high margins of safety for the FVIII preparation, particularly with respect to human immunodeficiency virus and hepatitis A, B, and C virus.

Patients receiving this pdFVIII were included in this combined NIS conducted by Biotest AG and Intersero GmbH, respectively.

Setting

The observation period for each patient was planned to be at least 12 months. The studies were conducted at 34 German and 8 Hungarian hemophilia centers. Final data from the two studies were merged; they covered nearly the same observation period (May 1998 to December 2015 and January 1998 to January 2014). Observation plans and case report forms (CRFs) were highly comparable for the two studies. The studies were approved by ethics committees, and informed consent was obtained from the study patients from 2013 onwards.

Study Design

The study was a prospective, multicenter, binational, long-term NIS of the safety and efficacy of pdFVIII in hemophilia A patients.

Participants

Patients with severe or nonsevere hemophilia A were included who were receiving treatment with pdFVIII on the basis of the recommendations in the Summary of Product Characteristics for pdFVIII. There were no specific exclusion criteria, to avoid any selection.

This final analysis includes all data acquired from January 1998 until December 2015 (inclusive). Patients were classified as previously treated patients (PTPs) or previously untreated patients (PUPs). PUPs are defined as those patients who had never been treated with clotting-factor concentrates.

Variables and Data Sources

Treatments with pdFVIII and visits were at the discretion of the physician. However, patients were required to attend for at least one annual visit at the hemophilia treatment centers. Most of the patients had three to four routine visits per year.

Variables as described below were documented in paper CRFs and are summarized in more detail in ►**Supplementary Table 1**. In addition, the doses administered of pdFVIII, bleedings, and reasons for administration were recorded in patient diaries.

Data Sources and Measurement

Data were entered from the CRFs and diaries into the clinical database at Metronomia Clinical Research GmbH (Munich, Germany). In addition, safety data for all patients were documented at the safety department at Biotest AG.

Assessment of Effectiveness

Effectiveness was analyzed by the annual bleeding rate (ABR), by assessing the expected therapeutic effect and by global assessment of effectiveness, ease of use, the patient's condition, and joint status as judged by the investigator and a global hemostatic assessment by the patient. Documented bleedings and calculations of the ABR included traumatic and spontaneous bleedings. "Bleeding" was not predefined. Documented days with bleedings in the patient diaries, bleeding episodes per month, per year, per 3- to 4-year period (1998–2002, 2003–2007, 2008–2012, and 2013–2015), and over the whole study period, taking into account the actual treatment, were used for analyzing the ABR.

For global assessment of effectiveness, the possible ratings were "very good" (1), "good" (2), "moderate" (3), "poor," (4) and "none" (5). For global assessment of ease of use, these were "very good" (1), "good" (2), "satisfactory" (3), "adequate," (4) and "poor" (= 5).

Treatment and effectiveness data obtained from patients during immune tolerance induction (ITI) treatment were not included in the summaries of effectiveness.

The scores from global assessments by investigators and patients were analyzed as continuous variables. For each patient a mean of all ratings over the whole study was first calculated, and this was then summarized for all patients.

Safety and Tolerability Assessment

Adverse events (AEs)—including any bleedings due to suspected lack of efficacy of prophylactic treatment, aggravation of underlying or newly diagnosed diseases, and laboratory assessments of selected clinical, biochemical, and hematological variables—were documented. They were assessed (and, if clinically relevant, commented on) by the physician; any deviation from normal range, clinical relevance, severity, seriousness, and pdFVIII relationship were taken into account. In contrast to most studies, the presence of a FVIII inhibitor was already documented after the first positive result (above the normal range ≥ 0.6 Bethesda units, BU, per mL, depending on the center-specific sensitivity of the assay) without the need for confirmatory testing.

A global assessment of tolerability was performed by both the investigator and the patient using the possible ratings: "very good" (1), "good" (2), "moderate," (3) or "poor" (4). The same scale was used by physicians to assess the subjective conditions of the patients.

Treatment Regimen

Dosage and frequency of the pdFVIII administration were based on the Summary of Product Characteristics. pdFVIII was administered at the physician's discretion. Reasons for pdFVIII treatment were classified by the physician as "prophylaxis," "bleeding," "follow-on treatment" (on demand), or "surgery" ("surgery" as an option for treatment was not available at the start of the study, but was introduced with a CRF update in 2013); there were no study-specific definitions for the different treatment regimens, which were analyzed as documented by the investigator.

Statistical Analysis

Descriptive statistical methods were applied for final analysis. Generally, categorical variables were summarized by number of observations (*N*), frequency count (*n*), and percentage of patients at each level of response. Continuous variables were summarized by *N*, mean, standard deviation (SD), median, minimum, and maximum values. Data were analyzed by means of the SAS system version 9.4 (SAS Institute Inc.; Cary, North Carolina, United States).

Results

Patients and Treatment

Starting in January 1998, a total of 199 male patients were enrolled and 198 were analyzed until the end of 2015. One patient was excluded from effectiveness analysis because no treatment data were recorded. Of the remaining 198 patients, 52 were treated at Hungarian and 146 at German centers. Thirty patients were enrolled as PUPs and 168 as PTPs. A subgroup of 160 patients had severe hemophilia A (FVIII residual activity $\leq 1\%$); 137 patients with severe hemophilia A were enrolled as PTPs and 23 patients as PUPs (►**Table 1**).

During the 18-year observation period, the mean (±SD) documentation time for all patients was 7.3 ± 5.1 years; for patients with severe hemophilia A it was 7.8 ± 5.0 years.

The mean number of infusions for all patients was 665.9 ± 580.8.

A total of 1,418 patient-years (including 1,215 patient-years in patients with severe hemophilia A) were documented.

Table 1 Demographics—status of treatment, severity of hemophilia A, age, treatment modality at inclusion, and treatment modality at the end of study

Age at inclusion (y)	PTP ($N=168$)[a]		PUP ($N=30$)[b,c]		All ($N=198$)	
	Severe ($n=137$)[d]	Nonsevere ($n=31$)	Severe ($n=23$)	Nonsevere ($n=7$)	Severe ($n=160$)[d]	Nonsevere ($n=38$)
	n (%)		*n* (%)		*n* (%)	
<1	3 (2.2)	0 (0.0)	16 (69.6)	1 (14.3)	19 (11.9)	1 (2.6)
1	1 (0.8)	0 (0.0)	3 (13.0)	1 (14.3)	4 (2.5)	1 (2.6)
2–5	8 (5.8)	1 (3.2)	2 (8.7)	3 (42.8)	10 (6.3)	4 (10.5)
6–11	11 (8.0)	1 (3.2)	2 (8.7)	1 (14.3)	13 (8.1)	2 (5.4)
12–17	20 (14.6)	3 (9.7)	0 (0.0)	0 (0.0)	20 (12.5)	3 (7.9)
18–64	94 (68.6)	22 (71.0)	0 (0.0)	0 (0.0)	94 (58.7)	22 (57.9)
65–74	0 (0.0)	3 (9.7)	0 (0.0)	1 (14.3)	0 (0.0)	4 (10.5)
Over 74	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.6)
Treatment modality at inclusion						
Prophylaxis	62 (45.3)	16 (51.6)	8 (34.8)	3 (42.9)	70 (43.8)	19 (50.0)
On demand	75 (54.7)	15 (48.4)	15 (65.2)	4 (57.1)	90 (56.2)	19 (50.0)
Treatment modality at the end of study						
Prophylaxis	96 (70.1)	18 (58.1)	23 (100.0)	4 (57.1)	119 (74.4)	22 (57.9)
On demand	41 (29.9)	13 (41.9)	0 (0.0)	3 (42.9)	43 (26.9)	16 (42.1)

Abbreviations: ED, exposure day; PTP, previously treated patient; PUP, previously untreated patient.
[a]Including minimally treated patients with fewer than 50 EDs and 150 EDs.[22]
[b]One PUP received at the very first treatment a different pdFVIII product, before starting treatment with pdFVIII.
[c]One PUP with severe hemophilia A was treated for 24 EDs during the study and was then lost to follow-up.
[d]Missing for 2 patients at inclusion and 1 patient throughout the study period; presented as patient with severe hemophilia A.

Twenty-four patients were followed up for more than 15 years, of whom 21 were PTPs and 3 (initially) PUPs.

Demographic Data

All patients were male and almost all were Caucasian, with 4 exceptions: 2 were black, 1 was Asian, and 1 was Arabian. At the time of inclusion in the NIS, patients had a mean age of 25.1 ± 18.7 years, with ages ranging from 0 to 80 years. PTPs were aged 28. 9 ± 17.0 years, ranging from 0 to 80 years, and PUPs were aged 3.5 ± 11.8 years (median 1.0 years), ranging from 0 to 65 years (details in ►Table 1). The oldest PTP turned 88 years old during the study.

Treatment Data

A total of 213,471,262 international units (IU) of pdFVIII were administered in 130,595 treatments during the study period; of these 70.5% were given as prophylactic treatment and 29.1% as bleeding or follow-on treatment.

On average, 31.6 ± 15.2 IU/kg body weight (BW) were administered per exposure. Patients received on average 8.3 ± 5.1 treatments per month (►Table 2).

Effectiveness

The ABR was considerably lower for patients who were treated prophylactically (mean ABR of 5.4 ± 6.3; median 3.1; range 0.0–30.5) compared with those patients treated on demand (mean ABR of 26.1 ± 19.2; median 21.9; range 0.0–113.3) for the entire study period. Spontaneous and traumatic bleedings were included in calculating the ABR. The mean dose of prophylaxis (31.5 ± 15.4 IU/kg BW; median 28.9 IU/kg BW; range 9.1–120.4 IU/kg BW; see ►Table 2) and the mean frequency per month (6.4 ± 5.9; median 5.7; range 0.0–30.0; $n=156$) of prophylactic treatments varied substantially between the patients. Overall, considering all patients, the ABR decreased over time, from a median ABR of 20.7 (mean 23.7 ± 23.6; $n=90$) in the period 1998 to 2002 to 5.2 (mean 13.2 ± 18.8; $n=123$) in the period 2008 to 2012 and finally to 2.6 (mean 7.2 ± 10.4; $n=70$) in the period 2013 to 2015. In parallel, the proportion of patients receiving prophylactic treatment increased during the long-term treatment with pdFVIII from 41.1% (37/90 patients) in 2003 to 52.3% (46/88) in 2008, to 63.6% (42/66) in 2013, and finally to 65.7% (44/67) in 2015. These results were similar for patients with severe and nonsevere hemophilia A. The increasing proportion of prophylaxis during the whole study period can also be seen when comparing the treatment modalities of patients at inclusion ($N=198$) with their treatment modalities at the end of their study periods (►Table 1). A higher increase of prophylaxis was observed in patients with severe hemophilia A and PUPs at inclusion compared with patients with nonsevere hemophilia A and PTPs at inclusion.

As presented in the interim analysis,[9] the type of treatment varied considerably between patients treated in Germany or Hungary. These initial country-specific differences in regimens and dosing decreased during the study period.

The joint status was documented for 57 patients from 2010 onwards. Of these patients, 53 had affected joints, of

Table 2 Treatment and dosing data; annual bleeding rate

Mean ± SD	$N = 198$[a]	n (severe) = 160[a]	n (nonsevere) = 38
Total doses administered (IU)	1,089,139 ± 1,028,721	1,197,545 ± 1,035,781	691,213 ± 908,873
Doses administered per year (IU/y)	164,965 ± 123,343	173,159 ± 122,918	133,951 ± 123,141
Doses administered per year and body weight (IU/kg/y)	3,277 ± 3,381	3,557 ± 3,623	2,131 ± 1,764
No. of infusions administered per year	99.9 ± 61.1	106.5 ± 62.7	69.7 ± 44.8
No. of infusions	665.9 ± 580.8	744.6 ± 593.5	366.7 ± 415.3
Percentage of infusions, on-demand (%)[b]	34.9 ± 36.6	33.6 ± 35.9	41.7 ± 39.8
Percentage of infusions, prophylaxis (%)[c]	64.3 ± 37.3	66.1 ± 35.8	55.6 ± 42.7
Percentage of infusions, surgery (%)	0.8 ± 3.7	0.3 ± 0.8	2.7 ± 8.0
Dose per infusion (IU/kg)	31.6 ± 15.2	31.78 ± 15.7	31.68 ± 13.0
On-demand[b]	33.4 ± 17.4	33.7 ± 18.5	32.6 ± 12.4
Prophylaxis[c]	31.5 ± 15.4	32.3 ± 15.8	28.4 ± 13.3
Surgery	60.7 ± 37.6	62.9 ± 41.1	51.9 ± 17.1
ABR[d] during prophylaxis[c] (median)	5.4 ± 6.3 (3.1)	5.8 ± 6.7 (3.3)	3.4 ± 3.5 (2.2)
ABR[d] during on-demand[b] treatment (median)	26.1 ± 19.2 (21.9)	30.8 ± 18.4 (29.3)	7.9 ± 7.4 (5.6)

Abbreviations: ABR, annual bleeding rate; ED, exposure day; SD, standard deviation.
[a]Data derived from 40–196 (32–156/8–38) patients as applicable and available.
[b]"On-demand" means either primary treatment of a bleeding episode or follow-on treatment, if needed, for the same episode.
[c]Prophylaxis was not predefined and varied in administered dosages and their frequencies.
[d]Including traumatic and spontaneous bleedings.

which 47 patients had an impact on their daily life. Walking impairment and total knee replacement was documented for 24 and four patients, respectively.

Overall, 156 surgical procedures were performed successfully in 79 patients under long-term therapy with pdFVIII; of these 145 were performed in 72 PTPs and 11 in 8 PUPs. Twenty-nine procedures were successfully performed in 18 patients with nonsevere hemophilia A (of these five were in two PUPs).

Investigators assessed the therapeutic effect of treatment as "successful" for nearly all (99.4%) treatments.

The majority of both investigators and patients rated the global effectiveness as "very good": mean assessment by investigators was 1.3 ± 0.4 (range 1.0–2.5; $n = 162$) and by patients 1.4 ± 0.5 (range 1.0–3.5; $n = 160$).

On average, patients judged their overall satisfaction with their hemophilia A treatment as "good" (1.7 ± 0.5; range 1.0 to 3.0; $n = 162$). The physicians on average assessed the subjective condition of 163 patients as "good" (1.7 ± 0.5; range 1.0–4.0).

The global ease of use was assessed as "good" or "very good" by the majority of both investigators (1.4 ± 0.5; range 1.0–3.0; $n = 162$) and patients (1.5 ± 0.5; range 1.0–3.0; $n = 160$).

Safety and Tolerability

During the study period (January 1998–December 2015), within 1,418 patient-years, 10 AEs in seven patients were considered pdFVIII-related, resulting in an adverse drug reaction (ADR) rate of <0.1 per patient, 7.1 per 1,000 patient-years, and 0.1 per 1,000 administrations.

All ADRs involved FVIII inhibitor formation documented in 7 patients with severe hemophilia A (3 PUPs and 4 PTPs: "PUP1–3" and "PTP1–4"; see details in ►**Supplementary Table 2**). All seven patients recovered from the 10 ADRs. In two patients (PUP3, PTP4), the inhibitor formation was recorded twice and in PUP3 an increased bleeding tendency was additionally documented.

From January 1998 to December 2015 (inclusive), no pdFVIII-related thrombosis, thromboembolic event, or hypersensitivity reaction and no suspected viral transmission related to pdFVIII was documented.

During the study, no unexpected information on the safety and tolerability of pdFVIII was acquired.

The incidence of all FVIII inhibitor formation in PUPs with severe hemophilia A was 13% (3/23), and 4% (1/23) for high-titer FVIII inhibitors.[3,10]

One PUP (PUP2) of African ethnicity[11,12] developed a high-titer (≥5 BU/mL) and persistent inhibitor with a peak titer of 320 BU/mL (ED 6).[10] After 11 months of ITI (Bonn Protocol) with pdFVIII, inhibitor test results remained negative.

The other two PUPs showed transient and low-titer inhibitors (peak titers of 0.9 and 1.6 BU/mL; ED 16 and ED 27).[3,10] The first pdFVIII exposure of PUP1/PUP2 was linked with a severe bleeding and surgical procedure requiring concomitant red blood cell transfusion and ≥10/≥5 consecutive days of intensive pdFVIII treatment.[11–22]

PUP3 was first exposed to pdFVIII at an early age (6 months) and suffered form an intron-22 inversion.[11,12,23] Inhibitor results remained negative in PUP1/PUP3 after a few days/months of three times weekly doses of approximately 50 IU/kg pdFVIII.

During the study period, overall four PTPs with severe hemophilia A developed inhibitors, thereof three high-titer inhibitors, resulting in frequencies of 3.3 inhibitors and 2.5

high-titer inhibitors in 1,000 patient-years with severe hemophilia A.[3,10] Three PTPs developed transient FVIII inhibitors without any change in treatment. All three PTPs had single high-titer inhibitor results (5–8 BU/mL) that were not confirmed. Consecutive low-titer inhibitor results were documented for only two of these three PTPs. A fourth PTP (PTP3) developed a low-titer and persistent inhibitor.[10] During follow-up observation after the study period, inhibitor titer did not increase but a high-dose ITI with pdFVIII was initiated. After 1 month and an initial booster to 30 BU/mL inhibitor, results remained negative (details in ►Supplementary Table 2).

For only one patient (PUP3) with inhibitor formation, an increased bleeding tendency was documented (►Supplementary Table 2). No other bleeding within the study was documented in relation to FVIII inhibitor formation. However, for five patients with FVIII inhibitor development (PUP1, PUP2, PTP2, PTP3, and PTP4), severe bleedings with coexisting inflammation, requiring ≥3 up to ≥10 consecutive days of pdFVIII treatment, were identified as a risk factor for inhibitor formation. In addition, two PTPs with inhibitor formation were treated on demand or had been treated on demand in the past.[11–13,18–20,24–27]

Overall, 16 PUPs with severe hemophilia A suffered from a total of 32 severe bleeding episodes requiring peak treatment moments within their first 50 EDs. Two of these patients (PUP1, PUP2) needed treatment with packed erythrocytes concomitantly with pdFVIII.[3,11–13,20,21]

Two further suspected pdFVIII-related nonserious AEs (joint bleeding, hematoma; cause not documented) were documented by the physician for one adolescent patient with severe hemophilia A. Both AEs were assessed by the sponsor as not pdFVIII-related, but rather as symptoms of the underlying hemophilia A and probably low prophylactic doses (15 IU/kg BW) and their low frequency (~1–2 per week). Neither the dosage or the frequency of pdFVIII therapy nor the patient's concomitant medication was changed. No FVIII antibody was diagnosed and the therapeutic effectiveness of pdFVIII was judged to be "very good" by the physician.

Overall, tolerability was assessed globally as "very good" or "good" by both investigators (1.2 ± 0.4; range 1.0–2.0; $n = 162$) and patients (1.3 ± 0.4; range 1.0–2.0; $n = 160$).

Discussion

To our knowledge, this is the longest NIS of a single FVIII concentrate conducted so far, investigating real-life effectiveness and safety in clinical daily routine of prophylactic or on-demand treatment of hemophilia A and its influence on patient health.[6,28–31] Within this unique study, a broad range of patients with severe as well as nonsevere hemophilia A, covering all age groups (postnatal up to 88 years), was investigated.

Within the study period of nearly two decades, pdFVIII was effective and well tolerated, and no pharmacological safety concern was raised. FVIII inhibitor development within the study was mainly transient, with low titers and without clinical relevance, and was manageable.

In addition to the severe form of hemophilia A, all patients with inhibitor formation showed various risk factors for inhibitor formation.[25,27] Several risk factors are known or currently under discussion: these include the regimen and intensity of factor replacement, age at the start of treatment, coexisting inflammation, type of FVIII product, F8 mutation type, ethnicity, family history of inhibitors, and severity of hemophilia A.[5,11–14,16–21,25,32–37] Inflammatory danger signals, such as recurrent joint bleeding and severe bleeding episodes (including surgical procedures) requiring peak treatment moments, seem to increase the risk of developing mainly transient and low-titer inhibitors without clinical relevance in both PUPs and PTPs treated on demand with pdFVIII.[3,11–14,18–20,24–27,37]

Overall, rates of inhibitor formation in PUPs and PTPs were low, and lower than or similar to those reported for other pdFVIII products, including the comparisons between plasma-derived and recombinant FVIII products.[5,6,11,22,36] The higher inhibitor incidence observed in the SIPPET study (27%) for pdFVIII concentrates, in contrast to the overall inhibitor incidence in the present study (13%), may be explained *inter alia* by the different ethnic origins of the patients and the different study settings. The SIPPET study was a randomized, controlled trial with defined inhibitor assessments.[22,38] However, the incidence of inhibitor formation in PUPs with severe hemophilia A in the present study (overall 13.0%; high-titer 4.3%) was comparable to the incidence reported for another vWF-containing pdFVIII product (overall 11.1%; high-titer 8.9%)[38] and lower compared with incidences reported in the EUHASS,[39] RODIN, and CANAL studies.[12,15] Considering applied definitions of inhibitors,[10] for PTPs, inhibitor incidences were similar to those observed in the EUHASS study or reported by Kempton et al[40] and Xi et al,[5] but lower than those observed in the United Kingdom (>5.3).[6]

There were limitations in data acquisition due mainly to the long observational period, especially at the beginning of the study, mostly on account of items documented at the start of the NIS two decades earlier. Therefore, underreporting of AEs, such as those related to bleeding (e.g., pain or causes of [traumatic] bleeding), elective procedures and underlying hemophilia A, related comorbidities (e.g., arthropathy) and their aggravation cannot be ruled out. However, the documentation and reporting of clinically relevant events, e.g., FVIII inhibitor formation or thromboembolic events, are assumed to have been documented completely.

In addition, no especial attention was given to the development of joint status over time, but only the status of affected joints and their impact on daily life was documented from 2010 onwards. ABRs were calculated on the basis of bleedings (for which no definition was prespecified) and were documented in patient diaries, without the requirement that they be medically confirmed.

Notably, in this study "real-life patients"—i.e., including high-risk patients—instead of carefully selected patients were observed.

Conclusions

This study covered 18 years of observation and documented 1,418 patient-years. Patients with hemophilia A received long-term prophylactic and on-demand therapy with a pdFVIII concentrate containing vWF. The study has demonstrated that

this treatment was effective, safe, and well tolerated, with a low incidence of inhibitor formation in PTPs and PUPs.[5,11,38]

Novel therapies, including nonfactor therapeutic agents and gene therapy, are currently being established in hemophilia treatment. Their long-term outcome will need to be established. To date, all novel treatments still rely on some FVIII replacement therapy, i.e., in cases of bleeding. Thus, data on efficacy and safety obtained during long-term therapy with pdFVIII reaffirm that there is no unexpected adverse effect on the health of hemophilia A patients.

What is known about this Topic?

- Collecting real-life safety and effectiveness data during long-term hemophilia A treatment from clinical practice and daily routine to investigate the treatment's influence on the health of patients of all age groups is essential to prove the long-term tolerability of the FVIII concentrates used and to ensure consistency with the outcomes of clinical studies.
- In long-term noninterventional studies "real-life" patients (including high-risk ones) are included, rather than carefully selecting the patient population.
- Development of FVIII inhibitors remains the most serious complication of the therapy with FVIII concentrates in patients with hemophilia A.

What does this Paper Add?

- Within the 18 years covered by this noninterventional study, investigating patients with severe as well as nonsevere hemophilia A and aged postnatally up to 88 years, therapy with pdFVIII was assessed as being very effective and well tolerated.
- No unexpected adverse effect on the health of patients, no pdFVIII-related thrombosis, thromboembolic event, or hypersensitivity reaction, and no suspected viral transmission related to pdFVIII were documented.
- FVIII inhibitor development was mainly transient, with low titers, without clinical relevance, and comparable to postmarketing data on other pdFVIII concentrates.

Authors' Contributions

L.N. and C.K. contributed with a significant number of patients to this NIS. A.B. and T.B. designed the Biotest NIS. A.B., J.S., T.B., and S.F.K.K. performed the research. P.D. was responsible for data management and provided statistical support. S.F.K.K., A.B., T.B., and J.S. analyzed the data. The main writing was done by S.F.K.K. Analyses were discussed with W.M., C.K., H.R., and L.N.

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Supplementary Table 1 Variables of the study

Variables	Doc. at inclusion	Contin. doc. at each visit
Demographic data: age, sex, ethnicity	X	–
Body measurements: body height and weight	X	X
History of hemophilia (treatment): date of the initial diagnosis, FVIII baseline activity, previous hemophilia treatment	X	–
Family history of parents: immune deficiencies or infections after previous transfusion	X	–
Hepatitis vaccination: hepatitis A and B (if yes: current titer)	X	–
Consumption habits of: alcohol, tobacco, and recreational drugs	X	–
Treatment regimen and reason for treatment: incl. FVIII dose (IU/kg, IU total) and no. of doses per week	X	X
Therapeutic effect: yes or no	X	X
Global assessment of effectiveness by patient and physician[a]	X	X
Overall assessment on patients' satisfaction with hemophilia treatment by patient[a]	X	X[b]
Global assessment of ease of use by patient and physician[c]	X	X
Annual bleeding rate: bleeding days/episodes per treatment regime per month, year, and 3 to 4-year period; site of bleeding	X	X
Joint status: if yes: affected joints, impact on daily life, walking impairment, TKR implanted?	X[d]	–
Subjective condition of the patient by physician[e]	X	X
Concomitent/intercurrent disease and concomitant medication	X	X
Status of possible FVIII inhibitors (BU)	X	X
Clinical chemistry and hematology parameters: bilirubin, AP, SGPT, SGOT, γ-GT, LDH, hematocrit, platelets, CD4- and CD8-positive lymphocytes	X	X
Serological parameters: anti-HBs, HBs-Ag, anti-HBc, anti-HCV, anti-HAV, IgG, IgM, anti-HIV-1/2	X	X
Global assessment of tolerability by patient and physician[e]	X	X
Adverse events (diagnosis or signs/symptoms): including date of onset, course, intensity, duration, outcome, seriousness, corrective action/treatment, pdFVIII-relationship	X	X

Abbreviations: Contin., continuous; Doc., documentation; TKR, total knee replacement.
[a]Possible ratings were "very good" (1), "good" (2), "moderate" (3), "poor," (4) and "none" (5).
[b]Only at the last visit.
[c]Possible ratings were "very good" (1), "good" (2), "satisfactory" (3), "adequate," (4) and "poor" (5).
[d]Documented with CRF update only from January 2010 onwards.
[e]Possible ratings were "very good" (1), "good" (2), "moderate," (3) or "poor" (4).

Supplemental Table 2 Details of PUPs/PTPs with FVIII inhibitor formation during the study period

PUP/ PTP no.	Onset of development (ED, age) Type of antibody; Peak of titer Number of consecutive positive antibody tests Clinically relevant according to the physician	Corrective treatment Outcome
PUP1	ED 16, 15 months Transient, low-titer, peak titer of 0.9 BU/mL; 2 consecutive positive tests within 1 week, approx. 1 week later negative results. No (reportedly, no increased bleeding tendency exists).	Resolved through the start of prophylaxis 3 times weekly 500–1,000 IU, later 250 IU. After a few days and pdFVIII substitutions, FVIII antibody was no more detectable.
PUP2	ED 6, 11 months Persistent, high-titer, peak titer of 320 BU/mL; 3 consecutive positives tests: 1st high-titer (320 BU/mL), 2nd after approx. 5 months after start if ITI (low-titer: 1.74 BU/mL), and 3rd after a further month (low-titer); afterwards negative results. Yes.	Resolved through successful ITI performed with pdFVIII including bypassing agents: after port catheter insertion twice daily 1,000 IU pdFVIII and twice daily 500 IU bypassing agent. After approx. 11 months after antibody development first negative antibody test (0 BU/mL).
PUP3	ED 27, 10 months Transient, low-titer; peak titer of 1.6 BU/mL; 3 consecutive positive tests within approx. 8 weeks. No (first 2 results documented as clinically not relevant, no judgement of relevance of the 3rd result); only 1 bleeding, a traumatic laceration at the left eyebrow 3 months after 1st positive result; on-demand pdFVIII on 2 consecutive days (effectiveness of pdFVIII as "very good“ by physician). Overall assessed as not clinically relevant by the authors.[3]	Resolved through “intensified prophylaxis” (55 IU/kg 3 ×/wk); neither the dosage nor the frequency of pdFVIII therapy was changed after traumatic laceration; “Borderline“ test result approx. 3.5 months after first positive test. After approx. 8.5 months after first positive result, all antibody tests remained negative (0 BU/mL).
PTP1/2	14 years/12 years Transient, high-titer; peak titer of 5 BU/mL; according to the laboratory, not significantly increased. 2 consecutive positive results/1 single positive test measured on the same day with the same result as for PTP1 (his brother) in the same laboratory; afterwards all results negative. No (no increased bleeding tendency).	None, prophylactic dose and frequency unchanged; Negative at next regular control approx. 6 months after peak titer. The physician documented that owing to the prior polypragmasy an unequivocal causality assignment to one of the administered preparations was not possible.
PTP3	42 years Low-titer antibody, persistent; peak titer of 3.3 BU/mL; 3 consecutive positive test results, each after approx. 6 months. No (within study period, physician assessed as not clinically relevant and “low responder“/no increased bleeding tendency).	None within course of the study. Approx. 2.5 years after antibody appearance (approx. 0.5 years after last study visit), ITI successfully performed with pdFVIII: after a Port-A-Catheter insertion, start daily doses of 2 × 8,000 IU of pdFVIII; initial booster to 30 BU/mL; ∼1 month later negative test results. Reportedly, no increased inhibitor titer was observed before the start of ITI.
PTP4	52-year-old: 2 consecutive positive tests (8.0 BU/mL, 2 weeks later drop to 2.3 BU/mL), 59-year-old: 1 positive test (2.4 BU/mL), and 60-year-old: 1 positive test (3.5 BU/mL), next all negative. Transient, low-titer, and fluctuating FVIII antibody (recurrence). No (reportedly, the patient is known to be a low-titer transient alloantibody patient of no clinical relevance).	Reportedly, no change in treatment was necessary in this patient. Patient recovered after 4 months.

Abbreviations: ABR, annual bleeding rate; ED, exposure day; ITI, immune tolerance induction; pdFVIII, plasma-derived FVIII concentrate; Haemoctin/ Faktor VIII SDH Intersero; PTP, previously treated patient; PUP, previously untreated patient.

4. Long-term analysis of the benefit of prophylaxis for adult patients with severe or moderate haemophilia A

4.1 Abstract

Introduction: Early prophylaxis with factor VIII concentrates in children with haemophilia A is the current standard of care. The benefit of prophylactic treatment for adult haemophilia A patients is not commonly accepted.

Aim: To investigate the benefit of prophylaxis over on-demand treatment in adult and elderly patients with severe or non-severe haemophilia A in a real-life setting.

Methods: Data from 163 patients comprising 1202 patient-years were evaluated for 7.5 ± 5.3 years. The effects on the annual bleeding rate (including spontaneous and traumatic bleedings) of treatment with a plasma-derived factor VIII concentrate, the patient's age and disease severity were investigated. The effect of changing the treatment from on demand to continuous prophylaxis on the patients' annual bleeding rates was further analysed.

Results: Prophylaxis had the greatest effect on the annual bleeding rates of patients of any age with severe or non-severe haemophilia A. The difference in annual bleeding rate of all patients treated on demand (median 31.4; interquartile range 27.6, *N*= 83) compared with those treated prophylactically (median 1.3; interquartile range 3.6; *N*= 122) was statistically significant ($p< 0.05$), even for patients with non-severe haemophilia A (median 8.4; interquartile range 15.5 (*N*= 11) versus median 1.5; interquartile range 4.2 (*N*= 17)). Patients, aged up to 88 years, switching from on demand to continuous prophylaxis showed the lowest median annual bleeding rate (1.1; *N*= 51) after their regimen change.

Conclusion: Any (even low frequent) prophylaxis is better than no prophylaxis. Switchers benefitted most, irrespective of age or haemophilia A severity. Therefore, prophylactic treatment and the switch to it is the regimen of choice for patients of any age, including elderly patients, with severe or non-severe haemophilia A.

4.2. Introduction

The severity of bleedings, as the main clinical feature of haemophilia A, is generally correlated with the level of clotting factors.[1] In particular, patients with a residual factor VIII activity of 1–5 % may have frequent spontaneous bleedings that appear to be clinically severe.[2,3]

Factor VIII concentrates are used for factor VIII replacement therapy of haemophilia A.[1] The superiority of prophylactic factor VIII replacement over episodic (on-demand) treatment has been demonstrated for severe haemophilia A.[1,4,5] Starting prophylaxis early in children with severe haemophilia A can wholly or largely prevent life-threatening bleedings, chronic arthropathy and disability, thereby reducing the need for surgical interventions and contributing to improved health and social well-being for haemophilia A patients. Currently, early-started individualised prophylactic regimens for children have become the standard of care to prevent joint bleeding and chronic arthropathy.[1,6-12]

In contrast, the superiority of prophylactic factor VIII replacement over on-demand treatment in adult patients and in patients with non-severe haemophilia A is not generally accepted. Many middle-aged and elderly patients neither start with prophylaxis nor receive prophylactic factor VIII substitutions, and they thus experience limitations in their activities of daily life.[12]

A Cochrane review from 2011 concluded that there was insufficient evidence from randomised controlled trials to confirm that prophylaxis decreased bleedings and related complications in patients with existing joint damage. Therefore, that review suggested that further studies were needed to establish the best preventative regimen, dose frequency and minimum effective dose.[7]

More recently, retrospective and prospective studies have established that even delayed prophylaxis decreases the number of bleedings, the severity of arthropathy and the patient's physical and psychological restrictions, while improving quality of life. These studies have revealed the benefit of (adherent) prophylaxis over on-demand treatment in adolescents and adults with haemophilia A.[9,13-29]

However, less information is available about the benefit of tertiary prophylaxis for reducing the annual bleeding rate and annual joint bleeding rate. Furthermore, although follow-up documentation is limited, especially from real-life settings, opinion is shifting toward recommending life-long prophylaxis.[1,4,5,30-33]

The present prospective long-term non-interventional study was conducted under real-life conditions to investigate the influence of prophylactic factor VIII treatment or the switch to this regimen on the annual bleeding rate of haemophilia A patients of all age groups and haemophilia A severities, and the long-term effects of this regimen on the patients' annual bleeding rates.

4.3. Materials and methods

4.3.1. Study setting and design

The non-interventional study was conducted as a prospective, non-interventional, multicentre, binational, long-term, safety and efficacy study with haemophilia A patients (males of all ages) at 25 German and eight Hungarian haemophilia centres. The study was approved by the relevant Ethics Committees and informed consent was obtained from 2013 onwards.

4.3.2. Study treatment

The plasma-derived factor VIII concentrate administered in this study (study drug) is marketed as Haemoctin® and is manufactured by Biotest Pharma GmbH (Dreieich, Germany). Study drug is produced from human plasma in a manner that complies with the relevant European Pharmacopoeia monograph. The factor VIII molecule is present in a physiological complex with von Willebrand factor without added artificial stabilisers.

All patients were divided into the following three groups depending on their treatment regimen(s) throughout their observation period during the study: (i) patients with only on-demand treatment, (ii) patients with only prophylactic treatment and (iii) those whose treatment regimen changed during their observation time ("switchers"). Patients whose treatment regimen was changed

continuously from on-demand treatment to continuous prophylaxis during the observation period were further investigated, because these switches allowed within-patient comparison regarding the development of annual bleeding rates. Continuous prophylaxis was defined following the World Federation of Hemophilia guideline[1] as prophylactic administration for at least 45 consecutive weeks. From 2010 onwards, the patient's joint status and prophylaxis protocol (primary, secondary or tertiary) was documented in the case report form. In addition, tertiary prophylaxis was assumed if the patients suffered from affected joints and chronic haemarthrosis. Additionally, the frequency and dosing of the patients treated prophylactically were assessed.[34]

The development of the annual bleeding rate of the patients in these three groups was further analysed considering the patients' ages and their haemophilia A severity. Four age groups were chosen (≤ 17, 18–39, 40–64, and ≥ 65 years), based on those used by Scott *et al*.[3]

4.3.3. Variables and data sources

Study drug treatments and visits were at the discretion of the physician. However, the patients were required to attend their haemophilia treatment centre at least once a year. Most of the patients had 3–4 routine visits per year.

The variables described below were documented in paper case report forms. In addition, the doses of study drug administered, bleedings and reasons for administration were recorded in patient diaries.

4.3.4. Data sources and measurement

Data were entered from the case report forms and diaries into the clinical database at Metronomia Clinical Research GmbH (Munich, Germany). In addition, safety data for all patients were documented at the safety department of Biotest AG.

4.3.5. Assessment of effectiveness

Effectiveness was analysed on the basis of the annual bleeding rates and documented bleedings in patient diaries taking into account the actual treatment

regimen. “Bleeding” was not predefined. The annual bleeding rate calculations included both traumatic and spontaneous bleedings. A mean annual bleeding rate was first calculated for each patient and then summarised for all patients. Further methodological details are described elsewhere.[35]

4.3.6. Statistical analyses

Standard descriptive statistical methods were used. The data were analysed by using the SAS system version 9.4 (SAS Institute Inc.; Cary, NC, USA).

The effect of prophylaxis on annual bleeding rate was analysed by Poisson regression with annual bleeding rate as the dependent variable, and the influence of age and/ or the haemophilia A severity was investigated.

Median differences of annual bleeding rates between treatment regimens were evaluated by using the non-parametric Wilcoxon signed-rank test with each test performed at a 5 % alpha level and Bonferroni-adjusted for multiple testing, with a *p* value < 0.05 considered statistically significant.

The following comparisons between treatment regimens were made (Figure 1, page 50):

(1) On demand (*N*= 32) versus prophylaxis (*N*= 71);

(2) On demand before switch (on demand → prophylaxis; *N*= *51*) versus prophylaxis (*N*= 71);

(3) On demand before switch (on demand → prophylaxis; *N*= *51*) versus prophylaxis after switch (on demand → prophylaxis; *N*= 51);

(4) On demand before switch (on demand → prophylaxis) + on demand (*N*= *51+32= 83;* Table 5, page 49) versus prophylaxis + prophylaxis after switch (on demand → prophylaxis; *N*= 51+71= 122; Table 4, page 48);

(5) Prophylaxis (*N*= 71) versus prophylaxis after switch (on demand → prophylaxis; *N*= 51).

This was done for all patients, followed by these with non-severe haemophilia A only.

4.4. Results

4.4.1. Patients and treatment

Long-term data were collected from 164 enrolled patients between May 1998 and December 2015 (Figure 1, page 50). Three patients were excluded from efficacy analysis. For one patient no treatment was documented, for the second only an immune tolerance induction was documented and the third suffered from acquired haemophilia A. Thus, 161 patients were included in the analyses of the effect of the treatment regimen on the annual bleeding rate.

Thirty-two of the 161 patients received study drug as an on-demand regimen throughout their observation period, comprising 21 patients with severe and eleven with non-severe haemophilia A. Seventy-one patients received study drug as a prophylactic regimen throughout their observation period, comprising 54 patients with severe and 17 with non-severe haemophilia A. The remaining 54 patients changed their treatment regimen during the study. Of these switchers, 51 were treated on demand at their inclusion in the study and changed to a continuous prophylaxis with study drug, and the remaining three switched *vice versa*. Four patients with non-severe haemophilia A received study drug during the study only during peri-surgical procedures.

In this study a total of overall 1202 patient years were documented for all patients; of these 1024 were documented for patients with severe haemophilia A. On average, all patients were documented for a time period (± standard deviation) of 7.5 ± 5.3 years (median 6.0; range 1.0 days to 16.5 years). On average, patients were followed up during one continuous treatment regimen for approximately 5–6 years. The 51 patients who switched from on-demand to prophylactic treatment were followed for an above-average period, with a documented duration of 11.5 ± 4.3 years.

4.4.2. Demographic data

Overall, patients with an on-demand regimen were included at greater ages (median 40.5 years) than patients under continuous prophylaxis (median 16.0 years), and patients with non-severe haemophilia A were older at inclusion than

those with severe haemophilia A (median 39.5 versus 20 years; Table 3). For the 51 patients whose therapy changed to continuous prophylaxis, the median age at switching was 30 years, and the maximum age was 82 years.

4.4.3. Treatment data

A total of 185,694,262 IU and 3,354,958 IU/kg study drug were administered in 110,078 treatments during the study period. On average, for all patients the reason for treatment was prophylaxis in 62.4 % (median 80.2%) of infusions and bleeding or follow-on in 36.9% (median 19.5 %) of infusions ranging from zero to 100 %.

At patient level, the mean number of administrations was 683.7 ± 605.9 (median 473.0; range 1.0–2528.0). Patients with severe haemophilia A tended to have more study drug administrations (744.2 ± 623.7; median 517.0).

The combined median prophylactic doses, their frequencies per week and the annual consumption of study drug (in IU per kg body weight) of patients with a continuous prophylactic regimen since inclusion (*N*= 71) and after a switch from an initial on-demand regimen (*N*= 51) are shown in Table 4 (page 48). The combined annual bleeding rate and annual consumption of patients who received on-demand therapies with study drug at inclusion, including those who continued with that regimen (*N*= 32) and those who later switched to prophylaxis (*N*= 51), are displayed in Table 5 (page 49).

4.4.4. Analyses of the effect of treatment regimen on annual bleeding rate

Among the 163 analysed patients, a strong relationship was found between prophylactic treatment and mean annual bleeding rate (Poisson regression, β= –0.02, $p< 0.001$). That means, when administering X prophylactic administrations more per year, the annual bleeding rate decreases by 2X %. Thus, the more prophylactic administrations a patient received, the lower his annual bleeding rate. No differences in this relationship were detected between patients with severe and non-severe haemophilia A or between age groups.

Notably, patients with non-severe haemophilia A who continuously received prophylactic study drug doses had similar or even higher median annual bleeding rates than patients with severe haemophilia A treated continuously prophylactic with study drug (Table 4 and Figure 2, pages 48 and 51).

Overall, the median annual bleeding rate of the patients receiving study drug on demand was considerably higher than the median annual bleeding rate of patients treated prophylactically with study drug during the observation period of this study (Tables 4,5 and Figure 2, pages 48, 49 and 51). Considering all patients with severe and non-severe haemophilia A, the differences in annual bleeding rate were statistically significant ($p< 0.05$) for patients treated on demand compared with those treated continuously prophylactic with study drug (see comparisons 1–4, and comparison 4 for patients with non-severe haemophilia A, described in section “4.3.6. Statistical analyses”, page 38). Although, this difference was higher in patients with severe haemophilia A than in those with non-severe haemophilia A, the difference was also statistically significant ($p< 0.05$) considering all patients with non-severe haemophilia A. A remarkable drop, and an even greater and statistically significant ($p< 0.05$) difference/ benefit, was observed for patients whose on-demand regimen was changed to continuous prophylaxis (Figure 2, page 51). The benefit of the change to continuous prophylaxis over on-demand treatment was demonstrated for all age groups, with high reductions in annual bleeding rate in all haemophilia A patients irrespective of their haemophilia A severity (Figure 3, page 52). For adult and elderly patients, a reduction in median annual bleeding rate of approximately 40 was observed for patients with severe haemophilia A and up to 29 in patients with non-severe haemophilia A.

A very large benefit of switching the regimen to prophylaxis was demonstrated for the oldest patient with moderate haemophilia A, as shown in Figure 4 (page 53).

4.4.5. Haemophilic arthropathy

Joint status was documented for 57 patients, starting in 2010. Fifty-three patients suffered from affected joints, which had an impact on daily life for 47 of

these patients. In total, 27 patients had a walking impairment and four patients underwent a total knee replacement as a result of their affected joints.

Of these 53 patients, 30 switched their treatment regimen from on demand to prophylaxis, five were treated continuously on demand, and 17 were treated prophylactically; of these 17 only three received study drug regularly in respect of dose (20–40 IU/kg) and frequency (2–3 per week). One of these two patients was inhibitor-positive before the observation period, as he had received another factor VIII therapy. The remaining patient received study drug only for immune tolerance induction due to an inhibitor he had received during another factor VIII therapy.

In 28 of the 30 switchers their affected joints resulted in an impact upon their daily lives; 15 experienced walking impairment and one received a total knee replacement. Of the five “on demand“ patients, for four an impact on daily life and in addition for three of these four patients walking impairment was documented. Fourteen of the 17 patients who received continuous prophylaxis suffered from an impact on their daily life, and/ or nine from walking impairment, and three received a total knee replacement because of their affected joints.

From 2010 onwards, for 15 patients (including nine switchers) a tertiary prophylaxis was documented, thereof three with non-severe haemophilia A (including one switcher).

4.4.6. Patients with no or one bleeding per year

Overall, for the 51 switchers, reductions of their annual bleeding rates started directly after switching to a prophylactic regimen (for an example see Figure 4, page 53). Twenty-five (49 %) of the 51 patients whose treatment regimen was changed from on demand to continuous prophylaxis suffered on average from less than one bleeding per year after the switch, and of these 13 (25 %) did not suffer from any bleeding (annual bleeding rate= 0.0), including respectively two and one patients with moderate haemophilia A (2 %). Notably, nine switchers with affected joints suffered from zero bleedings after switching and five additional switchers suffered from less than one bleeding per year.

Of the 71 patients treated by continuous prophylaxis, 25 patients (35 %) on average suffered from less than 1.0 bleedings per year, thereof eleven patients (15 %) suffered from no bleeding at all, including respectively six patients and one patient with moderate haemophilia A. Four additional of these 71 patients had a mean annual bleeding rate of 1.0.

None of the patients treated under the on-demand regimen suffered from ≤1.0 bleedings per year (minimum= 2.9).

4.4.7. Safety and tolerability

A detailed safety analysis is presented elsewhere.[35] Inhibitors were found in 13 % (3/23) and high-titre inhibitors in 4 % (1/23) of previously untreated patients with severe haemophilia A. Four previously treated patients with severe haemophilia A developed inhibitors, thereof 3 high-titre inhibitors (3.3 and 2.5 high-titre inhibitors in 1000 patient-years).[35]

Severe bleedings, requiring treatment peaks of ≥ 3 and up to ≥ 10 consecutive days of on-demand treatment, were identified as a risk factor for inhibitor formation.[36-42] In addition, for previously treated patients on-demand regimen was identified as a potential risk factor.[35,37-39,41-47]

4.5. Discussion

Prophylaxis is the gold standard for the treatment of severe haemophilia A during childhood and adolescence. Recent recommendations state that prophylaxis is the treatment of choice for all haemophilia A patients at any age on account of the improvements in their quality of life. Therefore, this treatment should be continued life-long.[1,4,9,13-28,48-53] However, data on adult patients benefitting from prophylaxis are very limited.

The results of the present analysis using real-life data collected prospectively over a nearly 18-year period support this recommendation and strengthen comparable results of previous studies revealing the superior benefit of prophylaxis compared with on-demand treatment in adolescents and adults with haemophilia A.[9,13-29] This prospective study is the longest non-interventional study of a single factor VIII concentrate reported so far in which

treatment data were collected from the daily treatment of haemophilia A patients.[35] In a broad range of patients with severe as well as non-severe haemophilia A, covering all age groups up to 88 years, long-term prophylaxis with study drug reduced the annual bleeding rate remarkably, in some cases down to zero. Annual bleeding rates of patients receiving continuous prophylaxis were statistically significantly different ($p< 0.05$) from those of patients treated on demand, even for patients with non-severe haemophilia A. This beneficial effect of prophylaxis, including tertiary prophylaxis, on the annual bleeding rate was further confirmed by assessing patients whose treatment changed to continuous prophylaxis and thus could be considered “in-patient controls”. Irrespective of their ages and the severity of their underlying haemophilia A, these switchers experienced the highest median reduction in their annual bleeding rate, which were even lower than the median annual bleeding rate of patients who received continuous prophylaxis from the start of documentation onwards. The reason for switching to prophylaxis was not documented in the case report form. The reason for this can in most cases be assumed to be the high annual bleeding rate during on-demand therapy, including severe bleedings and related consequences, and also (in Hungary) the increasing availability of prophylaxis.

The median frequency of prophylactic doses in the patients with non-severe haemophilia A during continuous prophylaxis was slightly lower than that recommended.[34] This discrepancy might be the reason for their remarkably high annual bleeding rates compared with those of patients with severe haemophilia A. This finding is in line with data recently published by Scott *et al*.,[3] which revealed that patients with severe or moderate haemophilia A suffered from unexpectedly high annual bleeding rates. In contrast to those authors' results, an increase in annual bleeding rate with age was not observed in the present study. However, overall annual bleeding rates as reported by Scott *et al*. were comparable to those reported here.

Overall, the treatment regimen seems to require individualisation with regard to frequency and dosage.[34] Concomitant diseases – including haemophilia-related and non-haemophilia-related comorbidities, such as age-

related diseases – may be one important factor to be considered here, especially in older haemophilia A patients, who are at higher risk of bleeding owing to their comorbidities and ageing.[31,54]

In addition, treatment peaks due to recurring joint bleeding and severe bleeding episodes (including surgical procedures) were identified as risk factors for inhibitor development in both previously treated and untreated patients treated on demand with study drug.[35,37-47]

Therefore, more long-term data should be acquired under real-life conditions, to establish the best prophylaxis regimen, dose frequency and minimum effective dose.[7,32,33,55]

These data, to which the present report may contribute, will help to improve haemophilia A therapy and the quality of life of patients with haemophilia A in everyday clinical practice.[25] Beside non-factor treatment options such as Emicizumab,[56] prophylaxis with factor VIII concentrates can still be considered as an option for helping haemophilia A patients to attain low annual bleeding rates or even a bleeding-free life.

This study had limitations in data acquisition that were due mainly to the long observational period, especially at the beginning of the study, mostly on account of items documented at the start of the non-interventional study two decades earlier. In addition, no special attention was given at the outset to the development of joint status over time; instead, the status of the type of prophylaxis, affected joints, and their impact on daily life were documented only from 2010 onwards. The annual bleeding rates were calculated on the basis of bleedings (for which no definition was prespecified), including spontaneous and traumatic as well as joint and non-joint bleedings, and were documented in patient diaries without the requirement that they be medically confirmed.

Individual prophylactic treatment and its frequency varied substantially within the study, reflecting real-life conditions in daily clinical practice. Notably, in this study “real-life patients” instead of carefully selected patients were observed. Therefore, this data cannot be compared with treatment data from (randomised) clinical trials.[35] However, median annual bleeding rate under continuous prophylaxis in the present study (1.3) was comparable with that

observed in clinical trials with other factor VIII products (0–2) with observation periods of about six months.[23,57-61]

4.6. Conclusion

This unique study of “real-life” long-term follow-up documentation of haemophilia A therapies, including regimen switches, allows one to assess the benefit of prophylaxis over on-demand treatment by within-patient comparisons regarding the development of annual bleeding rates. The analysis revealed a statistically significant benefit of prophylactic factor VIII therapy compared with on-demand factor VIII therapy in daily clinical practice for patients of any age (up to 88 years) with severe or non-severe haemophilia A. Long-term prophylaxis with plasma-derived factor VIII in daily routine was shown to be highly effective, reducing annual bleeding rates to approximately one including all bleedings and patients with haemophilic arthropathy. This benefit was greatest in patients (irrespective of age and severity of haemophilia A) who switched to continuous prophylaxis. Prophylaxis had the greatest effect on the annual bleeding rate of all patients: In about 50 % of the switchers, their average annual bleeding rate decreased to less than one, and 25 % suffered from zero bleedings.

Thus, beside other promising haemophilia A therapy options, prophylaxis with plasma-derived factor VIII and the switch to it even at high age may be considered an effective and safe option, resulting in very low or zero annual bleeding rates.

4.7. Tables

Table 3. Treatment regimen sorted by factor VIII residual activity and age at inclusion

Factor VIII–Activity [%]	**Prophylaxis *N*= 71 (44 %)**	**On demand *N*= 32 (20 %)**	**Switcher OD→ PX *N*= 51 (32 %)**		**Switcher PX→ OD *N*= 3 (2 %)**	**Surgery *N*= 4 (2 %)**	**Total *N*= 161 (100 %)**
Median age (range) [years]	*n* (%)	*n* (%)	*n* (%)		*n* (%)	*n* (%)	*n* (%)
≤ 1 (Severe)	**57 (80)**	**25 (78)**	**47 (92)**		**2 (67)**	**0 (0)**	**131 (81)**
Age [years]	15 (0–59)	32 (18–63)	22 (0–59)	30 (0–69)[a]	25 (9–48)	NA	20 (0–59)
≥ 2 (Non-severe)	**14 (20)**	**7 (22)**	**4 (8)**		**1 (33)**	**4 (100)**	**30 (19)**
Age [years]	21 (2–63)	42 (1–74)	49 (2–80)	59 (3–82)[a]	48	58 (47–65)	40 (0–80)
Total age [years]	**16 (0–63)**	**401 (1–74)**	**22 (0–80)**	**30 (0–82)[a]**	**41 (9–48)**	**58 (47–65)**	**22 (0–80)**

PX, patients with prophylactic regimen; OD, patients with on-demand regimen.

a Age at switch to continuous prophylaxis

Table 4. Annual bleeding rate and annual study drug consumption of patients with continuous prophylaxis at inclusion and after switch

Age groups [years]	**≤ 17**	**18–39**	**40–64**	**≥ 65**	**Total (*N=122=71+51*)**
Severity of haemophilia A	**Median annual bleeding rate; IQR, range (*n*)**				
Severe	2.2; 0.0–19.6 (*43*)	0.8; 0.0–17.6 (*40*)	0.4; 0.0–13.5 (*19*)	3.6; 1.2–6.0 (*2*)	**1.2; IQR 3.5; 0.0–19.6 (*104*)**
Non-severe	3.8; 1.4–9.8 (*6*)	0.3; 0.0–4.5 (*7*)	5.6; 0.5–5.8 (*3*)	0.2; 0.0–0.5 (*2*)	**1.5; IQR 4.2; 0.0–9.8 (*17*)**
Total	**2.3; 0.0–19.6 (*49*)**	**0.7; 0.0–17.6 (*47*)**	**0.5; 0.0–13.5 (*22*)**	**0.8; 0.0–6.0 (*4*)**	**1.3; IQR 3.6; 0.0–19.6 (*122*)**
	Median prophylactic study drug dose; range [IU/kg] (median prophylactic applications per week)				
Severe	31; 5–120 (3.1)	29; 12–48 (2.2)	29; 12–54 (2.1)	27; 25–28 (1.4)	**30; 12–120 (2.5)**
Non-severe	25; 20–45 (2.4)	34; 16–57 (1.2)	25; 12–35 (1.1)	43; 41–45 (4.0)	**28; 12–57 (1.8)**
Total	**30; 15–120 (3.0)**	**29; 12–57 (1.9)**	**28; 12–54 (1.9)**	**35; 25–45 (2.4)**	**29; 12–120 (2.4)**
	Median study drug consumption per year; range [IU/kg]				
Severe	4,595; 485–25,357	3,384; 516–229,000	3,341; 468–7,675	1,991; 1,822–2,161	**3,683; 468–229,000**
Non-severe	2,939; 1,732–7,991	2,023; 818–6,705	1,208; 841–2,478	9,587; 9,099–10,074	**2,478; 818–10,074**
Total	**4,419; 485–25,357**	**2,930; 516–229,000**	**2,368; 468–7,675**	**5,630; 1,822–10,074**	**3,547; 468–229,000**

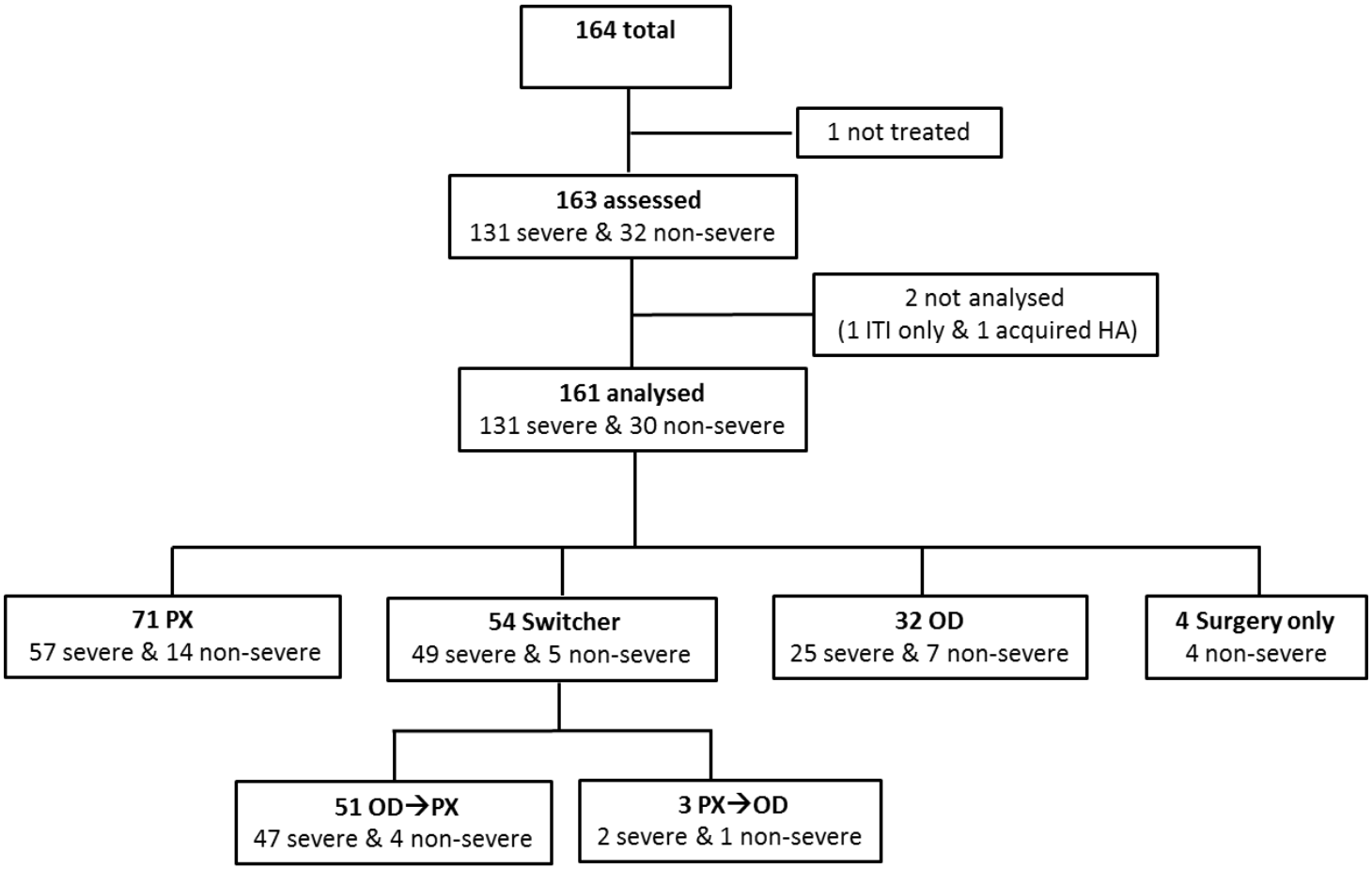
Table 5. Annual bleeding rate and annual study drug consumption of patients with on-demand regimen at inclusion

Age groups [years]	**≤ 17**	**18–39**	**40–64**	**≥ 65**	**Total (*N=83=32+51*)**
Severity of haemophilia A	**Median annual bleeding rate; IQR; range (*n*)**				
Severe	30.4; 1.3–76.0 (*18*)	38.0; 6.2–64.6 (35)	29.2; 2.9–130.2 (19)	-	**33.7; IQR 27.3; 1.3–130.2 (*72*)**
Non-severe	25.4; 21.5–29.3 (*3*)	15.8 (*1*)	6.9; 5.3–9.1 (*4*)	6.0; 2.9–48.8 (*3*)	**8.4; IQR 15.5; 2.9–48.8 (*11*)**
Total	**28.5; 1.3–76.0 (*21*)**	**37.5; 6.2–64.6 (*36*)**	**18.3; 2.9–130.2 (*23*)**	**6.0; 2.9–48.8 (*3*)**	**31.4; IQR 27.6; 1.3–130.2 (*83*)**
	Median study drug consumption per year (range) [IU/kg]				
Severe	895; 27–2,121	1,337 (182–3,583)	1,065 (173–5,176)	-	**1,099 (27–5,176)**
Non-severe	3,059 (2,177–3,941)	1,313	545 (442–1,031)	1,817 (746–2,327)	**1,172 (442–3,941)**
Total	**924 (27–3,941)**	**1,326 (182–3,583)**	**1,020 (173–5,176)**	**1,817 (746–2,327)**	**1,099 (27–5,176)**

IQR, Interquartile range.

4.8. Figures

Figure 1. Patient distribution by treatment regimen



HA, haemophilia A; ITI, immune tolerance induction; PX, patients with prophylactic regimen; OD, patients with on-demand regimen.

Figure 2. Median annual bleeding rates of patients by treatment regimen and severity of haemophilia A

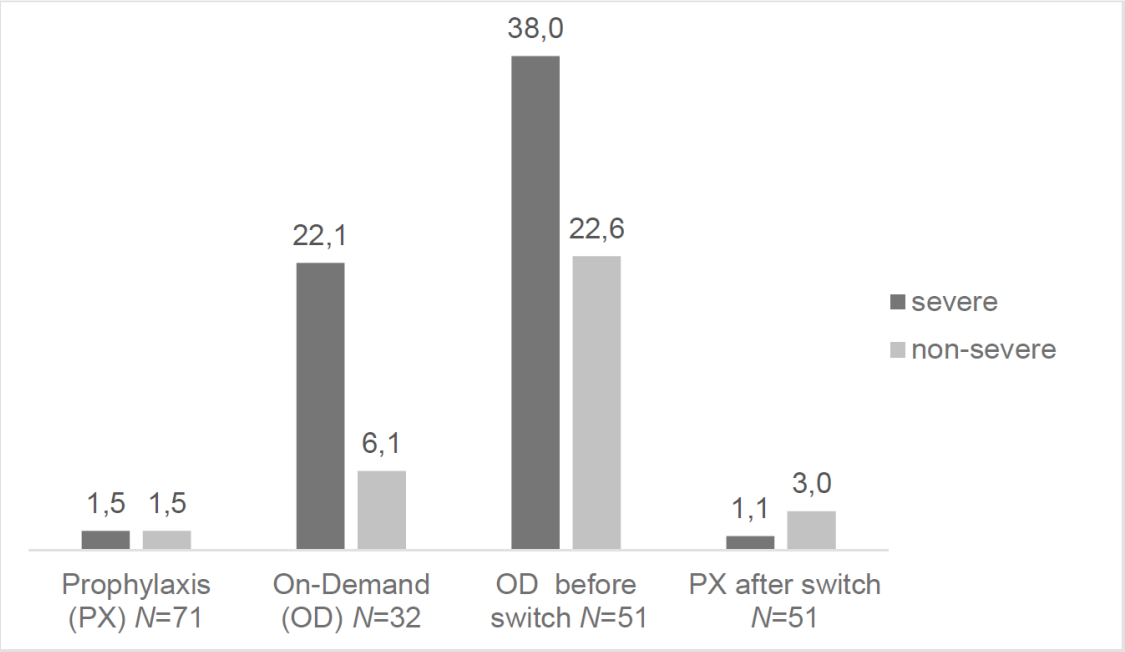
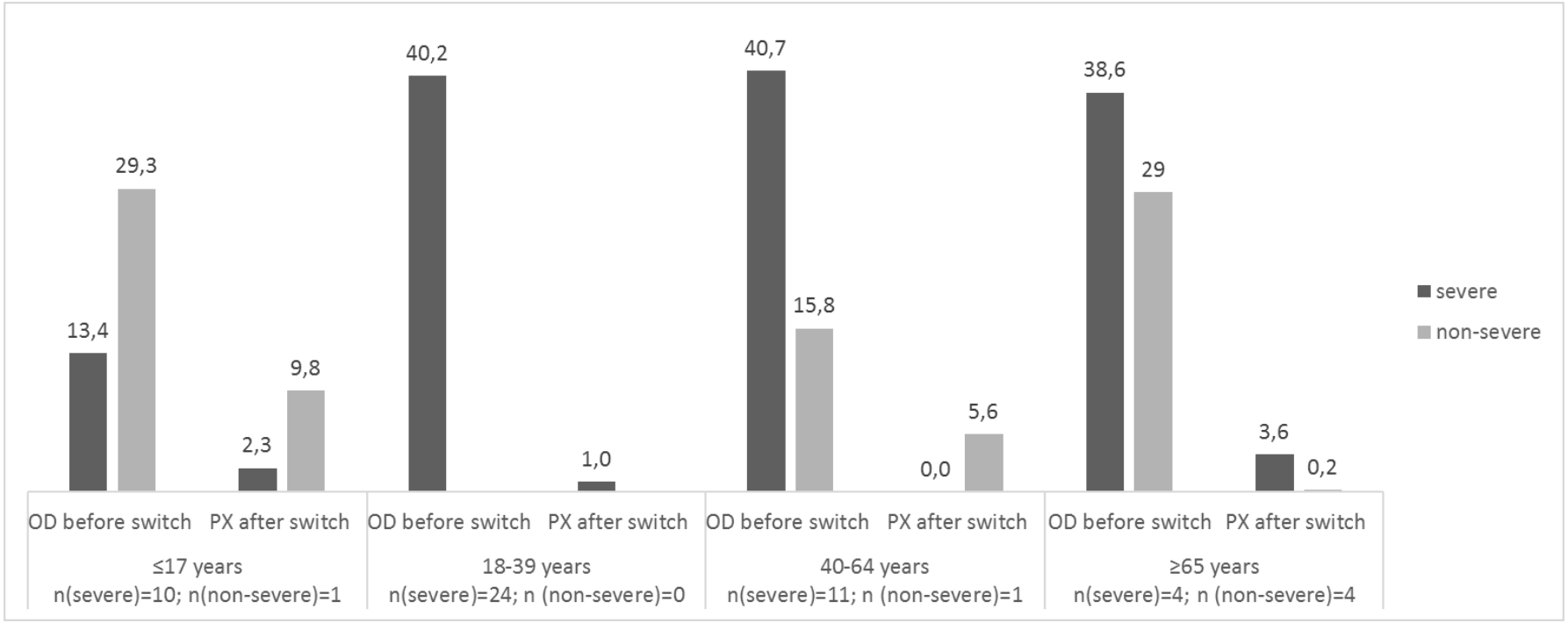
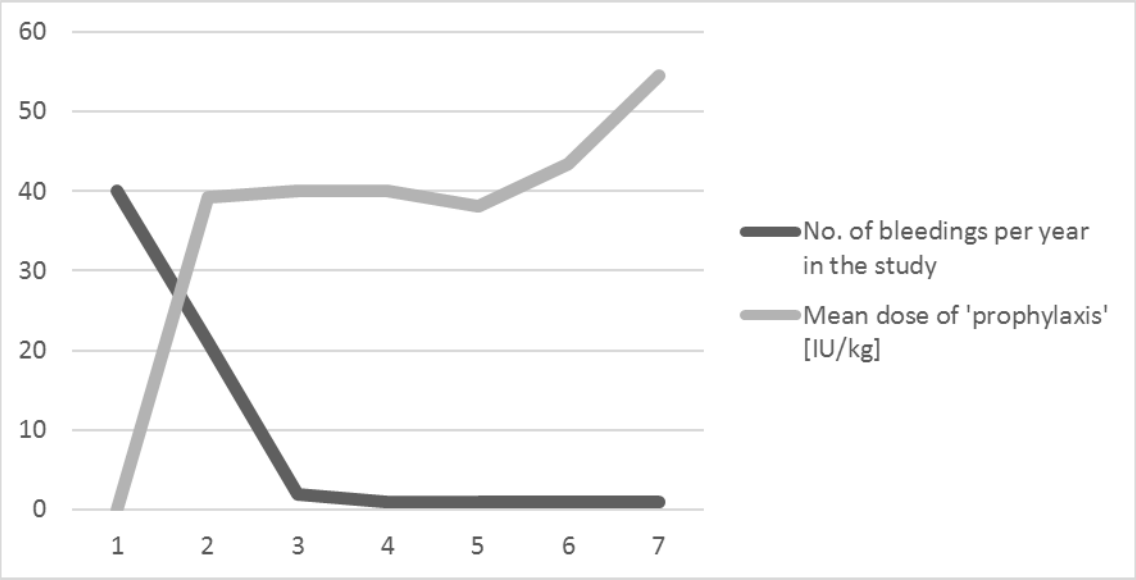


Figure 3. Median annual bleeding rates of switchers (On demand → prophylaxis; *N*= 51) by age group at onset of switch and severity of haemophilia A



PX, prophylaxis; OD, on demand.

Figure 4. Development of the annual bleeding rate and mean prophylactic dose per calendar year in an elderly patient (≥ 65 years; 81–88 years old) with moderate haemophilia A



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5. Own achievements for the manuscripts

5.1. Data collection, review, verification, and combination

Source documents (case report forms) were available of overall 1418 patient-years from 198 patients, which were included between 1998 until end of 2015 in two studies. These efficacy and safety data included all health records documented by the treating physicians and by the patients in patient diaries. During each visit, the physician documented all variables, such as treatment data, adverse events, concomitant diseases and medications, and laboratory results. Most of the patients had three or four routine visits per year at their haemophilia centre, resulting in health records of several thousands visists. All variables of both studies were summarised and presented in Supplemental Table 1 (page 32). In addition, between these visits, patients continuously documented treatment data of their individual long-term therapies in their diaries, resulting in treatment data in diaries of overall 1418 patient-years.

Own achievements, which are described in the following, included the collation and review of the pdf-scans of the paper-based case report forms. In addition, programmed listings of treatment and safety data were reviewed. Medical plausibility checks were performed and data were verfied, focusing on documented treatment data, adverse events (especially factor VIII inhibitor formation), the classifications of all 198 patients as previously treated or previously untreated, and their haemophilia A severity.

Furthermore, these data of the two studies, including 163 and 35 treated patients, were compared with each other to verify a potential combination of the data from both studies. These data included the observation plans, describing the conduct and documentation procedures of each study, and case report forms including all study variables. This verification was done in close cooperation with data management and statistics. As a result, to have a lager data record and to increase the validity, data from both studies were combined for the main analysis on the influence of the long-term treatment on the patients` health presented in the published manuscript. A merge was not possible of the data needed for the additional analyses presented in the submitted manuscript, because these data were available in different databases with different electronic formats.

5.2. Hypothesis and key messages formulation

On the basis of the large data record from 198 patients during 18 years of observation time, the hypothesis was formulated and the key messages of the two manuscripts were identified together with the coauthors.

5.3. Data preparation for (statistical) analyses

The own achievements included the descriptive preparation of parts of the collated and combined data, as described in the following, before transferring the data to a data management and statistical specialist, who after consultation and close cooperation, performed the statistical analyses and created the figures.

All 198 patients were sorted according to their age at inclusion in age classes and according to their haemophilia A severity and presented in Table 1 (page 27). Treatment modalities of all patients at the beginning and at the end of the study documentation period of each patient were retrieved from source documents and presented in Table 1 (page 27). In addition, to combine the data of both studies for the main analysis, treatment, dosing, and bleeding data of both studies were prepared in excel for statistical analyses, and to create Table 2 (page 28).

Adverse drug reaction rates and frequencies of inhibitor formations were calculated. All peak treatment moments, including bleedings which led to the hospitalisation of a patient (serious bleeding event) and surgical procedures were retrieved from source documents of all 198 patients; for previously untreated these were summarised and presented in Table 8 (page 80).

To perform the analyses presented in the submitted manuscript, 163 patients were divided into the three following groups depending on their treatment regimen(s) throughout their observation period during the study: (i) patients with only an on-demand or (ii) prophylactic treatment regimen and (iii) those whose treatment regimen changed during their observation time (=”switchers”). Additionally, the development of mean frequencies and dosages of prophylactic administrations were calculated per patient and then summarised per regimen group. Treatment and bleeding data of patients whose treatment regimen(s) changed continuously from on-demand treatment to

continuous prophylaxis or *vice versa* were further divided into periods of the respective regimen(s). The (development of) annual bleeding rates during each individual therapy regimen were calculated and its increase or decrease in percentage before and after each regimen change. In addition, switchers were divided in age groups depending on when their treatment regimen changed. All patients with the same treatment regimen considering the patients` ages at onset of their switches and haemophilia A severity were divided in four age groups. Tables 3,4, and 5 (pages 47 to 49), Figure 1 (page 50), and the data set to create Figures 2-8 (pages 51 to 53; 82 to 85), were prepared.

5.4. Analyses and discussions

A literature search was performed to retrieve all studies investigating the efficacy and safety of other factor VIII concentrates. A further detailed literature search was performed on risk factors for the development of factor VIII inhibitors.

In detail, all documented adverse events were analysed and assessed with regard to their seriousness, severity, and causal relationship to the study drug considering underlying diseases, concomitant medications, potential contributing factors/ risk factors, and alternative explanations. A more comprehensive analysis of risk factors for the development of neutralising factor VIII antibodies was conducted for all patients and discussed (Supplemental Table 2, page 33, Tables 6 and 7, pages 76 and 78). In addition, all peak treatment moments of all patients due to severe bleedings and/ or surgical procedures were analysed (concerning previously untreated patients, see Table 8, page 80). Surgical procedures were analysed taking into account for example the severity of the underlying haemophilia A of each individual patient and the amount of administered study drug and concomitant medications.

For the discussion, findings from scientific literature were compared with the presented study results. The investigated risk factors and the rate of inhibitor formation were compared with rates of inhibitor formations investigated in (post-marketing surveillance) studies and pharmacovigilance surveillance programmes with other single or various plasma-derived and recombinant factor VIII products.

To evaluate the efficacy of the plasma-derived factor VIII concentrate, the development of the annual bleeding rates and treatment modalities (Table 1, page 27) of all patients were analysed. In addition for the submitted manuscript, the influence of prophylactic factor VIII treatment or the switch to this regimen on the annual bleeding rate of haemophilia A patients of all age groups and haemophilia severities, and the long-term effect of prophylaxis on the patients' annual bleeding rates were analysed (Tables 4,5, pages 48, 49, and Figures 2 to 4, pages 51 to 53). Additionally, the frequencies and dosages of prophylactic administrations were assessed by comparing these with prophylactic dosages and frequencies recommended in the summary of product characteristics of study drug as "regular" or not. It was verified, if a patient received a continuous or intermittent prophylaxis. Treatment data of patients whose treatment regimen changed continuously from on-demand treatment to prophylaxis or *vice versa* were further investigated, because these switches allowed within-patient comparison regarding the development of annual bleeding rates (see in addition highlighted examples in section 7.1, page 82 and subsequent pages).

For the discussion, findings from scientific literature and annual bleeding rates observed in studies of other factor VIII concentrates were compared with the presented study results.

The formulated hypothesis was tested and proven descriptively, and the results of the analyses were discussed with the coauthors.

5.5. Manuscript preparations

Both manuscripts including the tables and figures were written. The manuscripts were reviewed and modified by the coauthors, and the language style was improved by a medical writer.

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7. Appendices

Table 6. Previously untreated patients with factor VIII inhibitor formation during the study period—Analysis of risk factors

Patient number **Age at haemophilia A diagnose/ first factor VIII (study drug) treatment** **Age at onset** **Treatment regimen before inhibitor detection**	**Risk factors at first factor VIII exposure (environmental risk factors)[a]** **and other risk factors[b]**
PUP1 15 months/ 15 months 15 months Haemophilia A diagnosed in context of severe bleeding, which required intensive treatment	First factor VIII exposure linked to severe bleeding caused by fall with tongue bite, which required hospitalisation and surgical procedure, infusion of erythrocyte concentrate (haemoglobin level 4.5 g/dl), and ten consecutive exposure days of treatment with study drug Severe haemophilia A
PUP2 Eleven months/ eleven months Eleven months Early start of intensive on-demand treatment Haemophilia A diagnosed in context of surgery and severe bleeding, which required intensive treatment	First factor VIII exposure linked to surgical procedure (circumcision), which required five consecutive exposure days of treatment with study drug and blood transfusion due to severe bleeding First factor VIII exposure with eleven months Black African Positive family history of haemophilia A Severe haemophilia A

PUP3	Intron-22 inversion
Zero months/ six months	Early treatment start (with six months)
Ten months	Positive family history of haemophilia A
Prophylaxis 28 IU/kg body weight three times per week	Severe haemophilia A
	Vaccinated 3.5 months prior to inhibitor detection

Abbreviations: PUP1-3, previously untreated patient number 1-3.

[a] Linked peak treatment episode including surgical procedures and/ or severe bleedings.[31,32,35,39,40,46,48,49,58]

[b] Factor VIII gene defect,[30,37] family history of haemophilia A, ethnic origin,[29,30] age at first exposure to factor VIII.[31,32,35,38,47]

Table 7. Previously treated patients with factor VIII inhibitor formation during the study period—Analysis of risk factors

Patient number **Age at haemophilia A diagnose** **Previous administered factor VIII products**[60] **Duration of study drug treatment** **Treatment regimen before inhibitor detection**[45] **Age at onset**	**Environmental risk factors**[a] **and other risk factors**[b]
PTP1 Zero years Including cryoprecipitate, other factor VIII products Brother of PTP2 Approximately three years Switched to prophylactic treatment three times weekly over a year ago 14 years	Positive family history of haemophilia A Polypragmasy of the pretreatment with factor VIII containing products (including cryoprecipitate) Hepatitis C and hepatitis B Obesity In the past: on-demand treatment Severe haemophilia A
PTP2 Zero years Including cryoprecipitate, other factor VIII products Brother of PTP1 44 months Prophylactic treatment three times weekly 12 years	Bleeding due to bone fracture eight days before inhibitor detection, which required five consecutive days of treatment with study drug Positive family history of haemophilia A Polypragmasy of the pretreatment with factor VIII containing products (including cryoprecipitate) Severe haemophilia A

PTP3 Three years Including other factor VIII products 14 months Switch of factor VIII treatment to study drug 14 months ago On-demand treatment 42 years	Within the last two months three bleedings due to recurrent joint bleedings, which required three to five consecutive days of treatment with study drug Recurrence of inflammatory joint bleedings Hepatitis B vaccination ten months before Switch of factor VIII product On-demand treatment Severe haemophilia A
PTP4 Two years Including fresh frozen plasma, cryoprecipitate, other plasma-derived factor VIII product Approximately one year at first occurrence On demand 52 years, at recurrence 59 and 60 years	About 3.5 months before last recurrence of factor VIII inhibitor a gastrointestinal bleeding with subsequent hypotension and anaemia required hospitalisation, five consecutive days of treatment with study drug, and concomitant treatment with red blood cells (about one month before this bleeding last negative inhibitor test) Severe haemophilia A On-demand treatment Steroidal antiphlogistic drug (diclofenac) Chronic hepatitis C, hay fever, arthritic pain

Abbreviations: PTP1-4, previously treated patient number 1-4.

[a] Linked peak treatment episode including surgical procedures and/ or severe bleedings.[31,32,35,39,40,46,48,49,58]

[b] Factor VIII gene defect,[30,37] family history of haemophilia A, ethnic origin,[29,30] age at first exposure to factor VIII.[31,32,35,38,47]

Table 8. Environmental risk factors of all 23 previously untreated patients with severe haemophilia A[31,32,35,38-40,46-49,55,58]

	Number of patients (%)	Number of peak treatment episodes
Risk factors at first exposure with study drug		
Age: less than six months old	3 (13.0)[a]	-
Age: 6–11 months	6 (26.1)[b, c]	-
Age: at least 12 months	14 (60.9)	-
Peak treatment episode (≥ 3 consecutive exposure days)	8 (34.8)	8
Peak treatment episode (≥ 5 consecutive exposure days)	6 (26.1)[b]	6
Peak treatment episode (≥ 10 consecutive exposure days)	2 (13.0)[d]	2
First exposure linked to surgical procedure (≥ 3 exposure days)	1 (4.3)[b]	1
First exposure linked to severe bleeding episode	7 (30.4)[d]	7
Risk factors within first 50 exposure days[e]		
History of peak treatment episode (≥ 3 consecutive exposure days)	13 (52.2)	24
History of peak treatment episode (≥ 5 consecutive exposure days)	4 (17.4)	5
History of peak treatment episode (≥ 10 consecutive exposure days	3 (13.0)	3
History of surgical procedure (≥ 3 consecutive exposure days)	4 (17.4)[f]	5
History of severe bleeding episode	10 (43.5)[f]	19
Total number of severe bleeding episodes during first 50 exposure days	**16 (69.6)**	**32**

a Two previously untreated patients received their first factor VIII/ study drug treatment postnatally.

b PUP2 with inhibitor was of African ethnicity.[29,30]

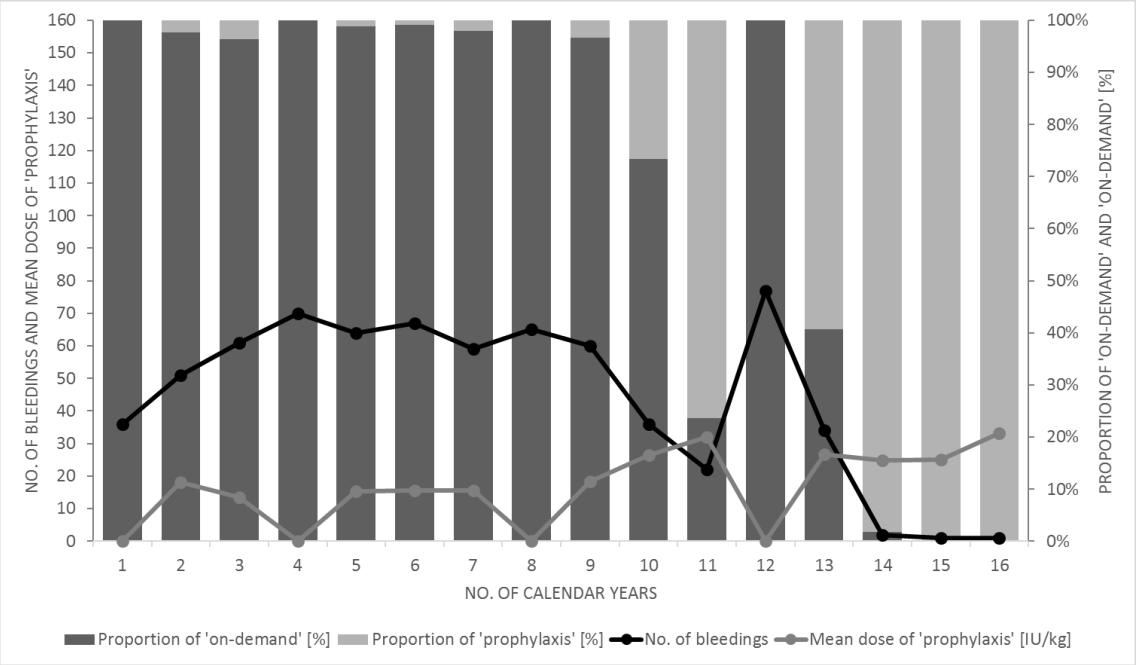
c PUP3 with inhibitor; intron-22 inversion documented as risk factor by the physician.[31-33]

d PUP1 with inhibitor.

e Excluding bleedings linked with first study drug exposure.

7.1. Highlighted case reports of haemophilia A patients whose treatment regimen changed continuously

Figure 5. Development of number of bleedings, treatment regimen and mean prophylactic dose per calendar year in an adolescent patient with severe haemophilia A

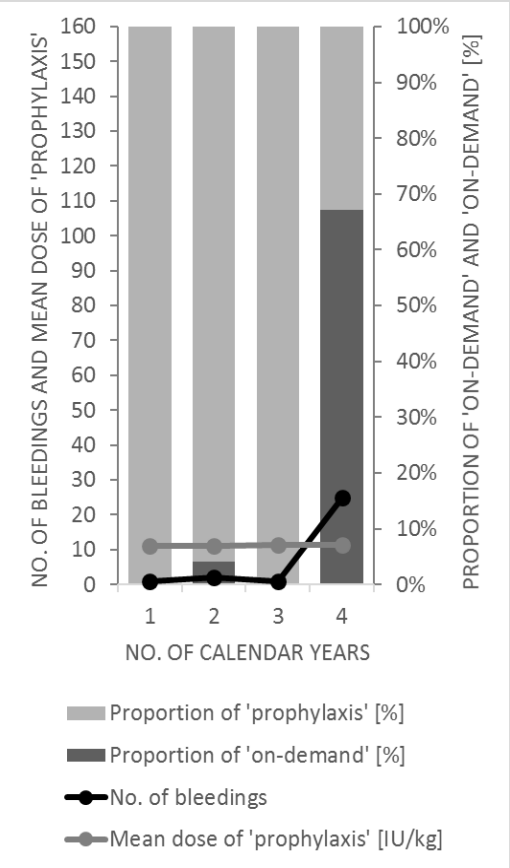


This patient with severe haemophilia A was included in the study with 14 years (169 cm, 51 kg) for a total documentation period of 15.6 years. He was pretreated with cryoprecipitate and other plasma-derived factor VIII concentrates. His underlying diseases included hepatitis C infection and chronic arthropathy. Within the first ten years in the study he was treated on demand with study drug and suffered during these ten years form 591 bleedings. Most of these bleedings, approximately 565, were joint bleedings. After these ten years, he was hospitalised due to an arthroscopy of his left knee. Afterwards, for approximately nine months, he intermittently received a regular prophylaxis while suffering from no further bleeding. The following 1.3 years, his therapy regimen changed back to on demand. During this time, he suffered from 109 joint bleedings and a second arthroscopy of his right knee was performed. Within the last 3.5 years in the study, he continuously administered regular prophylactic substitutions of study drug. During the first of these last 3.5 years, he suffered from four bleedings (all into joints) during prophylaxis. Within the next and last years, he suffered form no further bleeding. Radiosynovectomies

of his right ankle and left elbow were performed within the last six months of his study period.

Overall under prophylaxis, the patient received mean doses of 27.6 IU/kg per body weight on average 3.2 times per week. During his regular and continuous prophylaxis, he suffered from four joint bleedings resulting in a mean annual (joint) bleeding rate of 1.0, compared with a mean annual bleeding rate of 62.0 under on demand. His mean overall annual bleeding rate was on average 98.4 % lower under prophylaxis than under on-demand treatment.

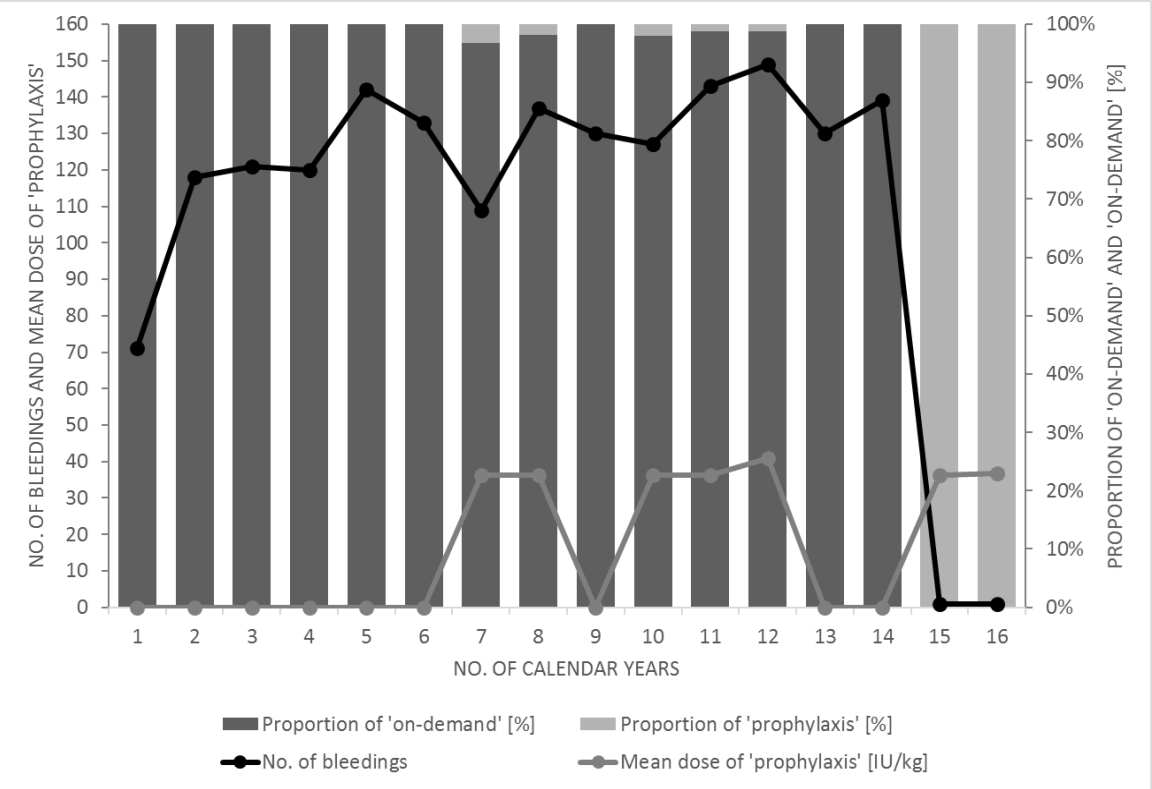
Figure 6. Development of number of bleedings, treatment regimen and mean prophylactic dose per calendar year in an adult patient with moderate haemophilia A



This patient with moderate haemophilia A (2 % factor VIII residual activity) was included in the study with 48 years (178 cm, 90 kg) for a total documentation period of 3.1 years. He was pretreated with other factor VIII concentrates. His underlying diseases included hepatitis C infection, chronic arthropathy, and arterial hypertension. Within his first 2.4 years in the study, he was treated

prophylactically with study drug. He received mean prophylactic doses of 11.4 IU/kg per body weight on average 1.5 times per week while suffering from two joint bleedings (mean annual (joint) bleeding rate of 0.8). Within the last calendar year of his observation, after the change to an on-demand regimen at the age of 51 years, his mean annual (joint) bleeding rate increased by 97.9 % from 0.8 to 37.5 (25 joint bleedings in 0.7 years/ 8 months).

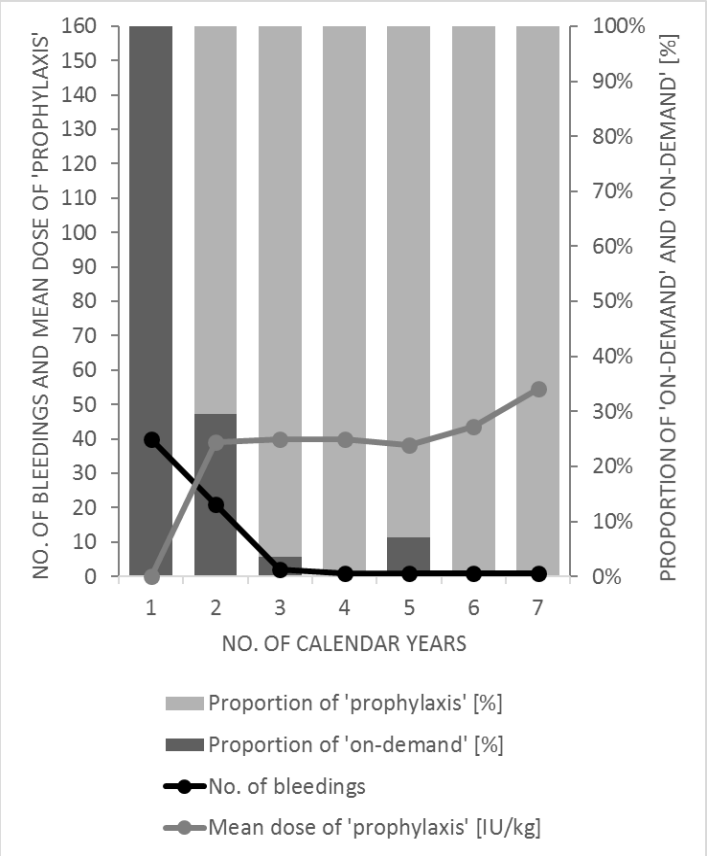
Figure 7. Development of number of bleedings, treatment regimen and mean prophylactic dose per calendar year in an adult patient with severe haemophilia A



This patient with severe haemophilia A was included in the study with 44 years (165 cm, 55 kg) for a total documentation period of 15.6 years. He was pretreated with cryoprecipitate and another plasma-derived factor VIII concentrate. His underlying diseases included hepatitis C infection, chronic arthropathy, hypertension, and cholelithiasis. During his first 13.6 years in the study, he was treated on demand with study drug while suffering from overall 1769 bleedings, including approximately 1669 joint bleedings. During these 13.6 years, his mean overall annual bleeding rate was 130.2. At the age of 57 years,

two years before study end, his therapy regimen was changed to a tertiary prophylaxis with study drug. He received a continuous and regular prophylaxis with mean doses of 36.3 IU/kg per body weight on average 3.8 times per week. Within the last two calendar years of observation, after the regimen change from on demand to prophylaxis, his mean annual (joint) bleeding rate decreased by 100.0 % to 0.0.

Figure 8. Development of number of bleedings, treatment regimen and mean prophylactic dose per calendar year in an elderly patient with moderate haemophilia A



This patient with moderate haemophilia A (2 % factor VIII residual activity) was included in the study with 81 years (176 cm, 77 kg) for a total documentation period of 6.5 years. He was previously treated with other factor VIII products, fresh frozen plasma, and cryoprecipiate. His underlying diseases included essential hypertension, ischaemic heart disease, hepatitis C and hepatitis B infection, gastrooesophageal reflux disease, nephrosclerosis, chronic renal failure, hyperuricaemia, renal anaemia, cerebrosclerosis, and chronic

haemarthrosis. Within approximately his first year in the study, he received an on-demand regimen. During this period, he suffered from 61 bleedings, thereof 57 joint bleedings (mean annual bleeding rate of 61 and mean annual joint bleeding rate of 57). After this period, the patient was hospitalised due to a gastrointestinal bleeding which required corrective treatment with study drug (intensified on-demand treatment), four units fresh frozen plasma, and nine units packed red blood cells. The patient recovered completely. Afterwards, at the age of nearly 83 years, his treatment regimen was changed to a tertiary prophylaxis. Within the first 1.2 years after this regimen change, the patient suffered from two none-joint bleedings into the gum and epidermis. Within the remaining years, the patient suffered from no further bleeding during his study period.

After the regimen change from on demand to prophylaxis, he received a regular prophylaxis with mean doses of 40.6 IU/kg per body weight on average 3.4 times per week. The regimen change resulted in decreases of his annual bleeding rate and of his annual joint bleeding rate by 99.0 % and 100.0 %.

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Schriftliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die dem Fachbereich Medizin der Johann Wolfgang Goethe-Universität Frankfurt am Main zur Promotionsprüfung eingereichte Dissertation mit dem Titel

Langzeitbeobachtung der Therapie von Hämophilie A-Patienten mit einem humanen Faktor VIII-Konzentrat

in der/dem DRK-Blutspendedienst Baden-Württemberg-Hessen gemeinnützige GmbH, Institut für Transfusionsmedizin und Immunhämatologie und Biotest AG unter Betreuung und Anleitung von PD Dr. Dr. Jörg Schüttrumpf mit Unterstützung durch Prof. Dr. Dr. Erhard Seifried ohne sonstige Hilfe selbst durchgeführt und bei der Abfassung der Arbeit keine anderen als die in der Dissertation angeführten Hilfsmittel benutzt habe. Darüber hinaus versichere ich, nicht die Hilfe einer kommerziellen Promotionsvermittlung in Anspruch genommen zu haben.

Ich habe bisher an keiner in- oder ausländischen Universität ein Gesuch um Zulassung zur Promotion eingereicht*. Die vorliegende Arbeit wurde bisher nicht als Dissertation eingereicht.

Vorliegende Ergebnisse der Arbeit wurden (oder werden) in folgendem Publikationsorgan veröffentlicht:
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