Haemophilia A

In pursuit of optimised outcomes via personalised treatment

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LUND
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Abstract:

Haemophilia A (HA) is a hereditary bleeding disorder, characterised by deficiency of coagulation factor VIII (FVIII). Repeated joint bleeds can lead to permanent joint damage. FVIII replacement therapy has a high cost and can reduce but not completely prevent bleeding. This thesis aims to promote personalised treatment and optimised outcomes through a clinical and pharmacokinetic characterisation. **Paper I** compared the PK estimations by two population-PK tools, MyPKFiT and WAPPS-Hemo**,** in a cohort of male patients with severe HA treated with octocog alfa. Both web tools were able to overcome assay discrepancy and produced similar FVIII half-life estimations. However, WAPPS-Hemo generated significantly longer estimations of time to various FVIII trough levels, and as a result, significantly lower dosing proposals than MyPKFiT, with possible clinical implications. **Paper II** investigated a cohort of patients with severe and moderate HA in Malmö and Oslo, after the switch from standard half-life (SHL) FVIII products to BAY 81-8973. The median annualised bleeding rate was 0 before and after the switch, despite the presence of arthropathy and mostly intermediate intensity dose regimens. Treatment adherence was excellent. The Oslo centre had significantly lower annual FVIII consumption. We concluded that personalised prophylaxis and good adherence can reduce FVIII consumption and maintain haemostatic efficacy. **Paper III** investigated the underlying reasons for the difference in FVIII consumption between the Malmö and Oslo cohorts in Paper II. This analysis showed that most patients in Oslo were on secondary prophylaxis with intermediate dose intensity, whereas most patients in Malmö were on primary prophylaxis. Secondary prophylaxis prevents bleeds but at a cost of more arthropathy and reduced health-related quality of life, compared to higher intensity primary prophylaxis. Additionally, non-null *F8* genotypes may allow lower factor consumption with similar haemophilia joint health score (HJHS) and bleeding rates, compared to null genotypes. In **Paper IV**, the long-term joint outcomes, bleeding phenotype, and prophylaxis implementation in childhood were examined in patients born after 1980, with severe HA on primary prophylaxis. This study showed that primary prophylaxis is effective in delaying but does not completely prevent the gradual development of arthropathy in severe HA, with total HJHS rising to a median of 4 at 35-40 years. We concluded that joint assessments should begin at an early age and prophylaxis escalation should proceed expeditiously to prevent bleeds.

Key words: Arthropathy, Bleeding, Coagulation, *F8* gene variants, FVIII consumption, Haemophilia A, MyPKFiT, Pharmacokinetics, Quality of Life, Treatment Adherence, WAPPS-Hemo.

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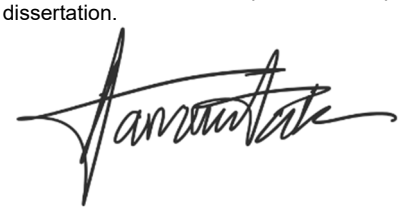
Signature  Date 2024-03-14

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Coverphoto provided courtesy of the documentary film “Bombardier Blood", www.bombardierblood.com.

Mr Chris Bombardier, who has severe haemophilia, has climbed Mount Everest and the other highest peaks of the seven continents, known as the Seven Summits. “Bombardier Blood” depicts his inspirational story.

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MADE IN SWEDEN

“I’m going on an adventure”,

from the book “The Hobbit” by J.R.R. Tolkien

Table of Contents

Abstract

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Arvanitakis A, Berntorp E, Astermark J.
A comparison of MyPKFiT and WAPPS-Hemo as dosing tools for optimizing prophylaxis in patients with severe haemophilia A treated with Octocog alfa.
Haemophilia. 2021 May;27(3):417-424. doi: 10.1111/hae.14295.

II. Arvanitakis A, Holme PA, Berntorp E, Astermark J.
Clinical outcome and adherence rate in Scandinavian patients with intermediate-intensity prophylaxis before and after the switch of standard half-life FVIII products to BAY 81-8973.
Haemophilia. 2022 Mar;28(2):223-229. doi: 10.1111/hae.14489.

III. Arvanitakis A, Holme PA, Berntorp E, Astermark J.
Impact of timing of prophylaxis commencement, *F8* genotype and age on factor consumption and health-related quality of life in patients with severe haemophilia A.
Haemophilia. 2023 Jul;29(4):1032-1038. doi: 10.1111/hae.14806.

IV. Arvanitakis A, Jepsen C, Andersson NG, Baghaei F, Astermark J.
Primary prophylaxis implementation and long-term joint outcomes in Swedish haemophilia A patients.
Manuscript.

Abbreviations

AAV: Adeno-Associated Virus
ABR: Annualised Bleeding Rate
AHG: Antihaemophilic globulin
AJBR: Annualised Joint Bleeding Rate
aPC: activated Protein C
aPTT: activated Partial Thromboplastin Time
BPA: bypassing agents
BU/mL: Bethesda Units per millilitre
Chr: Chromogenic (assay)
CNS: Central Nervous System
COX2: Cyclooxygenase-2
DDAVP: Desmopressin
EHL: Extended Half-life
FII: Prothrombin
FV: Factor V
FV(a): (activated) Factor FV
FVII: Factor VII
FVIII: Factor VIII
FVIII(a): (activated) Factor VIII
FVIII:C: FVIII plasma activity
FIX: Factor IX
FIX(a): (activated) Factor IX
FX(a): (activated) Factor X
FXI(a): (activated) Factor XI
FXII: Factor XII
FXIII: Factor XIII
HA: Haemophilia A
HB: Haemophilia B
HCV: Hepatitis C Virus
HEAD-US: Haemophilia Early Arthropathy Detection with Ultrasound
HGVS: Human Genome Variation Society

HIV: Human Immunodeficiency Virus
HJHS: Haemophilia Joint Health Score
HRQoL: Health-related Quality of Life
HMWK: High Molecular Weight Kininogen
IQR: Interquartile Range
ITI: Immune Tolerance Induction
MRI: Magnetic Resonance Imaging
NBA: Nijmegen modification of the Bethesda Assay
NSAID: Non-Steroidal Anti-inflammatory Drugs
OS: One-Stage (assay)
PAI-1: Plasminogen Activator Inhibitor-1
PCR: Polymerase chain reaction
pd-aPCC: plasma-derived activated Prothrombin Complex Concentrate
pdFVIII: plasma derived FVIII
PEG: Polyethylene glycol
PK: Pharmacokinetics
PwH(A/B): People with haemophilia (A/B)
rFVIIa: recombinant activated FVII
rFVIII: recombinant FVIII
SHL: Standard Half-life
SiRNA: Small interfering RNA
SVP: Subcutaneous venous port
TAFI: Thrombin Activated Fibrinolysis Inhibitor
TF: Tissue Factor
TFPI: Tissue Factor Pathway Inhibitor
tPA: tissue Plasminogen Activator
TXA: Tranexamic acid
uPA: urokinase
VWF: Von Willebrand factor
VWF:Ag: Von Willebrand Factor antigen
WFH: World Federation of Haemophilia

Author's contribution to the papers

Paper I

I contributed to designing the research study and writing the ethics application. I collected clinical data from medical records. I performed the pharmacokinetic (PK) analysis with MyPKFiT and WAPPS-HEMO. I analysed and interpreted the clinical data, performed the statistical analysis, and wrote the paper.

Papers II and III

I contributed to designing the research study and writing the ethics applications. I collected clinical data from medical records. I performed the PK analysis with WAPPS-HEMO. I analysed and interpreted the clinical data, performed the statistical analysis, and wrote the paper.

Paper IV

I contributed to designing the research study and writing the ethics application. I collected clinical data from medical records. I analysed and interpreted the clinical data, performed the statistical analysis, and wrote the paper.

Introduction

Haemostasis

The word “haemostasis” is derived from the Greek words “αίμα”, which means “blood”, and “στάσις”, which means “arrest of flow”. The ancient Greek philosopher Plato noticed that blood changes its character after it leaves the body, becoming thread-like, and coined the term “fibrin”, meaning “thread”. Even though the ancient Greeks could never fathom out the intricacies of haemostasis, they would surely appreciate the drama of it all, a balancing act on a razor’s edge.

The waterfall/cascade model for haemostasis[1,2] was introduced in the 1960s and proposed a stepwise sequence of conversion of inactive proenzymes to active enzymes by the upstream activated factor.[3] The cascade model consisted of two independent pathways: the contact (intrinsic) and the tissue-factor (extrinsic) pathway, converging in the common pathway, which results in the generation of activated factor X (FXa) and, subsequently, thrombin and fibrin (Figure 1).[4]

The cascade model has been fundamental in developing the coagulation tests that are used routinely today to assess the intrinsic, extrinsic, and common pathways and illustrating how the enzymatic reactions are interconnected with every reaction becoming amplified.[2] However, the cascade model failed to reflect the dynamic interplay between the endothelium, vascular and cell surfaces, coagulation factors and platelets that occurs *in vivo,*[4,5] and could not explain why deficiency of the intrinsic pathway components factor VIII (FVIII) or factor IX (FIX) caused a bleeding diathesis, despite the existence of a “parallel” pathway that can generate thrombin.

The model’s proposal of two redundant parallel pathways was therefore insufficient to explain the observed clinical complexity.[4,6]

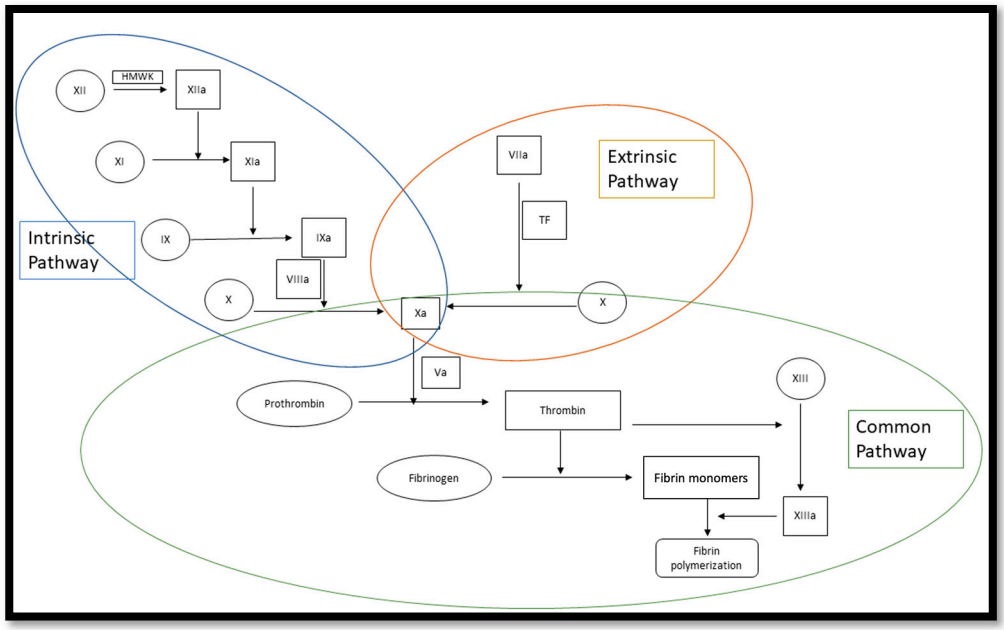


Figure 1. The waterfall/cascade model for haemostasis.

The cell-based model of coagulation (Figure 2), which was introduced in 1992,[7] highlights the importance of platelets and tissue factor (TF)-bearing cells, which provide the surfaces for the coagulation reactions, while exponentially increasing their efficacy.[4,5] According to the cell-based model, the occurrence of vascular injury leads to vasoconstriction and the activation of platelets, which undergo a shape change and secrete their granules.[4,8,9] During the *initiation* phase, expressed TF in subendothelial TF-bearing cells comes into contact with factor FVII (FVII) in the bloodstream and the TF/FVII complex is formed,[6,10] which then activates FIX and FX into activated factor IX (FIXa) and activated FX (FXa), respectively. The prothrombinase complex is formed by FXa and its cofactor activated factor V (FVa) to convert prothrombin to thrombin.[5] As a result of inhibition by tissue factor pathway inhibitor (TFPI) and antithrombin, only trace amounts of thrombin are generated at the *initiation* phase.[4,5] At the consequent *amplification* phase, thrombin augments platelet activation[9] and accelerates the formation of FVa and activated factor XI (FXIa) on the platelet surface.[11] The activated platelets build the initial platelet plug.[8] The disassociation of the FVIII/von Willebrand factor (VWF) complex leads to VWF-mediated platelet adhesion and aggregation, and thrombin activates FVIII to FVIIIa.[12]

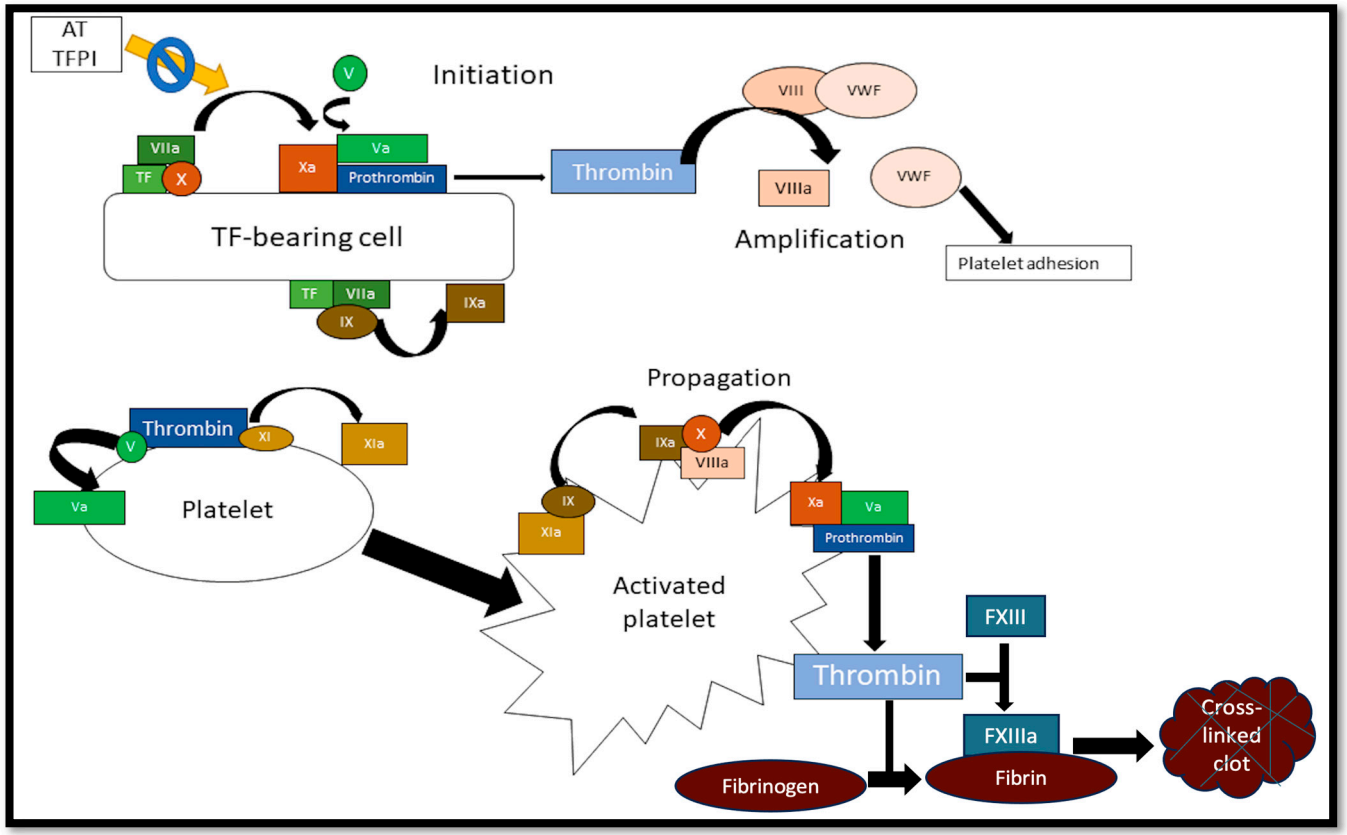


Figure 2. The cell based model of coagulation

Platelets also provide the location of the *propagation* phase,[3] in which FXIa activates FIX to FIXa. Consequently, FIXa and FVIIIa build the "tenase" complex to activate factor X (FX) to FXa.[4,9] The "prothrombinase" complex consists of FXa and FVa and converts prothrombin to thrombin. Soluble fibrinogen is, in turn, cleaved by thrombin to insoluble fibrin monomers. The thrombin-activated factor XIII (FXIIIa) polymerises and stabilises the platelet plug into a fibrin clot,[6,13] by forming cross-links between fibrin strands.[8,14]

Fibrinolysis prevents fibrin formation from arresting blood flow, while still minimising blood loss.[15] Additionally, clot formation is limited to the site of injury by intact adjacent endothelial membranes.[8] The plasminogen activators tissue plasminogen activator (tPA) and urokinase (uPA) lead to the generation of plasmin, which cleaves fibrin, in the process generating fibrin degradation products.[4]

The clotting process is regulated in more ways than fibrinolysis. Thrombin binds to thrombomodulin, which inhibits the actions of fibrin in activating platelets, FVa and FVIIIa. Simultaneously, thrombin activates protein C, which, together with its co-factor protein S, can inactivate factors FVa and FVIIIa.[16] Finally, the natural anticoagulant antithrombin III, whose function is augmented by heparin, inhibits thrombin, FXa and FIXa.[17] The excess creation of plasmin is inhibited by plasminogen activator inhibitor-1 (PAI-1) and A2-antiplasmin, prohibiting hyperfibrinolysis.[16,18] The premature degradation of the fibrin clot is prohibited by thrombin activated fibrinolysis inhibitor (TAFI).[19]

In haemophilia A, FVIII deficiency leads to inadequate generation of thrombin by the FIXa/FVIIIa complex at the platelet surfaces. Even though the "redundant" TF/FVIIa pathway exists, it cannot compensate for the FVIII deficiency, as antithrombin and TFPI inhibit the diffusion of the FXa produced on TF-bearing cells into the bloodflow.[6] Thus, the meticulously maintained balance between clot formation and dissolution is gravely upset.

A brief history of haemophilia

The first mention of haemophilia can be found in Jewish rabbinical writings from 2AD, where it is written that male babies should not be circumcised if their two brothers had previously died as a result of bleeding after this procedure.[20] Albucasis, the great Arabian surgeon of the 11th century, observed that there were many patients with bleeding tendency in a single village, that the disease was restricted to males, that these males could die of trivial injuries, and suggested catheterisation of the wound to stop the bleeding.[21,22]

The Jewish physician Maimonides in the 12th century made the decision to prohibit circumcision in the case of the third son of a woman, whose first two sons had died,

even though she was married twice and the sons had different fathers. Maimonides' decision signifies he suspected a connection between the mother and her sons' affliction.[23]

In the modern age, haemophilia was first described by the American physician John Conrad Otto, from Philadelphia. In 1803 he published *"Über die Hämophilie oder die erbliche Anlage zu tödtlichen Blutungen"*, which described the bleeding tendency of affected male family members.[24,25] The first use of the word "Haemophilia" is found in an essay by Hopff and Schönlein at the University of Zurich.[20]

The first account that bleeding in haemophilia predominantly targets the joints was first given in 1890, whereas previous assumptions were that the joints of patients with haemophilia were afflicted by other types of arthritis instead, such as tuberculosis or rheumatism.[26] In the 1900s, haemophilia was initially thought to be a platelet disorder, but it was eventually shown that the addition of donor platelets did not correct the clotting time.[26] It was in the 1940s when it was discovered that the addition of "anti-haemophilic globulin (AHG)" corrected clotting times in haemophilia.[27] AHG later received its modern name of factor VIII in 1962, by an international committee in the nomenclature of coagulation factors.[28]

Haemophilia was also known as "The Royal Disease", as Queen Victoria of England was a carrier of haemophilia, and passed the disease to her son Leopold, who suffered many bleeds and died of a brain haemorrhage aged 31 years. Through Victoria's daughters the genes transferred to the Royal houses of Germany, Spain and Russia.[25] Analysis of the Romanov family remains by Rogaev et al. in 2009 showed that the Royal Disease was, in fact, haemophilia B, as a result of FIX deficiency.[29]

A brief etymology of the term "Haemophilia"

The term "haemophilia" originates from the Greek words "αίμα" and "φιλία", which translates as "affinity for blood". Thus, "haemophilia" could be considered by the linguistically pedantic as a less than apt description of the disease. Such a person would prefer the more correct term "haemorophilia", which also contains the Greek word "ροή", changing the meaning to "propensity for the blood to flow". Records suggest that even Schönlein may have preferred the later term,[25], but a monography by Grandidier in 1855 solidified the easier term "haemophilia",[30,31] which has been used ever since.

Clinical characterisation of haemophilia A

Epidemiology and classification

Haemophilia A is more common than haemophilia B and accounts for 80-85% of haemophilia cases.[32]

The incidence of HA has recently been estimated to be 1 per 5000-6000 live male births,[33] with prevalence of approximately 12 cases per 100,000 males.[33,34] However, the prevalence of haemophilia is influenced by both life expectancy and access to treatment,[35-37] which results in higher prevalence in high-income compared to low-income countries.[38]

HA is classified in different degrees of severity depending on plasma levels of FVIII activity:[39]

- Severe (< 1% of normal activity or < 1 IU/dL)
- Moderate (1-5% of normal activity or 1-5 IU/dL)
- Mild (> 5% and < 40% of normal activity
  or > 5 IU/dL and < 40 IU/dL)

In the last published annual report of the national Swedish haemophilia registry (2022), approximately 42.1% of patients living with HA in Sweden had severe HA, 13.9% had moderate HA and 44% had mild HA, respectively,[40] which is a similar distribution of HA severity to those reported from the Netherlands and the United States of America.[33,41]

Pathophysiology and genetics

The absence or deficiency of FVIII, caused by pathogenetic variants in the *F8* gene,[42] results in inability to activate FXa adequately, thus compromising the production of thrombin and causing failure of the early clot.[37] There is no platelet dysfunction in haemophilia. Thrombin production is, however, compromised secondary to FVIII deficiency, the haemostatic platelet plug cannot strengthen, and the bleeding diathesis ensues.[43]

A thousand years ago, the aforementioned Maimonides and Albucasis deducted the probable hereditary nature of their patients' bleeding disease, but they would have nonetheless been astonished by the hidden complexity behind their observations.

Haemophilia A is caused by recessive pathogenic variants on the *F8* gene, located on the long arm of the X chromosome (Xq28) and overwhelmingly affects males who have inherited an affected X chromosome from their mother.[32]

Family history can be identified in approximately 70% of haemophilia patients,[44] whereas 30% of cases are sporadic. Genetic testing of the sporadic cases reveals that 70% of the mothers are carriers of haemophilia.[45] In the remaining 30% of sporadic cases, *de novo* variants or genetic mosaicism of the mother can be detected by modern molecular polymerase chain reaction (PCR) technique.[45,46] Mothers with genetic mosaicism would have previously been classified as non-carriers. As haemophilia has historically led to excess mortality for affected persons, the rise of *de novo* variants can explain the disease's persistence in modern times. To paraphrase the British-Indian geneticist J.B.S. Haldane: "if there were no *de novo* variants, all Englishmen at the time of the Norman conquest would need to have had haemophilia".[30]

The pathogenic *F8* variant determines the plasma FVIII activity and, thus, the severity of HA.[47] A pronounced genetic heterogeneity can be found across the different severity grades of HA.

The most common variant in severe HA is intron 22 inversion, found in approximately 40-52% of different cohorts of patients with severe HA.[48,49] Other pathogenic variants found in severe HA include frameshift, missense, nonsense, large structural deletions, splice site variants, promoter site variants and intron 1 inversions.[47,49,50]

The most common variants in non-severe HA are missense gene variants (91% in moderate and 95% in mild HA, respectively) followed by splice site variants (in 3.5% of moderate and 1% of mild HA cases, respectively).[49]

The pathogenetic *F8* gene variants can be classified as null or non-null, based on the assumption that residual FVIII production is present in patients bearing non-null variants, even if not detectable on laboratory assays[51] (Table 1). Null variants have been associated with an earlier onset of bleeding and diagnosis of haemophilia[51] and have a higher risk for the development of FVIII inhibitors compared to non-null variants.[52,53] In an Italian cohort study, pathogenic variants causing a null-allele genotype were found in 80%, 15% and fewer than 1% of patients with severe, moderate, and mild HA, respectively.[50]

Pathogenic *F8* gene variants also influence the risk of developing inhibitors to FVIII, with the most disruptive null variants posing the greatest risk for inhibitor (risk of large deletions > nonsense variants > intron 22 and 1 inversions > missense variants).[37,54]

Table 1. Classification of *F8* gene variants according to assumed resting FVIII production.

Pathogenic *F8* gene variants	
Null	**Non-null**
• intron 22 and intron 1 inversions • nonsense variants • large deletions • small deletions or insertions outside poly-A runs • splice site variants involving conserved nucleotides	• missense variants • small deletions or insertions inside poly-A runs • splice site variants involving nonconserved nucleotides

FVIII structure and function

Factor VIII (FVIII) is a glycoprotein synthesised primarily in hepatocytes; other sites of FVIII synthesis are the kidneys, endothelial cells in the liver and lung, and lymphatic tissue.[43,55] The *F8* gene is one of the largest genes (186,000 base-pairs),[56,57] located on the X chromosome (Xq28), and is comprised of 26 exons.[43] Synthesis of FVIII generates a polypeptide chain of 2351 amino acids (a signal peptide of 19 amino acids and the mature FVIII protein of 2332 amino acids)[56-58]. The amino acid sequence of FVIII forms six domains: A1-*a1*-A2-*a2*-B-*a3*-A3-C1-C2, which create a heavy chain of 200 kDa (contiguous A1-A2-B domains) and a light chain of 80 kDa (contiguous A3-C1-C2 domains), which are interconnected by a covalent bond[43,59] (Figure 3).

The FVIII heterodimer circulates as a noncovalent complex with VWF that regulates platelet aggregation and clot formation. Free FVIII (3-5%) is cleared rapidly and FVIII half-life is reduced six-fold in the absence of VWF.[55] The VWF/FVIII protects FVIII from proteolytic clearance and degradation,[60] inhibits binding of FVIII to negatively charged phospholipid surfaces and FIXa, and prevents the cellular uptake of FVIII.[61] Consequently, VWF-bound FVIII (95-97%) has a much longer half-life of approximately 12 hours, though with significant inter-individual variation.[60]

Factor VIII activation occurs through limited proteolysis by thrombin or FXa, during which the B-domain is released.[62]

The activation of FVIII to FVIIIa leads to the exposure of sites that interact with phospholipids (the C2 domain with the help of the C1 domain), FIXa (regions within the light chain, mostly A3 domain but even the A2 domain in the heavy chain) and FX (the acidic region *a2*), in the presence of calcium ions.[43,61,63] FVIIIa thus becomes a part of the tenase complex and accelerates, by an order of magnitude of 10^5, the activation of FXa.[43]

Inactivation and loss of procoagulant function of FVIII occurs after proteolysis and inactivation by activated protein C (aPC) or spontaneous disassociation of A2-*a2*.[62]

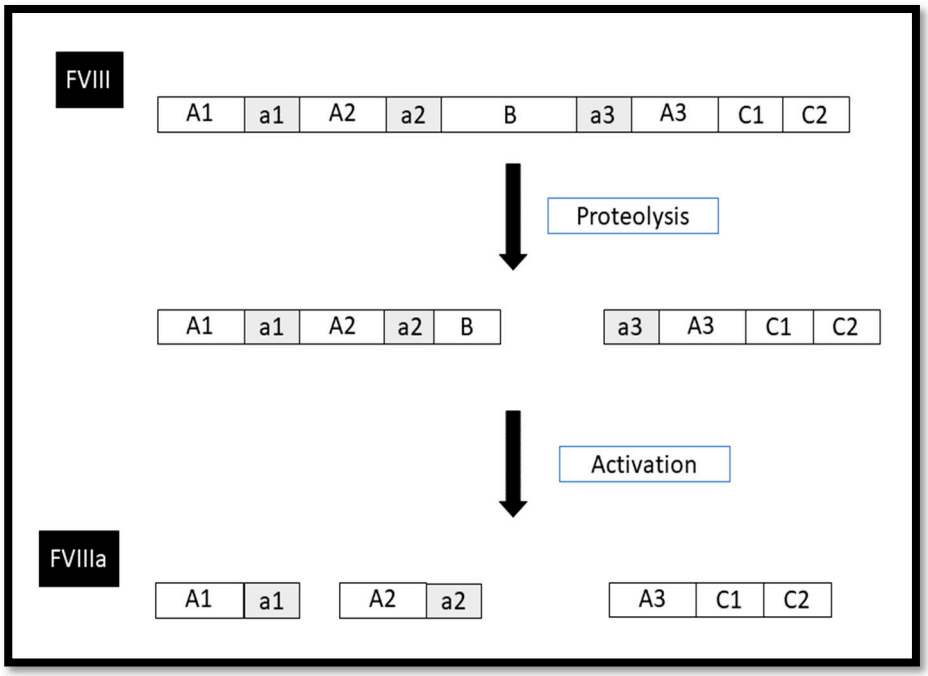


Figure 3. FVIII structure, proteolysis and activation.

Diagnosis

The diagnosis of haemophilia is suspected on the basis of abnormal bleeding tendency, pathological coagulation tests or a positive family history. Severe and moderate haemophilia is usually diagnosed before the age of 2 years,[37] whereas mild haemophilia in an individual without a positive family history is usually diagnosed later, at a median age of 5.3 years, and in some cases can remain undiagnosed until adulthood.[37,64]

A prolonged activated partial thromboplastin time (aPTT) test is not an uncommon finding. The mixing test can then be performed to elucidate if the prolonged aPTT is the result of a coagulation factor deficiency or the presence of an inhibitor, such as lupus anticoagulant.[65] Importantly, the aPTT can fail to detect some cases of mild HA, as some aPTT reagents lack sensitivity for FVIII levels above 30%[66] and FVIII levels can temporarily rise in the context of acute phase reactions.[67] The aPTT reagent is still how practically all cases of acquired (antibody-mediated and non-hereditary) haemophilia are identified.

Diagnosis of HA thus requires a FVIII activity assay. The one-stage (OS) and chromogenic (Chr) assays are the assays in clinical use today.[32]

One-Stage Assay

The OS FVIII activity assay is based on the aPTT test.[68] The OS assay examines whether test plasma can normalise the coagulation defect of FVIII-free plasma (in the case of HA), in the presence of the aPTT reagent, a standardised amount of phospholipids and calcium ions. The results are then compared to those calculated with standard reference plasma, with known FVIII concentrations, and correlated to FVIII activity on a logarithmic/linear scale graph paper.[37,69]

Chromogenic Assay

The Chr FVIII activity assay, which is a variation of the much less commonly used two-stage assay,[70] consists of two steps. The first step is based on the incubation of test plasma with optimal concentrations of FIX, FX, phospholipids, calcium ions, and thrombin. FVIIIa is activated and then contributes to the generation of FXa. The second step consists of the hydrolysation of a chromogenic substrate by the activated FXa. The cleavage of the chromogenic substate releases a chromophore (*p*-nitroaniline), which absorbs light at a specific wavelength. The produced colour intensity is measured and correlated to the amount of FXa, which in turn is correlated to the amount of FVIII in the sample.[69,70]

Assay discrepancy

Discrepancy between the OS and Chr assays has been observed in approximately 30% of mild HA cases and can, in some cases, result in misdiagnosis.[71] Furthermore, some cases of moderate HA can be misclassified as mild HA, if only the OS assay is used.[69]

Assay discrepancies, where either the OS assay (discrepant mild HA) or the Chr assay (inverse discrepancy) provide higher results,[69,72] have been observed and correlate to different pathogenetic *F8* gene variants.[69,71,73] Furthermore, assay discrepancy has been observed in recovery values after FVIII treatment, both for plasma-derived and recombinant-FVIII products.[69] In contrast to the assay discrepancy in mild HA, the Chr assay more commonly results in higher levels of factor concentrate potencies than the OS assay, in testing of the patient after FVIII factor infusion.[74] Compared to the OS assay, the Chr assay can measure up to 40-50% higher FVIII:C activity for recombinant and 17-25% higher for plasma-derived FVIII products, respectively.[75,76] Significant assay discrepancy has been observed with B-domain deleted rFVIII products, where the Chr assay can yield 30% or higher results than the OS assay.[77]

Clinical phenotype

The severity of the haemorrhagic diathesis in haemophilia A is dependent on the plasma FVIII activity level.[62] Severe HA is characterised by spontaneous bleedings, primarily into joints (haemarthrosis) or muscles, but bleeding can occur at more sites. Moderate HA is characterised by occasional spontaneous bleeding. However, prolonged bleeding after a haemostatic challenge (trauma, surgery) can occur. Finally, patients with mild HA can suffer severe bleeding mostly following trauma or surgery, and spontaneous bleeding is rare.[32,78]

The clinical hallmark of haemophilia, an acute joint bleed (haemarthrosis), is usually manifested by "aura", i.e. an unusual sensation in the joint, together with pain, swelling, warmth over the joint and decreased range of motion compared to the patient's baseline. In infants and young children, the sole feature of haemarthrosis may be the child's unwillingness to use the affected limb.[39] Haemarthrosis constitutes 70-80% of all bleedings in haemophilia, and usually affects the knees, ankles and elbows, with less frequent affected joints being the shoulders, wrists and hips.[32,78]

Other important bleeding manifestations include muscle bleeds (frequency 10-20%), central nervous system (CNS) bleeds (< 5%), and bleeding at other sites (15-20%).[32,78] Intracranial bleeds, bleeds near the neck/throat regions and gastrointestinal bleeds can be life-threatening.[32] Bleeding into confined areas, such as the calf, forearm and hip can threaten the local circulation and innervation and, at worst case, cause necrosis (compartment syndrome). In such cases, aggressive management, which can include fasciotomy and joint aspiration, may be required.[79]

The severity of the bleeding diathesis varies greatly among patients with severe HA.[80] The age of the first bleed is indicative of the severity of the phenotype and can range from 0.2 to almost 6 years,[81,82] whereas late onset bleeding may predict a milder phenotype, with less factor concentrate requirement to prevent bleeds.[81] In moderate HA and FVIII:C levels less than 3 IU/dL, younger age at first bleed predicts a more severe phenotype.[83] The bleeding severity in haemophilia can be influenced by the presence of prothrombotic factors, such as co-inheritance of FV Leiden variant[84] or other thrombophilias,[85] the type of *F8* variant (e.g. null vs. non-null)[51], the inter-individual variation in FVIII pharmacokinetics, and the presence of blood group O (leading to lower von Willebrand factor antigen (VWF:Ag) levels by approximately 30%) which contributes to decreased FVIII half-life.[86,87] There is, therefore, no predetermined trajectory of joint outcomes in severe and moderate HA, despite similar baseline FVIII activity levels.[88]

Target joint and haemophilic arthropathy

Recurrent joint bleeding over time results in joint damage. A "target joint" has had at least three or four bleeds within a 3-6 month period.[39] Target joints and joints with repeated bleedings during a longer time period are at risk of developing haemophilic arthropathy.[89] Historically, the knee and ankle joints, as weight-bearing joints, have been most affected by haemophilic arthropathy. With modern therapy, however, the ankle joint is now considered to be most at risk.[37] The risk of joint bleeding is believed to be increased as a result of low intraarticular expression of tissue factor.[90]

Haemophilic arthropathy is the culmination of a process characterised by joint bleeds, synovial hypertrophy and the subsequent destruction of the cartilage and bone of the affected joint.[91] This is the result of repeated episodes of haemarthrosis, which affect the articular cartilage directly. The release of free haemoglobin and iron depositions (haemosiderin) in the joint lead to chronically inflamed and hypertrophic synovium, with increased vascular perfusion as a result of synovial neoangiogenesis (synovitis).[91,92]

Haemophilic arthropathy is a major cause of morbidity in haemophilia and can lead to chronic pain and decreased range of motion. The resultant physical inactivity leads to muscle atrophy, subsequent joint instability and further increased risk of bleeding.[93] At the end stage, a fibrotic and stiff joint has minimal range of motion as a result of joint contraction, but the pain usually subsides.[89] The degree of haemophilic arthropathy can be determined by physiotherapeutic assessment and radiological methods (Table 2).

Persons with haemophilia are also at risk for developing osteoporosis, both because of haemophilic arthropathy, but also secondary to low physical activity, the presence of hepatitis C virus (HCV)- or human immunodeficiency virus (HIV)-infection, and a possible protective effect of FVIII and VWF against osteoclastogenesis.[94-98]

Subclinical bleeding, i.e. the detection of radiological abnormalities suggestive of joint damage in the absence of clinical overt bleeding, is an important and possibly underdiagnosed contributor to haemophilic arthropathy.[99,100] Magnetic resonance imaging (MRI)-assisted investigations have shown evidence of subclinical bleeding in 16%-26% of joints without reported bleeds in patients with severe HA,[101,102] but subclinical bleedings have been detected even in non-severe haemophilia.[41] The presence of subclinical CNS bleeds in approximately 2.5% of children with severe HA has been suggested by MRI findings, but there was no control group of children without haemophilia.[103]

Table 2. Summary of methods for assessing haemophilic arthropathy.

Method	Benefits	Drawbacks
Physiotherapeutic assessment (HJHS 2.1)[104]	Good availability. Practical for follow up. Can be performed in children and teenagers.	Influenced by acute bleed, inflammation. Operator-dependent. Cannot be reassessed.
X-Ray[105]	Low cost. Good availability. Established staging system. Can be reassessed.	Cannot detect early joint damage. Cannot visualize soft tissues. Radiation.
Musculoskeletal Ultrasound (HEAD-US)[106]	Easy to use and low cost. Practical for follow-up. Detects early joint changes in bone and cartilage and can visualize synovitis. Can distinguish between bleeds and effusion.	Operator-dependent. The evaluation cannot be reassessed. Cannot visualize internal bone changes, bone marrow edema and ligaments.
MRI[107]	Detects early changes in joint and hemosiderin deposits. The images can be reassessed. Visualizes deeper structures (internal structure of bones, ligaments, muscle, and bone marrow edema).	High cost. Uneven availability. Requires sedation in children and intravenous contrast. Cannot detect fluids in the joint. Not practical for follow-up.

Health-related Quality of Life

The concept of well-being has been important for people ever since ancient times. The ancient Greek Aristotle wrote about the state of "ευδαιμονία", derived from the words "εύ", meaning "good, well" and "δαίμων", meaning "spirit", and referring to a state of happiness, bliss.[108] The concept of health-related quality of life (HRQoL) is a modern concept but reflects a thousand years' need to understand the human condition and how it is affected by disease.

The effect of haemophilia on HRQoL assesses the burden of this disease on the patients' lives. HRQoL has thus become an important outcome measure and part of management.[109,110] A review from 2012 showed a negative impact of haemophilia upon HRQoL, employment and management,[111] and there is correlation between haemophilic arthropathy and the reduction of HRQoL outcomes.[112-114] Furthermore, haemophilia patients are still more likely to suffer from anxiety and depression and that risk increases with disease severity.[115]

However, Swedish patients with haemophilia have overall high HRQoL results,[116] and Danish haemophilia patients matched the general population in education level and marriage/cohabitation.[117]

Women and neonates with haemophilia

For every man with haemophilia, there are approximately 1.6 female somatic carriers.[118] The random inactivation of the X chromosome in women, a process called lyonisation, results in 28% of female carriers having FVIII/FIX levels under 40%,[119] thus meeting requirements for the diagnosis of haemophilia.[32] Other mechanisms than can cause haemophilia in women are homozygosity or compound heterozygosity for *F8* pathogenic variants, and chromosome X monosomy (Turner syndrome).[37]

The bleeding phenotype of women with haemophilia can include heavy menstrual and mucocutaneous bleeding. Women with haemophilia are also at risk of clinical and subclinical joint bleeding, and earlier onset of arthropathy, compared to healthy controls.[119-122]

Haemophilia carriers are at risk of reproductive tract bleeding and bleeding, both at the time of delivery (13-22% of women), and secondary bleeding from 24 hours until 6 weeks post-partum (9-20%), respectively.[123-125] As a result of this risk, women with FVIII plasma activity (FVIII:C) < 50% should receive treatment with FVIII replacement therapy or desmopressin (DDAVP) at the time of delivery. However, DDAVP should be used with caution and avoided in cases of preeclampsia or eclampsia.[32,118] The World Federation of Haemophilia (WFH) recommends against instrumental delivery.[32]

Approximately 30% of neonates with HA have a *de novo* mutation, and two/thirds are the result of inheritance of a pathogenic *F8* variant from their carrier mother.[118] The incidence of bleeding during the first month of life in affected boys is 35%.[126] During the first 30 days of life, bleeding after circumcision was the location of the first bleed in 28.4%, extracranial bleeding in 17.2%, intracranial haemorrhage in approximately 6%, bleeding after heel stick in 7% and after intramuscular injection in 3.6%, respectively, for boys with HA of all severity degrees.[126] Most cases of HA can be diagnosed after birth, as FVIII:C in the newborn is at normal levels or slightly increased.[32] However, a result in the lower normal range can occur in mild HA and the test should be repeated when the infant is 6 months old.[127]

Treatment of Haemophilia A

Organisation of haemophilia care

In the Nordic countries, persons with haemophilia (PwH) mainly receive treatment at comprehensive care centres, with lifetime management under the care of a multidisciplinary team, which includes physicians, nurses, physiotherapists and orthopaedic surgeons, all with expertise in haemophilia. There is close cooperation with associated specialised laboratories and on-call service provides aid to patients and medical personnel in emergency situations and in case of surgery or trauma.

Factor replacement therapy

Factor replacement therapy with administration of the deficient coagulation factor (FVIII in HA) aims to correct the haemostatic defect, leading to restored haemostasis. This treatment can be administered after the bleeding occurred, i.e. *on demand*, or in order to prevent a bleeding event from taking place, i.e. *prophylactic therapy* in the absence of bleeding.[42] Through preventing joint bleeds, prophylactic therapy aims to prevent joint destruction and preserve normal function of the musculoskeletal apparatus.[128]

Prophylactic therapy was introduced in Sweden by Professor Inga Marie Nilsson in the 1950s,[129] resulting in the amelioration of bleedings and reduction of joint damage.[130]

The rationale for the initiation of prophylactic treatment was the observation that patients with moderate haemophilia with factor activity levels 1-5% had a lower frequency of bleeding episodes than those with severe haemophilia.[131] Thus, prophylactic therapy aimed to convert the severe into a moderate phenotype, by keeping factor plasma levels above 1%.[132] Time with residual FVIII activity below 1%, while on prophylaxis, correlates with an increased number of total bleeds and haemarthroses, which are called “breakthrough bleeds”.[133]

The benefits of prophylaxis compared to on-demand treatment were shown in randomised clinical trials, both in younger and older children (the Joint Outcome[134] and ESPRIT[135] studies), and adults (the SPINART study,[136] and later studies such as LEOPOLD II[137]), which showed the value of prophylaxis in reducing bleeds and protecting joint health.

The start of prophylaxis earlier in life, before the age of 3 years, is associated with fewer bleeding events (as measured by the annualised bleeding rate [ABR]) and better joint outcomes in severe haemophilia.[138] Prophylaxis cannot reverse established joint damage, but it can slow down its progression and reduce morbidity by preventing new bleeding events.[139]

Types of prophylaxis in haemophilia

Depending on the timing of start of prophylaxis, the WFH has defined regular continuous prophylaxis as primary, secondary, or tertiary.[32]

Primary prophylaxis is started:

- Before the age of 3 years.
- Before the second clinically evident joint bleed.
- In the absence of documented joint disease, as determined by physical examination and/or imaging studies.

Secondary prophylaxis is started:

- After two or more joint bleeds.
- Before the onset of documented joint disease, as determined by physical examination and/or imaging studies.
- Usually at 3 or more years of age.

Tertiary prophylaxis is started:

- After the onset of documented joint disease, as determined by physical examination and/or imaging studies.
- Mostly in adulthood.

In Sweden, the traditional prophylaxis regimen of FVIII replacement therapy in severe HA consists of administration of FVIII product with a dose of 25-40 IU/kg three times a week or every other day. This high-dose regimen results in fewer bleeds and better joint outcomes than an intermediate-dose Dutch prophylaxis model, but at 66% higher cost and similar HRQoL.[140] Another prophylaxis model (the Canadian model) starts with prophylaxis once weekly and the frequency of treatment is escalated according to the bleeding phenotype.[141] It also leads to lower factor consumption but more bleeds than the Swedish model.

Pharmacokinetics

Pharmacokinetics (PK) studies the fate of a drug in the organism, after the drug's administration, which is the result of the drug's absorption, distribution, metabolism, and excretion in the organism.[142] In haemophilia, dosing of factor concentrates is weight-based and their haemostatic efficacy is highly related to their concentration in the blood. The amount of administered factor, the frequency of infusions, and the PK response after administration, will determine the concentration of the factor product over time and govern the factor trough level and time above a certain level. All currently available factor concentrate products are given intravenously, which means that absorption does not influence the PK of factor replacement.[143] Table 3 lists and explains the most frequently used PK parameters in the management of factor replacement therapy.

Table 3. PK parameters.
Adapted from Hermans and Dolan, Ther Adv Hematology, 2020.[144]

PK parameter	Description
Peak Level (C_{max})	Maximum clotting factor concentration following infusion
Half-life (t½)	Time taken for 50% reduction of clotting factor concentration after reached equilibrium (e.g. from 100% to 50%, from 50% to 25%)
Trough level	Minimum clotting factor concentration following infusion, before the administration of the next dose
CL	Clearance. Volume of plasma cleared of clotting factor per unit time (mL/h/kg)
Vss	Volume of distribution at steady state. Apparent volume (mL) in which the coagulation factor is distributed at equilibrium after infusion
IVR	*In Vivo* Recovery. Peak factor activity following infusion divided by expected peak of factor activity
AUC	Area under the Curve. The integral of the concentration-time curve, which relates to the total exposure over time

The PK of FVIII follows a two-compartment model, where an initial distribution phase is followed by an elimination phase.[145] Interestingly, peak FVIII:C usually occurs 10-15 minutes after infusion, and in some cases 1-2 hours post-infusion.[146] Factors that influence the PK of exogenous FVIII include the size of the molecule, the binding of the FVIII molecule to VWF, and modifications to the molecule, such as PEGylation or FC fusion, that affect the molecules' distribution and elimination.[147] However, the PK response after FVIII product infusion is not uniform as weight- and age-based dosing cannot predict factor activity values that would prevent bleeding,[148] there is significant inter-individual variation,[149] and difference in PK between children and adults.[145,150] Interestingly, intra-individual variation, i.e. variation within the same person at different time points, is considerably less pronounced.[145]

The application of pharmacokinetics in factor replacement treatment in haemophilia can allow for individualised dosing and more effective treatment, with lower FVIII consumption that may still maintain the haemostatic effect.[151,152] However, a traditional PK analysis is cumbersome, requiring rich sampling of 10-11 samples, taken over 32-48 hours, in order to estimate the PK response after infusion of a FVIII product.[153] In contrast, population PK models can estimate PK data for an individual patient by using FVIII/FIX data from a large group of patients with a sparse drug sampling of 2-3 samples, taken over a period of 48 hours (preferably at 4, 24 and 48 hours).[154,155]

The population-PK pharmacokinetics model uses Bayesian analysis, based on the theorem proposed by the Reverend Thomas Bayes in 1764,[156] and allows the estimation of individual PK parameters based on knowledge of a relevant patient population; the model's PK estimates are adjusted by the introduction of patient data. Thus, population-PK deals with the inter-personal variability in PK parameters

by including relevant covariates (such as age, body mass, VWF levels) in a multivariable regression model.[155,157] Additional covariates usually include age and weight,[155] and extrinsic factors, such as the assay method used.[142] Bayesian analysis does not require washout[158] and samples can be drawn after separate infusions on different occasions and analysed together.[159] Finally, the Bayesian software allows for estimations of the expected effect in factor levels after dose modifications and suggestions of dosage when targeting a specific level at a predetermined dosing frequency.[159]

FVIII products in haemophilia A

FVIII products are classified as SHL or Extended Half-life (EHL), depending on the product's expected half-life after infusion. SHL FVIII products have an estimated expected half-life of 8-12 hours, whereas EHL FVIII products have an expected improvement of half-life of approximately 1.5 times compared to SHL products and allow for either reduced frequency of administration, or higher trough levels if the same dosing frequency is maintained.[160]

SHL FVIII products are further classified as plasma-derived (pdFVIII) or recombinant (rFVIII) products. Plasma-derived products, which were developed in the 1970s, are manufactured from human plasma.[78] Tragically, contaminated plasma-derived factor products led to the infection of haemophilia patients with HIV and HCV during the 1970s and 1980s.[161] Modern viral inactivation techniques have made pdFVIII products safer, but the theoretical risk of contamination with viruses such as viral Creutzfeldt-Jacob disease remains.[128,162] Treatment with SHL products in HA can be classified as high, intermediate or low intensity, according to dosing and administration frequency of the prophylactic regimen (Table 4).

Recombinant SHL FVIII products, which are safe from the risk of blood-borne pathogen transmission, were introduced during the 1990s.[161] The manufacturing process of modern, third-generation, rFVIII products excludes plasma components and animal-derived proteins, minimising the risk of viral and prion infection.[163] A newer class of SHL rFVIII products are manufactured with single-chain technology, where the light and heavy chains are bound together, which increases the stability and VWF affinity, thus potentially improving half-life.[164]

The strive for better half-life of FVIII products has resulted in using bioengineering to modify rFVIII products.[37] These strategies include:

- PEGylation: conjugation to polyethylene glycol (PEG), which reduces rFVIII susceptibility to proteolysis and clearance.[165,166]
- Fc-fusion or albumin-fusion: fusion of rFVIII to the Fc-region of IgG or albumin, which delays lysosomal degradation of the fusion protein and recycles them to the circulation.[167,168]

The extension of half-life of FVIII products has so far been limited by the half-life of VWF, the FVIII carrier protein. However, a recently developed EHL product, efanesoctocog alfa, which was designed to decouple recombinant FVIII from endogenous VWF, could overcome the half-life restrictions that VWF imposed and exhibits a mean t½ of 47 hours, which may allow for once weekly dosing.[169]

Table 4. Dosing intensity of treatment with SHL FVIII products in haemophilia A.
Adapted from Srivastava A et al, *Haemophilia.* 2020[32]

Intensity of Prophylaxis	Ccmmon dose and Frequency of Administration	Estimated yearly FVIII Consumption
High-dose prophylaxis	25-40 IU/Kg every 2 days	> 4000 IU/Kg/Year
Intermediate-dose prophylaxis	15-25 IU/Kg 3 days per week	1500-4000 IU/Kg/Year
Low-dose prophylaxis	10-15 IU/Kg 2-3 days per week	1000-1500 IU/Kg/Year

Adherence to treatment

Adherence to the prescribed prophylaxis regimen is an essential factor for its efficiency.[170,171] The need for frequent intravenous infusions in FVIII concentrate treatment, as well as venous access issues, can cause significant treatment burden in haemophilia and antagonise adherence.[172] In haemophilia, adherence can be influenced by the patients' understanding of their disease, understanding the rational and benefits of prophylactic factor replacement treatment, planning capability, and mastering of the correct injection technique.[173]

Transition to adolescence/young adulthood can be associated with worsened adherence to treatment and requires the development of strategies to facilitate self-management during this period.[171]

Poor adherence is associated with more self-reported bleeding episodes for adults and days off school for children[174]. Regular undertreatment can thus lead to haemarthrosis, subsequent joint damage and arthropathy.[175] It is therefore of importance that the treatment team tries to identify PwH at risk of reduced adherence, by assessing the patients' treatment perceptions, psychosocial circumstances and support, and the outcomes patients hope to achieve.[176,177]

Inhibitors to FVIII

Inhibitors are high-affinity polyclonal IgG antibodies that neutralise the procoagulant activity of a coagulation factor.[128] In HA, these antibodies specifically target FVIII. Approximately 30% of patients with severe HA develop an inhibitor, usually within the first 10-20 days of treatment with factor replacement.[54,178] The risk of inhibitor development is lower in moderate and mild HA, with an incidence of 2.7-13%.[179] The

presence of an inhibitor renders replacement therapy ineffective, making bleeding episodes more difficult to control.[180] Multiple risk factors for inhibitor development have been identified, both genetic and environmental[181] (Table 5).

Table 5. Summary of main risk factors for development of inhibitors.

Genetic Factors	Environmental Factors
Causative *F8* pathogenic variant of null type (especially large deletion and nonsense), but also certain missense variants[53,182]	Factor VIII concentrate (higher risk with rFVIII than pdFVIII[54] , lower risk with 3rd generation rFVIII than older generation)[183]
Higher risk if positive family history for inhibitors[184]	Higher risk with *on demand* treatment than prophylaxis and with intensive treatment during surgery[178]
Immune response gene polymorphisms (e.g. higher risk with IL-10 polymorphism)[185] and *F8* haplotype (higher risk with H3 or H4)[184]	"Danger signals": increased risk at moments of inflammation with high amount of exposed antigen[184]
Ethnicity (higher risk with African and Latin ancestry) [186]	Intensive treatment at first exposure (higher risk with many exposure days and high doses)[187]

The presence of FVIII inhibitors can be suspected by the prolongation of the aPTT, and confirmed with analysis of FVIII:C and the inhibitor titre, as assessed by the Nijmegen modification of the Bethesda assay.[188] The inhibitor titre can vary greatly and range between 0.5 to >100 BU/mL (the inhibitor titre is above 100 BU/mL in approximately 10% of cases).[189] One BU is defined as the amount of inhibitor that results in 50% residual FVIII activity.[190]

Inhibitors can be classified according to their titre and management of acute bleeds differs accordingly:

Low titre: The inhibitor titre remains low (0.5-5 BU/mL) despite repeated exposures. These patients can be treated with higher doses of FVIII concentrate in order to saturate the inhibitor and provide haemostasis.[191]

High titre: The inhibitor titre is > 5 BU/mL. The strategy to saturate the inhibitor is not feasible as a result of high inhibitor titres. Haemostatic products called bypassing agents (BPA) have to therefore be used to achieve haemostasis.[192] Two BPA are available: rFVIIa (Novoseven, Novo Nordisk) and plasma-derived activated prothrombin complex concentrate (pd-aPCC) (FEIBA, Takeda Pharma), with an efficacy of 80-90% in managing bleeds. There is, however, heterogeneity of response, and no way of predicting whether patients will respond to one agent better than the other.[193]

Immune Tolerance Induction (ITI) therapy aims to eradicate high-titre inhibitors by re-inducing tolerance of the immune system towards exogenous FVIII, restore normal PK after FVIII infusion and, thus, re-establish the factor product's ability to restore haemostasis and treat or prevent bleeding.[180] This is achieved by the use of supratherapeutic dosing, occasionally with the addition of immunomodulation,

according to established ITI treatment protocols.[194,195] Success of ITI is defined as a negative inhibitor titre, normal FVIII recovery (≥ 66% of normal), normal FVIII half-life (≥ 6 hours after 72-hours FVIII washout), and absence of anamnesis (rising inhibitor titre to > 5 BU) after re-exposure to FVIII.[196] Predictors of ITI success include pre-ITI titre of < 5 BU, peak titre < 200 BU and peak titre during ITI < 100 BU.[197]

Prophylactic therapy for patients with high-titre FVIII inhibitors in HA, i.e. with no expected effect of FVIII concentrates, consists of two main strategies:

Prophylaxis with BPA can reduce joint bleeding and the risk of arthropathy in patients who have not yet achieved a response to or have failed to respond to ITI.[198] Both rFVIIa and pd-aPCC can be used, alone, sequentially, or even in combination at low doses.[195]

Emicizumab (Hemlibra, Roche) is a bispecific monoclonal antibody that mimics and restores the function of FVIIIa, by bridging FIXa and FXa, and can, consequently, only be used in patients with HA. Emicizumab, which is administered subcutaneously, is effective in reducing bleeding in patients with HA, with and without FVIII inhibitor.[199,200] Even though emicizumab treatment is effective in reducing bleeds in patients with HA and inhibitors, it cannot tolerise the patient, nor can it completely prevent breakthrough bleeds.[195] The combination of emicizumab and rFVIIa has been shown to be safe. However, the combination of emicizumab with pd-aPCC at doses of > 100 IU/kg can lead to the development of thrombotic microangiopathy[199], most likely as the result of synergistic thrombin formation.[195] ITI treatment protocols in combination with emicizumab have been introduced.[201]

Non-factor replacement therapy

Management of haemophilia can also include non-factor replacement-based treatment, with different modes of action, that can be used to both increase coagulative potential and decrease anti-coagulative potential.[200]

Desmopressin

Desmopressin (DDAVP) is an effective haemostatic agent for preventing and treating bleeds in mild and moderate HA.[202] DDAVP increases plasma levels of FVIII, VWF and tPA in the circulation and enhances platelet adhesion, thus producing a pro-haemostatic effect in patients with mild and moderate HA, healthy individuals, and people with already elevated VWF and FVIII levels as a result of other illness.[203] Unfortunately, DDAVP has no clinical effect in severe HA, as there is no FVIII to release.[204] DDAVP can be administered subcutaneously, intravenously or intranasally.[205] Because clinical effect varies and cannot be predicted by the patients' baseline FVIII:C levels,[205] a test infusion to assess the

response to DDAVP in the case of bleeding or before elective procedures should be performed.[205] Repeated administration of DDAVP during 12-24 hours can lead to tachyphylaxis, i.e. a progressively worse response or a lack of response, as a result of depletion of FVIII from cellular storage.[206] Finally, as a result of the antidiuretic properties of DDAVP, repeated administration can lead to hyponatraemia[207] and fluid restriction is recommended during repeated administration.[208] As DDAVP does not raise FIX levels, it has no effect in persons with haemophilia B.[205]

Tranexamic acid

An antifibrinolytic agent, tranexamic acid (TXA) is a synthetic reversible competitive inhibitor to the plasminogen lysine receptor, which inhibits plasminogen's binding to fibrin and its activation to plasmin.[209] TXA can be useful in treating soft tissue and mucosal bleeds, such as epistaxis and menorrhagia, and in the setting of dental surgery, but has no value in preventing haemarthrosis.[32,209-211]

Emicizumab

The bispecific antibody emicizumab mimics the function of FVIIIa and has shown to be effective in both adults and older children[199] and paediatric patients[212] with HA and inhibitors. Subsequent clinical studies also demonstrated the clinical efficiency of emicizumab in adults and children with severe HA without inhibitors,[213,214] and in patients with mild or moderate HA.[215]

Treatment with emicizumab resulted in significantly reduced frequency of bleeds (annualised bleeding rate [ABR] approaching zero), compared to both on-demand and prophylactic treatment with FVIII concentrates,[213] and this efficacy was maintained with different dosing intervals.[215,216]

Treatment with emicizumab can convert a severe HA phenotype to one that corresponds to a mild HA phenotype, with estimated corresponding FVIII:C levels of approximately 9-20%.[217,218] Consequently, emicizumab can potentially prevent arthropathy by protecting against subclinical bleeds, promote adherence as a result of the ease of subcutaneous administration, and allow for a very early start of treatment.[219] However, emicizumab does not lead to FVIII:C activity peaks that may be needed for high-risk activities.[219] Furthermore, breakthrough bleeds can still occur and treatment with a FVIII concentrate or BPA is then needed, as in cases of elective and emergency surgery.[220-222] Finally, theoretical concerns about other important non-coagulative functions of FVIII exist,[223] especially in bone health,[224,225] and long-term data for joint outcomes with non-factor replacement is needed.

Other non-factor replacement treatments

A different approach in rebalancing haemostasis in haemophilia is by targeting natural anticoagulants, such as antithrombin and TFPI, aiming for a renewed haemostatic equilibrium.

Fitusiran, a small interfering RNA (siRNA) which inhibits antithrombin,[226] concizumab, a monoclonal antibody against TFPI,[227] and SerpinPC, a serine protease inhibitor (SERPIN) that inhibits aPC,[228] have shown positive effects in protecting against bleeds in PwH with and without inhibitors.[227] The safety profiles of these agents regarding the risk for thrombosis are being evaluated in ongoing clinical studies.[200,228,229]

In a cohort of HA and HB patients with inhibitors, treatment with fitusiran resulted in fewer bleeds compared to treatment with BPA, with 5% incidence of thromboembolism in the fitusiran arm.[230]

Concizumab treatment led to a significant reduction in ABR in patients with HA and HB and inhibitors. The concizumab clinical study had previously been halted temporarily as a result of thromboembolic events, but no thromboembolism episodes occurred after study resumption with risk mitigation strategies.[231,232]

SerpinPC aims to prolong the activity of the prothrombinase complex through inhibition of aPC, thus promoting haemostasis.[228]

Recently presented data showed that SerpinPC treatment resulted in a median ABR of 1 in patients with severe haemophilia A and B without major adverse events.[233]

These "rebalancing" agents can therefore be valuable in the care of patients with haemophilia A and B, with or without inhibitors. This is of importance, as emicizumab is not an option for PwHB.[234]

Gene Therapy for Haemophilia A

The arrival of gene therapy has ignited the hope for a potential cure of haemophilia. Gene therapy in haemophilia A uses AAV vectors as a means of introducing a normal copy of the FVIII cDNA into hepatocytes, thus restoring endogenous FVIII production.[235] The presence of pre-existing neutralising antibodies against the AAV-vector has therefore been an exclusion criterion for treatment.[236,237] The size of FVIII (280 kDa) did not initially allow for insertion into the AAV-vector, which led to the use of B-domain deleted or truncated FVIII.[238] Treatment with FVIII gene therapy leads to increased production of circulating FVIII, which can ameliorate the bleeding phenotype, and make the need for FVIII infusions obsolete in almost all patients.[239,240] However, the therapeutic response is variable, and this is more pronounced in gene therapy for haemophilia A.[241] There is also uncertainty in the assessment of therapeutic efficacy, as significant assay discrepancy has been noted: the OS assay estimated 1.65 times higher FVIII:C levels than the Chr assay.[242] Concerns exist regarding the durability of response with decreasing trend in FVIII expression during the following years after treatment.[242,243] Hepatotoxicity is an additional issue, as the AAV-vector infects the liver.[235] A mainly theoretical concern, for the time being, is that the AAV vector could theoretically integrate into the genome, leading to a risk of oncogenesis.[244]

Gene therapy treatments for both HA and HB have been approved in North America and Europe and PwH have already received these treatments outside a clinical study setting.

The promise of gene therapy can therefore not be denied. However, the durability of gene expression and long-term treatment efficiency is still uncertain,[245,246] and gene therapy is still unavailable for the majority of PwH.

Treatment of haemophilic arthropathy

Haemophilic arthropathy is associated with symptoms of pain, decreased range of motion, muscle spasm and decreased proprioception, and can be treated conservatively or surgically.[247,248] Acute pain in haemophilia usually results from haemarthrosis and is treated by administration of coagulation factor concentrate.[32] Pharmacological management of acute and chronic pain includes paracetamol, which is the most usually used pain medication in Europe,[249] anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and cyclo-oxygenase-2 (COX2) inhibitors,[249] weak opioids, such as tramadol or codeine,[250] and strong opioids for severe pain.[250] Non-pharmacological options can include rest, ice, elevation, physical therapy, and methods such as acupuncture.[251]

Chronic synovitis can be treated with synovectomy, which can stop the downward spiral of repeated bleeds and the resulting vascular hypertrophy and inflammation. Radiosynovectomy, in which radioisotopes are injected into the intra-articular shape, has a 75% average success rate and can preserve range of motion.[247] Chemical and surgical synovectomy are alternative options, although they are used less frequently.[252]

Physical therapy and muscle strengthening is the prepared method of management of haemophilic contractures, which usually present as equinous deformity of the ankle or flexion contracture of the knee or elbow.[247] The application of corrective devices and surgical procedures are reserved for more severe cases.[247]

Arthrodesis of the ankle joint is effective in reducing pain, preventing bleeding and correcting deformity in haemophilia,[253] with a lower risk of revision and lower complication rates than total ankle replacement.[253]

Total knee arthroplasty is indicated when pharmacological therapies, physical therapy and intra-articular injections of hyaluronic acid fail to lead to clinical improvement, in the presence of a destroyed joint as a result of arthropathy.[254] Complications such as infection, postoperative bleeding, and need for revision can occur.[254] Postoperative physiotherapy sessions can help restore the range of motion, improve proprioception, and assist in muscular strengthening.[255]

Aims of this thesis

This thesis' *raison d'être* is to promote personalised treatment and optimised outcomes through a clinical and PK characterisation.

Specifically, this thesis aims to examine the impact of variables such as genotype, prophylaxis implementation patterns and early bleeding phenotype, FVIII PK, type of prophylaxis and choice of rFVIII product, and adherence to treatment, on essential clinical outcomes such as bleeding, development of arthropathy, FVIII product consumption and HRQoL.

Specific aims of the thesis include:

Paper I

Investigate how two population-PK based web tools, MyPKFiT and WAPPS-Hemo, can be used to generate PK estimates for treatment optimisation in severe HA, using both the Chr and OS assays. In addition, to compare the generated PK estimates and dose recommendations by the population PK tools and assess their potential impact on treatment.

Paper II

Examine whether the switch from SHL FVIII products to BAY 81-8973, while maintaining the same dose and infusion frequency, affects the bleeding phenotype of patients with severe and moderate HA, treated at the haemophilia centres of Malmö, Sweden and Oslo, Norway. Characterise and consider the cohorts' arthropathy, FVIII product consumption and adherence to treatment.

Paper III

Elucidate potential reasons underlying the differences in FVIII product consumption, with similar bleeding phenotype and arthropathy, between the centres of Malmö and Oslo, as shown in Paper II. Evaluate the impact of *F8* gene variants, patient age at inclusion, and timing of start of prophylaxis, on the clinical outcomes of bleeding events, arthropathy development, HRQoL, and FVIII consumption.

Paper IV

Evaluate long-term joint-health outcomes in Swedish patients with severe HA born after 1980 and treated with primary prophylaxis at the comprehensive haemophilia

care centres of Malmö and Gothenburg, Sweden. Correlate joint and bleeding outcomes to how prophylaxis was implemented in childhood, i.e. the initial treatment provided, time until prophylaxis was escalated, and to the final regimen's intensity, and adherence to treatment.

Methods

Study designs and study cohorts

Paper I

This was a randomised, non-intervention, open-label single-centre cohort study, conducted at the haemophilia centre of Malmö, Sweden.

This study enrolled persons with severe HA (FVIII:C < 1 IU/dL), treated with regular factor replacement prophylaxis with the rFVIII product octocog alfa (Advate, Takeda Pharma). All patients had received FVIII prophylaxis for more than 50 exposure days. Exclusion criteria were the presence or history of inhibitory FVIII antibodies, as measured by the Nijmegen-modified Bethesda assay, and the use of another FVIII product during the 30 days prior to inclusion.

Papers II and III

This was an open-label, non-interventional, single arm double-centre study, conducted at the haemophilia centres of Malmö, Sweden and Oslo, Norway.

This study enrolled male patients ≥ 12 years of age, with moderate HA (FVIII:C 1-5 IU/dL) and severe HA (FVIII:C < 1 IU/dL), who had switched or were planning to switch to BAY 81-8973 (octocog alfa, Kovaltry, Bayer) from another SHL FVIII product. All patients had received FVIII prophylaxis for more than 50 exposure days and had been treated with their previous FVIII product for at least 30 days, before the switch to BAY 81-8973. Patients with current FVIII inhibitor, as measured by the Nijmegen-modified Bethesda assay, were excluded.

Paper IV

This was a retrospective double-centre study conducted at the haemophilia centres of Malmö and Gothenburg, Sweden.

This study enrolled male patients ≥ 18 years of age and born after 1980, with severe HA (FVIII:C < 1 IU/dL) and treated with primary prophylaxis, defined as prophylactic factor replacement therapy that started before the age of 3 years and the second joint bleed. Patients with a current or history of FVIII inhibitor were excluded.

Pharmacokinetic Assessment

FVIII and VWF:Ag assays (Papers I, II)

Factor VIII and VWF:Ag levels were estimated with the BCS XP analyser (Siemens Healthcare Diagnostics) according to the manufacturer's instructions for both the Chr and OS methods, at the coagulation laboratory associated with the Malmö treatment centre.

The OS assay was performed with the PTT-Automat (Stago), whereas the Chr assay was performed with the Coatest reagent (Chromogenix) according to local guidelines. The VWF:Ag assay (Siemens Healthcare) was used for assessment of VWF:Ag levels.

Population-PK analysis with MyPKFiT (Paper I) and WAPPS-Hemo (Papers I, II)

Web-Accessible Population Pharmacokinetic service–Haemophilia (WAPPS-Hemo)[256] and MyPKFiT[257] are web-based population-based applications which can be used for population PK calculations and dosing estimations with only sparse sampling, compared to the rich sampling required by conventional PK analysis.

MyPKFiT is product-specific and can be used for octocog alfa (Advate, Takeda Pharma) and rurioctocog alfa pegol (Adynovi, Takeda Pharma). In contrast, WAPPS-Hemo can be used for all currently available factor replacement products.

Both programs require a limited number of two or three samples, taken within 4-48 hours after factor infusion, along with information on previous administered doses, information on age and weight of the subject, and, optionally, other co-variates such as VWF:Ag levels.[257,258]

The PK Models used by MyPKFiT and WAPPS-Hemo for Advate (octocog alfa, Takeda Pharma) are both two-compartment models using PK-dense data as the basis for the model, with different co-variates (age, fat-free mass) depending on the product and FVIII assay used.[257-259] The WAPPS-Hemo PK model for Kovaltry (octocog alfa, Bayer) is a two-compartment model, using fat-free mass and age as co-variates, independent of the assay used.[259]

Assessment of treatment outcomes

Annualised bleeding rate and annualised joint bleeding rate (AJBR) (Papers I, II, III, IV)

The bleeding phenotype was assessed with the ABR and AJBR, which were defined as the number of reported bleeding episodes and joint bleeding episodes, respectively, divided by the observation period in months multiplied by 12.

Target joint (Papers I, II, III, IV)

A target joint was defined as the patient having more than three bleeds in that joint during a 6-month period.

Haemophilia Joint Health Score (Papers I, II, III, IV)

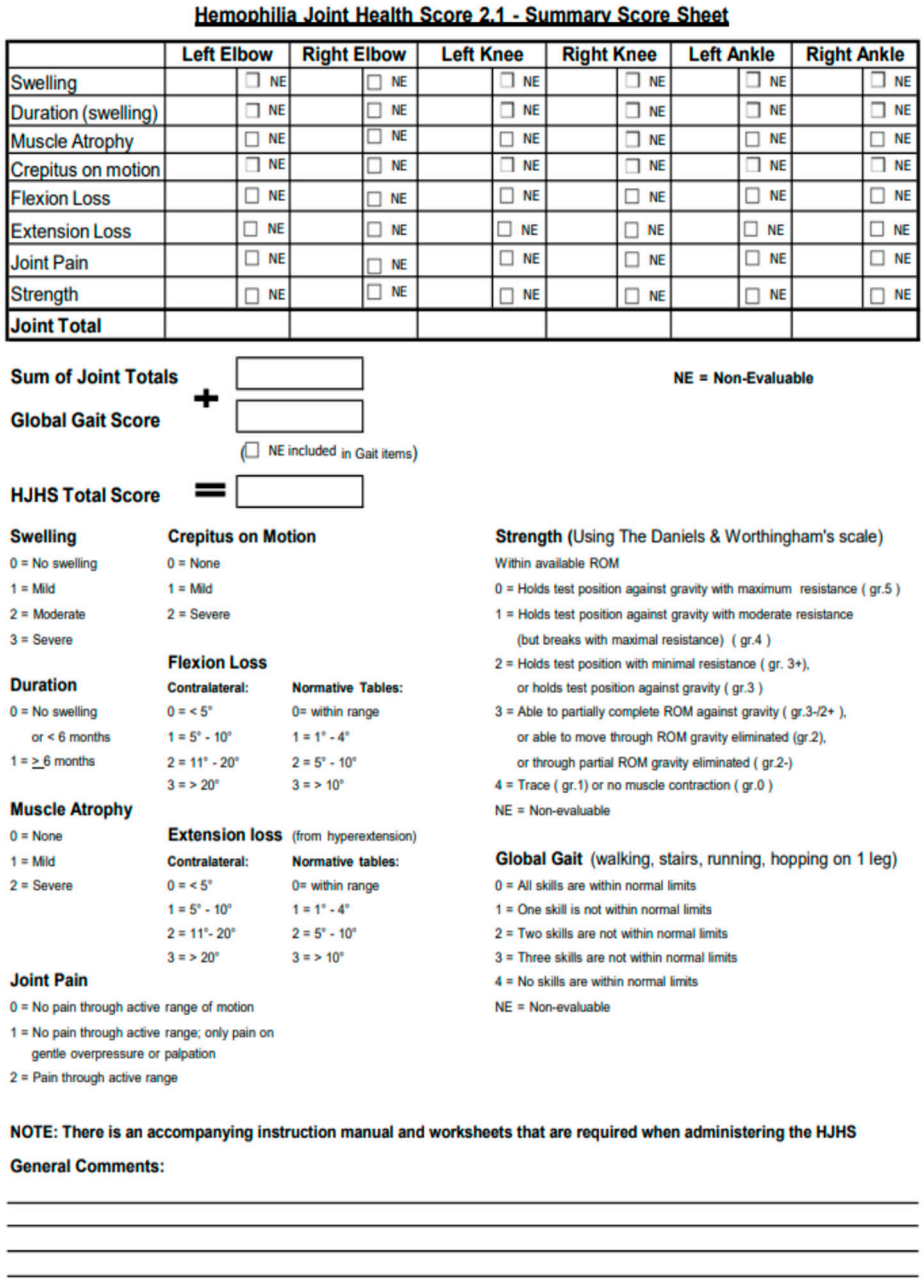
The validated scoring tool HJHS 2.1,[104] performed by a physiotherapist at each participating centre, was used to assess joint impairment in the elbow, knee and ankle joints. The HJHS 2.1 was initially developed for use in paediatric patients, but has since also been validated for use in adult patients.[260] HJHS 2.1 assesses joint structure and function, and exhibits good intra-rater and inter-rater reliability.[261]

The items assessed in HJHS 2.1 are: swelling (none/mild/moderate/severe, score 0-3), duration of swelling (less/more than 6 months, score 0-1), muscle atrophy (none/mild/severe, score 0-2), crepitus on motion (none/mild/severe, 0-2), flexion loss (<5°/5-10°/11-20°/>20°, score 0-3), extension loss from hyperextension (<5°/5-10°/11-20°/>20°), joint pain (no pain through active range of motion ± gentle overpressure or palpation/pain through active range of motion, score 0-2), strength (depending on the patient holding the test position against gravity with maximum/moderate/minimum resistance or only partially holding against gravity or, most severely, no muscle contraction, score 0-4). Finally, the global gait is examined (walking, stairs, running, hopping on one leg) and the number of skills within normal limits is assessed (score 0-4).

The HJHS 2.1 evaluation then generates a score ranging from 0-124, with higher scores indicating worse joint status.[260] The HJHS 2.1 summary score is shown in Figure 4.

WFH Orthopaedic Joint Score (Paper IV)

The WFH Orthopaedic Joint Score[262] (Gilbert score) is a haemophilia-specific grading tool that predates the HJHS and, similarly to the HJHS, evaluates the joint health of the knee, elbow and ankle joints. Depending on the degree of arthropathy, the joint receives a score (0-10 for elbows and 0-12 for knees and ankles). The parameters assessed are joint swelling (0-2), crepitus on motion (0-1), muscle atrophy (0-1), flexion contracture (0-2), range of motion (0-2) and instability (0-2). For the knee and ankle joints, the axial deformity (0-2) was also evaluated. The scores ranged from 0-68, with higher scores signifying more severe joint damage.[263]



Hemophilia Joint Health Score 2.1 - Summary Score Sheet

	Left Elbow	Right Elbow	Left Knee	Right Knee	Left Ankle	Right Ankle
Swelling	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE
Duration (swelling)	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE
Muscle Atrophy	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE
Crepitus on motion	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE
Flexion Loss	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE
Extension Loss	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE
Joint Pain	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE
Strength	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE	☐ NE
Joint Total						

Sum of Joint Totals +

Global Gait Score

(☐ NE included in Gait items)

HJHS Total Score =

NE = Non-Evaluable

Swelling
0 = No swelling
1 = Mild
2 = Moderate
3 = Severe

Duration
0 = No swelling or < 6 months
1 = ≥ 6 months

Muscle Atrophy
0 = None
1 = Mild
2 = Severe

Crepitus on Motion
0 = None
1 = Mild
2 = Severe

Flexion Loss

	Contralateral:	Normative Tables:
0	< 5°	0= within range
1	5° - 10°	1 = 1° - 4°
2	11° - 20°	2 = 5° - 10°
3	> 20°	3 = > 10°

Extension loss (from hyperextension)

	Contralateral:	Normative tables:
0	< 5°	0= within range
1	5° - 10°	1 = 1° - 4°
2	11°- 20°	2 = 5° - 10°
3	> 20°	3 = > 10°

Joint Pain
0 = No pain through active range of motion
1 = No pain through active range; only pain on gentle overpressure or palpation
2 = Pain through active range

Strength (Using The Daniels & Worthingham's scale)
Within available ROM
0 = Holds test position against gravity with maximum resistance (gr.5)
1 = Holds test position against gravity with moderate resistance (but breaks with maximal resistance) (gr.4)
2 = Holds test position with minimal resistance (gr. 3+), or holds test position against gravity (gr.3)
3 = Able to partially complete ROM against gravity (gr.3-/2+), or able to move through ROM gravity eliminated (gr.2), or through partial ROM gravity eliminated (gr.2-)
4 = Trace (gr.1) or no muscle contraction (gr.0)
NE = Non-evaluable

Global Gait (walking, stairs, running, hopping on 1 leg)
0 = All skills are within normal limits
1 = One skill is not within normal limits
2 = Two skills are not within normal limits
3 = Three skills are not within normal limits
4 = No skills are within normal limits
NE = Non-evaluable

NOTE: There is an accompanying instruction manual and worksheets that are required when administering the HJHS

General Comments:

Hemophilia Joint Health Score 2.1 , © The Hospital for Sick Children, Centre Hospitalier Universitaire Sainte Justine, the Regents of the University of Colorado, Karolinska Hospital, University Medical Center Utrecht, 2009. Used under license by The Hospital for Sick Children

Figure 4. Summary Score for Haemophilia Joint Health Score 2.1.
Reprinted with permission from the Haemophilia Joint Health Score team, the Hospital for Sick Children, Toronto, Ontario,Canada.

HEAD-US (Paper IV)

The Haemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) system[106] was developed for use by non-imaging specialists at a point-of-care setting, to assess for changes indicative of haemophilia arthropathy. The HEAD-US system assesses the elbow, knee, and ankle joints for the presence of synovial hypertrophy and damage in the cartilage or bone.

Hypertrophic synovium is graded in three steps (0=absent, 1=mild/moderate, 2=severe). Haemophilic synovium appears hypovascular in Doppler, which was therefore excluded from analysis.[106]

The damage in articular cartilage is graded in five steps (0=normal, 1= echotexture abnormalities and focal loss involving < 25% of the target surface, 2=partial/full thickness loss of cartilage involving up to 50% of the target surface, 3=partial/full thickness loss of cartilage involving > 50% of the target surface, 4=complete cartilage destruction or absent visualisation of articular cartilage on the target surface).

The damage of subchondral bone is graded in three steps (0=normal, 1=mild irregularities with/without initial osteophytes around the joint, 2=damaged subchondral bone with/without erosions and presence of prominent osteophytes around the joint). Each joint thus receives a point of 0-8, with a score of 8 signifying the worst damage. The HEAD-US system does not assess for effusion, subchondral cysts, or depositions of haemosiderin.

The HEAD-US findings correlate to assessment by the HJHS, but HEAD-US can detect abnormalities in the joints of PwH with a low/normal HJHS (0-2).[264] A limitation of the HEAD-US system is that it can miss synovial hypertrophy in approximately 20% of cases, as it does not examine the posterior aspect of the joint.[265] It is also an operator-dependant investigation and the pictures can usually not be reassessed. The HEAD-US scoring protocol is shown in Figure 5.

Disease activity (synovitis)	Scale
Hypertrophic synovium	
0. Absent/Minimal	0
1. Mild/Moderate	1
2. Severe	2
Disease damage (articular surfaces)	
Cartilage	
0. Normal	0
1. Echotexture abnormalities, focal partial/full-thickness loss of the articular cartilage involving <25% of the target surface*	1
2. Partial/full-thickness loss of the articular cartilage involving at least ≤50% of the target surface*	2
3. Partial/full-thickness loss of the articular cartilage involving >50% of the target surface*	3
4. Complete cartilage destruction or absent visualization of the articular cartilage on the target bony surface*	4
Bone	
0. Normal	0
1. Mild irregularities of the subchondral bone with/without initial osteophytes around the joint	1
2. Deranged subcondral bone with/without erosions and presence of prominent osteophytes around the joint	2
Note: Elbow: anterior aspect of the distal humeral epiphysis, Knee: femoral trochlea; Ankle: anterior aspect of the talar dome.	

Figure 5. HEAD-US scoring protocol.
Reprinted from Martinolli et al.[106] from permission from Georg Thieme Verlag.

Adherence to treatment (Papers II, IV)

Adherence to therapy was measured with the VERITAS-PRO questionnaire.[266] VERITAS-PRO is a validated measure of adherence to prophylactic treatment of haemophilia, which is filled out by the patient or their caregiver.[266] VERITAS-PRO provides a total score reflecting overall adherence but can also examine outcomes of six different sub-dimensions with relation to adherence (Time, Dose, Plan, Remember, Skip and Communicate)[266]. Every category can be scored from 1 to 5 (never/rarely/ sometimes/often/always). The minimum score is 24 and the maximum 120.[267] Higher VERITAS-PRO scores signal worse adherence to treatment and a cut-off score of 57 defines non-adherence.[266,268] The VERITAS-PRO questionnaire is shown in Figures 6A & 6B.

Health-related quality of life (Paper III)

HRQoL was assessed by the generic self-filled questionnaire EQ-5D-5L.[269] EQ-5D-5L consists of two parts. The first part is the descriptive system, which consists of five dimensions describing different health states: mobility, usual activities, self-care, pain, and anxiety/depression. Each dimension has five levels of severity: no, slight, moderate, severe, and extreme problems, which are graded from 1 to 5, respectively. A score of 11111 thus signifies no problems in any of the dimensions, while 55555 signifies extreme problems in all dimensions. The EQ-5D-5L dimensions are converted to an index value that ranges from 0 to 1 and is based on the health preferences of the general population of a country or region. An EQ-5D-5L index of 1 is the best possible value and 0 the worst.[270] The Swedish time to trade-off valuation scores was used to calculate the index value.[271] The second part of EQ-5D-5L consists of the Visual Analogue Scale (EQ VAS), where the patient assesses his individual state of health at the day of the questionnaire. EQ VAS score ranges from 0 to 100 (worst to best possible health state, respectively).[270] The EQ-5D-5L questionnaire is shown in Figures 7A (EQ-5D-5L Index) and 7B (EQ-5D-5L VAS).

VERITAS-Pro®

Managing hemophilia is a challenging task. The questions below ask about how you manage hemophilia and prophylaxis. We'd like to get an idea of how often you have done each of these things in the **past three months**. There are no right or wrong answers. The most important thing is for you to answer each question as honestly as possible. Please answer each question using the following scale:

Always – all of the time, 100% of the time
Often – most of the time, at least 75% of the time
Sometimes – occasionally, at least 50% of the time
Rarely – not often, 25% of the time
Never – not at all, 0% of the time

Timing

1. I do prophylaxis infusions on the scheduled days.

   Always Often Sometimes Rarely Never

2. I infuse the recommended number of times per week.

   Always Often Sometimes Rarely Never

3. I do prophylaxis infusions in the morning as recommended.

   Always Often Sometimes Rarely Never

4. I do infusions according to the schedule provided by the treatment center.

   Always Often Sometimes Rarely Never

Dosing

5. I use the doctor-recommended dose for infusions.

   Always Often Sometimes Rarely Never

6. I infuse at a lower dose than prescribed.

   Always Often Sometimes Rarely Never

7. I increase or decrease the dose without calling the treatment center.

   Always Often Sometimes Rarely Never

8. I use the correct number of factor boxes to total my recommended dose.

   Always Often Sometimes Rarely Never

Planning

9. I plan ahead so I have enough factor at home.

   Always Often Sometimes Rarely Never

10. I keep close track of how much factor and how many supplies I have at home.

    Always Often Sometimes Rarely Never

Figure 6A. Sample copy of VERITAS-PRO questionnaire (page 1).
Reprinted with permission from the Indiana Hemophilia and Thrombosis Center Inc, Indianapolis, USA

11. I run out of factor and supplies before I order more.

Always Often Sometimes Rarely Never

12. I have a system for keeping track of factor and supplies at home.

Always Often Sometimes Rarely Never

Remembering

13. I forget to do prophylaxis infusions.

Always Often Sometimes Rarely Never

14. Remembering to do prophylaxis is difficult.

Always Often Sometimes Rarely Never

15. I remember to infuse on the schedule prescribed by the treatment center.

Always Often Sometimes Rarely Never

16. I miss recommended infusions because I forget about them.

Always Often Sometimes Rarely Never

Skipping

17. I skip prophylaxis infusions.

Always Often Sometimes Rarely Never

18. I choose to infuse less often than prescribed.

Always Often Sometimes Rarely Never

19. If it is inconvenient to infuse, I skip the infusion that day.

Always Often Sometimes Rarely Never

20. I miss recommended infusions because I skip them.

Always Often Sometimes Rarely Never

Communicating

21. I call the treatment center when I have questions about hemophilia or treatment.

Always Often Sometimes Rarely Never

22. I call the treatment center when I have hemophilia-related health concerns or when changes occur.

Always Often Sometimes Rarely Never

23. I make treatment decisions myself rather than calling the hemophilia center.

Always Often Sometimes Rarely Never

24. I call the treatment center before medical interventions, such as dental extractions, colonoscopies, visits to the emergency room, or hospital stays.

Always Often Sometimes Rarely Never

Figure 6B. Sample copy of VERITAS-PRO questionnaire (page 2).
Reprinted with permission from the Indiana Hemophilia and Thrombosis Center Inc, Indianapolis, USA

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about ❑
I have slight problems in walking about ❑
I have moderate problems in walking about ❑
I have severe problems in walking about ❑
I am unable to walk about ❑

SELF-CARE

I have no problems washing or dressing myself ❑
I have slight problems washing or dressing myself ❑
I have moderate problems washing or dressing myself ❑
I have severe problems washing or dressing myself ❑
I am unable to wash or dress myself ❑

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

I have no problems doing my usual activities ❑
I have slight problems doing my usual activities ❑
I have moderate problems doing my usual activities ❑
I have severe problems doing my usual activities ❑
I am unable to do my usual activities ❑

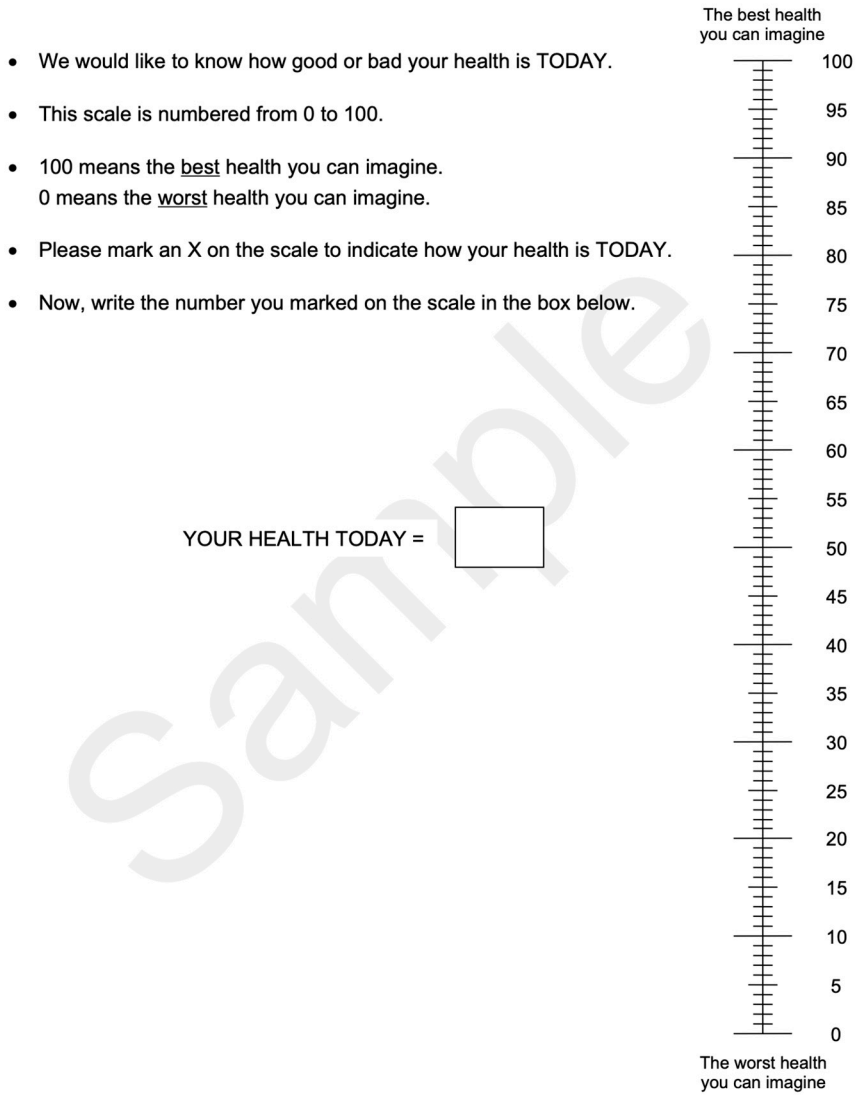
PAIN / DISCOMFORT

I have no pain or discomfort ❑
I have slight pain or discomfort ❑
I have moderate pain or discomfort ❑
I have severe pain or discomfort ❑
I have extreme pain or discomfort ❑

ANXIETY / DEPRESSION

I am not anxious or depressed ❑
I am slightly anxious or depressed ❑
I am moderately anxious or depressed ❑
I am severely anxious or depressed ❑
I am extremely anxious or depressed ❑

Figure 7A. Sample copy of EQ-5D-5L questionnaire (EQ-5D-5L Index)
Reprinted with permission from the EuroQol Research Foundation.



- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Please mark an X on the scale to indicate how your health is TODAY.
- Now, write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐

3

Figure 7B. Sample copy of the EQ-5D-5L questionnaire (EQ-5D-5L VAS).
Reprinted with permission from the EuroQol Research Foundation.

Genetic characterisation

Paper III

Characterisation of the causative *F8* gene variants was performed using routine methods, as part of the diagnostic work-up, at the genetic laboratories associated with the haemophilia care centres in Malmö and Oslo. All variants were classified according to the recommendations of the Human Genome Variation Society (HGVS).

Inversions, nonsense variants, small deletions and insertions outside poly-A-runs, splice site variants within conserved regions, large deletions and deletions of the promoter were defined as null variants, as described previously.[51]

Missense variants, small deletions and insertions inside poly-A-runs and splice site variants of non-conserved nucleotides were characterised as non-null variants.[51]

Statistics

Paper I, II, III, IV

Descriptive statistics were used. Median and interquartile ranges (IQR 25^{th}-75^{th} percentile) described continuous variables and the data are presented as median (IQR) throughout this text. All statistical analyses were performed with SPSS software, version 25. (SPSS, IBM, Chicago, I, USA). A p value of < 0.05 was considered to be statistically significant.

Paper I

The assay results (Chr vs. OS) and estimated half-life and time to 1%, 2%, 3% and 5% by MyPKFiT and WAPPS-Hemo for the Chr and OS assays, respectively, were compared with the Wilcoxon signed-rank test for paired non-parametric variables. The Spearman's correlation test was used to assess the correlation of estimated FVIII half-life to VWF:Ag levels at the time of sampling.

Paper II

The comparison between the bleeding phenotype before and after the switch to BAY 81-8973 was performed with the Wilcoxon signed-rank test for paired non-parametric variables. The comparison between the clinical outcomes at the Malmö and Oslo centres was performed with the Mann-Whitney U test for unpaired non-parametric variables. Spearman's correlation test for non-parametric variables was used to correlate the FVIII half-life to VWF:Ag levels at the time of sampling. Fisher's exact test was used to correlate the presence of a positive bleeding phenotype (ABR > 0) to severity of arthropathy (defined as HJHS > 10).

Paper III

Comparison of clinical outcomes between the primary and secondary prophylaxis groups and between the null vs. non-null *F8* variant groups was performed with the Mann-Whitney U test for unpaired non-parametric variables. Kendall tau-b correlation was to assess the relationship between age of the patients at study enrolment to their age at the start of prophylaxis.

Paper IV

The Mann-Whitney U test for unpaired non-parametric variables was used to compare the groups with and without a subcutaneous port. The Spearman's ρ correlation for non-parametric variables was used to assess the correlation between clinical variables referring to patterns of prophylaxis implementation, bleeding and joint health outcomes.

Ethics

Paper I and IV

These studies were approved by the Regional Ethics Review Board of Lund University, Lund, Sweden. The study subject (Papers I and IV) or his legal representative (Paper I) provided written informed consent before entering the study.

Papers II and III

This study was approved by the Regional Ethics Review Board of Lund University, Lund, Sweden and Oslo University, Oslo, Norway. The study subject or his legal representative provided written informed consent before entering the study.

Results and Discussion

Paper I

Patient and treatment characteristics

Fourteen adult patients on regular prophylaxis with octocog alfa, with a median age of 38 years (30.8-48.5 years) were included. Baseline clinical characteristics are shown in Table 6. The regular dose of octocog alfa was between 17.4 to 28.8 IU/kg with a median dose of 24.4 IU/kg. Six patients had ABR > 0 and four patients had AJBR > 0. Median HJHS was 10 (3.5-30.5). Despite the presence of arthropathy with HJHS ≥ 10 in seven out of 14 patients, only two patients in this cohort experienced spontaneous joint bleeds. However, five of the patients with bleeds had FVIII trough levels ranging from <1% to 2.2%, indicating a need for treatment modification to achieve higher trough levels.[134,272]

Assay discrepancy

The Chr and OS assays were used to calculate FVIII:C at the two sampling points. At the first sampling point, the median FVIII level was 34% (27-39%) with the OS assay and 43% (37-52%) with the Chr assay, respectively. The Chr assay thus produced significantly higher results ($p = 0.001$), than the OS assay (Figure 8). At the second sampling point (25 to 31 hours post-infusion), the measured FVIII levels were similar at 7% (5.8-9%) and 8% (6-10%) for the OS and Chr assays, respectively.

At sampling point 1, the OS:Chr ratio ranged from 0.4 to 0.94, with an average OS/Chr ratio of 0.72. Assay discrepancy has been defined as an OS:Chr ratio of ≤ 0.6 or ≥ 1.5, but this definition applies primarily to discrepancy in diagnostic testing of patients with non-severe haemophilia.[273,274]

Table 6. Patient characteristics.

BMI: body mass index, EOD: every other day, M/TH: Monday and Thursday, ABR: annual bleeding rate, AJBR: annual joint bleeding rate, HJHS: Haemophilia Joint Health Score, S: spontaneous bleed, T: traumatic bleed.

Pat ID	Age (yrs)	BMI (kg/m2)	Regular total dose (IU)	Regular Dose (IU/kg)	Regular dosing interval	ABR	AJBR	HJHS Score
1	30	21.8	2000	24.4	EOD	0	0	4
2	41	37.1	3000	26.1	M/TH	1(T)	0	18
3	67	17.2	1500	28.8	Daily	4(2T,2S)	4(2T,2S)	38
4	31	28.1	2000	21.3	EOD	0	0	2
5	53	26.1	2000	25	3 times weekly	1 (T)	0	28
6	71	26.2	1500	18.1	Daily	0	0	47
7	31	23.7	2000	26.7	3 times weekly	0	0	14
8	47	24.5	2000	24.4	EOD	5 (5S)	5 (5S)	47
9	43	31.9	2000	17.4	3 times weekly	0	0	4
10	31	26.1	2000	27.8	EOD	0	0	6
11	35	23.7	2000	24.4	EOD	2 (2T)	2 (2T)	5
12	42	25.2	2000	22.2	EOD	0	0	2
13	27	22.7	1500	18.3	M/TH	1(T)	1 (T)	1
14	20	27.5	2000	22.3	EOD	0	0	18

Assay discrepancy in the setting of monitoring the effects of treatment has primarily been described for B-domain deleted rFVIII products (mostly moroctocog alfa, ReFacto, Pfizer)[275,276], and single chain BDD-rFVIII products (lonoctocog alfa, Afstyla, CSL Behring).[277] In both cases, the OS assay gives significantly lower results. However, varying degrees of assay discrepancy, with the OS producing lower results than the Chr assay, seem to apply for most rFVIII concentrates, and may be dependent on the choice of phospholipids in the OS assay.[278]

Pharmacokinetic analysis with MyPKFiT and WAPPS-Hemo

The co-variates used in the population-PK model of both MyPKFiT and WAPPS-Hemo were age (year and quarter of birth), height and weight, baseline FVIII:C, and information about the latest two octocog alfa infusions (timing of infusion in relation to sampling and factor concentrate dose in IU).

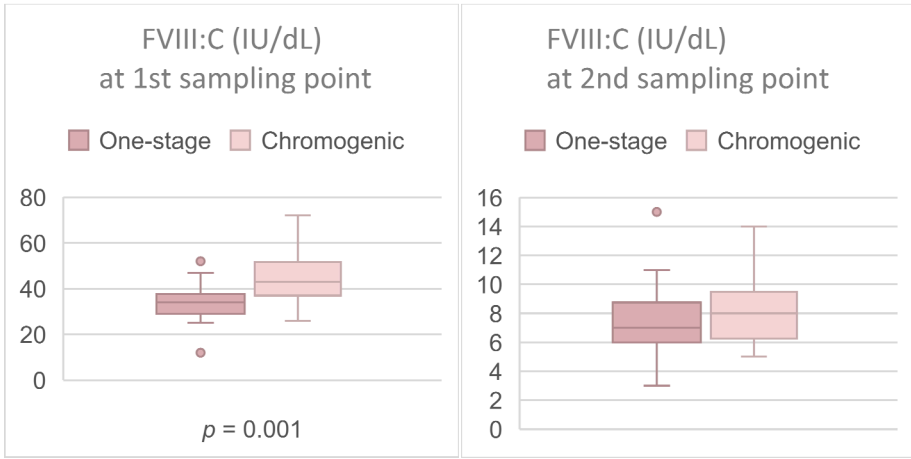


Figure 8. Assay Discrepancy between Chromogenic and One-stage assays.
FVIII:C (IU/dL) at first and second sampling point.

In contrast to traditional PK analysis, no wash-out was performed. Two post-infusion samples were collected following the patient's last regular prophylaxis dose. The time range for the first sample was 4-9 hours post-infusion and the time range for the second sample was 25-31 hours post-infusion, which allowed for more accurate pharmacokinetic estimations according to the MyPKFiT user guide.[257] In one patient (#8), sampling was performed after two separate infusions and the samples were then merged for the analysis, which was permitted by both PK algorithms. This broad sampling window and no need for wash-out speaks for the applicability of these tools in the real-world setting, allowing for flexibility to accommodate the life situation of each patient.

Pharmacokinetic analysis was performed to examine whether the significantly higher results with the chromogenic assay at the first sampling point led to a difference in the calculated PK parameters, i.e. if estimations of t½ and time to reach FVIII:C trough levels of 1%, 2% and 5% yielder higher results with the chromogenic assay. Both MyPKFiT and WAPPS-Hemo yielded median t½ values ranging from 10.5 to 11.2 hours. The choice of assay did not affect the PK estimations of half-life or any of the evaluated analyses regarding time to FVIII trough (Table 7). VWF:Ag levels were within the normal range at the time of sampling.

Table 7. Pharmacokinetic estimations by MyPKFiT and WAPPS-Hemo.

A comparison between the estimated half-lives and time to troughs of 1%, 2% and 5% by each PK algorithm using the chromogenic and the one-stage assay, respectively.

	CHROMOGENIC				
	MYPKFIT		WAPPS-HEMO		*P*-value
Parameter	Median	IQR	Median	IQR	
T½ (hrs)	11.2	10.1-12.1	10.5	9.1-12.7	0.93
Time to 1% (hrs)	58	50.5-65.3	68.2	59.8-80.8	0.003
Time to 2% (hrs)	47.5	41.8-55	51.5	45.2-60.6	0.019
Time to 5% (hrs)	32	28-36.3	35	30.4-41.7	0.017
	ONE-STAGE				
	MYPKFIT		WAPPS-HEMO		*P*-value
Parameter	Median	IQR	Median	IQR	
T½ (hrs)	11.1	10.4-12.5	10.5	9.1-12.7	0.55
Time to 1% (hrs)	55.5	51.5-65.3	67.5	61.7-83.2	0.001
Time to 2% (hrs)	45	41.8-52.8	50.5	45.6-61.4	0.013
Time to 5% (hrs)	31	28-36.3	35	30.4-41.7	0.048

Both population-PK tools could therefore overcome differences in assay results and generate similar estimations for t½ and time to the evaluated trough levels 1-5%. This result means that both PK tools can be used regardless for the assay used and signals the strengths of the population PK model, where knowledge of a relevant patient population can adjust for heterogeneity in specific co-variates. The estimated PK curve for patient #14, as analysed by WAPPS-Hemo and MyPKFiT, is shown in Figures 9A and 9B, respectively.

Even though MyPKFiT and WAPPS-Hemo generated similar results in their estimations of t½, there were significant differences in the estimations made by the two PK algorithms in time to reach a trough level of 1%, 2%, 3% and 5%, with both the Chr and OS assays (Figure 10A and 10B). WAPPS-Hemo generated consistently longer times to the assessed trough levels than MyPKFiT. This difference was most pronounced for the 1% trough, where the estimations differed on an average of 11-12 hours, depending on the assay used, which would impact upon clinical decision making. The differences regarding the trough of 3% and 5% were less pronounced at ≤4 hours, which may be less clinically important.

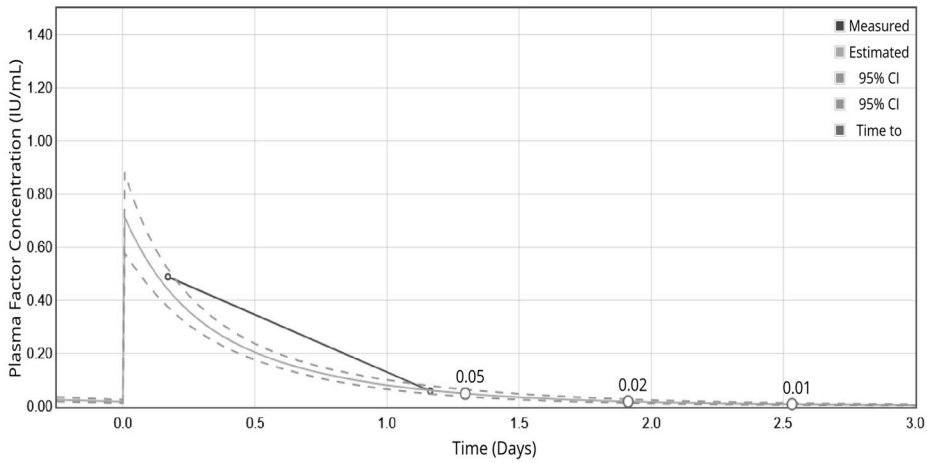


Figure 9A. PK estimation by WAPPS-HEMO, patient #14

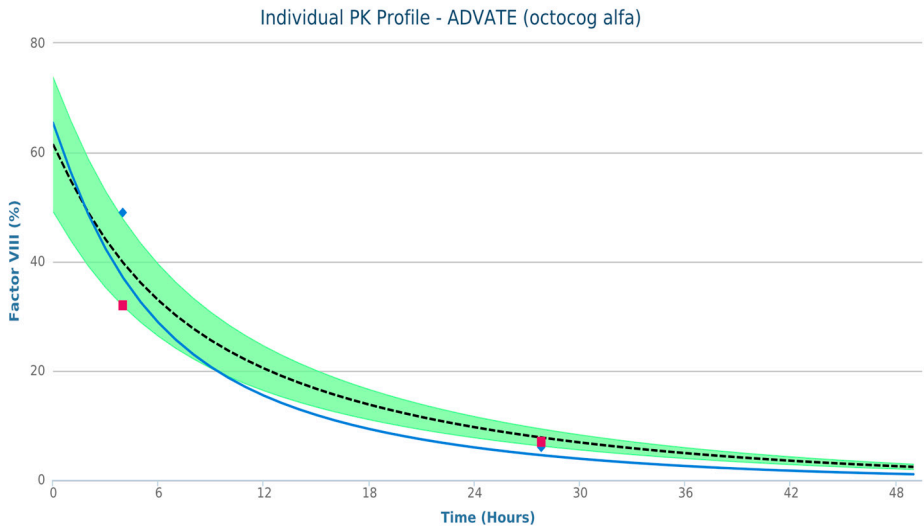


Figure 9B. PK estimation by MyPKFiT, patient #14

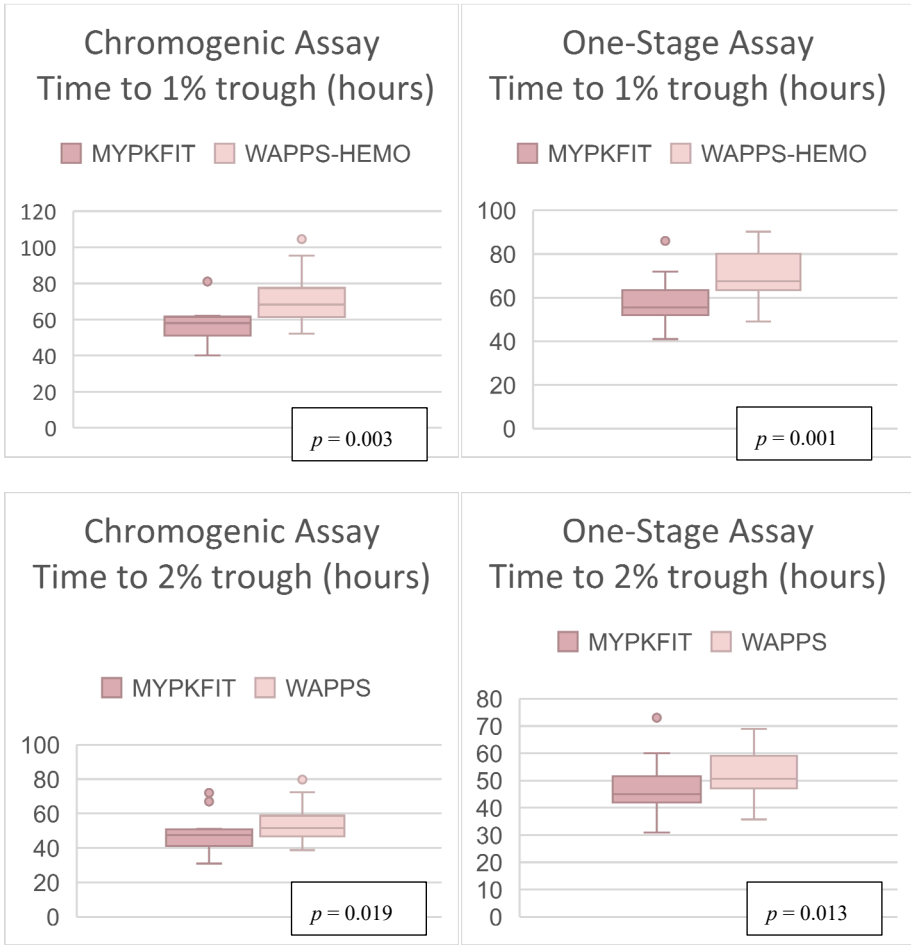


Figure 10A. Differences in PK estimations by MyPKFiT and WAPPS-Hemo (continues on next page).
PK estimations by MyPKFiT and WAPPS-Hemo of time to reach trough 1%,3% and 5% by the chromogenic and one-stage-assays.

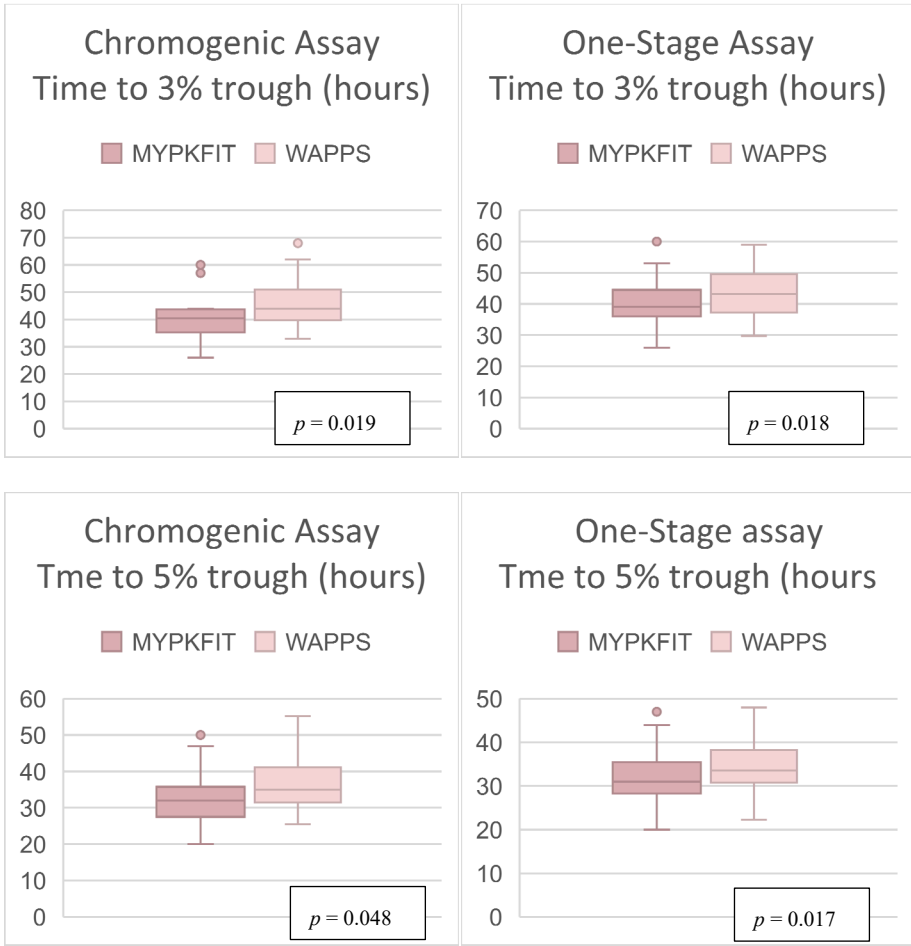


Figure 10B. Differences in PK estimations by MyPKFiT and WAPPS-Hemo (Continued). Pharmacokinetic estimations by MyPKFiT and WAPPS-Hemo of time to reach trough 1%,3% and 5% by the chromogenic and one-stage-assays.

Why were these differences in the PK estimates observed? In a similar study by Prejers et al., significant variation between PK estimations by MyPKFiT and WAPPS-Hemo was also seen, which was interpreted as a result of the individual PK parameters used in each tool.[279]

In our evaluation, pre-infusion levels were not available and instead were estimated by the PK algorithms. Different estimations of pre-infusion levels by the two PK models may have influenced the estimated times to trough, despite the similar half-life values. Inter-patient variability within the Bayesian analysis and the different impact of co-variates in each PK model may also have contributed to the discordant PK estimates seen in our study. Of note, the WAPPS-Hemo tool generated different

PK estimations for each patient, classified as "balanced", "optimistic" or "conservative". The "balanced" estimation, which we deemed would be the preferred choice of most clinicians in the absence of validating data, was used for the comparisons to the PK estimations by MyPKFiT.

Dosing Proposals by MyPKFiT and WAPPS-Hemo

The discrepancy in the estimations by MyPKFiT and WAPPS-Hemo in the time required to reach troughs of 1%-5%, resulted in significant differences in the dosing proposals suggested by the PK algorithms. (Table 8). WAPPS-Hemo proposed consistently lower octocog alfa doses to achieve a trough of 1% with dosing every 48 hours. The differences in dosing proposals were observed regardless of the assay used (Figure 11).

Table 8. Dosing proposals by MyPKFiT and WAPPS-Hemo for patients with bleeds.

Dosing proposal by MyPKFiT and WAPPS-Hemo for the six patients with bleeding manifestations on their current prophylactic treatment. The recommendations are based on the measurements made by the chromogenic assay, and with a target trough of 3% and 5%, respectively, using a 48-hour (every other day) schedule. The percent difference between the estimations is also depicted.

Pat ID	Observed trough level on current regimen	Trough 3%			Trough 5%		
		MyPKFiT (IU)	WAPPS-HEMO (IU)	Percent differ-ence (%)	MyPKFiT (IU)	WAPPS-HEMO (IU)	Percent differ-ence (%)
2	1%	3000	2750	8.7	5000	5000	0
3	13.5%	1250	750	50	2000	1250	46.2
5	<1%	4000	2750	37	6500	5000	26.1
8	2.2%	3000	1750	52.6	5000	3250	42.4
11	1.9%	2500	2000	22.2	4250	3750	12.5
13	<1%	2250	1000	68.7	3500	2000	54.5

Dosing estimations for target troughs of 3% and 5% were calculated for the patients with bleeding episodes and troughs under 3%. In these cases, treatment intensification may be desired, as an increase of FVIII trough levels from 1% to 3% is expected to decrease the expected ABR from two bleeds to one bleed per year.[280] Even in this scenario, the dosing estimations by WAPPS-Hemo were significantly lower than those by MyPKFiT to achieve the troughs of 3% and 5%.

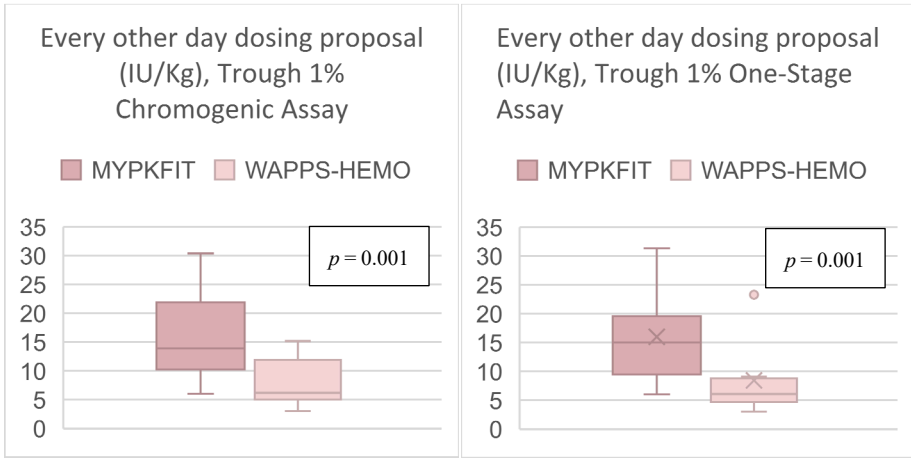


Figure 11. Dosing proposals by MPKiT and WAPPS-Hemo.
Dose proposals by MyPKFiT and WAPPS-Hemo for reaching a trough level of 1% after 48 hours in each patient, i.e. for every other day dosing schedule.

Consequently, the median annual consumption required to reach a trough of 3% using every other day infusion schedule would be 2.69 x 10^6 IU and 1.87 x 10^6 IU for MyPKFiT and WAPPS-Hemo, respectively, based on the Chr assay. For the trough of 5%, the difference between the higher MyPKFiT- and lower WAPPS-Hemo-estimated annual FVIII consumption would be a median of 0.96 x 10^6 IU. As higher trough levels are increasingly becoming a target of treatment,[281] future research is needed to assess whether the observed differences in PK assessments for the SHL product octocog alfa in this study would be seen in PK estimations for EHL factor concentrates.

Strengths and limitations

This study has some limitations, primarily the relatively small number of study subjects, the retrospective design, and subjective reporting of bleeding events. In addition, no pre-infusion levels were collected and there was no *in vivo* validation of the different PK estimations. Strengths of this study include the evaluation of PK tools in the real-world setting, with all patients treated at a single centre, and all analyses were performed in one laboratory. Additionally, this study was one of the first to correlate PK estimations to clinical data of bleeding phenotype and joint health of the patients. The findings of this study signal that choice of PK tool may influence PK estimations and dosing proposals to achieve the desired trough level, which clinicians should be aware of.

Paper II

Patient and treatment characteristics

This study included 40 patients who switched to BAY 81-8973, corresponding to all patients who switched in Malmö and half of the patients in Oslo. Eighteen patients were included at the Malmö centre and 22 at the Oslo centre. Two patients at the Oslo centre had moderate HA with baseline FVIII:C of 2 IU/dL and 3 IU/dL, respectively. The remaining 38 patients had severe HA. None of the patients had a current inhibitor to FVIII.

The cohort's median age was 40.5 years (26.0-48.8 years) and the median BMI was 26.6 (23.1-29.6). The median dose of infused FVIII before the switch was 20.4 IU/kg (12.9-26.2) and all patients received regular prophylaxis. The frequency of infusion was daily (N=4), every other day (N=14), three times weekly (N=14), two times weekly (N=6), and once weekly or less (N=2). All patients continued with the same dose and infusion frequency after the switch to BAY 81-8973, except for two patients (#19 and #26), whose infusion frequency increased slightly, from three times weekly to every other day. Median dosing and annual FVIII consumption were otherwise the same prior to and after the switch. The median FVIII consumption for the cohort on BAY 81-8973 was 3345 IU/kg/year (1944-4463).

The cohort had a median HJHS of 14 (5.5-27.0). Patients with high HJHS were scored on decreased mobility in the elbow, knee, and ankle joints, decreased muscle strength, and gait problems. Crepitus on motion was a common cause of scoring in patients with low HJHS. Crepitus may indicate cartilage damage, but no functional impairment was observed in those cases. There were no detected target joints, which may, in cases of patients with high HJHS, be the result of advanced arthropathy and fibrotic degeneration.

Differences in bleeding phenotype after the switch to BAY 81-8973

The median ABR was 0 (0-1.5) before and remained 0 (0-0) after the switch to BAY 81-8973, corresponding to a median AJBR of 0 (0-0), both before and after the switch (Figure 12). The mean ABR was 1.1 vs. 0.4 ($p = 0.136$) and the mean AJBR 0.7 vs 0.3 ($p = 0.194$), before and after the switch, respectively. The median ABR of the 10 patients with reported bleeds prior to the switch to BAY 81-8973 was reduced from 4 (0-6) to 0 (0-0.25) ($p = 0.007$) and the median AJBR was reduced from 2 (0-6) to 0 (0-0) ($p = 0.017$), respectively, after the switch.

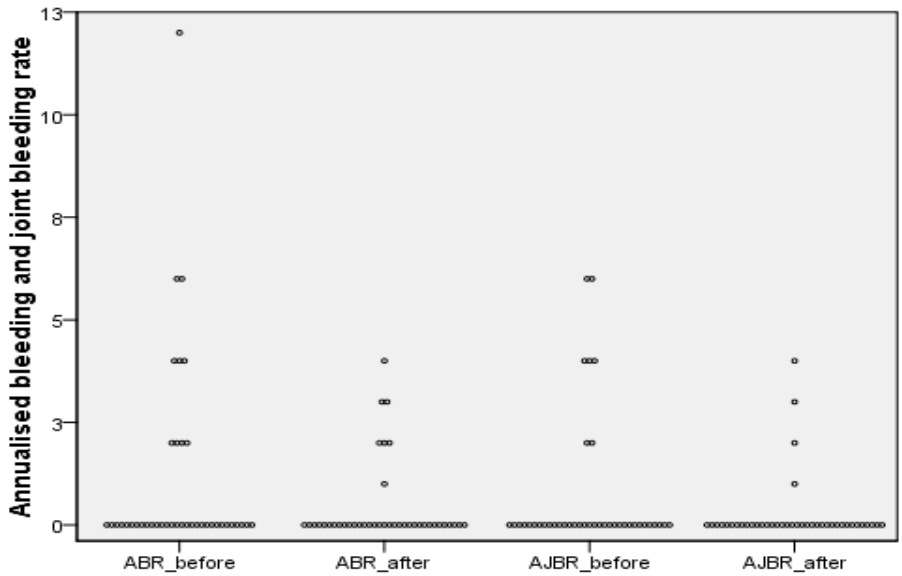


Figure 12. ABR and AJBR before and after the switch to BAY 81-8973.
2D-dot plot showing ABR and AJBR before and after switch to BAY 81-8973, respectively. Each dot symbolises one patient.

There was no correlation (p = 0.525) between bleeding events during the study period (ABR and AJBR > 0) and arthropathy, defined by an HJHS of ≥10 as in previous studies.[140,282] Figure 13 visualises the relative difference in ABR and AJBR after the switch in each patient in every patient in the cohort.

As a result of very low bleeding rates observed during the study period in this well-treated cohort, this study could not assess whether the switch to BAY 81-8973 influences the bleeding phenotype. Thus, even though there was a minor reduction in mean ABR and AJBR rates after the switch to BAY 81-8973, while maintaining the same dose and dosing frequency, this was not statistically significant.

Interestingly, the very low bleed rates in this cohort were observed despite the presence of significant arthropathy in 62.5% of majority of patients in our study. Furthermore, even though the prophylaxis regimen had intermediary intensity (1500-4000 IU/kg/year)[32] in 60% of patients, it could still maintain a median ABR of 0. Therefore, this study supports the benefits of individualised dosing for medical outcome and factor consumption, which is in agreement to the findings of a previous study comparing Swedish and Dutch dosing regimens.[140] However, there was no additional individualisation of the treatment regimen after the switch to BAY 81-8973, as the pre-switch median ABR was 0 and the treatment decisions were not protocol guided, but decided by the treating physicians in a personalised manner according to the patient's clinical phenotype.

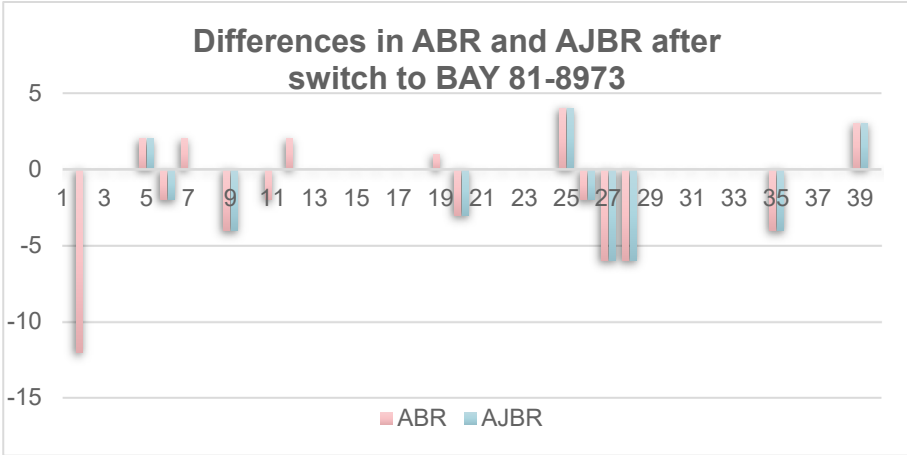


Figure 13. Difference in bleeding phenotype after switch to BAY 81-8973.
Bar chart showing the difference in ABR and AJBR in all 40 patients of the cohort after the switch to BAY 81-8973. Negative values indicate a reduction in ABR and AJBR after the switch, whereas positive values indicate increase, respectively. These differences were not statistically significant.

Adherence to treatment

The patients' compliance to treatment was assessed with the validated questionnaire VERITAS-PRO in 34 of 40 included patients. The median total VERITAS-PRO score was 40 (30.8-47). Low scores were observed in "dosing", "planning", "skipping" and "remembering" with a median of 4-6, and IQR 4-8, as shown in Figure 14. The worse adherence results in this cohort were seen in the sub-category of "communication", with a higher median score of 9 (6-12). When a cut-off of 57 points was used to define non-adherence, only one patient scored above that threshold, signifying 97% adherence in the cohort. The adherence rate in this Scandinavian cohort was comparable to that of a German cohort (adherence 93.1%),[268] and higher than the American cohort in the original validation study (adherence 82%).[266]

All the patients in our cohort had their follow-up at a specific haemophilia centre, a strong predictor of adherence[268]. Our results also support the previously described association between good adherence and low reported bleeding events.[283]

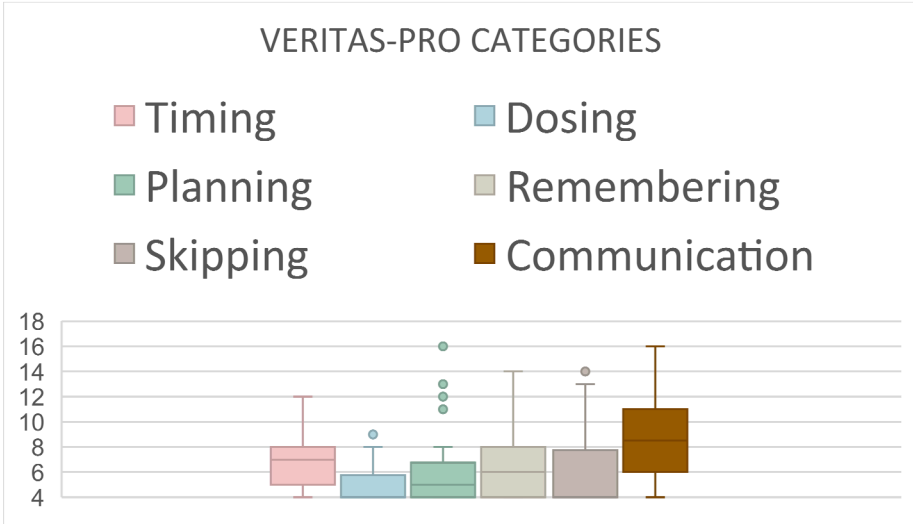


Figure 14. Boxplots showing the results of the VERITAS-PRO categories.

Pharmacokinetic analysis after switch to BAY 81-8973

In a subset of 14 patients from the Malmö cohort, a pharmacokinetic analysis was performed. Analysis was based on two sparse samples collected at least 12 hours apart, with no wash-out, between 4 and 48 hours after BAY 81-8973 infusion, according to the ISTH guidelines.[284] The WAPPS-Hemo estimated median t½ for BAY 81-8973 was 15.15 hours (11.5-21.3 hours) and the median estimated time to 1% was 96.5 hours (71.9-145.2 hours), as shown in Figure 15. The estimated half-life of 15.15 hours was longer than reported for other SHL products.[285-288] Interestingly, a similar range of 9.95 to 22.2 hours was seen in the study by Shah et al.[285] However, inter-study differences in design, FVIII wash-out and dosing, and the low number of included patients in the analysis, are important factors to consider and there was no control group. As expected, there was a significant correlation between VWF:Ag levels and FVIII half-life ($p = 0.01$).

When three patients with the longest t½ (patients #1, #8 and #16) with VWF:Ag levels ≥ 170 IU/dL were excluded from analysis, the remaining 11 patients had a median t½ of 13.4 hours (11.5-16.5 hours). This shows the importance of considering VWF levels when interpreting FVIII PK data and the need for head-to-head cross-over studies when comparing different products.

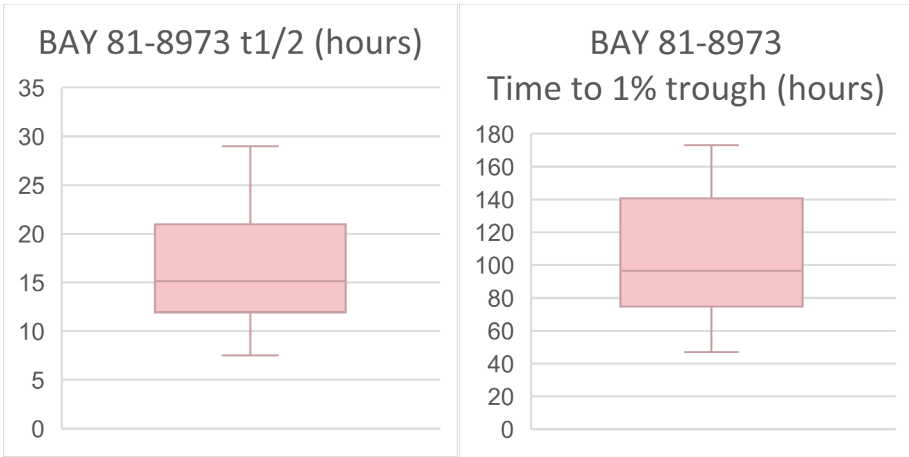


Figure 15. Pharmacokinetic estimations by WAPPS-Hemo.
Boxplots showing WAPPS-Hemo estimations of t½ and time to trough 1%, using the chromogenic assay, in 14 patients treated with BAY 81-8973 at the Malmö Haemophilia Centre.

Comparison between the Malmö and Oslo sub-cohorts

A comparison of the differences in clinical outcomes between the patients with severe HA treated at the haemophilia centres of Malmö (N=18) and Oslo (N=20) was performed (Table 9). The median age of the Malmö cohort was 35 years (IQR 20.5-44) and median BMI 26.4 (IQR 22.2-28.7). The patients of the Oslo cohort had a median age of 44 years (IQR 34-56), with a median BMI of 25.6 (IQR 23.7-29.2). The median FVIII dose per injection was 21.3 IU/kg in Malmö (IQR 14.5-26.4), and the frequency of injections was 182 per year (IQR 156-227.8). The corresponding numbers in Oslo were 20 IU/kg (IQR 12.2-25.1) and 156 (IQR 143-182), respectively.

Table 9. A comparison of clinical outcomes between the patients from Malmö and Oslo after the switch to BAY 81-8973.
ABR: annual bleeding rate, AJBR: annual joint bleeding rate, HJHS: Haemophilia Joint Health Score

	Malmö (N=18)		Oslo (N=20)		P-value
Parameter	**Mean**	**Median (IQR)**	**Mean**	**Median (IQR)**	
ABR	0.33	0 (0–0)	0.42	0 (0–0)	0.945
AJBR	0.11	0 (0–0)	0.26	0 (0–0)	0.617
HJHS	17.7	9.5 (3–35)	17.1	14 (12–19.8)	0.411
VERITAS-Pro	39.5	40 (28.5–47.5)	40.0	40 (31.8–46)	0.885
FVIII Consumption (IU/kg BW/Year	4018	3862 (3174–4860)	2891	2337 (1843 –3912)	0.006

There were no significant differences in the clinical outcomes of ABR, AJBR, arthropathy as assessed by HJHS, and adherence to treatment as assessed by VERITAS-PRO, between the severe HA sub-cohorts in Malmö and Oslo. In contrast, the Malmö cohort had a median FVIII consumption of 3862 IU/kg/year, compared to 2337 IU/kg/year in the Oslo cohort ($p = 0.006$), as visualised in Figure 16.

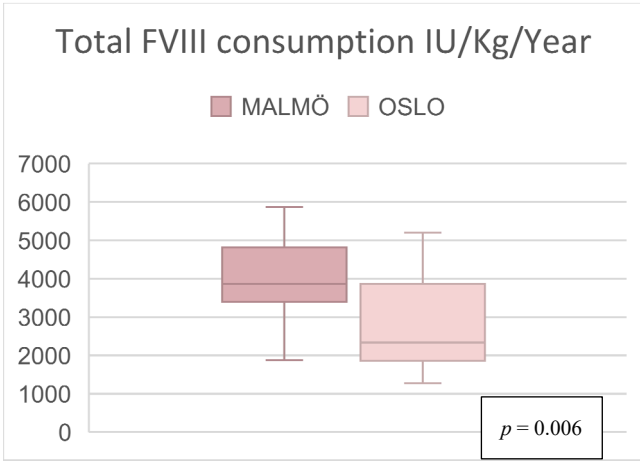


Figure 16. Annual total FVIII consumption (IU/kg/year) in Malmö and Oslo.

The Malmö centre had a lower absolute number of mean ABR and AJBR, but the difference was not significant and, as mentioned, there was no difference in arthropathy. The significant difference in factor consumption, which was observed despite both centres applying the same Nordic guidelines, was mainly the result of an overall more frequent administration and shorter dosing intervals in the Malmö cohort. However, the two groups were not matched, and recruitment bias cannot be ruled out, since all patients on prophylaxis with BAY 81-8973 were enrolled at the Malmö centre, but only one-half of those in Oslo. Furthermore, anonymous capture of register data in Oslo suggested overall slightly higher consumption in Oslo than observed in our enrolled sub-cohort. These findings nevertheless indicate the value of treatment individualisation and that more cost-efficient treatment strategies can still achieve the goal of treatment at both centres, which is an ABR and AJBR of zero bleeds. Our findings also show that population PK can identify patients with favourable PK, where treatment can be revised with either lower dosing or extended interval between doses, thus reducing treatment intensity without necessarily jeopardising the haemostatic efficacy.

Strengths and limitations

Limitations of this study include the retrospective design and subjective paper-based reporting of bleeds. Additionally, differences in how documentation is implemented at different centres may have influenced how bleeds were registered. Some selection bias cannot be ruled out as a result of the degree of enrolment at the Oslo centre. There were no pre-infusion levels collected for the PK analysis on BAY 81-8973 and no validation step was performed to confirm the PK estimates. In this well-managed cohort with very low reported ABR and AJBR and as a result of the sample size, any correlations between bleeding rates and arthropathy or adherence to treatment could not be detected. However, this may also be a consequence of the compensating influence of a personalised treatment plan and close follow-up of the patients. This study has several strengths, such as the comprehensive and thorough investigation of a homogeneous cohort of patients with moderate and severe HA which allowed for the correlation of clinical outcomes to pharmacokinetic parameters, adherence to treatment and FVIII consumption. In conclusion, this study's findings are of interest for the pursuit of treatment optimisation, as it shows that patients who have traditionally received high-dose prophylaxis regimens[140] can achieve favourable outcome rates despite the use of mainly intermediate-intensity regimens and the presence of haemophilic arthropathy.

Paper III

Patient and treatment characteristics

This study further investigated the severe HA cohort of Paper II, with the intention to elucidate the underlying reasons for the differences in annual FVIII consumption observed in Paper II. This analysis therefore included the 38 patients with severe HA who switched to BAY 81-8973 treated at the centres of Malmö and Oslo. Eighteen patients were treated at the Malmö haemophilia centre and 20 at the Oslo centre.

Impact of timing of prophylaxis commencement and patient age

Primary prophylaxis was defined as continuous regular prophylaxis commenced at least once weekly with SHL or EHL FVIII products before the patient reached 3 years of age, and before the second joint bleed or manifest joint disease.[39] Secondary prophylaxis was continuous regular prophylactic treatment, which did not fulfil the criteria of primary prophylaxis. The term "secondary prophylaxis" was chosen for the entire non-primary cohort, as in previous publications,[289-291] to avoid potential misclassification, because we did not have a complete data set on joint status at the start of prophylaxis. However, many patients in the secondary group probably had tertiary prophylaxis, i.e. prophylaxis initiated after the onset of documented joint disease.[32]

Data on the timing of start of prophylaxis and type of prophylaxis were available for 37 of the 38 enrolled patients. Of these, 15 patients, with a median age at study enrolment of 26 years (18-35 years) started primary prophylaxis, and 22 patients with a median age at enrolment of 45 years (40.8-59.8 years) were on secondary prophylaxis (Table 10). The median age was 1.25 years (1-2 years), and 31.5 years (10.5-42.8 years) at the start of primary- and secondary prophylaxis, respectively. The median ABR and AJBR after the switch to BAY 81-8973 for both the primary and secondary prophylaxis group was 0 (0-0). There were significant differences between the primary and secondary prophylaxis groups in HJHS and FVIII consumption (Figure 17) with a median HJHS of 4 (2-11) and 20 (12.5-35.5), respectively ($p < 0.001$). Median annual FVIII consumption was 3883 IU/kg/year (3319-4853) in the primary vs. 2737 IU/kg/year (1896-3909) in the secondary group ($p = 0.02$). Patient age at study enrolment correlated to age at start of prophylaxis ($p = 0.001$). Two patients in the primary prophylaxis and seven patients in the secondary prophylaxis group reported the use of pain medication.

Table 10. Clinical characteristics and outcomes of primary and secondary prophylaxis group.

	Primary Prophylaxis N = 15	**Secondary Prophylaxis N = 22**	***p***
Age at inclusion (Years)	26 (18-35)	45 (40.8-59.8)	
Patients with null-mutation, N (%)	9 (60)	15 (68.2)	
Age at start of prophylaxis (Years)	1.25 (1-2)	31.5 (10.5-42.8)	
ABR	0 (0-0)	0 (0-0)	0.960
AJBR	0 (0-0)	0 (0-0)	0.939
HJHS	4 (2-11)	20 (12.5-35.5)	< 0.001
FVIII Consumption (IU/Kg/Year)	3883 (3319-4853)	2737 (1895-3909)	0.02
EQ-5D-5L Index	0.9647 (0.934-0.9755)	0.904 (0.8332 -0.9647)	0.022
EQ-5D-5L VAS	87 (80-93.5)	75 (60-82.5)	0.01

Twenty-two patients in the study cohort had secondary prophylaxis; 68.2% (n=15) of these patients were treated at the Oslo centre. Reflecting changes in clinical practice over the last decades, there was a strong correlation between the current age of the patients, and the type of prophylaxis at start. The primary group had a significantly lower HJHS with a median score of 4, compared to 20 in the secondary group illustrating the benefits of starting primary prophylaxis at an early age, when joints are more susceptible to bleedings.[132,136,138,292] Interestingly, despite more arthropathy, the secondary prophylaxis group had a very low AJBR with a median of 0, despite FVIII consumption of a median of 2737 IU/kg/year, compared to 3883 IU/kg/year in the primary prophylaxis group. As most patients on secondary

prophylaxis were treated at the Oslo centre, this was a probable contributing factor to the observed difference in FVIII consumption between the two centres, as seen in Paper II.

These findings show that primary prophylaxis is beneficial in avoiding progressive joint damage, that the intensity of prophylaxis may be successfully lowered in adults without significantly jeopardising the bleeding phenotype and illustrate the importance of personalised treatment in haemophilia with the goal of improved outcomes. Through close patient follow-up and treatment adjustments according to bleeding phenotype, the treatment goal of zero bleeds was pursued through different dosing intensity regimens at the two centres but resulted nonetheless in a median ABR of 0.

Without doubt, the difference in median age at inclusion of almost 20 years between the primary and secondary prophylaxis groups may have been of importance for these outcomes, but age-matched comparisons regarding prophylaxis type are not possible in a Scandinavian cohort as all severe HA patients born in the last decades are on primary prophylaxis.

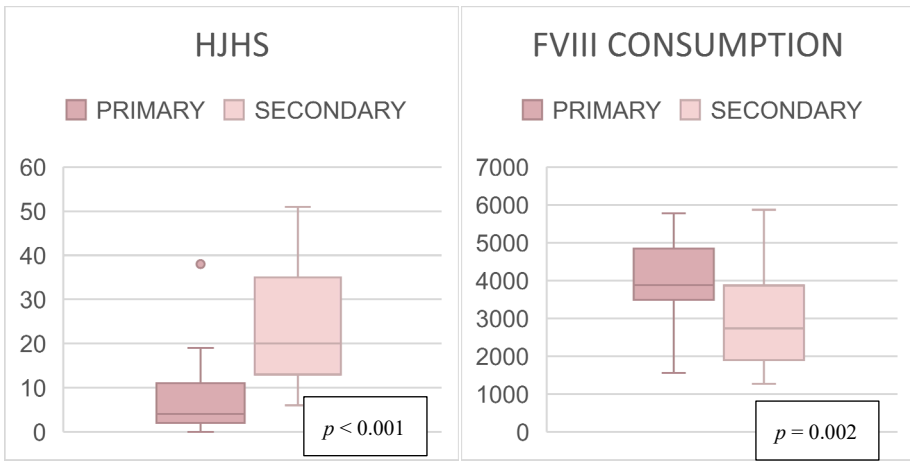


Figure 17. HJHS and FVIII consumption in primary and secondary group.
Boxplots showing difference in HJHS (A) and FVIII consumption (IU/kg/year) (B) between primary and secondary group.

Importantly, the subgroup that commenced secondary prophylaxis during childhood, between 3-9 years, but still had a higher median HJHS of 15 compared to a median HJHS of 4 in the primary prophylaxis group, despite moderate to high median FVIII consumption of 3872 IU/kg/year, signalling that high treatment intensity cannot compensate for a delayed prophylaxis start regarding the risk of developing arthropathy. Assessment of arthropathy with HJHS may be influenced by acute bleeds or inflammation. However, the HJHS was performed by

experienced physiotherapists at both centres and the absence of bleeds in the cohort implies that the evaluations of the joints were mostly performed at steady state.

Impact of F8 genotype

F8 gene variants were identified in all patients, i.e. inversions (N=13), missense variants (N=11), small deletions (N=8) and nonsense (N=5) variants. One patient had a splice variant (Table 11).

Table 11. *F8* gene variants found in the study cohort.

PAT-ID	Mutation Type / Effect	HGVS cDNA	HGVS protein	Mutation Group	Exon/Intron (FVIII Domain)
		NM_000132.4	NP_000123.1		
1	Substitution / Missense	c.902G>A	p.(Arg301His)	Non-null	Exon 7 (A1)
2	Substitution / Missense	c.902G>A	p.(Arg301His)	Non-null	Exon 7 (A1)
3	Deletion inside poly-A run/ Frameshift	c.3637delA	p.(Ile1213Phefs*5)	Non-null	Exon 14 (B)
4	Duplication / Frameshift	c.5116_5117 dupAG	p.(Ser1706Argfs*26)	Null	Exon 14 (a3)
5	Substitution / Nonsense	c.6590T>A	p.(Leu2197*)	Null	Exon 24 (C2)
6	Substitution / Nonsense	c.471G>A	p.(Trp157*)	Null	Exon 4 (A1)
7	Inversion 22			Null	
8	Small deletion outside poly-A-run/ Frameshift	c.954_955delCT	p.(Leu319Aspfs*18)	Null	Exon 7 (A1)
9	Duplication / Frameshift	c.6360dupT	p.(Ile2121Tyrfs*5)	Null	Exon 22 (C1)
10	Substitution / Missense	c.1795G>C	p.(Asp599His)	Non-null	Exon 12 (A2)
11	Small deletion outside poly-A-run/ Frameshift	c.954_955delCT	p.(Leu319Aspfs*18)	Null	Exon 7 (A1)
12	Inversion 1			Null	
13	Inversion 22			Null	
14	Substitution / Missense	c.6563G>A	p.(Cys2188Tyr)	Non-null	Exon 23 (C1)
15	Small deletion outside poly-A-run / Nonsense	c.1599delA	p.(Val534*)	Null	Exon 11 (A2)
16	Inversion 22			Null	
17	Small deletion inside poly-A run/ Frameshift	c.3637delA	p.(Ile1213Phefs*5)	Non-null	Exon 14 (B)
18	Substitution / Missense	c.902G>A	p.(Arg301His)	Non-null	Exon 7 (A1)
19	Inversion 22			Null	
20	Inversion 22			Null	
21	Inversion 22			Null	
22	Inversion 22			Null	
23	Small deletion outside poly-A-run/ Frameshift	c.205_206delCT	p.(Leu69Valfs*13)	Null	Exon 2 (A1)
24	Inversion 22			Null	
25	Substitution / Nonsense	c.3175A>T	p.(Lys1059*)	Null	Exon 14 (B)
26	Substitution / Missense	c.5825G>A	p.(Gly1942Asp)	Non-null	Exon 18 (A3)
27	Substitution / Nonsense	c.5883G>A	p.(Trp1961*)	Null	Exon 18 (A3)
28	Substitution /Missense	c.6273G>C	p.(Lys2091Asn)	Non-null	Exon 21 (C1)
29	Substitution / Missense	c.6278A>T	p.(Asp2093Val)	Non-null	Exon 22 (C1)

PAT-ID	Mutation Type / Effect	HGVS cDNA	HGVS protein	Mutation Group	Exon/Intron (FVIII Domain)
		NM_000132.4	NP_000123.1		
30	Substitution / Missense	c.5624T>G	p.(Leu1875Arg)	Non-null	Exon 17 (A3)
31	Inversion 22			Null	
32	Inversion 22			Null	
33	Inversion 22			Null	
34	Substitution / Missense	c.5825G>A	p.(Gly1942Asp)	Non-null	Exon 18 (A3)
35	Inversion 22			Null	
36	Substitution / Splice-cite change within conserved region	c.6115+5G>A		Null	Intron 19
37	Substitution / Nonsense	c.2440C>T	p.(Arg814*)	Null	Exon 14 (B)
38	Substitution / Missense	c.6545G>T	p.(Arg2182Leu)	Non-null	Exon 23 (C1)

Twenty-five variants were classified as null, and thirteen as non-null (Figure 18). The distribution of null variants in the primary and secondary prophylaxis group was 60% and 68.2%, respectively (Table 10). In the entire cohort, there was no difference between the null and non-null groups in HJHS, ABR, AJBR, FVIII consumption, start at age or prophylaxis, EQ-5D-5L index or EQ VAS. However, in the secondary prophylaxis group, there was a trend towards lower consumption in the non-null group with a median FVIII consumption of 1926 IU/kg/year (1867-2737), compared to 3370 IU/kg/year (2333-4021) in the null group ($p = 0.139$), while maintaining median ABR 0 vs. 0 and similar HJHS of 17 vs. 21, respectively (Figure 19).

Figure 18. Distribution and classification of null and non-null *F8* gene variants.

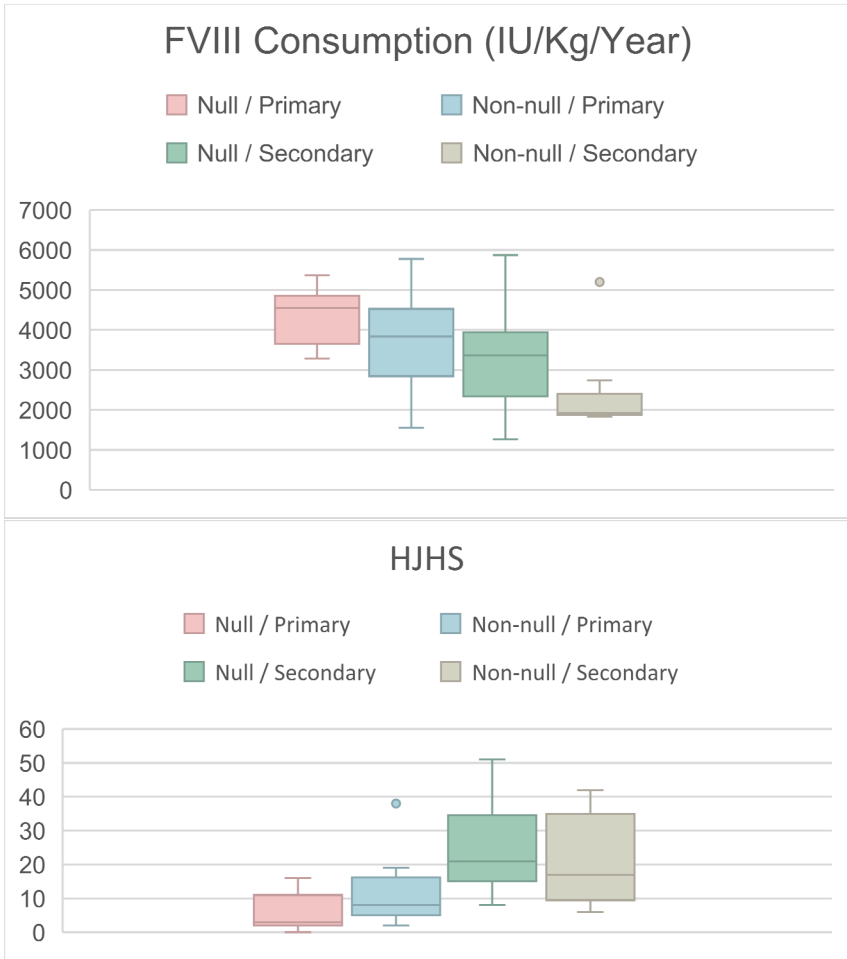


Figure 19. FVIII consumption and HJHS according to genotype and prophylaxis type.
Boxplots showing differences in FVIII consumption (IU/kg/year) and HJHS, according to FVIII variant status (non/non-null) and type of prophylaxis (primary/secondary).

Previous studies in paediatric cohorts[51,52] have shown that the type of *F8* genotype can influence the start of the first bleed, which, in turn, may impact upon the timing for start of prophylaxis. As a result of the small sample size, statistical analysis did not show any significant differences in the impact of the null and non-null groups upon start of prophylaxis and risk of developing arthropathy. However, subgroup analysis showed a trend towards lower FVIII consumption in the secondary prophylaxis group in the presence of non-null variants, with similar HJHS and ABR to the higher consumption null group. This finding could indicate that circulating trace amounts of FVIII may impact upon the bleeding phenotype[51] and subsequent development of arthropathy. Therefore, dose reductions of factor replacement in

non-null mutations in the secondary prophylaxis setting could be considered, but further studies are needed.

Health related quality of life assessment

The EQ-5D-5L questionnaire was completed by 34 patients, 13 in the primary and 21 in the secondary prophylaxis group. HRQoL in the entire study cohort was high with a median EQ-5D-5L index above 0.9 and median VAS 80. However, as shown in Table 10 and Figure 20, there were significant differences in the median EQ-5D-5L Index value and EQ VAS between the younger (median age 26 years) primary prophylaxis group and the older (median age 45 years) secondary prophylaxis group with median EQ-5D-5L Index 0.9647 (0.934-0.9755) vs. 0.904 (0.8332-0.9647) (p = 0.022) and EQ VAS 87 (80-93.5) vs. 75 (60-82.5), (p = 0.01), respectively.

These small but significant differences in HRQoL outcomes between the primary and secondary group underscore both the influence of age and the value of primary prophylaxis. The presence of a disability paradox, where haemophilia patients report higher health state evaluations than otherwise healthy peers cannot be excluded.[293] Comparable HRQoL outcomes were seen between the older delayed prophylaxis cohort and other published European cohorts.[294,295] Furthermore, the absence of bleeding episodes in this cohort can be expected to exert beneficial affects against the development of synovitis and further progression or arthropathy[89] and is another reflection of the benefits of individualised prophylaxis, which has been the treatment goal for Scandinavian patients with HA since at least the 1990s.[296]

When the distinct dimensions results were dichotomised into “no problems” vs. “any problems”, more patients experienced problems in the secondary group in all dimensions (Figure 21).

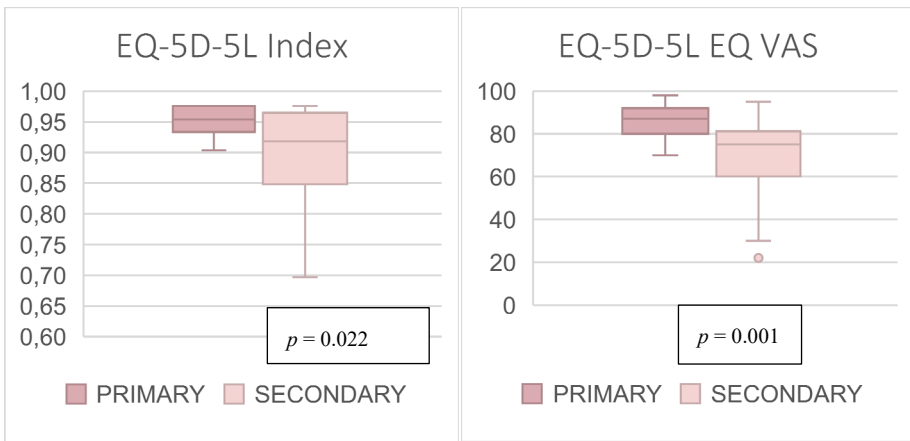


Figure 20. Health-related quality of life results according to prophylaxis type.
Boxplots showing differences in EQ-5D-5L Index value and EQ VAS between the primary and secondary prophylaxis group.

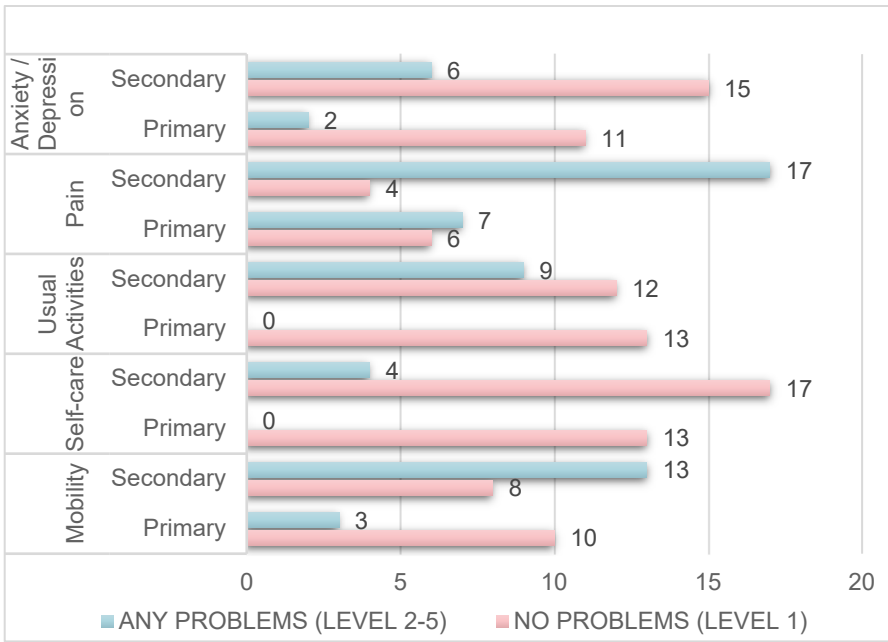


Figure 21. EQ-5D-5L dimension results.
Bar chart showing dimension results of EQ-5D-5L after dichotomisation in "no problems" (level 1) and "any problems" (levels 2-5).

In the dimension "pain", 7 of 13 patients (53.8%) in the primary-, whereas 17 of 21 (80.9%) in the secondary prophylaxis group experienced problems. This contrasted with the use of medication for pain management, mostly anti-inflammatory drugs (NSAID or COX-2 inhibitors), by two (13.3%) and seven (35%) patients in the primary and secondary prophylaxis group, respectively. This discrepancy between reported pain and use of painkillers, seen in both the primary and secondary prophylaxis groups may imply undertreatment of pain problems, as reported elsewhere.[297] However, no one in the secondary prophylaxis group reported the use of opioids and the highest EQ-5D-5L score in the pain dimension was 3, signifying moderate pain even in patients with relatively advanced arthropathy. Even though these findings are based on few patients, they suggest the benefit of prophylaxis against severe pain, possibly as a result of a reduction in subclinical bleeds and synovitis.[298,299]

Strengths and limitations

Limitation of this study include the small sample size, which may have contributed to a risk of recruitment/selection bias and influenced the significance of the analyses, such as the impact of null vs. non-null genotype. Additionally, this study did not investigate how possible differences in the dosing regimens and the bleeding

phenotype at treatment start and over the years, may have influenced clinical outcomes.

This study's main strength was the examination of a severe HA cohort who had received individualised prophylaxis for many decades, with the goal of bleeding freedom in mind. This study was thus able to investigate the impact of non-primary prophylaxis on bleeding, arthropathy and HRQoL in adults, and the differences for younger patients on primary prophylaxis. Furthermore, this study investigated the impact of genotype upon the relevant clinical outcomes of factor consumption and arthropathy in an adult cohort, whereas previous studies primarily examined paediatric cohorts and the genotype's impacts on bleeding and inhibitor development.[51,52,300] This may prove of value with a global perspective in mind, as the majority of adult patients with severe HA in developing countries on FVIII replacement therapy have secondary prophylaxis and knowledge of the genotype can assist in treatment personalisation and optimising the management of economic resources.

Paper IV

Patient and treatment characteristics

Thirty-five adult male patients treated at the comprehensive haemophilia centres in Malmö and Gothenburg were eligible for study inclusion and 30 patients were enrolled. All patients were born after 1980 and had severe HA with primary prophylaxis and no history of FVIII inhibitors.

At study inclusion, the median age was 33.5 years (24.3-38 years) and median BMI was 24.8 (22.9-28.9). A positive family history of haemophilia was present in 11 patients. Genetic characterisation revealed null *F8* genotype in 26 patients and non-null genotype in four patients. There was no documentation of prophylaxis interruption in any patient.

Early bleeding phenotype and prophylaxis start in childhood.

Prophylaxis with a once-weekly regimen commenced in childhood at a median age of 1.2 years (1-1.3 years). Transition to the full-dose escalated prophylaxis regimen occurred at a median age of 1.7 years (1.3-1.8 years).

Before the initiation of prophylaxis, a median of 0 joint bleeds (0-0) and one non-joint bleed (1-3), requiring FVIII concentrate infusion, were documented. Median FVIII dose at prophylaxis start was 47.8 IU/kg (33.9-54.2) with median infusion once weekly, as illustrated in Figure 22.

During the period after the start of once-weekly prophylaxis and prior to the escalation to the final prophylaxis regimen, a median of 0 (0-0) of both joint and

non-joint bleeds was documented. Median FVIII dose at transition to escalated prophylaxis was 41.7 IU/kg (37.2-45.6) with median infusion frequency thrice weekly (range twice weekly to daily), signifying that most patients were on high-dose regimens with annual FVIII consumption above 4000 IU/kg/year.[32]

In 14 patients, a subcutaneous venous port (SVP) was installed, which did not impact the age at the start of prophylaxis (median 1.2 years with SVP vs. 1.3 years without). However, the presence of a SVP correlated with a significantly shorter time between start and escalated prophylaxis (median 0.3 years vs 0.7 years, p = 0.024) and fewer non-joint bleeds (ρ = 0.542, p = 0.004) during that period. Additionally, there was significant correlation between the time from the start to escalated prophylaxis and the incidence of joint ($\rho = 0.470$, $p < 0.018$) and non-joint ($\rho = 0.703$, $p < 0.001$) bleeds, as seen in Figure 23. Higher patient age at inclusion correlated with higher age at transition to the final prophylaxis regimen ($\rho = 0.687$, $p < 0.001$).

A shorter time to escalated prophylaxis seen in younger patients and those with SVP may signify changing treatment practice over time. With the finding that it shortens the escalation period and fewer bleeds in mind, insertion of an SVP or switch to non-factor replacement therapies should therefore be considered at an early stage if the administration of factor replacement poses a challenge.

No impact of the *F8* genotype (null vs non-null) or knowledge of positive heredity upon bleeding or prophylaxis start patterns was shown. The significance of these findings is uncertain as a result of the study's sample size.

Treatment characteristics in adulthood

These data were collected at study inclusion and based on documentation at the last regular visit to the study centre. The median ABR was 0 (0-0) and AJBR was 0 (0-0.2). No target joints were reported. Median annual FVIII consumption was 4277 (3622-4672) IU/kg/year.

Assessment of adherence to treatment was performed with VERITAS-PRO.[266] Median VERITAS-PRO score was 35 (30-42). A score < 56 was seen in 32 of 33 patients, signifying an adherence rate of 96.9%. Best results were observed in sub-categories of "dosing" and "skipping"; worse results were observed in "timing", "remembering" and "communication" (Figure 22).

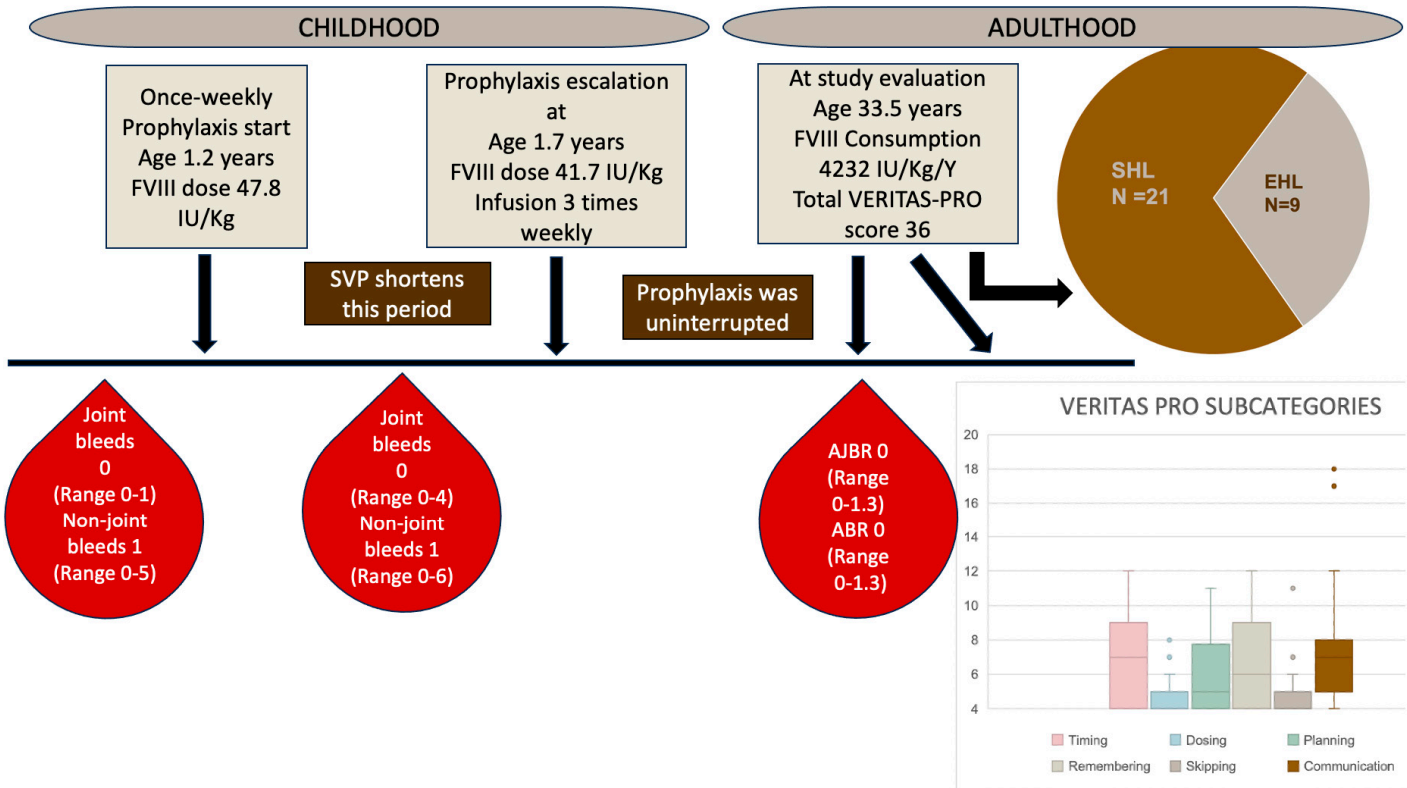


Figure 22. Clinical and treatment characteristics at study inclusion and prophhylaxis start.

At their last visit, 21 patients were treated with SHL products and nine with EHL products (Figure 22). The median age was 34 years (31.5-39 years) vs. 25 (21-35) years (p=0.05) and median FVIII consumption was 4386 (3622-4672) vs. 4056 (3441-4056) IU/kg/year for the SHL vs. EHL groups, respectively. Median ABR was 0 (0-0) and median HJHS was 2 for both the SHL and EHL groups. The choice of product type did not impact upon adherence. The high number of patients treated with SHL products may be explained by the influence of age in FVIII pharmacokinetics, which may potentiate the efficiency of treatment with SHL produts,[144] the reluctancy of physicians and patients to implement regimen changes during the previous pandemic years, and some patients' participation in ongoing or recently completed clinical studies with SHL products. The very low bleeding rates in adulthood may also be related to the cohort's excellent adherence rate of 100%, as all patients had a VERITAS-PRO total score that was under the cut-off score of 57.[283]

Joint health from childhood to adulthood

The patients' joint health development through the decades was examined by the assessment of repeated HJHS examinations performed throughout the years. HJHS examinations performed at 3-5 years intervals were assessed, starting at childhood and continuing into adulthood. As the patients' ages spanned from 18 to 42 years at inclusion, the number of HJHS assessments available per patient differed, but at least three assessments were documented per patient.

Joint assessments were performed using the HJHS by physiotherapists or physicians with experience in haemophilia. Prior to 2006, the WFH Orthopaedic Joint Score[262] was used, and those scores were converted to the corresponding HJHS by a physician or physiotherapist for the purposes of this study. All the assessments that were selected for inclusion in this study were performed at a non-bleeding state, according to available documentation in the medical records.

This analysis showed that the HJHS increases slowly through the decades despite high-intensity primary prophylaxis. The median HJHS was 0 until the 20-25 years period, when it increased to a median of 1. Median HJHS continued to increase gradually afterwards, reaching a median of 4 at 35-40 years (Figure 24A and 24B). There may have been an impact of ageing on the joint outcomes of this cohort. Additionally, HJHS increases with age despite prophylaxis.[301] However, the observed median HJHS of 4 at 35-40 years in the cohort is higher than that seen in age-matched non-haemophiliacs,[302] and is likely representative of arthropathy. These findings show that primary prophylaxis is undoubtably effective in delaying the onset of haemophilic arthropathy but cannot completely prevent it. Once it has debuted, with time, the degree of arthropathy will gradually increase.

Multiple significant correlations between HJHS in youths (15-20 years) and later in life, i.e. at 20-25 years (ρ=0.716, p<0.001), 25-30 years (ρ=0.629, p=0.02) and 35-

40 years (ρ=0.651, *p*=0.022), were identified. The worst HJHS value for each patient correlated to their age at transition to the final prophylaxis regimen (ρ=0.498, *p*=0.007), as shown in Figure 23.

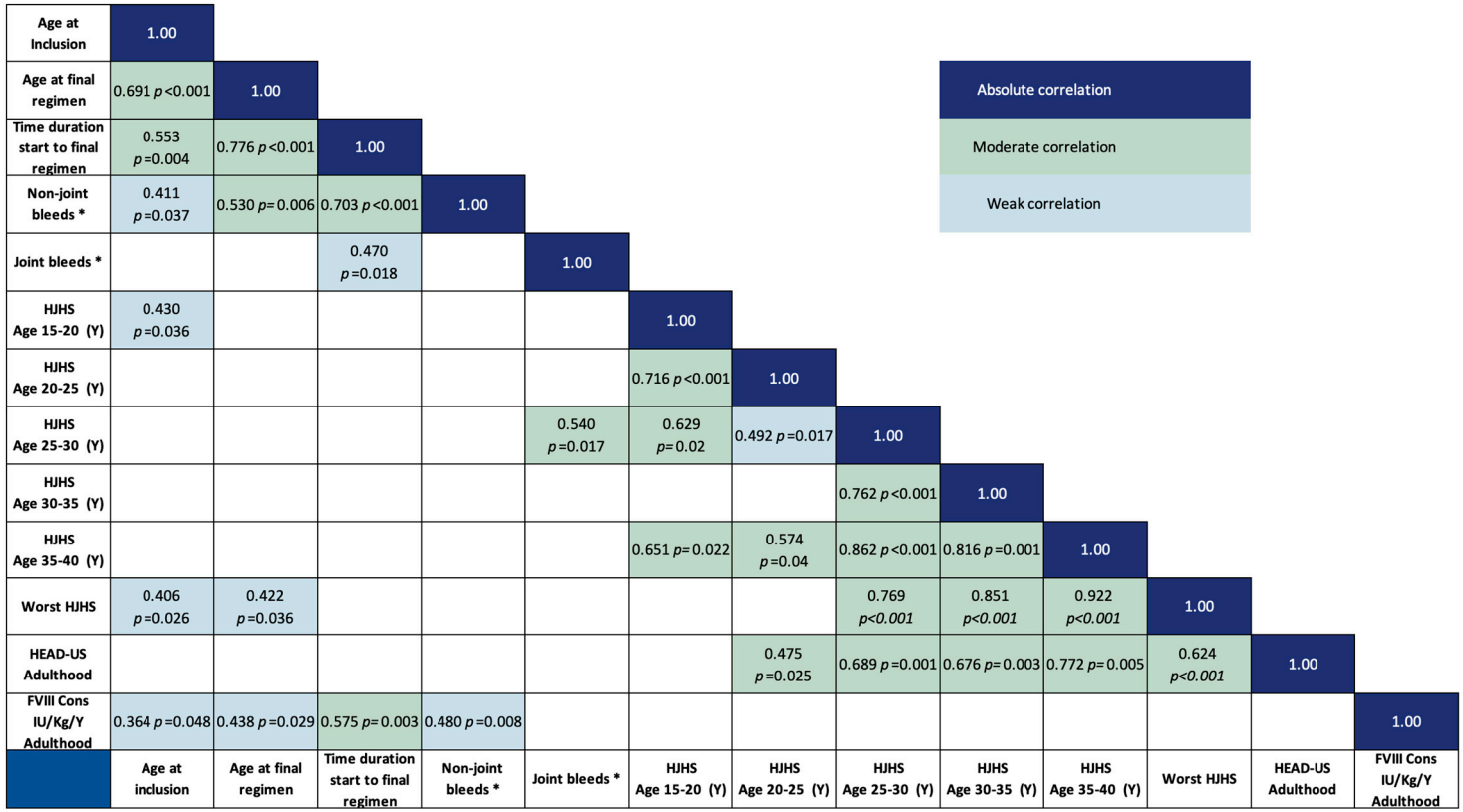
This study's findings thus signify the need for an early start of joint assessments, as they can be indicative of future joint outcomes. In contrast, bleeds prior to prophylaxis did not impact upon joint outcomes, which was probably caused by the sparsity of joint bleeds prior to prophylaxis start in this primary prophylaxis cohort.

Evaluation of joint health was also performed by ultrasound analysis, according to the HEAD-US (Haemophilia Early Arthropathy Detection with Ultrasound) protocol.[106] Twenty-six patients were evaluated at a median age of 32 years (21.5-36). The median total HEAD-US score was 1 (0-2). The median score was 0 (0-0) for the elbow, knee, and ankle joints (Table 12). Bone or cartilage changes were identified in six right (23.1%) and five left (19.2%) ankle joints, respectively. The HEAD-US score correlated to HJHS at the 20-25 (ρ = 0.475, *p* = 0.025), 25-30 (ρ = 0.689, *p* = 0.001), 30-35 (ρ = 0.676, *p*=0.003), and 35-40-years periods (ρ = 0.722, *p* = 0.005), as shown in Figure 23. The complete HEAD-US data are shown in Table 13.

Based on the combined findings by HJHS and HEAD-US, the assessed joints were classified as pristine, if the joint HJHS was below four and HEAD-US did not show signs of bone or cartilage damage, as published previously.[303] A joint with HJHS above four was classified as non-pristine, even if HEAD-US was not available.

The right ankle joint was the most affected, as 76% of joints (19/25) were classified as pristine, whereas 84% (21/25) of left ankle, and 86.5% (45/52) of all knee joints were pristine. This is consistent with a recent magnetic resonance imaging (MRI) evaluation of a younger (mean age 23.5 years) Swedish moderate and severe haemophilia cohort that showed osteochondral changes in the ankle but not the knee joints.[304] All left elbow joints (28/28) and 84.6% (22/26) of right elbow joints were classified as pristine. HJHS and HEAD-US have shown good inter-rater reliability and correlation with MRI findings of synovial hypertrophy and osteochondral damage, even when performed by non-radiologists.[305-307] Nonetheless, our findings indicate that high-intensity primary prophylaxis delays arthropathy and 40% (10 of 26) of patients in the cohort had pristine joints.

These findings show that primary prophylaxis is effective in preserving knee joint health, which is also supported by MRI findings from another Swedish primary prophylaxis cohort.[304] The slightly worse findings in the right elbow were also seen in a recent study evaluating subjectively affected joints in a German haemophilia cohort,[308] maybe the result of a higher percentage of right-handed persons,[309] but this finding needs to be evaluated further.



	Age at inclusion	Age at final regimen	Time duration start to final regimen	Non-joint bleeds *	Joint bleeds *	HJHS Age 15-20 (Y)	HJHS Age 20-25 (Y)	HJHS Age 25-30 (Y)	HJHS Age 30-35 (Y)	HJHS Age 35-40 (Y)	Worst HJHS	HEAD-US Adulthood	FVIII Cons IU/Kg/Y Adulthood
Age at Inclusion	1.00												
Age at final regimen	0.691 p <0.001	1.00											
Time duration start to final regimen	0.553 p =0.004	0.776 p <0.001	1.00										
Non-joint bleeds *	0.411 p =0.037	0.530 p= 0.006	0.703 p <0.001	1.00									
Joint bleeds *			0.470 p =0.018		1.00								
HJHS Age 15-20 (Y)	0.430 p =0.036					1.00							
HJHS Age 20-25 (Y)						0.716 p <0.001	1.00						
HJHS Age 25-30 (Y)					0.540 p =0.017	0.629 p= 0.02	0.492 p =0.017	1.00					
HJHS Age 30-35 (Y)								0.762 p <0.001	1.00				
HJHS Age 35-40 (Y)						0.651 p= 0.022	0.574 p =0.04	0.862 p <0.001	0.816 p =0.001	1.00			
Worst HJHS	0.406 p =0.026	0.422 p =0.036						0.769 *p<0.001*	0.851 *p<0.001*	0.922 *p<0.001*	1.00		
HEAD-US Adulthood							0.475 p =0.025	0.689 p =0.001	0.676 p= 0.003	0.772 p= 0.005	0.624 *p<0.001*	1.00	
FVIII Cons IU/Kg/Y Adulthood	0.364 p =0.048	0.438 p =0.029	0.575 p= 0.003	0.480 p =0.008									1.00

Figure 23. Significant correlations between clinical variables of prophyaxls and joint outcomes.

Evaluation of joint health was also performed by ultrasound analysis, according to the HEAD-US (Haemophilia Early Arthropathy Detection with Ultrasound) protocol.[106] Twenty-six patients were evaluated at a median age of 32 years (21.5-36). The median total HEAD-US score was 1 (0-2). The median score was 0 (0-0) for the elbow, knee, and ankle joints (Table 12). Bone or cartilage changes were identified in six right (23.1%) and five left (19.2%) ankle joints, respectively. The HEAD-US score correlated to HJHS at the 20-25 ($\rho = 0.475$, $p = 0.025$), 25-30 ($\rho = 0.689$, $p = 0.001$), 30-35 ($\rho = 0.676$, $p=0.003$), and 35-40-years periods ($\rho = 0.722$, $p = 0.005$), as shown in Figure 23. The complete HEAD-US data are shown in Table 13.

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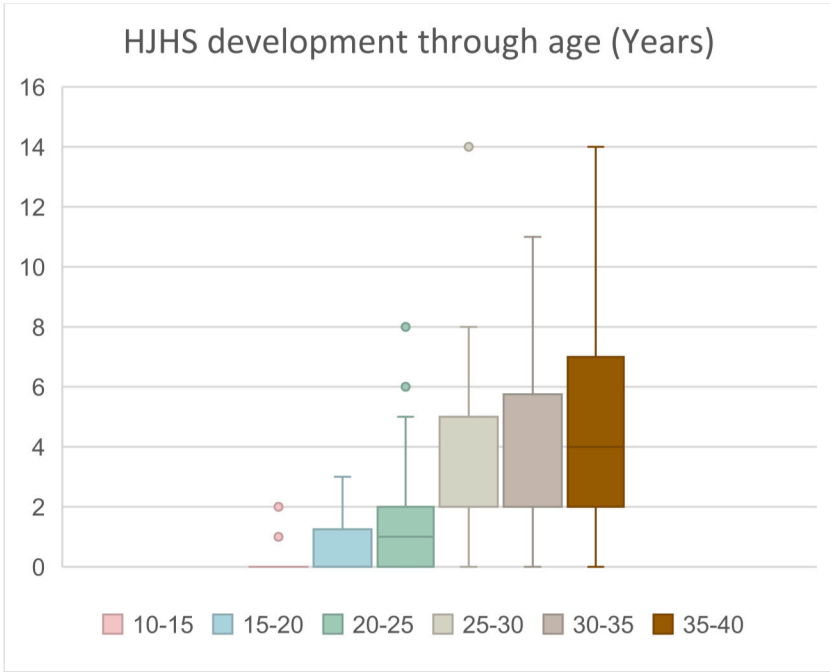
A



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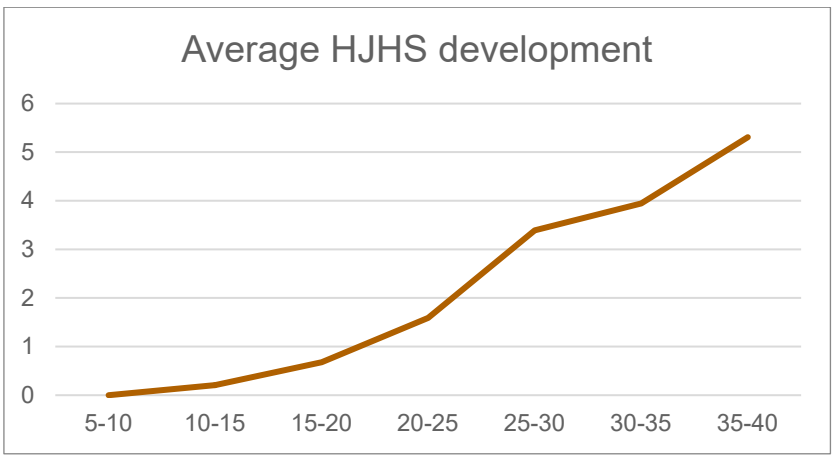

Figures 24A and B.
A. Median HJHS (Haemophilia Joint Health Score) development at progressive time periods of the patients' lives. B. Development of average value of cumulative HJHS through the years.

Five of the six patients who reported chronic pain used paracetamol or anti-inflammatory agents, except patient #24, who also used short-acting opioids. Three patients underwent orthopaedical interventions, i.e. two synovectomies with Yttrium-90 (patient #1 in the right knee at age 20 years and #26 in the right elbow at age 25 years) and one right elbow arthroscopy with synovectomy (#27 at age 34 years).

Table 12. Joint health development through progressive age periods.
Median (IQR) values.

Joint	HJHS in different age periods (years)							HEAD-US	Pristine joints n/N (%)
	5-10	10-15	15-20	20-25	25-30	30-35	35-40		
Right elbow	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-1)	0 (0-2.5)	0 (0-0)	22/26 (84.6%)
Left elbow	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	25/25 (100%)
Right knee	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	1 (0-1)	1 (0-1)	1 (0-1)	0 (0-0)	23/26 (88.4%)
Left knee	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	1 (0-1)	1 (0-1)	1 (0-1)	0 (0-0)	22/26 (84.6%)
Right ankle	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-1)	1 (0-2)	0 (0-0)	19/25 (76%)
Left ankle	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-0)	21/25 (84%)
Total HJHS	0 (0-0)	0 (0-0)	0 (0-1)	1 (0-2)	2 (2-5)	2 (2-6)	4 (2-7)	1 (0-2)	132/153 (86.3,%)

Strengths and limitations

Limitations of this study include the retrospective design, which did not allow for the documentation of bleeds during the early prophylaxis period that were not treated with factor concentrate. Joint health was assessed with HJHS and HEAD-US, which are dependent on operator skill.[310] MRI can show haemosiderin deposits and detect subclinical joint bleeds, occurring in approximately 16% of severe HA patients despite prophylaxis[311], which our study could not assess. In the HEAD-US analysis, they may have been some underdiagnosis of cartilage damage, possibly implying worse joint outcomes, despite the overall good repeatability of the HEAD-US protocol.[306] Secondary to the cohort size, this study was most likely underpowered to discover all significant correlations between the examined clinical variables and, as in a previous study,[312] no mathematical correction was applied for multiple comparisons. Moreover, we cannot be certain that treatment patterns remained unchanged for all patients during their lives, which may have impacted upon joint outcomes.

In contrast, a strength of this study is the well-documented assessment of joint health trough over a greater length of time. A thorough characterisation of the clinical phenotype and treatment practice was performed, both in childhood and adulthood. HJHS and HEAD-US are the most commonly used tools to assess joint health in haemophilia in clinical praxis, which strengthens the clinical relevancy of the findings. The assessment of adherence in a socioeconomically homogeneous cohort should be representative of overall Swedish practice. Additionally, the risk of selection bias is small, as 86.8% of eligible patients at the two centres were included. Finally, this study is one of the few to examine long-term outcomes in an adult population with primary prophylaxis.

Table 13. HEAD-US assessment at adulthood.

ROM: Range of motion. B: bone changes. C: cartilage changes S: synovial hypertrophy.

Patient ID	Age at analysis (Years)	Right elbow	Left elbow	Right knee	Left knee	Right ankle	Left ankle	Total Score
1	32	0	0	0	0	8 (no ROM)	0	8
2	31	0	0	1B	0	0	0	1
3	32	0	0	1B	1B	0	0	2
4	25	0	0	0	1C	0	0	1
5	37	0	0	0	0	0	0	0
6	29	0	0	0	0	0	1B	1
7								
8	31	0	0	0	0	0	1B	1
9	18	0	0	0	0	0	0	0
10	22	0	0	0	0	0	0	0
11	34	0	0	0	0	0	0	0
12	37	0	0	0	0	1B	0	0
13	18	0	0	0	0	1B	0	1
14	30	0	0	1B	1B	0	0	2
15	35	0	0	0	1B	0	0	1
16	38	0	0	0	1S	0	1S	2
17	34	1C	0	0	0	4BC	5BCS	13
18				0	0			
19								
20								
21	40	7BCS	0	0	0	1C	0	8
22	21	0	0	1S	0	0	0	1
23	19	0	0	0	0	2C	1C	3
24								
25	32	0	0	0	0	0	0	0
26	32	0	0	0	0	0	0	0
27	38	4BC	0	0	0	0	0	4
28	42	0	0	0	0	0	0	0
29	18	0	0	0	0	0	0	0
30	18	0	0	0	0	0	0	0

Conclusions

This thesis has investigated clinical, genetic and treatment aspects of importance in personalising treatment in haemophilia A in the pursuit of optimised outcomes.

In this context, this thesis assessed how heterogeneity in the genotype, clinical phenotype, type of prophylaxis, FVIII PK, and treatment intensity and adherence, impact upon the clinical outcomes of bleeding, joint health and the development of haemophilic arthropathy, HRQoL and FVIII concentrate consumption.

The key findings and conclusions derived of the papers comprising this thesis are as follows:

Paper I: Comparison of pharmacokinetic estimations and dose proposals by MyPKFiT and WAPPS-Hemo for octocog alfa (Advate)

- MyPKFiT and WAPPS-Hemo can overcome the discordance between the results of the Chr and OS assays in their estimations of t½ and time to target troughs.
- MyPKFiT and WAPPS-Hemo provide similar t½ estimations for octocog alfa, independent of the assay used (Chr or OS).
- The time to reach trough levels 1-5% was significantly longer in the estimations made by WAPPS-Hemo, compared to MyPKFiT, which resulted in significant differences in the dosing proposals to achieve target troughs.
- Clinicians should be aware of these discrepancies and consider them when making treatment decisions.

Paper II: Clinical outcomes before and after the switch to BAY 81-8973 and adherence rate

- The switch from other SHL FVIII concentrates to BAY 81-8973 preserved excellent bleeding rates with median ABR and AJBR of 0, both prior to and after the switch, using mostly intermediate dose regimens, signalling the importance of treatment individualisation.
- BAY 81-8975 has a favourable half-life of 15.15 hours, but there is significant correlation to VWF:Ag levels.

- The Oslo cohort had similar arthropathy and bleeding to the Malmö cohort, with significantly less annual FVIII consumption compared to the Malmö cohort, 2337 vs. 3862 IU/kg/year, respectively.
- Excellent adherence to treatment contributes to optimised bleeding outcomes despite the presence of arthropathy.

Paper III: Impact of prophylaxis timing and *F8* genotype on clinical outcomes and HRQoL

- Delayed prophylaxis start in older patients with severe HA can protect against bleeding but is correlated to more severe arthropathy and worse HRQoL, compared to younger patients on primary prophylaxis.
- A non-null *F8* genotype can predict the bleeding phenotype and may be associated with a reduced risk of arthropathy despite less intense prophylaxis.
- The majority of patients in Oslo were on secondary prophylaxis, whereas most patients in Malmö were on primary prophylaxis, which contributed to the discrepant FVIII consumption observed in Paper II.

Paper IV: Long-term joint health outcomes with primary prophylaxis and early prophylaxis patterns and clinical phenotype

- Primary prophylaxis in severe HA is effective in delaying the development of arthropathy but cannot completely prevent it from occurring.
- At prophylaxis implementation, escalation to the final regimen should occur as soon as possible to prevent bleeds.
- Assessment of joint outcomes should begin at an early age, as a higher HJHS in the adolescent period correlated with joint outcomes later in life.

Epilogue

Future perspectives - a bright tomorrow for all?

The cover of this book depicts an impressive accomplishment: Chris Bombardier, who has severe haemophilia, climbed the seven summits, culminating with him standing on the peak of Mount Everest in Nepal. This feat was made possible by human willpower and modern haemophilia therapy, which Mr Bombardier, who comes from the United States of America, has had access to all his life. However, such a feat is out of reach for the PwH who live in Nepal today. They, and many more PwH in developing nations, are suboptimally treated and their lives are affected by significant risk of bleeding and arthropathy. Approximately 75% of PwH living in low Human Development Index countries receive inadequate treatment.[313] Only 8% and 15% of the PwH living in Africa and South-East Asia, respectively, even receive a diagnosis.[314] For many PwH in developing countries, haemophilia is a life sentence.

Acknowledgement of this inequality was one of the reasons for the creation of the WFH in the 1960s.[315] Educational programmes and WFH-shepherded twinning programs aim to educate medical personal in developing countries, aiming to improve haemophilia management.[316,317]

Through aid programmes, PwH living in low and low-middle income countries have gained access to factor concentrates, and, more recently, novel therapies, such as FVIII-mimetics.[318] By the benefit of ease of administration, novel therapies can reduce the burden of disease for patients and/or their caregivers, while simultaneously relieving the strain in the usually scarce health-care resources of developing countries.[319] Novel therapies are, however, very expensive and presently out of reach for PwH in low-income countries, outside the setting of aid programmes. At present, low dose prophylaxis with both SHL and EHL products is a more financially viable, though imperfect, option for low- and lower-middle income countries.[320-322] Population PK tools such as MyPKFiT and WAPPS-Hemo can assist in the personalisation of these treatments, thus efficiently managing the available recourses and optimising outcomes. Knowledge of the underlying arthropathy and *F8* genotype could help identify patients for whom treatment intensity could be adjusted safely while still providing protection from bleeds, as the findings in this thesis suggest. However, this thesis has shown the benefits of

primary prophylaxis of high intensity. Low-dose prophylaxis must therefore be considered as a solution of necessity, which we should not be content with.

People living with HA in developed countries have access to modern effective factor replacement therapy. Many have already switched to non-factor replacement therapy with emicizumab, and additional treatments are incoming. Gene therapy has altered the lives of some PwH, relieving them from the burden of factor infusions. However, with a global perspective in mind, the reality is that many PwH today do not have access to sufficient treatment to prevent bleeding and delay the progress of arthropathy.

Therefore, the hope of optimised outcomes for all PwH once again must reside on medical progress. The arrival of the novel treatments, such as FVIII mimetics, rebalancing agents, and gene therapy, is causing a paradigm shift in haemophilia care of the same magnitude as when Professor Nilsson first introduced factor replacement prophylaxis in Malmö. Additionally, the lowering of the cost of factor concentrates would make treatment of adequate intensity available to all PwH regardless of where they live. There is, therefore, reason for optimism that both future generations and PwH living with the disease today will be able to reap the benefits of today's advancements.

The pursuit of effective personalised treatment providing freedom from bleeds and healthy joints for all people with haemophilia lives on.

Populärvetenskaplig sammanfattning

Hemofili A är den vanligaste formen av klassisk blödarsjuka och orsakas av medfödd brist på en koagulationsfaktor som heter faktor VIII (FVIII), vilket är att äggviteämne som behövs för att kroppen ska kunna stoppa och förebygga blödningar. Sjukdomen ärvs könsbundet recessivt och drabbar företrädesvis män. Det uppskattas att cirka 1 av 5000 pojkar föds med hemofili A. Blödningsbenägenheten vid hemofili A är framför allt beroende på nivån av FVIII i blodet. Hemofili A klassificeras som svår om FVIII-nivå ligger under 1%, det vill säga omätbart lågt, moderat vid FVIII nivå 1–5%, och mild vid FVIII nivå 5–40%.

Patienter med svår hemofili A har störst risk för spontana blödningar, följt av de allvarligare formerna av moderat hemofili. Svår hemofili A kännetecknas framför allt av blödningar i leder och muskulatur, vilket kan uppträda spontant eller utan tydligt trauma. De leder som framför allt kan drabbas av blödning är armbågarna, knäna och anklarna. Ledblödningar brukar uppkomma med debut vid cirka ett års ålder, i anslutning till att barnet börjar gå, och kan successivt leda till ledskador, med förändringar i ledkapseln, brosk och ben och tilltagande påverkan i ledernas mobilitet och funktion. Genom att höja nivåerna av FVIII med profylaktisk substitutionsbehandling kan man reducera blödningarna och på så vis försöka bevara ledhälsan.

Sverige har varit ett föregångsland när det gäller denna typ av behandling och i dagsläget erbjuds alla hemofilipatienter med svårare former av sjukdomen profylax med början vid cirka ett års ålder. I enighet med tidigare observation att patienter med FVIII> 1% hade färre blödningar och bättre ledhälsa jämfört med patienterna med FVIII <1%, har behandlingsmålet under decennier varit att upprätthålla en lägsta nivå av faktorn (s.k. trough) kring 1% efter infusion. Denna nivå räcker dock inte för att skydda alla mot blödningar. Patienterna brukar ta extra faktorbehandling vid manifest eller misstänkt blödning men subkliniska blödningar, dvs små blödningar som inte ger upphov till smärta eller symtom, noteras ej och blir därmed ej åtgärdade med extra faktorbehandling. Detta innebär att även patienter som haft profylaktisk behandling sedan barndomen riskerar att utveckla ledskador under livet.

Med hjälp av farmakokinetik (PK) kan man undersöka omsättningen av FVIII i kroppen efter administration av FVIII-innehållande läkemedel. Farmakokinetiken varierar mellan olika individer, vilket innebär att samma dos av faktor VIII kan ge

varierande skydd från patient till patient. En farmakokinetisk beräkning har genom åren krävt en omfattande provtagning och har därför varit svår att använda i klinisk praxis. En mer förenklad metod som heter populationsbaserad PK behöver endast enstaka provtagningar för att kunna beräkna individens PK-profil, det vill säga hur FVIII omsättes hos den enskilde patienten. Intresset har därför ökat för hur farmakokinetiken kan utnyttjas tillsammans med kunskap av patientens kliniska sjukdomsbild för att skräddarsy behandlingen för varje patient i syfte att optimera behandlingens effektivitet och tillåta bästa möjliga nyttjandet av tillgängliga resurser.

Denna avhandling syftar till att genom kartläggning av kliniska panoramat och behandlingen vid hemofili A (kunskap om blödningsbilden vid barndom och vuxen ålder, kartläggning av ledhälsan hos olika patientgrupper, följsamhet till behandling, hälsorelaterad livskvalitet, och underliggande genetiska förändringar) samt farmakokinetiska analyser efter infusion av FVIII-preparat, öka kunskapen om hemofili A, med målet att individanpassa och optimera behandlingen.

Avhandlingen bygger på tre delarbeten som givit upphov till fyra artiklar.

Delarbete I ligger till grund för artikel I och bygger på jämförelsen av farmakokinetiska beräkningar av två populationsbaserade verktyg, MyPKFiT and WAPPS-Hemo, på patienter med svår hemofili A som behandlades med FVIII produkten octocog alfa. Provtagning avseende FVIII nivåerna genomfördes med hjälp av två olika laboratorieanalyser, den s.k. kromogeniska analysen och enstegsanalysen. Vi upptäckte att trots signifikanta skillnader i resultaten av dessa två metoder kunde båda MyPKFiT och WAPPS-Hemo övervinna dessa skillnader vid sina PK-beräkningar, vilket betyder att MyPKFiT och WAPPS-Hemo genererade likvärdiga resultat, oberoende av analysmetoden. MyPKFiT och WAPPS-Hemo gjorde likvärdiga beräkningar vad gäller halveringstiden av octocog alfa, vilket betyder tiden det tar för halva mängden av läkemedlet att lämna kroppen. Det blev däremot signifikanta skillnader mellan de beräkningarna av MyPKFiT and WAPPS-Hemo i estimerade tiden tills FVIII nivåerna skulle sjunka till en trough av 1%, d.v.s. lägsta nivån inför nästa infusion av octocog alfa.

Signifikanta skillnader, dock mindre uttalade, fanns även vad gäller beräkningarna för trough 2%, 3% och 5%. Enligt WAPPS-Hemo skulle det ta längre tid för FVIII nivåerna att sjunka till lägsta nivån. MyPKFiT beräknade konsekvent att det skulle ta kortare tid att nå FVIII-trough. Som följd, WAPPS-Hemo beräknande att det skulle krävas signifikant lägre doser av octocog alfa för att uppnå samma FVIII-nivåer i blodet, jämfört med MyPKFiT, vilket skulle kunna påverka behandlingen.

Delarbete II gav upphov till artiklar II och III i avhandlingen. I denna studie inkluderades patienter med svår och moderat Hemofili A, som behandlades på hemofilicentra i Malmö, Sverige och Oslo, Norge. De inkluderade patienterna behandlades tidigare med annat FVIII produkt men genomgick behandlingsbyte till

FVIII produkten BAY 81–8973, antingen före studien eller under tiden studien pågick.

I artikel II undersöktes huruvida bytet till BAY 81–8973 påverkade behandlingsresultaten avseende blödningar, ledskador, och konsumtion av FVIII-produkt. Patientens följsamhet till behandlingen kartlagdes. Analysen visade att patienterna hade median ABR 0 (årlig blödningsincidens för alla blödningar) och median AJBR 0 (årlig blödningsincidens för ledblödningar) före och efter bytet till BAY 81–8973, trots förekomst av ledskador i gruppen och måttlig behandlingsintensitet, avseende doseringen av FVIII-läkemedel. Följsamhet till behandling var mycket bra, vilket sannolikt bidrog till de goda resultaten. Vid jämförelse av resultaten mellan patienterna med svår hemofili A som behandlades i Malmö respektive Oslo visade studien att patienterna i Oslo använde signifikant lägre FVIII-läkemedel jämfört med patienterna i Malmö, men hade trots detta liknande blödningsfrekvens (median ABR 0) samt grad av ledskador. Ett intressant fynd som borde undersökas vidare.

Målet med artikel III var därför att ytterligare undersöka orsakerna bakom fyndet att patienterna i Oslo hade lägre FVIII-konsumtion än patienterna i Malmö vid analysen i artikel II, men samtidigt liknande frekvens av blödningar och samma grad av ledskador. Vi upptäckte att 15 av 20 patienter i Oslo behandlades med s.k. sekundär profylax, det vill säga profylaktisk behandling som påbörjades efter tre års ålder eller efter mer än två ledblödningar. De flesta patienterna i Malmö hade däremot primär profylax, vilket påbörjades före tre års åldern och innan patienterna fick två ledblödningar. Analysen visade att primär profylax är kopplad till bättre ledhälsa och hälsorelaterad livskvalitet än sekundär profylax. Patienter på sekundär profylax hade emellertid liknande frekvens av blödningsepisoder och ledblödningar, trots signifikant lägre årlig FVIII konsumtion än primär-profylax gruppen.

Detta talar för att man kan optimera behandlingen i gruppen av sekundär profylax och möjligtvis sänka intensiteten (doseringen) av behandlingen, utan att öka risken för blödningar, vilket kan bidra till ökad kostnadseffektivitet. Eftersom de flesta patienterna i Oslo hade sekundär profylax var detta en stark bidragande faktor till skillnaden i FVIII konsumtion som noterades i artikel II. I studien utfördes även analys av bakomliggande genetiska förändringar och hur dessa kan påverka kliniska bilden. Alla patienter som analyserades för artikel III hade svår hemofili A med habituella FVIII-nivåer under 1%. Det finns dock olika sorters genvarianter som kan orsaka hemofili. Analysen visade att en grupp av genvarianter som kallas ”non-null” (vilket innebär att spår av FVIII kan finnas kvar i blodet) kan potentiellt tillåta lägre FVIII konsumtion, utan ökad blödningsrisk eller ledskador, jämfört med ”null” genvarianter (där produktionen av FVIII har upphört helt), hos vuxna patienter med sekundär profylax. Vetskap om patienternas bakomliggande genetik skulle därför kunna användas för att ytterligare optimera behandlingen.

Delarbete III ligger till grund för artikel IV, vars syfte var att kartlägga ledhälsan hos en grupp av vuxna patienter med svår hemofili A som var födda efter 1980 och behandlades på hemofilicentra i Malmö och Göteborg. Alla patienterna hade haft primär profylax sedan barndomen och utan anamnes av genomgången eller aktuell inhibitorisk antikropp mot FVIII, vilket kan utvecklas under behandling och negativt påverka dess effektivitet. Undersökningen av leder med fysioterapeutisk och ultraljudsanalys visade att primär profylax är effektiv på att fördröja debuten av artropati men kan inte helt förebygga dess utveckling. Utvärderingen av ledhälsan behöver därför påbörjas tidigt. Studien undersökte också blödningar inför och under startperioden av profylaktisk behandling vid barndom och upptäckte att behandlingen bör övergå till full-dos regim minst två gånger per vecka så snart som möjligt för att effektivt kunna förebygga blödningar.

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Paper I

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ORIGINAL ARTICLE

Clinical Haemophilia

A comparison of MyPKFiT and WAPPS-Hemo as dosing tools for optimizing prophylaxis in patients with severe haemophilia A treated with Octocog alfa

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Abstract

Introduction: MyPKFiT and the Web-Accessible Population Pharmacokinetic service—Hemophilia (WAPPS-Hemo) are web-based population-based applications developed for helping physicians individualize and optimize replacement therapy. Although MyPKFiT is intended for Octocog alfa and Rurioctocog alfa pegol use only, the WAPPS-Hemo is applicable to all factor VIII concentrates.

Aim: To compare MyPKFiT and WAPPS-Hemo as dosing tools for optimizing treatment of patients with severe haemophilia A on regular prophylaxis with Octocog alfa in a real-world setting.

Methods: Fourteen patients with severe haemophilia A (median age 30.8 years; range 20–71) were enrolled. The FVIII activity was measured twice after a regular dose of Octocog alfa by the chromogenic and the one-stage assays. PK analyses were performed using each tool and dosing estimations to reach trough levels of 1%, 3% or 5% after 48 h. Findings were calculated and compared.

Results: The two PK algorithms yielded similar $t½$ independent of the type of FVIII assay used. However, there were significant differences in the time to reach 1%, 3% and 5%. The WAPPS-Hemo generated 10–12 h longer time to a trough of 1% and up to 4 h for the troughs of 3% and 5%. Accordingly, the doses estimated by WAPPS-Hemo for a daily regimen were between 28% and 100% of those proposed by MyPKFiT.

Conclusions: MyPKFiT and WAPPS-Hemo provided similar half-life estimations for Octocog alfa independent of the FVIII assay used. The doses suggested by WAPPS-Hemo to reach specific troughs were overall lower, which may have implications for treatment optimization.

KEYWORDS
Advate, haemophilia, MyPKFiT, Octocog alfa, population pharmacokinetics, WAPPS-Hemo

1 | INTRODUCTION

Factor VIII (FVIII) prophylaxis has been the mainstay of treatment for severe haemophilia A for many decades.[1] Different dosing schedules have been used with significant reductions in the bleeding rate, but usually at high cost.[2] Importantly, however, the relationship between treatment provided and bleeding phenotype is not absolute, and large interpatient variability in pharmacokinetic parameters exists providing an obstacle to a 'one size fits all' prophylaxis regimen.[3,4]

Individualization and optimization of treatment by Bayesian pharmacokinetics, for example population-based assessment, has shown to be a useful approach without the demanding sampling requirements of conventional single-subject full PK analysis.[3,5] The Bayesian approach not only reduces the need for samples, but also minimizes the inter-individual variability by including covariates such as age, weight and the von Willebrand factor levels in a multivariable model of a relevant patient population.[6] Usually, only 2–3 samples from between 4 and 48 h post-infusion are required for standard half-life FVIII products and even single samples have been evaluated.[5,7]

The MyPKFiT and the Web-Accessible Population Pharmacokinetic service—Hemophilia (WAPPS-Hemo) are web-based population-based applications developed to assist clinicians in making treatment decisions. MyPKFiT was initially for use in conjunction with Octocog alfa (Advate, Takeda Pharma) only, but more recently for the pegylated form of this molecule (rurioctocog alfa pegol, Adynovi, Takeda Pharma) also. WAPPS-Hemo, however, can be used for all currently available replacement products. Estimated dosing and administration frequency to achieve specific target levels are provided for each. There are limited data about potential differences in outcome for the tools.[8] As clinicians and centres may use different methods, it is therefore of importance to evaluate whether PK algorithms using the same core data generate different estimates.

The MyPKFiT algorithm has been shown to be effective in optimizing clinical outcomes and factor consumption using either chromogenic or one-stage FVIII assays.[9-11] Whether inter-assay discrepancies impact outcome and therapeutic decision-making has not been fully evaluated.[12] The same is true for the WAPPS-Hemo.[13]

This study was designed to compare the generated PK profiles and dosing estimations by MyPKFiT and WAPPS-Hemo using chromogenic as well as one-stage assays for the optimization of prophylaxis in children and adult patients with severe haemophilia A treated with Octocog alfa.

2 | MATERIALS AND METHODS

2.1 | Patients

This is a non-randomized, non-intervention, open-label single-centre cohort study enrolling 18 persons with severe haemophilia A (FVIII:C < 1 IU/dl) on regular prophylaxis with Octocog alfa, a recombinant antihaemophilic FVIII indicated for use in children and adults with haemophilia A. The study was conducted between May 2017 and October 2018. To be eligible, patients must have had more than 50 exposure days (EDs) to factor replacement and be without a history of inhibitory FVIII antibodies, as measured by the Nijmegen-modified Bethesda assay. Any patient treated with another FVIII product during the prior 30 days was excluded. Two patients withdrew during the course of the study, and in two patients, adequate sampling within the time frame defined for PK analysis could not be achieved. Therefore, the final analyses included 14 patients.

2.2 | Factor VIII and VWF:Ag assays

Factor VIII and VWF:Ag levels were estimated at the local coagulation laboratory at Labmedicin Skåne with the BCS-XP analyser (Siemens Healthcare Diagnostics) according to the manufacturer's instructions for both the chromogenic and one-stage methods. The one-stage assay was performed with the PTT-Automat (Stago), whereas the chromogenic assay was performed with the Coatest reagent (Chromogenix) according to local guidelines. The VWF:Ag assay (Siemens Healthcare) was used for assessment of VWF:Ag levels.

2.3 | Pharmacokinetic analyses

WAPPS-Hemo and MyPKFiT are two-compartment models that use dense PK sampling data as the basis for the Octocog alfa PK model. The MyPKFiT model is based on a population of 152 patients, with age as a covariate.[14] The corresponding WAPPS-Hemo model is based on 79 patients with fat-free mass and age as covariates for the one-stage assay.[15] For the chromogenic assay, WAPPS-Hemo uses a brand-specific model derived from 91 patients with fat-free mass and age as covariates.[16] For the PK assessments, baseline FVIII activity, year and quarter of birth (age), body weight (BW) and height, timing of the last two administered doses of Octocog alfa, as well as the infused dose in IU, were collected. The dosing regimen for each patient was at the discretion of the treating physician. Following a regular prophylactic dose with no wash-out, two post-infusion samples were collected from each patient in citrated 4.5 ml tubes, centrifuged and processed at the coagulation laboratory in Malmö. The first sample was taken 4–9 h post-infusion and the second sample 25–31 h after the same infusion. Information about the date, time and dose of the latest two infusions was introduced to both algorithms. The MyPKFiT algorithm allows the treating physician to determine which blood samples and/or prior PK assessments to use in each evaluation,[6] while WAPPS-Hemo allows the patients to merge different infusions in a combined analysis.[17] In one patient (#8), for logistical reasons, two different infusions administered within the same year were used for sampling. There were no discrepancies in weight or height and no signs of concomitant infection or inflammatory processes at the time of either sampling. Dosing estimates targeting a trough of 1% in IU/kg BW with an infusion interval of 48 h independent of the current prophylactic regimen were calculated for comparison. The dose provided was also rounded up or down to the

nearest commercially available vial size of Octocog alfa provided the dose was decreased by <1 U/kg of the patient's BW compared to the estimated exact dose. In order to perform comparisons between MyPKFiT and WAPPS-Hemo, the same doses, infusion times, post-infusion samples and clinical parameters were used.

2.4 | Clinical data

Data including the annual bleeding rate (ABR), annual joint bleeding rate (AJBR), presence or absence of a target joint defined as ≥3 bleeding episodes in the same joint in the prior 6 months and use of any relevant concomitant medication(s) were documented. The ABR and AJBR were defined as the number of reported bleeding episodes and joint bleeding episodes divided by the observation period in months multiplied by 12. The bleeding events were collected retrospectively for the 6–8 month period preceding the study visit and used to calculate the bleeding rates. Joint health was assessed by the physiotherapist at the haemophilia centre according to the Hemophilia Joint Health Score (HJHS) version 2.1.[18] HJHS total scores range from 0 to 124, with higher scores indicating worse joint status. No patients with arthroplasty were included.

2.5 | Statistical analysis

Descriptive statistics were used. Median and interquartile ranges (IQR; 25th-75th percentile) describe continuous variables. The Wilcoxon signed rank test for paired non-parametric variables and the Spearman's correlation test were performed using SPSS software, version 25 (SPSS). A *p*-value of <.05 was considered statistically significant.

2.6 | Ethics

The study was approved by the Regional Ethics Review Board of Lund University, Lund, Sweden. The study subject or his legal representative provided written informed consent before entering the study.

3 | RESULTS

3.1 | Patient and treatment characteristics

Fourteen adult patients on regular prophylaxis with Octocog alfa were included in the final analyses. Baseline clinical characteristics and demographics are shown in Table 1.

The median age was 38 years (IQR 30.8–48.5), and the median BMI was 25.6 kg/m^2 (IQR 23.4–27.6). The regular dose of Octocog alfa was between 17.4 and 28.8 IU/kg with a median dose of 24.4 IU/kg. Two patients received daily infusions, whereas the others had infusions every other day (*N* = 7), three times weekly (*N* = 3) or less frequent and/or flexible (*N* = 2). None of the patients had a history of inhibitory antibodies against FVIII. The median ABR and AJBR were, in both cases, 0 (IQR 0–1.3). Two patients (patient #3 and #8) reported spontaneous bleeding events despite FVIII trough levels of 14 and 2%, respectively. Two additional subjects reported single traumatic joint bleeding with trough levels of <1 and 2%. The

TABLE 1 Patient characteristics

Pat ID	Age (years)	BMI (kg/m^2)	Regular total dose (IU)	Regular dose (IU/kg)	Regular dosing interval	ABR	AJBR	HJHS score
1	30	21.8	2000	24.4	EOD	0	0	4
2	41	37.1	3000	26.1	M/TH	1 (T)	0	18
3	67	17.2	1500	28.8	Daily	4 (2 T,2S)	4 (2 T,2S)	38
4	31	28.1	2000	21.3	EOD	0	0	2
5	53	26.1	2000	25	3 times weekly	1 (T)	0	28
6	71	26.2	1500	18.1	Daily	0	0	47
7	31	23.7	2000	26.7	3 times weekly	0	0	14
8	47	24.5	2000	24.4	EOD	5 (5S)	5 (5S)	47
9	43	31.9	2000	17.4	3 times weekly	0	0	4
10	31	26.1	2000	27.8	EOD	0	0	6
11	35	23.7	2000	24.4	EOD	2 (2 T)	2 (2 T)	5
12	42	25.2	2000	22.2	EOD	0	0	2
13	27	22.7	1500	18.3	M/TH	1 (T)	1 (T)	1
14	20	27.5	2000	22.3	EOD	0	0	18

Note: None of the patients had a target joint.

Abbreviations: ABR, annual bleeding rate; AJBR, annual joint bleeding rate; BMI, body mass index; EOD, every other day; HJHS, Hemophilia Joint Health Score; M/TH, Monday and Thursday; S, spontaneous; T, traumatic.

remaining 10 (71.4%) patients experienced no joint bleeding during the study period. None had target joints.

The joint health scores for each patient are shown in Table 1. The HJHS revealed significant arthropathy in seven of 14 patients with scores ranging from 14 to 47. Two of the three subjects with ABR ≥4 also had relatively high HJHS scores. Patients with high scores exhibited variation in their trough levels, from 1% to 14% calculated by MyPKFiT and 1%–18% by WAPPS-Hemo, as well as bleeding phenotype. The majority of the patients were over 40 years of age. The median HJHS score was 10 (IQR 3.5–30.5). Those with higher HJHS scores primarily exhibited crepitations in multiple joints, decreased muscle strength and joint motility and, in some instances, muscle atrophy, mainly in the biceps and quadriceps muscles.

The median FVIII level at the first sampling point was 34% (IQR 27–39) with the one-stage method and 43% (IQR 37–52) with the chromogenic method (p = .001; Table 2). The corresponding FVIII levels measured 25–31 h post-infusion were 7% (IQR 5.8–9) and 8% (IQR 6–10), respectively.

3.2 | Comparison of the PK outcome with MyPKFiT and WAPPS-Hemo

MyPKFiT and WAPPS-Hemo yielded similar $t½$, independent of the type of FVIII assay used (Table S1), despite the significant assay discrepancy at the first sampling point. The median $t½$ value ranged from 10.5 to 11.2 h in all settings. All patients had VWF:Ag levels within normal range at the time of sampling (Table S1) and the $t½$ correlated to the actual VWF:Ag level at the time of sampling (not shown). The calculated times to a specific trough level were, overall, slightly longer for both MyPKFiT and WAPPS-Hemo when using the chromogenic FVIII method compared to the one-stage, but the differences were not statistically significant (Table S2).

Only minor discrepancies in the calculated $t½$ were observed between the two population-based PK algorithms using either the chromogenic or the one-stage assay (Table 3). The median $t½$ calculated with the chromogenic assay was 11.2 h for WAPPS-Hemo and 10.5 h with MyPKFiT. When the one-stage assay was used, the median $t½$ was 11.1 h for WAPPS-Hemo and 10.5 h for MyPKFiT. None of these differences were statistically significant. With respect to the time to a trough of 1%, 2% and 5%, however, significant differences were observed with WAPPS-Hemo exhibiting longer time to the various troughs regardless of the assay used (Table 3). In the case of troughs of 3% and 5%, the differences were ≤4 h and may be less clinically important. However, for a trough of 1%, the differences were between 10 and 12 h, which could impact clinical management.

3.3 | Dosing estimations

Dosing estimates in IU/kg BW to reach a FVIII trough level of 1% after 48 h using an every other day regimen based on either the one-stage or the chromogenic assay are shown in Table 4. Overall, the WAPPS-Hemo system provided generally lower dose estimates than MyPKFiT in all patients independent of the assay used. The doses suggested were on average 50% of those calculated by MyPKFiT.

The estimated doses in IU/kg BW were also rounded up or down to the nearest vial size of Octocog alfa with a focus on the six patients reporting bleeding episodes shown in Table 1. In one of these patients (#3), both traumatic and spontaneous bleeding episodes were reported despite daily treatment and a high estimated trough of 14%. The remaining five patients had troughs <3%. Dosing estimates by each algorithm targeting trough levels of either 3% or 5% with an every other day regimen for these patients using the commercially available vial sizes are shown in Table 5. The doses estimated by WAPPS-Hemo were on average 70% of those estimated by MyPKFiT for both troughs of 3% and 5%, but varied between 40% and 100%, that is the same amount.

4 | DISCUSSION

The aim of this study was to compare the outcomes of two population-based PK algorithms for optimizing prophylactic treatment with a relatively broad sampling window that should be practical in the clinical setting and accommodate the life and work demands of the patient. We also wanted to evaluate any potential impact of the FVIII assay used. To this end, we studied a cohort of patients treated with Octocog alfa and prophylaxis for many years. Several of the patients had severe haemophilic arthropathy, but did not experience any bleeding episodes. However, six patients reported bleeding while on prophylaxis and in five of these cases the trough levels were from <1% to 2.2%, indicating a need for a revision in treatment and/or higher trough levels.[19,20] In the remaining patient (#3), both traumatic and spontaneous bleeding episodes were reported despite daily treatment and a high trough. The reason for this is not clear, but may partly be due to either synovitis, with an increased bleeding tendency, and/or the difficulties in identifying actual bleeds versus symptoms of arthropathy.

Despite significant discordances between the chromogenic and one-stage assays as previously described,[21,22] the two methods generated similar results for the half-life and time intervals to reach troughs of 1%, 2% and 5%, respectively, which is both important and promising. On the other hand, we observed significant differences when comparing the two PK algorithms regarding time to reach a specific trough level for each type of FVIII assay. In the majority of cases, the difference was up to 4 h, which may be less clinically important. For a trough of 1%, however, this difference was as great as 10–12 h, which could impact clinical management. In addition, we observed major differences in the dosing estimates by each PK tool for reaching a specific trough level—and not only for the trough of 1% (Tables 4 and 5). According to these estimations, more or less all of our patients would consume significantly more FVIII concentrate based on the proposals provided by MyPKFiT compared to those of

TABLE 2 Sampling time in hours post-infusion for each patient and the corresponding FVIII levels measured by the one-stage and the chromogenic methods at each time point

	Sample 1			Sample 2		
Pat ID	Time (h post-inf)	Chromogenic (%)	One-stage (%)	Time (h post-inf)	Chromogenic (%)	One-stage (%)
1	4	39	34	28	5	6
2	5	72	52	26	12	15
3	5	56	47	31	14	11
4	5	41	37	25	8	8
5	6	51	42	26	8	7
6	4[a]	54	35	25[a]	14	11
7	9	26	12	26	6	4
8	5	52	38	31	8	7
9	4	36	34	25	5	5
10	7	37	15	30	7	3
11	8	36	25	28	8	8
12	4	45	32	27	8	6
13	5	37	28	28	10	9
14	4	49	32	25	6	7

[a]The sampling points were after two separate infusions.

TABLE 3 A comparison between the estimated half-lives and time to troughs of 1%, 2% and 5% by each PK algorithm using the chromogenic and the one-stage assay

	Chromogenic				
	MyPKFiT		WAPPS-Hemo		*p*-value
Parameter	Median	IQR	Median	IQR	
*T*½ (h)	11.2	10.1–12.1	10.5	9.1–12.7	.93
Time to 1% (h)	58	50.5–65.3	68.2	59.8–80.8	.003
Time to 2% (h)	47.5	41.8–55	51.5	45.2–60.6	.019
Time to 5% (h)	32	28–36.3	35	30.4–41.7	.017
	One-stage				
	MyPKFiT		WAPPS-Hemo		*p*-value
Parameter	Median	IQR	Median	IQR	
*T*½ (h)	11.1	10.4–12.5	10.5	9.1–12.7	.55
Time to 1% (h)	55.5	51.5–65.3	67.5	61.7–83.2	.001
Time to 2% (h)	45	41.8–52.8	50.5	45.6–61.4	.013
Time to 5% (h)	31	28–36.3	35	30.4–41.7	.048

WAPPS-Hemo. This was true for the patients in our study reporting bleeds while on their present treatment and a need for revised schedules. Among the five cases with current troughs below 3%, the annual consumption required to reach a trough of 3% using an every other day infusion schedule based on the commercially available vial sizes would be 2.69 x 10^6 and 1.87 x 106 IU for MyPKFiT and WAPPS-Hemo, respectively, based on the chromogenic assay, and 4.43 x 10^6 and 3.47 x 106 IU to reach a trough of 5% (Table 5). The reasons for these major differences are not clear, but may be multifactorial. As both algorithms are population PK-based, the extent of interpatient variability within the Bayesian analysis may influence the outcomes. The estimated baseline level and/or the pre-infusion levels may also impact the calculations generated by the PK tool. Altogether, our findings show the importance of follow-up and monitoring of the actual achieved levels and correlate these to the bleeding phenotype.

Our results are consistent with those of previous studies demonstrating the potential of MyPKFiT in the clinical management of patients with haemophilia.[10,11] However, our data also call attention to further potential differences between the population-based PK tools and differences that may be significant from both medical and health-economic perspectives. Prejers et al. compared different population-based PK tools, that is NONMEM, MyPKFiT and WAPPS-Hemo and found that doses estimated by MyPKFiT and

TABLE 4 Dose estimates in IU/kg body weight (BW) by each PK algorithm for reaching a trough level of 1% after 48-h in each patient, that is for an every other day dosing schedule

		Chromogenic assay		One-stage assay	
Pat ID	Body weight (kg)	Mypkfit (IU/kg BW)	WAPPS-Hemo (IU/kg BW)	MyPKFiT (IU/kg BW)	WAPPS-Hemo (IU/kg BW)
1	82	24.4	15.2	18.3	9.1
2*	115	8.7	4.3	8.7	4.3
3*	52	9.6	4.8	9.6	4.8
4	94	18.6	8.0	16.0	5.3
5*	80	15.6	6.3	15.6	6.3
6	83	6.0	3.0	6.0	3.0
7	75	23.3	13.3	23.3	23.3
8*	82	12.2	6.1	12.2	6.1
9	119	30.4	13.0	30.4	8.7
10	72	13.9	13.9	31.3	24.3
11*	82	12.2	6.1	12.2	6.1
12	90	13.9	5.6	16.7	8.3
13*	82	9.1	3.0	9.1	3.0
14	87	23.0	8.6	14.4	5.7

Note: Patients with bleeding episodes reported during the study period are marked with an asterisk (*) in the first column.

TABLE 5 Actual dose estimates in IU/kg body weight (BW; *italic*) and actual total dose estimates in IU by MyPKFiT and WAPPS-Hemo, rounded up or down to the nearest commercially available vial size as mentioned in Material and Methods for the patients with bleeding manifestations during the study on their prophylactic treatment at enrolment. The estimates are based on the measurements made by the chromogenic assay and with a target trough of 3% and 5%, respectively, using a 48-h (every other day) schedule

		Trough 3%		Trough 5%	
Pat ID	Observed trough level on the current prophylactic regimen	MyPKFiT IU *IU/kg BW*	WAPPS-Hemo IU *IU/kg BW*	MyPKFiT IU *IU/kg BW*	WAPPS-Hemo IU *IU/kg BW*
2	1%	3000 *26.1*	2750 *23.9*	5000 *43.5*	5000 *43.5*
3	14%	1250 *24.0*	750 *14.4*	2000 *38.5*	1250 *24.0*
5	<1%	4000 *50.0*	2750 *34.4*	6500 *81.3*	5000 *62.5*
8	2.2%	3000 *36.6*	1750 *21.3*	5000 *61.0*	3250 *39.6*
11	1.9%	2500 *30.5*	2000 *24.4*	4250 *51.8*	3750 *45.7*
13	<1%	2250 *27.4*	1000 *12.2*	3500 *42.7*	2000 *24.4*

WAPPS-Hemo were higher than those estimated by NONMEM.[8] Moreover, similar to our findings, significant variation in the range of dosing proposals made by MyPKFiT and WAPPS-Hemo was observed. An example of this was a range between 10.0 and 57.3 IU/kg to reach a trough of 1% after 48 h proposed by MyPKFiT. Prejers et al did not report the individual dosing proposals, and a direct comparison between the two tools was not performed. The authors hypothesize that any differences were due to the individual PK parameters used in each tool.

The ability to individualize treatment according to each patient's requirements and circumstances is of paramount importance in precision medicine. Population PK tools, such as MyPKFiT and WAPPS-Hemo, clearly facilitate this goal and assist physicians in customizing treatment for each patient. Both tools offer a less burdensome evaluation than an individualized, rich PK sampling and overcome many pitfalls. Our study, however, shows that the choice of PK tool and type of assay may influence the outcome and, accordingly, the proposed dosing.

Our study has limitations including a relatively small number of study subjects, the retrospective design and subjective reporting of bleeding events. In addition, no pre-infusion levels were collected and there was no in vivo validation of the PK estimations performed. The primary focus, however, was to compare the outcome of the two PK approaches in a practical real-world setting using the same sampling points. As such, the single-centre design offers distinct advantages, with all analyses performed at our local specialized laboratory.

To conclude, MyPKFiT and WAPPS-Hemo appear to overcome assay discrepancies. They provide similar PK outcomes with the one-stage and chromogenic assays. However, the tools calculate different times to a specific trough level, hence providing different dose estimations which may have a significant impact on clinical outcome and factor consumption. We believe clinicians need to be aware of these discrepancies and take them into account while making clinical decisions, as well as closely monitoring the activity levels achieved by each dosing schedule.

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CONFLICT OF INTEREST

AA has received research grants from Takeda/Shire and Bayer. Consultant for Ablynx/Sanofi, Sobi, Chiesi. EB has acted as paid consultant to Bayer, CSL Behring, Octapharma, Sobi, Takeda, and has received funding for research from Sobi and Bioverativ. JA has received research grants from Sobi, CSL Behring, Takeda/Shire, and Bayer. Speakers fee and consultant for Octapharma, Novo Nordisk, Pfizer, Bayer, Sobi, CSL Behring, Takeda/Shire.

AUTHOR CONTRIBUTIONS

AA and JA designed the study, acquired, analysed and interpreted the data, drafted and finalized the manuscript. EB designed the study, interpreted the data and finalized the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Table S1.
Calculated half-life (t½) by each PK algorithm based on either the chromogenic or the one-stage method and the corresponding VWF:Ag level at the sampling time for each patient. VWF:Ag: Von Willebrand Factor antigen, hrs: hours, MD: missing data

Pat ID	ONE-STAGE		CHROMOGENIC		
	MYPKFiT (hrs)	WAPPS-Hemo (hrs)	MYPKFiT (hrs)	WAPPS-Hemo (hrs)	VWF:Ag (kIE/L)
1	10.5	10.3	9.7	8.8	0.68
2	13.4	12.8	13.3	11	1.12
3	12.3	13.3	13.6	15.5	1.70
4	10.9	10.5	10.7	10.3	0.76
5	10.7	9.8	11.2	10.3	0.93
6	14.4	13.8	17.2	14.8	1.25
7	9.9	8.5	10	8.8	MD
8	11.9	11.8	12	12	1.23
9	10.1	9.5	10.1	8.5	0.82
10	9.2	8.3	11.2	9.3	1.05
11	12.3	12	11.7	11.3	0.79
12	10.8	10.5	11.2	10.8	0.90
13	13	13.8	12.3	14.8	1.90
14	11.3	11	10.1	9.5	0.70

Table S2.
A comparison between the chromogenic and one-stage assay-based estimations of half-life and time to troughs of 1%, 2% and 5% by each PK algorithm.

	MYPKFIT				
	CHROMOGENIC		ONE-STAGE		*P*-value
Parameter	Median	IQR	Median	IQR	
t½ (hrs)	11.2	10.1-12.1	11.1	10.4-12.5	0.38
Time to 1% (hrs)	58	50.5-65.3	55.5	51.5-65.3	0.70
Time to 2% (hrs)	47.5	41.8-55	45	41.8-52.8	0.55
Time to 5% (hrs)	32	28-36.2	31	28-36.2	0.41
	WAPPS-Hemo				
	CHROMOGENIC		ONE-STAGE		*P*-value
Parameter	Median	IQR	Median	IQR	
t½ (Hours)	10.5	9.1-12.7	10.5	9.1-12.7	0.98
Time to 1% (hrs)	68.2	59.8-80.8	67.5	61.7-83.2	0.36
Time to 2% (hrs)	51.5	45.2-60.6	50.5	45.6-61.4	0.23
Time to 5% (hrs)	35	30.4-41.7	35	30.4-41.7	0.12

Paper II

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ORIGINAL ARTICLE

Clinical outcome and adherence rate in Scandinavian patients with intermediate-intensity prophylaxis before and after the switch of standard half-life FVIII products to BAY 81–8973

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Abstract

Introduction: Treatment optimization in haemophilia A can be achieved by choice of FVIII product and knowledge of pharmacokinetics (PK), phenotype and adherence. A favourable PK profile of BAY 81–8973 (octocog alfa) (Kovaltry, Bayer AB) compared to other standard half-life (SHL) FVIII products has been suggested.

Aim: To evaluate whether the switch to BAY 81–8973, using the same dosing schedule, impact factor consumption and bleed rates, taking arthropathy and adherence into account

Methods: Forty patients on prophylaxis with SHL (median age 40.5 years) attending the haemophilia treatment centres in Malmö and Oslo were enrolled. The annualised bleeding rate (ABR) and joint bleeding rate (AJBR) before and after the switch to BAY 81–8973 was calculated. PK analyses were performed with WAPPS-Hemo. Joint health status and treatment adherence were assessed.

Results: The median ABR and AJBR was 0 before and after the switch, at both centres. The median yearly factor consumption was 3,345 IU/Kg/year in the entire study group corresponding to intermediate-intensity prophylaxis in most patients and with significantly more used in Malmö (3,862 IU/Kg/year), compared to Oslo (2,337 IU/Kg/year) (*P* .006). There was no correlation between arthropathy and bleeding. The median BAY 81–8973 t½ was 15.15 h (range 7.5–29 h), with significant correlation to VWF levels, and 13.4 h after exclusion of VWF outliers. Adherence to treatment was 97%.

Conclusions: Concentrate switch, using mainly intermediate-intensity regimens with high adherence rates, preserves excellent prophylaxis outcome using standard half-life FVIII products, indicating the value of individualized prophylaxis and close follow-up.

KEYWORDS
BAY 81—8973, haemophilia, Kovaltry, Octocog alfa, population pharmacokinetics, WAPPS-Hemo

1 | INTRODUCTION

Haemophilia A (HA) is caused by the deficiency or absence of factor VIII (FVIII) and characterized by bleeding diathesis, with joint bleeding as clinical hallmark.[1,2] Prophylactic replacement therapy in HA aims to reduce the risk of bleeding by raising FVIII levels,[1,2] thus preventing the development of haemophilic arthropathy.[3] Different FVIII products, with distinct manufacturing methods,[4,5] molecular[6] and pharmacokinetic[7] properties, have been used in the treatment of HA. BAY 81–8973 (octocog alfa, Kovaltry, Bayer AB) is a standard half-life (SHL) recombinant FVIII product,[8] with a suggested favourable pharmacokinetic profile compared to octocog alfa (Kogenate, Bayer AB and Advate, Takeda Pharma).[9,10] Interpersonal variability in factor VIII pharmacokinetics influences; however, the FVIII levels post-infusion and the outcome of treatment.[11] Population PK models, such as the Web-Accessible Population Pharmacokinetic service – Haemophilia (WAPPS-Hemo)[12] take this into account in a multivariable model of a relevant patient population, while requiring minimal sampling when compared to a conventional PK analysis.[13,14] This can help optimize treatment[15,16] and illuminate differences in the pharmacokinetic profiles of specific FVIII products.

Haemophilic arthropathy is the result of repeated hemarthroses, mainly affects the knee, elbow and ankle joints,[17,18] and can reduce quality of life.[19] The chronic synovitis and vascular fragility in haemophilic arthropathy can both predispose to bleeding and mimic its symptoms.[20] Furthermore, independent of the treatment regimen used, adherence to the prescribed treatment is essential for its effectiveness. Poor adherence is associated with more self-reported bleeding episodes for adults and days off school for children.[21]

Historically, significant differences in the clinical management of HA patients have existed between the Scandinavian countries. For example, whereas prophylaxis has been standard of care in Sweden since the 1970s, it became available in Norway during the 1990s.[22] However, haemophilia management in Scandinavia has since been harmonized, with the development of Nordic guidelines.[23,24]The aim of this study was to study and compare whether the switch between SHL products with slightly different PK properties with the same dosing schedule, have influenced the clinical outcome in terms of factor consumption and bleeds of patients attending two of the larger Scandinavian Haemophilia centres taking arthropathy and adherence into account.[25]

2 | METHODS

2.1 | Study design

This is an open label, non-interventional, single arm double-centre study, enrolling male patients above 12 years of age with moderate (FVIII:C 1–5 IU/dl) and severe HA (FVIII:C < 1 IU/dl), who had switched or were planned to switch to BAY 81–8973 from another SHL FVIII product. The patients had prophylaxis for more than 50 exposure days, had their previous FVIII product for at least 30 days prior to switching to BAY 81–8973, and no current inhibitor, as measured by the Nijmegen-modified Bethesda assay. Patients fulfilling the inclusion criteria were eligible. PK analysis with WAPPS-HEMO was performed on a subset of patients from the Malmö cohort to evaluate the pharmacokinetics of BAY 81–8973 and phenotype changes after the switch. The study was conducted between March 2017 and February 2020.

2.2 | Clinical data

Clinical data regarding age, height, weight, bleeds, and joint bleeds were collected. The bleeding events prior the switch to BAY 81–8973 were documented retrospectively the year preceding the study visit with a median duration of 12.5 months (IQR 10–13 months), after which the switch to BAY 81–8973 occurred. The bleeding events after the switch to BAY 81–8973 were documented for the period after the switch and prior the final study visit. The recording of bleeds was paper based after oral or written report from the patient or his caregiver. The annual bleeding rate (ABR) and annual joint bleeding rate (AJBR) were defined as the number of reported bleeding episodes and joint bleeding episodes divided by the observation period in months multiplied by 12. A target joint was defined as > 3 bleeding episodes in the same joint during 6 months. Joint health was assessed by the physiotherapist at the haemophilia centre according to the Haemophilia Joint Health Score (HJHS) version 2.1.[26] A cut off score 10 was applied to dichotomize the results between arthropathy and non-arthropathy, as used in previous studies.[27,28]

Adherence to therapy was measured with the validated VERITAS-PRO questionnaire[29] and filled out by the patient or their caregiver.[29] A cut-off score 57 defined non-adherence, as in previous studies.[29,30]

2.3 | FVIII and VWF: Ag assays and pharmacokinetic analysis

PK analysis was performed in 14 patients with severe HA, who were treated at the Coagulation Centre in Malmö. Analysis was based on two sparse samples collected at least 12 h apart, with no wash-out, between four and 48 h after BAY 81–8973 infusion, according to the ISTH guidelines.[31] FVIII levels with use of the chromogenic assay and VWF:Antigen (VWF:Ag) levels were estimated at the coagulation laboratory at Labmedicin Skåne according to local routines.

The WAPPS-HEMO web-based algorithm was used for population PK analysis. For the PK assessments, baseline FVIII activity, age, body weight, height, timing of the last two administered doses and infused dose were collected. The dosing regimen for each patient was at the discretion of the treating physician. The first sample was collected 4—8 h and the second sample 20–30 h post-infusion, respectively (Table 1).

2.4 | Statistical analysis

Descriptive statistics in the form of mean, median and interquartile ranges (IQR 25^{th}–75^{th} percentile) described continuous variables.

TABLE 1 Sampling time in hours post infusion for each patient and the corresponding FVIII levels measured by the chromogenic method at each time point

	Sample1		Sample 2		WAPPS-HEMO PK Estimations		
PAT-ID	Time (hrs post infusion)	FVIII level (%)	Time (hrs post infusion)	FVIII level (%)	t1/2 (hrs)	Time to 1% (hrs)	VWF:Ag (IU/dl)
1	5	63	23	31	24.0	159.8	193
3	5	41	25	11	13.5	85.5	MD
5	4	58	28	10	13.3	87.5	94
6	5	38	29	2	7.5	47.0	50
7	4	65	29	17	16.0	108.0	77
8	5	76	29	23	21.3	143.3	171
9	5	24	26	6	14.3	75.0	95
11	7	35	28	11	18.0	105.5	131
13	4	87	25	35	21.5	150.8	92
14	6	19	24	6	11.5	63.5	85
15	4	60	27	23	20.0	133.0	150
16	5	51	29	20	29.0	173.0	170
17	4	33	24	5	11.5	64.0	51
18	5	59	26	9	11.3	74.5	65

Calculated t1/2, time to troughs of 1% by WAPPS-Hemo, and VWF:Ag level at the sampling time for each patient are provided.
Abbreviations: VFW:Ag, Von Willebrand Factor antigen; PK, pharmacokinetic; hrs, hours; MD, missing data.

Statistical tests used were the Wilcoxon signed rank test, Mann-Whitney U test, Fischer's exact test and Spearman's correlation. All tests were performed using SPSS software, version 25 (IBM, Chicago, IL, USA). A *P*-value of $< .05$ was considered statistically significant.

2.5 | Ethics

The study was approved by the Regional Ethics Review Board of Lund University, Lund, Sweden and Oslo University, Oslo, Norway. The study subject or his legal representative provided written informed consent before entering the study.

3 | RESULTS

3.1 | Patient and treatment characteristics

Forty-three patients were enrolled corresponding to all patients who switched to BAY 81–8973 prophylaxis at the Malmö centre and one half of those in Oslo. One patient withdrew the day after inclusion for personal reasons and in one patient there was inadequate bleeding data. A third patient was excluded since he had been treated with extended half-life product efmoroctocog alfa (Elocta, Sobi), prior the switch to BAY 81–8973. Consequently, the final analysis included 40 patients (baseline clinical characteristics and demographics shown in Table S1), 18 patients treated at the Haemophilia centre in Malmö (#1-#18) and 22 patients treated at the Haemophilia centre in Oslo (#19-#40). All patients had severe Haemophilia A, except two patients who had moderate HA (#33 and #39 with baseline FVIII:C of 3 IU/dl and 2 IU/dl, respectively). There was no history of previous or current FVIII inhibitor. The type of SHL FVIII used prior to the switch to BAY 81–8973 was Kogenate in 21 patients, Helixate in 13, Advate in 5 pat and Refacto (moroctocog alfa, Pfizer) in 1. The median age of the entire cohort was 40.5 years (IQR 26.0–48.8) and the median BMI was 26.6 (IQR 23.1–29.6). The corresponding figures for the Malmö cohort were 35 years (IQR 20.5–44) with median BMI 26.4 (IQR 22.2–28.7) and for the severe HA patients of the Oslo cohort 44 years (IQR 34–56), with median BMI 25.6 (IQR 23.7–29.2). The median dose of infused FVIII before the switch was 20.4 IU/kg (IQR 12.9–26.2) and all patients received regular prophylaxis, either daily ($N = 4$), every other day ($N = 14$), three times weekly ($N = 14$) or two times weekly ($N = 6$). Two patients had a sparse infusion schedule of once weekly or less. All patients continued with the same dose and infusion frequency after the switch to BAY 81–8973, except for two patients (#19 and #26), whose infusion frequency was increased slightly, from three times weekly to every other day (Table S1). Dosing and median yearly FVIII consumption was otherwise essentially the same in both cohorts prior and after the switch. The median FVIII consumption for the entire cohort on BAY 81–8973 was 3345 IU/Kg/year (IQR 1944–4463) (Table S2). There was a significant difference in yearly factor consumption of BAY 81–8973, between the severe HA patients in the Malmö and Oslo cohort. The median FVIII dose per injection was 21.3 IU/Kg in Malmö (IQR 14.5–26.4), and the frequency of injections was 182 per year (IQR 156–227.8). The corresponding numbers in Oslo were 20 IU/Kg (IQR 12.2–25.1) and 156 (IQR 142–182), respectively. The Malmö cohort had median FVIII consumption of 3862 IU/Kg/year, compared to 2337 IU/Kg/year in the Oslo cohort (*P* .006)(Table 2).

TABLE 2 A comparison of clinical outcomes between the patients from Malmö ($n = 18$) and those from Oslo ($n = 20$) after the switch to BAY 81-8973

	Malmö ($N = 18$)		Oslo ($N = 20$)		
Parameter	Mean	Median (IQR)	Mean	Median (IQR)	*P*-value
ABR	.33	0 (0–0)	.42	0 (0–0)	.945
AJBR	.11	0 (0–0)	.26	0 (0–0)	.617
HJHS	17.7	9.5 (3–35)	17.1	14 (12–19.8)	.411
VERITAS-Pro	39.5	40 (28.5–47.5)	40.0	40 (31.8–46)	.885
FVIII Consumption (IU/Kg BW/Year	4,018	3,862 (3,174–4,860)	2,891	2,337 (1,843 –3,912)	.006

Abbreviations: ABR, annual bleeding rate; AJBR, annual joint bleeding rate; HJHS, Haemophilia Joint Health Score.

3.2 | Bleeding phenotype before and after the switch to BAY 81–8973

The median ABR was 0 (IQR 0–1.5) before and remained 0 (IQR 0–0) after the switch to BAY 81–8973. The corresponding median AJBR was 0 (IQR 0–0), both before and after the switch (Figure 1, Table S1). The mean ABR was 1.1 prior and .4 after the switch (*P* .136) and the mean AJBR .7 prior versus .3 after (*P* .194). The corresponding figures for the two subcohorts in Malmö and Oslo are shown on Table 2. Basically, the bleed rates are the same, although a slightly higher mean was observed for patients attending the Oslo centre.

Before the switch, 30 patients (75%) had an ABR of 0 and after the switch the corresponding number of patients was 33 (82.5%). The median ABR of the 10 patients with reported bleeds prior to the switch to BAY 81–8973 was reduced from 4 (IQR 0–6) to 0 (IQR 0–.25) (*P*. .007) and the median AJBR reduced from 2 (IQR 0–6) to 0 (IQR 0–0) (*P*. .017), respectively.

3.3 | Pharmacokinetic analysis of BAY 81–8973

As described, pharmacokinetic analysis with WAPPS-HEMO was performed in a subset of 14 patients from the Malmö cohort treated with BAY 81–8973. The median age was 33.5 years (IQR 18.8–43.3) and the median BMI 26.1 kg/m^2 (IQR 21.6–28.9). The median ABR and AJBR was 0 (IQR 0-0 for both). The WAPPS-Hemo estimated median t½ for BAY 81–8973 was 15.15 h (IQR 11.5–21.3) and the median estimated time to 1% was 96.5 h (IQR 71.9–145.2). As expected, there was a significant correlation between VWF:Ag levels and FVIII half-life (*P* .01) (Figure S1). Notably, the three patients with the shortest t1/2 (patient #6, #17 and # 18) had VWF:Ag levels 50–70 IU/dl and the three patients with the longest t1/2 (patient #1, #8 and #16) had VWF:Ag levels ≥ 170 IU/dl. If these outliers were excluded from analysis, the remaining eleven patients had a median t1/2 of 13.4 h (IQR 11.5–16.5). The data on half-life and time to 1% trough is presented on Table 1.

3.4 | Joint health status

Joint health data was available for 39 of 40 included patients (Table S1). The median HJHS score was 14 (IQR 5.5–27.0). The HJHS score revealed arthropathy, as defined by HJHS > 10, in 25 patients with median HJHS 19 (IQR 14.0–35.5). The high HJHS was predominantly due to decreased mobility in the elbow, knee, and ankle joints, decreased muscle strength, and gait problems., whereas patients with low HJHS scores received points for crepitations. Crepitus on motion may indicate cartilage damage, but no functional impairment was observed in those cases. There was no correlation (*P* .525) between bleeding events during the study period (ABR and AJBR > 0) and arthropathy (HJHS ≥10) (Table S3). None of the patients had target joints. No significant difference was observed in HJHS score between the severe HA patients of the Malmö and Oslo centres (Table 2).

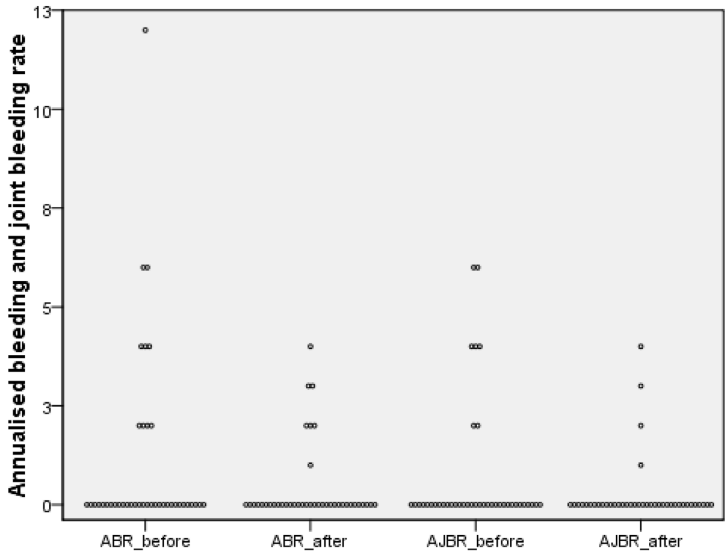
3.5 | Adherence to treatment

The complete VERITAS-Pro data is presented in Table S3. The VERITAS-Pro questionnaire was available in 34 of 40 included patients with a median score of 40 (IQR 30.8-47) (Table S1). Low scores were observed in "dosing", "planning", "skipping" and "remembering" (median 4–6, IQR 4–8). However, high scores were seen in "communication" (median 9, IQR 6–12). When a cut-off of 57 points was used to define non-adherence, only one patient scored above that threshold, signifying 97% adherence. No significant difference was observed in VERITAS-PRO score between the Malmö and Oslo centres (Table 2).

4 | DISCUSSION

The aim of this study was to examine whether the switch from a standard half-life FVIII products to BAY 81–8973, which has been reported to provide a beneficial PK compared to other SHLs[7,10] may influence clinical outcome in patients with HA. The PK analysis performed on 14 patients on BAY 81–8973 with WAPPS-HEMO confirmed a relatively favourable median half-life estimate of 15.15 h. for BAY 81–8973, which is longer than reported for other SHL products,[6,9,10,32] with a wide range of half-lives from 7.5 to 29 h (Table 1). Interestingly, a similar range of 9.95–22.2 h, was seen in the study by Shah et al.[9] However, inter-study differences in design, FVIII wash-out and dosing and the low subject number should be considered when interpreting these results. Additionally, due to the non-interventional design of the study,

A.



B.

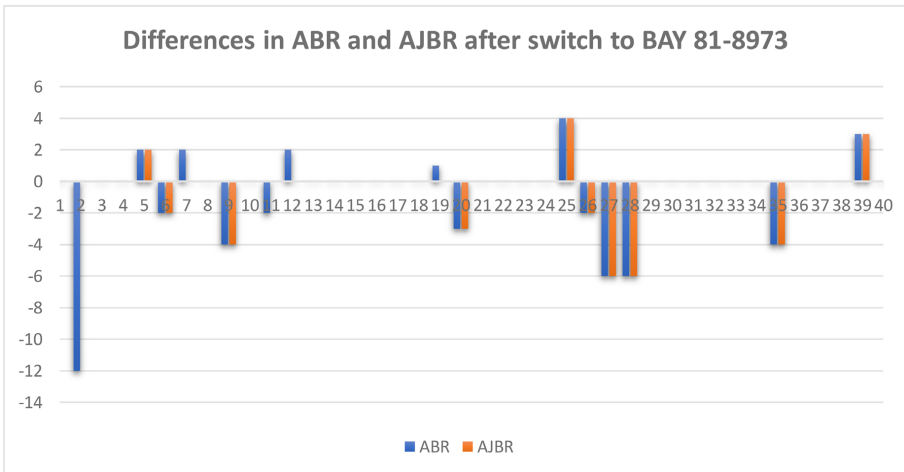


FIGURE 1 A. 2D-dot plot showing ABR and AJBR before and after switch to BAY 81-8973, respectively. Every dot symbolizes one patient B. Bar-chart showing difference in ABR and AJBR in all 40 patients of the cohort after switch to BAY 81-8973. Negative values indicate reduction in ABR and AJBR after the switch, whereas positive values indicate increase, respectively. These differences were not statistically significant.

a control group could not be evaluated. Furthermore, we observed a significant correlation to the VWF levels in our cohort,[25] and after exclusion of outliers with supranormal VWF levels, the median half-life was reduced to 13.4 h. This shows the importance to consider VWF levels when interpreting FVIII PK data and reinforces the use of head-to-head cross-over studies when comparing different products. Our data also showed that the patients overall were well treated with median ABR and AJBR of 0 both prior to and after the switch to BAY 81–8973. In absolute figures, there was a minor reduction in mean ABR and AJBR rates after the switch to BAY 81–8973, while maintaining the same

dose and dosing frequency, but this reduction was not statistically significant.

The majority of patients in our study (62.5%) had established arthropathy, but no target joints, which partly, in some cases, may be due to advanced arthropathy and fibrotic degeneration. There was no correlation between bleed rates, factor consumption and the degree of arthropathy Importantly, only 30% of our cohort were treated with high-dose prophylaxis, as defined by the World Federation of Haemophilia (WFH) cut-off of 4,000 IU/kg/year, indicating the benefits of individualized prophylaxis based on the observed individual bleeding phenotype.[23] Instead, 60% of the patients were receiving an intermediary-dose prophylaxis regimen (cut-off of 1500–4000 IU/Kg/year),[23] while still maintaining a median ABR of 0. The benefit of individualized dosing for medical outcome and factor consumption has previously been reported in a study comparing Swedish and Dutch dosing regimens[28] and these findings are further supported by our study. However, the switch to BAY 81–8973 did not lead to additional individualization of the treatment regimen.

The overall adherence to treatment in our cohort, as measured by VERITAS-Pro, was excellent, with 97% overall adherence and no difference was observed between the two centres. The adherence rate in our Scandinavian cohort was comparable to that of a German cohort (adherence 93.1%),[30] and higher than the American cohort in the original validation study (adherence 82%).[29] All the patients in our cohort had their follow-up at a specific Haemophilia centre, a strong predictor of adherence.[30] Our results also support the previously described association between good adherence and low reported bleeding events.[33]

Interestingly, we found significant differences in the yearly factor VIII consumption between the patients with severe HA treated at the two participating haemophilia centres, despite the use of the same Nordic guidelines. The Malmö centre had a lower absolute number of mean ABR and AJBR, but the difference was not significant and there was no difference in arthropathy. The difference in factor consumption was mainly due to an overall more frequent administration and shorter intervals in the Malmö cohort. However, the two groups were not matched, and recruitment bias cannot be ruled out, since all patients on prophylaxis with BAY 81–8973 were enrolled at the Malmö centre, but one half of those in Oslo. To appreciate any bias in the data collection from Oslo, the overall dosing regimen in patients on BAY 81–8973 was anonymously captured in the register. The treatment profile was similar besides more patients overall on every other day regimen instead of three times weekly. This may suggest that consumption in Oslo was overall slightly higher that observed in our enrolled subcohort. Nevertheless, our findings indicate that very low bleed rates can be achieved with relatively low FVIII consumption with entailed cost benefits. Furthermore, our findings indicate the value of PK estimations in optimizing treatment, as patients in Malmö with favourable PK profiles and low ABR could potentially extend the interval between doses.

Our study had several limitations, including the retrospective design and subjective paper-based reporting of bleeds, where potential differences in reporting and documentation practices between different centres may influence how bleeds are registered. Additionally, there was no control group and pre-infusion levels collected for the PK analysis and no validation step was performed to confirm the PK estimates. Due to the very low reported ABR and AJBR, our study was probably underpowered in detecting statistical correlations between bleeding rates and arthropathy or adherence to treatment. However, this may reflect the importance of an effective treatment plan and close follow-up of patients in ameliorating the impact of these variables on the bleeding phenotype. Finally, as previously stated, selection bias cannot be ruled out due to the relatively low enrolment at the Oslo centre.

In conclusion, in a cohort of previously well treated and well-adherent patients, the switch to BAY 81–8973, with a potential favourable half-life, achieved marginal improvements on already favourable outcome rates despite the use of mainly intermediate-intensity regimens. Our study also showed that a high degree of established arthropathy and lower annual FVIII consumption do not necessarily result in increased bleed rates. Instead, individualized prophylaxis regimens and close follow-up with high adherence to treatment, can reduce FVIII consumption while maintaining haemostatic efficacy. The data further underline that, not only the performance of the single product brand, but how to use the products is important for the outcome in the individual patient.

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AUTHOR CONTRIBUTIONS

AA and JA designed the study, acquired, analysed, and interpreted the data, drafted and finalized the manuscript. PAH recruited patients, interpreted the data and finalized the manuscript. EB designed the study, interpreted the data and finalized the manuscript.

CONFLICT OF INTEREST

AA has received research grants from Takeda/Shire and Bayer. Speaker's fee and consultant for Ablynx/Sanofi, Sobi, Chiesi, Amgen. PAH has received research grants to institution from Bayer, Octapharma, Pfizer and SOBI. Speaker's fee and consultant for Bayer, Takeda, Octapharma, Pfizer, NovoNordisk and SOBI. EB has acted as paid consultant to Bayer, CSL Behring, Octapharma, Sobi, Takeda, and has received funding for research from Sobi and Bioverativ. EB has acted as paid consultant to Bayer, CSL Behring, Octapharma, Sobi, Takeda, and has received funding for research from Sobi and Bioverativ. JA has received research grants from Sobi, CSL Behring, Takeda/Shire, and Bayer. Speaker's fee and consultant for Octapharma, Novo Nordisk, Pfizer, Bayer, Sobi, Sanofi, CSL Behring, Takeda/Shire, BioMarin and Uniqure.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Table S1. Patient characteristics.

BMI: body mass index. ABR: annual bleeding rate. AJBR: annual joint bleeding rate. HJHS: Hemophilia Joint Health Score. IU/Kg BW: International units per kilogram body weight, SHL: standard half-life. Patients with moderate hemophilia are marked with asterisk. None of the patients had a target joint.

PAT ID	Age (Years)	BMI (kg/m^2)	Regular Dose IU/Kg BW	Previous SHL FVIII product		After switch to BAY 81-8973		HJHS Total Score	Veritas-Pro Total Score
				AJBR	ABR	AJBR	ABR		
1	70	36.7	18.0	0	0	0	0	42	47
2	62	27.3	26.3	0	12	0	0	34	44
3	40	27.5	20.8	0	0	0	0	8	25
4	35	28.4	21.3	0	0	0	0	3	40
5	43	31.8	21.3	0	0	2	2	46	28
6	18	26.4	24.7	2	2	0	0	2	55
7	32	28.7	26.6	0	0	0	2	11	28
8	44	21.2	27.0	0	0	0	0	11	35
9	19	29.5	10.0	4	4	0	0	3	48
10	44	31.0	10.6	0	0	0	0	38	40
11	15	21.7	14.7	0	2	0	0	0	53
12	22	23.2	26.3	0	0	0	2	15	54
13	35	20.7	32.3	0	0	0	0	8	47
14	13	22.2	13.9	0	0	0	0	2	MD
15	38	27.5	21.1	0	0	0	0	33	27
16	59	25.7	26.0	0	0	0	0	51	34
17	26	23.5	13.0	0	0	0	0	8	29
18	21	19.2	31.7	0	0	0	0	4	37
19	25	24.0	18.1	0	2	0	3	2	46
20	45	MD	12.8	4	4	1	1	12	MD
21	63	25.9	24.4	0	0	0	0	19	MD
22	33	30.6	9.6	0	0	0	0	12	MD
23	45	26.9	22.5	0	0	0	0	13	30
24	27	22.6	26.7	0	0	0	0	16	37
25	13	21.9	29.2	0	0	4	4	2	31
26	49	30.4	10.3	2	2	0	0	12	42
27	59	28.7	18.5	6	6	0	0	38	34
28	40	26.8	11.8	6	6	0	0	6	26

29	64	24.4	12.3	0	0	0	0	36	40
30	66	22.1	28.6	0	0	0	0	17	55
31	41	27.7	25.8	0	0	0	0	20	40
32	53	24.7	24.4	0	0	0	0	MD	25
33*	16	22.5	12.8	0	0	0	0	2	39
34	41	31.2	20.0	0	0	0	0	15	46
35	42	31.9	20.0	4	4	0	0	7	60
36	44	25.3	12.2	0	0	0	0	25	MD
37	48	24.3	26.8	0	0	0	0	35	41
38	37	MD	14.3	0	0	0	0	22	47
39*	67	29.7	19.0	0	0	3	3	17	28
40	26	31.9	10.0	0	0	0	0	19	43

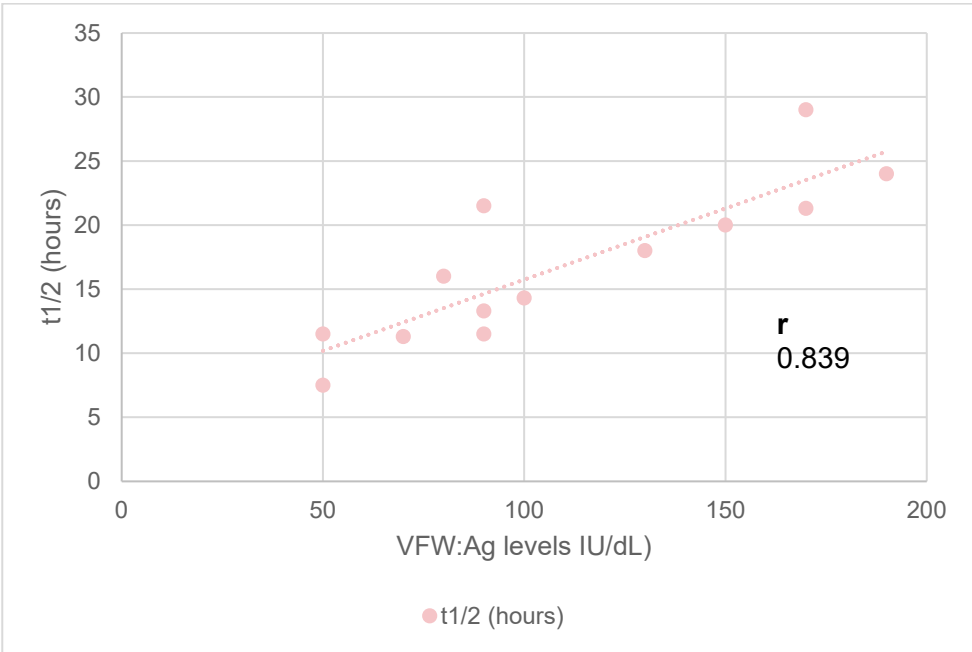

Figure S1.
Scatterplot showing correlation between t½ (hours) and VFW:Ag levels (IU/dL). r: Spearman's Correlation Coefficient, p: P-value, as assessed by Spearman's correlation.

Table S2. Treatment characteristics.

EOD: every other day, M/TH: Monday and Thursday, BW: body weight, IU: International Units, Kg: kilograms. Patients with increased dosing frequency after switch to Kovaltry are marked with asterisk. The patients' previous standard half-life (SHL) FVIII products were Helixate (octocog alfa, CSL Behring), Kogenate (octocog alfa, Bayer AB), ReFacto (moroctocog alfa, Pfizer Innovations) and Advate (octocog alfa, Takeda Pharma). ǂ Patients with moderate hemophilia A, who were excluded from the comparison of clinical outcomes and annual FVIII consumption between the Malmö and Oslo Hemophilia Centers.

CENTER	PAT ID	Regular total dose (IU)	Treatment with previous SHL FVIII product		Treatment with octocog alfa	
			Type of SHL FVIII product	Regular dosing interval	Regular dosing interval	Total FVIII consumption IU/Kg/Year
MALMÖ	1	2000	octocog alfa (Kogenate)	M/TH	M/TH	1873.9
	2	2000	octocog alfa (Helixate)	M/TH	M/TH	2736.8
	3	2000	rFVIII-FS (Kogenate)	EOD	EOD	3791.7
	4	2000	octocog alfa (Helixate)	3 times weekly	3 times weekly	3319.1
	5	2000	octocog alfa (Kogenate)	EOD	EOD	3872.3
	6	2000	octocog alfa (Kogenate)	3 times weekly	3 times weekly	3851.9
	7	2500	Octocog alfa (Helixate)	EOD	EOD	4840.4
	8	2000	octocog alfa (Kogenate)	EOD	EOD	4918.9
	9	1000	octocog alfa (Kogenate)	Daily	Daily	3650.0
	10	1000	Octocog alfa (Helixate)	Daily	Daily	3883.0
	11	1000	octocog alfa (Kogenate)	Daily	Daily	5367.6
	12	2000	octocog alfa (Kogenate)	M/TH	M/TH	2736.8
	13	2000	octocog alfa (Kogenate)	EOD	EOD	5871.0
	14	1000	Moroctocog alfa (ReFacto)	EOD	EOD	2527.8
	15	2000	octocog alfa (Kogenate)	EOD	EOD	3831.6
	16	2000	octocog alfa (Kogenate)	EOD	EOD	4727.3
	17	1000	octocog alfa (Kogenate)	Daily	Daily	4740.3
	18	2000	octocog alfa (Kogenate)	EOD	EOD	5777.8

OSLO	19*	1500	octocog alfa (Advate)	3 times weekly	EOD	3289.2
	20	1000	octocog alfa (Kogenate)	EOD	EOD	2333.3
	21	2000	octocog alfa (Helixate)	Once weekly	Once weekly	1268.3
	22	1000	octocog alfa (Advate)	3 times weekly	3 times weekly	1491.4
	23	2000	octocog alfa (Kogenate)	M/TH	M/TH	2337.1
	24	2000	octocog alfa (Kogenate)	EOD	EOD	4853.3
	25	2000	octocog alfa (Helixate)	3 times weekly	3 times weekly	4554.7
	26*	1000	octocog alfa (Kogenate)	3 times weekly	EOD	1866.7
	27	2000	octocog alfa (Helixate)	EOD	EOD	3370.4
	28	1000	octocog alfa (Kogenate)	3 times weekly	3 times weekly	1835.3
	29	1000	octocog alfa (Helixate)	3 times weekly	3 times weekly	1925.9
	30	2000	octocog alfa (Helixate)	EOD	EOD	5200.0
	31	2500	octocog alfa (Advate)	3 times weekly	3 times weekly	4020.6
	32	2000	octocog alfa (Helixate)	3 times weekly	3 times weekly	3804.9
	33‡	1000	octocog alfa (Advate)	3 times weekly	3 times weekly	2000.0
	34	2000	octocog alfa (Kogenate)	3 times weekly	3 times weekly	3120.0
	35	2000	octocog alfa (Kogenate)	M/TH	M/TH	2080.0
	36	1000	octocog alfa (Helixate)	3 times weekly	3 times weekly	1902.4
	37	2000	octocog alfa (Helixate)	3 times weekly	3 times weekly	4187.9
	38	1000	octocog alfa (Helixate)	M/TH	M/TH	1485.7
	39‡	2000	octocog alfa (Kogenate)	once weekly	once weekly	990.5
	40	1000	octocog alfa (Advate)	3 times weekly	3 times weekly	1560.0

Table S3.
Veritas-PRO scores in subcategories and total point score. A cut-off of 57 points was used to define non-adherence (n=34) ‡ When the patient failed to answer a specific question, the maximum point score 5 was awarded.

Patient	Timing	Dosing	Planning	Remembering	Skipping	Communication	Total
1	9	5	6	4	8	15	47
2	10	6	4	8	8	8	44
3	4	4	5	4	4	4	25
4	8	4	7	8	7	6	40
5	8	4	4	4	4	4	28
6	7	6	12	14	5	11	55
7	7	4	5	4	4	4	28
8	6	4	5	5	4	11	35
9	6	4	13	7	11	7	48
10	8	7	6	5	7	7	40
11	5	4	16	8	4	16	53
12	10	8	11	8	6	11	54
13	8	4	8	8	7	12	47
15	4	4	5	4	4	6	27
16	5	7	5	4	4	9	34
17	4	4	4	6	4	7	29
18	9	4	4	8	4	8	37
19	12 ‡	4	8‡	9	8	5	46
23	6	4	4	4	4	8	30
24	5	5	4	8	4	11	37
25	6	4	4	7	4	6	31
26	4	5	4	6	7	16	42
27	8	4	4	4	4	10	34
28	4	4	4	5	4	5	26
29	6	5	4	5	4	16	40
30	7	4	4	11	13	16	55
31	4	4	12	4	4	12‡	40
32	5	4	4	4	4	4	25
33	7	4	6	10	4	8	39
34	8	6	4	5	8	15	46
35	12	6	8‡	14	10	10	60
37	6	7	5	6	6	11	41
38	7	9	4	9	9	9	47
40	8	4	4	8	14	5	43

Paper III

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Haemophilia WFH WILEY

ORIGINAL ARTICLE

Impact of timing of prophylaxis commencement, F8 genotype and age on factor consumption and health-related quality of life in patients with severe haemophilia A

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Abstract

Introduction: The timing of prophylaxis and *F8* genotype can impact treatment outcomes in adults with severe haemophilia A (HA).

Aim: To investigate how *F8* genotype, timing, and type of prophylaxis influence arthropathy, bleeding rates, factor consumption and health-related quality of life (HRQoL).

Methods: Thirty-eight patients with severe HA were enrolled. Bleeding events were recorded retrospectively during median 12.5 months. *F8* gene variants were classified as null or non-null. Joint health and HRQoL were assessed with HJHS and EQ-5D-5L, respectively.

Results: The median age at prophylaxis start was 1.25 years in the primary prophylaxis group ($N = 15$, median age 26 years) and 31.5 years in the secondary group ($N = 22$, 45 years), respectively. There were significant differences in the medians of HJHS (4 vs. 20, $p < .001$), EQ-5D-5L index (0.9647 vs. 0.904, $p = .022$), EQ VAS (87 vs. 75, $p = .01$) and FVIII consumption (3883 vs. 2737 IU/kg/year, $p = .02$), between the primary and secondary groups, respectively. Median annualized bleeding rate (ABR) was 0 for both groups. Twenty-five null and thirteen non-null *F8* gene variants were identified. In the secondary prophylaxis group, lower median FVIII consumption (1926 vs. 3370 IU/kg/year) was shown for non-null compared to null variants, respectively, with similar ABR and HJHS.

Conclusion: Delayed prophylaxis start with intermediate dose intensity prevents bleeds but at a cost of more arthropathy and reduced HRQoL, compared to higher intensity primary prophylaxis. Non-null *F8* genotype may allow lower factor consumption with similar HJHS and bleeding rates, compared to null genotype.

KEYWORDS
arthropathy, F8 gene variant, F8 genotype, health-related quality of life, haemophilia, non-null, null, primary prophylaxis, secondary prophylaxis

1 | INTRODUCTION

Replacement of the deficient factor VIII (FVIII) in haemophilia A (HA) aims to prevent or stop joint bleeding and subsequent development of arthropathy,[1,2] thus preserving the health-related quality of life (HRQoL) of people with haemophilia (PWH). In the Scandinavian countries this goal has been pursued, with varying start points, with the use of factor replacement prophylaxis since the 1970s.[3] A high dose-intensity regime, as defined by the World Federation of haemophilia (WFH) cut-off of 4000 IU/kg/year[4] has traditionally been used, with very good outcomes regarding annual bleeding rate and joint health, albeit at a higher cost compared to lower dose-intensity regimes.[5]

HA is caused by pathogenic *F8* gene variants, leading to impaired biosynthesis, expression, secretion, stability of FVIII and resulting in decreased plasma levels and function of FVIII.[6–8] In severe HA, which is defined as FVIII activity of <1%, *F8* gene variants can be categorized as null versus non-null, based on measurable levels of circulating FVIII antigen.[9–12] Null variants have been associated with earlier onset of bleeding and diagnosis of haemophilia[9] and have a higher risk for development of inhibitor compared to non-null variants.[13,14]

Our previously published study enrolled patients with severe HA, treated at the haemophilia centres of Malmö, Sweden and Oslo, Norway, and showed very low bleeding rates with median annualized bleeding rate (ABR) 0 with both intermediate and high intensity prophylaxis, with no difference in arthropathy between the study groups.[15] To further explore these findings, we wanted to evaluate the impact of *F8* genotype, patient age at study inclusion and timing of start of prophylaxis on clinical outcomes, including arthropathy development, bleeding, FVIII consumption and HRQoL.

2 | METHODS

This analysis was performed on data collected from patients above 12 years of age with severe HA (FVIII:C < 1 IU/dL), enrolled in the Scandinavian Switch Population-PK study at the haemophilia centres in Malmö, Sweden and Oslo, Norway, as described.[15] Only patients who had previously switched or were planned to switch to BAY 81–8973 (octocog alfa, Kovaltry, Bayer AB), from another standard half-life (SHL) FVIII product, were eligible. This study examined the difference in bleeding rates before and after the switch to BAY 81–8973 in a Scandinavian cohort treated at the two centres. In addition, arthropathy, FVIII consumption and compliance to treatment were characterized. Bleeding events were documented retrospectively for a median time period of 12.5 months, (IQR 10–13) after the switch to BAY 81–8973 and before the final study visit. The ABR and annualized joint bleeding rate (AJBR) were calculated.

The patient's age at inclusion and at start of prophylaxis with factor replacement therapy, weight, arthropathy according to haemophilia joint health score (HJHS 2.1)[16] and FVIII consumption (IU/kg/year) were documented. Primary prophylaxis was defined as continuous regular prophylaxis commenced at least once weekly with standard (SHL) or extended half-life (EHL) FVIII products before the completion of 3rd year of age, and before the second joint bleed or manifest joint disease, according to recent guidelines,[4] whereas secondary prophylaxis was continuous regular prophylactic treatment, which did not fulfil the criteria of primary prophylaxis. Patients with persistent inhibitors were excluded.

Characterization of the causative *F8* gene variants was performed using routine methods at the genetic laboratories associated with the haemophilia centres in Malmö and Oslo, respectively, as part of the clinical investigation of the patients. All variants were classified according to the recommendations of the Human Genome Variation Society (HGVS). Inversions, nonsense mutations, small deletions outside poly-A-runs, splice cite mutations within conserved regions, large deletions and deletions of the promoter were defined as null mutations, whereas missense mutations, small deletions inside poly-A-runs and splice cite mutations of non-conserved nucleotides were characterized as non-null mutations, as previously described.[9]

Health related quality of life was assessed by the generic self-filled questionnaire EQ-5D-5L.[17] EQ-5D-5L consists of two parts. The first part is the descriptive system, which consists of five dimensions describing different health states: mobility, usual activities, self-care, pain, and anxiety/depression. Each dimension has five levels of severity: no, slight, moderate, severe, and extreme problems, which are graded from 1 to 5, respectively. A score of 11111 thus signifies no problems in any of the dimensions, while 55555 signifies extreme problems in all dimensions. The EQ-5D-5L dimensions are converted to an index value that ranges from 0 to 1 and is based on the health preferences of the general population of a country or region. An EQ-5D-5L index of 1 is the best possible value and 0 the worst.[18] The Swedish time to trade-off valuation scores were used to calculate the index value.[19] The second part of EQ-5D-5L consists of the Visual Analogue Scale (EQ VAS), where the patient assesses his individual state of health at the day of the questionnaire. EQ VAS score ranges from 0 to 100 (worst to best possible health state, respectively).[18]

2.1 | Statistical analysis

Descriptive statistics in the form of median and interquartile ranges (IQR 25th -75th percentile) described continuous variables. The Mann-Whitney U test and Kendall Tau-b test for non-parametric variables were performed using SPSS software, version 25 (IBM, Chicago, IL). A *p*-value of <.05 was considered statistically significant.

3 | RESULTS

3.1 | Clinical data and timing of initiation of prophylaxis analysis

Demographics and clinical characteristics of the study population are summarized in Table 1 and Supplement Table S1. Data about the timing of start of prophylaxis and type of prophylaxis were available for 37 of the 38 enrolled patients. Of these, 15 patients, with median age

TABLE 1 Clinical characteristics and outcomes (median and IQR) of primary and secondary prophylaxis group

	Primary prophylaxis (*N* = 15)	Secondary prophylaxis (*N* = 22)	*p*
Age at inclusion (years)	26 (18-35)	45 (40.8-59.8)	
Patients with null-mutation, *N* (%)	9 (60)	15 (68.2)	
Age at start of prophylaxis (years)	1.25 (1-2)	31.5 (10.5-42.8)	
ABR	0 (0-0)	0 (0-0)	.960
AJBR	0 (0-0)	0 (0-0)	.939
HJHS	4 (2-11)	20 (12.5-35.5)	**<.001**
FVIII consumption (IU/kg/year)	3883 (3319-4853)	2737 (1895-3909)	**.02**
EQ-5D-5L index	0.9647 (0.934-0.9755)	0.904 (0.8332-0.9647)	**.022**
EQ-5D-5L VAS	87 (80-93.5)	75 (60-82.5)	**.01**

Bold values indicate statistically significant results ($p < .05$).

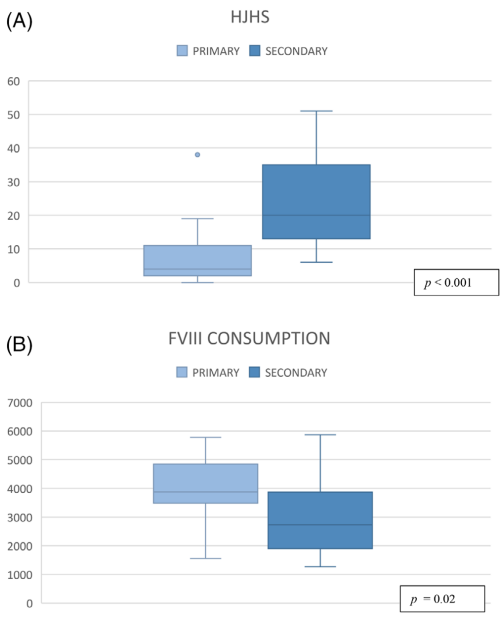

FIGURE 1 Boxplots showing difference in HJHS (A) and FVII consumption (IU/kg/year) (B) between primary and secondary group.

(IQR) at study enrolment of 26 years (18-35) started primary prophylaxis, and 22 patients with median age at enrolment of 45 years (40.8–59.8) were on secondary prophylaxis. The median age was 1.25 years (1–2 years), and 31.5 years (10.5–42.8 years) at the start of primary and secondary prophylaxis, respectively. The median ABR and AJBR after the switch to BAY 81–8973 for both the primary and secondary prophylaxis group was 0 (0-0). There were significant differences between the primary and secondary prophylaxis groups in HJHS and FVIII consumption (Figure 1) with a median HJHS of 4 (2-11) and 20 (12.5-35.5), respectively ($p < .001$). Median yearly FVIII consumption was 3883 IU/kg/year (3319-4853) in the primary versus 2737 IU/kg/year (1896-3909) in the secondary group ($p = .02$). A significant correlation between age of the patient at study enrolment and age at start of prophylaxis was seen ($p = .001$) (Supplement Figure S1). Five of the 22 patients of the secondary prophylaxis group started prophylaxis before the 10 years of age, while, in the remaining patients, prophylaxis commenced during adulthood. Patients who started secondary prophylaxis during childhood had median HJHS 15 (11.5-33) and median FVIII consumption 3872 IU/kg/year (2928-4946). Two patients in the primary prophylaxis and seven patients in the secondary prophylaxis group reported the use of pain medication (Supplement Table S1).

3.2 | *F8* gene variant analysis

F8 gene variants were identified in all patients, that is, inversions (*N* = 13), missense (*N* = 11), small deletions (*N* = 8) and nonsense (*N* = 5). One patient had a splice variant (Supplement Table S2). Twenty-five variants were classified as null, and thirteen as non-null (Figure 2). The distribution of null variants in the primary and secondary prophylaxis group was 60% and 68.2%, respectively (Table 1). In the entire cohort, there was no difference between the null and non-null groups in HJHS, ABR, AJBR, FVIII consumption, start at age or prophylaxis, EQ-5D-5L index or EQ VAS.

However, in the secondary prophylaxis group, there was a trend towards lower consumption in the non-null group with a median (IQR) FVIII consumption of 1926 IU/kg/year (1867-2737), compared to 3370 IU/kg/year (2333-4021) in the null group ($p = .139$) (Figure 3A), while maintaining median ABR 0 versus 0 and similar HJHS of 17 versus 21, respectively (Figure 3B).

3.3 | Health related quality of life analysis

The EQ-5D-5L questionnaire was completed by 34 patients, 13 in the primary and 21 in the secondary prophylaxis group. As shown in Table 1, there were significant differences in median EQ-5D-5L Index

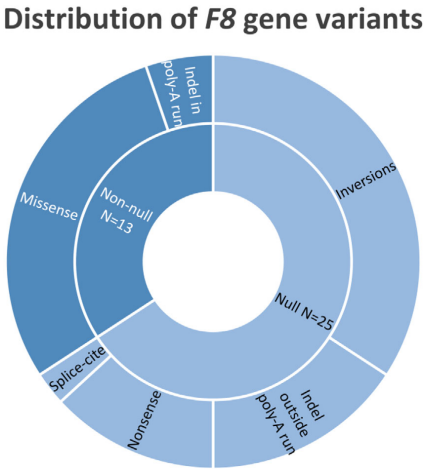

FIGURE 2 Distribution and classification of null and non-null FVIII variants.

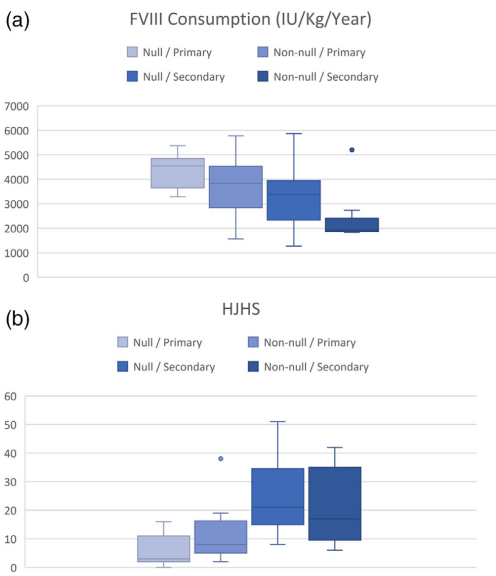

FIGURE 3 Boxplots showing differences in FVIII consumption (IU/kg/year) (A) and HJHS (B), according to FVIII variant status (non/non-null) and type of prophylaxis (primary/secondary).

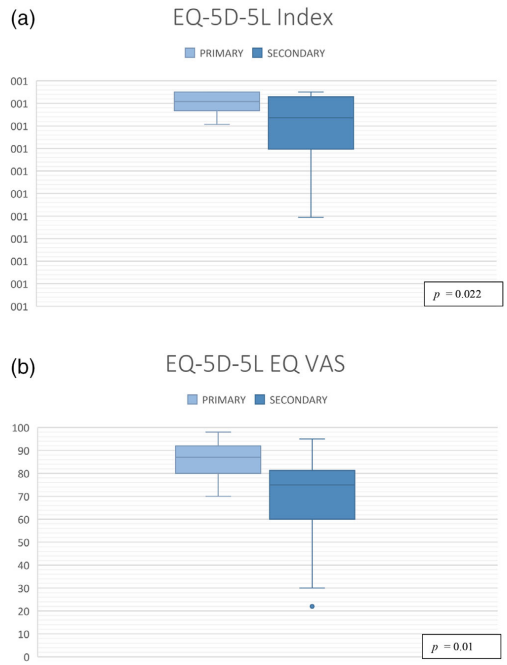

FIGURE 4 Boxplots showing differences in EQ-5D-5L Index value (A) and EQ VAS (B). Between the primary and secondary prophylaxis group.

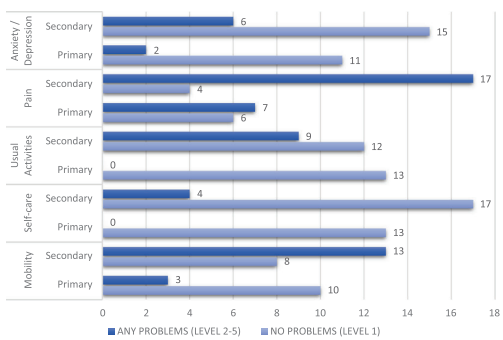

FIGURE 5 Bar chart showing dimension results of EQ-5D-5L after dichotomization in "no problems" (level 1) and "any problems" (levels 2-5).

value and EQ VAS between the younger (median age 26 years) primary prophylaxis group and the older (median age 45 years) secondary prophylaxis group with median Index 0.9647 (0.934-0.9755) versus 0.904 (0.8332-0.9647) ($p = .022$) (Figure 4A) and EQ VAS 87 (80-93.5) versus 75 (60-82.5) ($p = .01$), respectively. (Figure 4B). The complete EQ-5D-5L data is shown in Supplement Table S3.

When the distinct dimensions results were dichotomized in "no problems" versus "any problems," there were more patients that experienced problems in the secondary group in all dimensions (Figure 5). In the dimension "pain," seven of thirteen patients (53.8%) in the primary,

and 17 of 21 (80.9%) in the secondary prophylaxis group experienced problems. This contrasted with the use of painkillers by two (13.3%) and seven (35%) patients in the primary and secondary prophylaxis group, respectively.

4 | DISCUSSION

In our previous study,[15] we found a low bleeding rate and relatively modest degree of arthropathy in Norwegian patients despite the use of significantly lower factor consumption than the Swedish patients. To better appreciate these findings, the impact of the type and timing of prophylaxis and *F8* genotype were evaluated with a focus on the use of primary and secondary prophylaxis. In addition, we wanted to correlate the findings to health related QoL.

Twenty-two patients in the study cohort had secondary prophylaxis. The majority of these patients ($n = 15$) were treated at the Oslo centre. The median age at start of prophylaxis in the secondary prophylaxis group was 31.5 years, compared to 1.25 years in the primary prophylaxis group. Due to changes in clinical practice over the last decades, there was a strong correlation between the current age of the patients, and the type of prophylaxis at start. As expected, the comparison of the primary and secondary prophylaxis groups revealed significant differences in degree of arthropathy and HRQoL outcomes, but also regarding FVIII consumption. The primary group had significantly lower HJHS with median score 4, compared to 20 in the secondary group illustrating the benefits of starting primary prophylaxis at an early age with joints more susceptible to bleedings.[20–23] Interestingly, despite more arthropathy, the secondary prophylaxis group had a median low AJBR of zero and significantly lower yearly FVIII consumption of median 2,737 IU/kg/year, compared to 3,883 IU/kg/year in the primary group. Most patients on secondary prophylaxis were treated at the Oslo centre, which probably contributed to the observed difference in FVIII consumption between the two centres. Even though the goal of treatment at both centres was zero bleeds, this was pursued trough different dosing intensity regimens, resulting nonetheless in median ABR 0 at both centres. Accordingly, the intensity of prophylaxis may be successfully individualized and lowered in adults without significantly jeopardizing the bleeding phenotype. It should also be stated that the difference in median age at inclusion of almost 20 years between the primary and secondary prophylaxis group may have importance for these outcomes, but age-matched comparisons regarding prophylaxis type are not possible in a Scandinavian cohort as all severe HA patients born in the last decades are on primary prophylaxis. Unfortunately, we could not explore the exact prophylactic dosing regimen used at start in any of the two subgroups as well as the AJBR over the years, since this was outside the scope of the study. However, clearly, our data underlines the importance of starting primary prophylaxis to avoid progressive joint damage. Importantly, the subgroup that commenced secondary prophylaxis during childhood, between 3 and 9 years, had a high median FVIII consumption of 3872 IU/kg/year, but still had a higher median HJHS 15 compared to a median HJHS score of 4 in the primary prophylaxis group. These findings suggest that high treatment intensity cannot compensate for a delayed prophylaxis start regarding the risk of developing arthropathy, but the very low number of patients makes this finding uncertain.

Previous studies in paediatric cohorts[9,13] have shown that type of *F8* genotype influences start of first bleed and thus may influence when the providers decide to start prophylaxis. Due to the small sample size, statistical analysis could not show any significant differences between the null and non-null groups in timing for start of prophylaxis and risk for developing arthropathy. Subgroup analysis, however, showed a trend towards lower FVIII consumption in the secondary prophylaxis group in the presence of non-null variants, with similar HJHS and ABR to the higher consumption null group. This finding could indicate that circulating trace amounts of FVIII may have an impact on bleeding risk[9] and subsequent development of arthropathy and dose reductions of factor replacement in non-null mutations in the secondary prophylaxis setting could be considered, but further studies are needed to answer this hypothesis. Our study is one of the first to address the impact of non versus non-null variants in outcomes of an adult cohort, whereas previous studies primarily examined paediatric cohorts and the genotype's impacts in bleeding and inhibitor development.[9,13,24]

Health-related quality of in the entire study cohort was relatively high with a median EQ-5D-5L index of above 0,9 and median VAS 80. There were however small but significant differences in HRQoL outcomes between the primary and secondary group in both the Index value (median 0.9647 vs. 0.904) and VAS (median 87 vs. 75), which further underscores both the influence of age and the value of starting primary prophylaxis. The presence of a disability paradox, where haemophilia patients report higher health state evaluations than otherwise healthy pears cannot be excluded.[25] The older cohort with delayed prophylaxis start had comparable HRQoL outcomes to other published European cohorts[26,27] and the absence of bleeding exerts beneficial affects against the development of synovitis and further progression or arthropathy.[28] This reflects the benefits of individualized prophylaxis since at least the 1990s.[29] Analysis of the patient-reported use of medication for pain management, mostly anti-inflammatory drugs (NSAID or COX-2 inhibitors) and self-reported pain in the EQ-5D-5L questionnaire revealed a discrepancy between reported pain and use of painkillers, both in the primary and secondary prophylaxis groups, which may imply undertreatment of pain problems, as reported elsewhere.[30] However, none in the secondary prophylaxis group reported the use of opioids and the highest EQ-5D-5L score in the pain dimension was 3, signifying moderate pain even in patients with relatively advanced arthropathy. Even though these findings are based on few patients, they suggest benefit of prophylaxis against severe pain, possibly due to a reduction in subclinical bleeds and synovitis.[31,32]

Our study has limitations, mostly the small sample size which may have caused the lack of significance of, for example, the impact of *F8* genotype in the examined clinical outcomes. The risk of recruitment/selection bias can also not be ruled out, as patients on other SHL products attending the centres were not eligible. In addition, only 50% of Oslo patients treated with BAY 81–8973 were enrolled. The dosing regimens used over the years from start of prophylaxis to the

current regime may also have an impact on the outcome, but this was outside the scope of our study and needs to be addressed in a separate study. We did not have data on the date of first joint bleed, the number of joint bleeds prior to prophylaxis start and previous HJHS findings, which may be related to the severity of the bleeding phenotype. Because we did not have a complete data set on joint status at start of prophylaxis, we chose, as in previous publications,[33–35] to use the term secondary prophylaxis for the entire non-primary cohort to avoid potential misclassification. However, many patients in the secondary group probably had tertiary prophylaxis, i.e., prophylaxis initiated after the onset of documented joint disease.[4] Finally, arthropathy was assessed with HJHS, which may be influenced by acute bleeds or inflammation. However, the HJHS was performed by experienced physiotherapists at both centres and the absence of bleeds in the cohort implies that the evaluations of the joints were mostly performed at steady state. Even in this instance, some selection bias, where patients with recent bleeds and severe arthropathy symptoms were not included in the study, cannot be dismissed, even though our study included several patients with high HJHS. The strengths of our study consist of the life-long comprehensive follow-up of the study cohort which allowed for the investigation of an older patient population who has been receiving individualized prophylaxis for decades and the examination of multiple clinical outcomes within this cohort. Additionally, the cohort's socioeconomic homogeneity should suggest a high applicability of the HRQoL results. Finally, our study is one of few to correlate the findings of a thorough genetic characterization to the relevant clinical outcomes of factor consumption and arthropathy in an adult cohort.

In conclusion, our results indicate that delayed start of prophylaxis in an older cohort with severe HA can still achieve excellent bleeding control with intermediate-dose intensity, but at the expense of developing arthropathy and reduced HRQoL compared to a younger cohort on primary prophylaxis. To carry non-null *F8* gene variants may potentially predict the bleeding phenotype and thereby reduce the risk of severe arthropathy and improve QoL despite the use of less intense prophylactic regimes.

AUTHOR CONTRIBUTIONS

AA and JA designed the study, acquired, analyzed, and interpreted the data, drafted, and finalized the manuscript. PAH recruited patients, interpreted the data, and finalized the manuscript. EB designed the study, interpreted the data, and finalized the manuscript.

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CONFLICT OF INTEREST DISCLOSURES

AA has received research grants from Takeda/Shire and Bayer. Speaker's fee and consultant for Ablynx/Sanofi, Sobi, Chiesi, Amgen, Grifols. PAH has received research grants to institution from Bayer, Octapharma, Pfizer and SOBI. Speakers fee and consultant for Bayer, CSL Behring, Takeda, Octapharma, Pfizer, NovoNordisk, BioMarin and SOBI. EB has acted as paid consultant to Bayer, CSL Behring, NovoNordisk, Octapharma and Takeda. JA has received research grants from Sobi, CSL Behring, Takeda/Shire, and Bayer. Speakers fee and consultant for Octapharma, Novo Nordisk, Pfizer, Bayer, Sobi, Sanofi, CSL Behring, Takeda/Shire, BioMarin and Uniqure.

ETHICS STATEMENT

The study was approved by the Regional Ethics Review Board of Lund University, Lund, Sweden and Oslo University, Oslo, Norway. The study subject or his legal representative provided written informed consent before entering the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Table S1. Clinical characteristics and demographics.
MD: missing data. † COX-2 inhibitor. ‡ SSRI: selective serotonin reuptake inhibitor. § NSAID: non-steroidal anti-inflammatory drug.

CENTER	PAT-ID	Age at inclusion (Y)	Age at start of prophylaxis (Years)	Type of prophylaxis	HJHS	ABR	AJBR	Pain Medication	FVIII Consumpt IU/KG/YEAR
MALMÖ	1	70	50	Secondary	42	0	0	No	1874
	2	62	24	Secondary	34	0	0	Yes † ‡	2737
	3	40	1.67	Primary	8	0	0	No	3792
	4	35	1.67	Primary	3	0	0	No	3319
	5	43	3.92	Secondary	46	2	2	Yes†	3872
	6	18	1	Primary	2	0	0	No	3852
	7	32	1.25	Primary	11	2	0	Yes §	4840
	8	44	2.25	Primary	11	0	0	Yes†	4919
	9	19	0.92	Primary	3	0	0	No	3650
	10	44	2.17	Primary	38	0	0	No	3883
	11	15	1.17	Primary	0	0	0	No	5368
	12	22	4	Secondary	15	2	0	No	2737
	13	35	3.5	Secondary	8	0	0	No	5871
	14	13	1.08	Primary	2	0	0	No	2528
	15	38	31	Secondary	33	0	0	No	3832
	16	59	25	Secondary	51	0	0	No	4727
	17	26	1.25	Primary	8	0	0	Yes†	4740
	18	21	1.17	Primary	4	0	0	No	5778
OSLO	19	25	0.5	Primary	2	3	0	No	3289
	20	45	11	Secondary	12	1	1	No	2333
	21	63	45	Secondary	19	0	0	Yes §	1268
	22	33	MD	MD	12	0	0	MD	1491
	23	45	42	Secondary	13	0	0	No	2337
	24	27	1	Primary	16	0	0	No	4853
	25	13	2.5	Primary	2	4	4	No	4555
	26	49	28.7	Secondary	12	0	0	Yes†	1867
	27	59	26.8	Secondary	38	0	0	No	3370

	28	40	24.4	Secondary	6	0	0	MD	1835
	29	64	57	Secondary	36	0	0	MD	1926
	30	66	61	Secondary	17	0	0	Yes§	5200
	31	41	8	Secondary	20	0	0	No	4021
	32	53	42	Secondary	MD	0	0	No	3805
	33	41	9	Secondary	15	0	0	Yes†	3120
	34	42	37	Secondary	7	0	0	No	2080
	35	44	33	Secondary	25	0	0	No	1902
	36	48	26	Secondary	35	0	0	No	4188
	37	37	27	Secondary	22	0	0	Yes†	1486
	38	26	2	Primary	19	0	0	No	1560

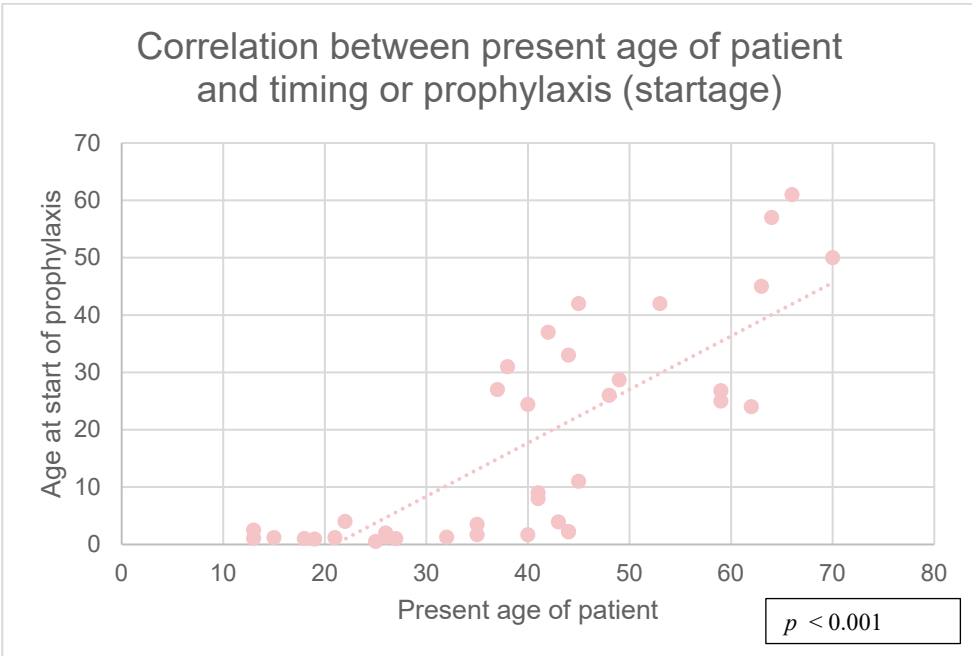

Figure S1.
Scatterplot showing correlation between age of patient at time of study and age at start of prophylaxis.

Table S2. *F8* gene variants found in the study cohort.

CENTER	PAT-ID	Mutation Type / Effect	HGVS cDNA	HGVS protein	Mutation Group	Exon/Intron (FVIII Domain)
			NM_000132.4	NP_000123.1		
MALMÖ	1	Substitution / Missense	c.902G>A	p.(Arg301His)	Non-null	Exon 7 (A1)
	2	Substitution / Missense	c.902G>A	p.(Arg301His)	Non-null	Exon 7 (A1)
	3	Deletion inside poly-A run/ Frameshift	c.3637delA	p.(Ile1213Phefs*5)	Non-null	Exon 14 (B)
	4	Duplication / Frameshift	c.5116_5117dupAG	p.(Ser1706Argfs*26)	Null	Exon 14 (a3)
	5	Substitution / Nonsense	c.6590T>A	p.(Leu2197*)	Null	Exon 24 (C2)
	6	Substitution / Nonsense	c.471G>A	p.(Trp157*)	Null	Exon 4 (A1)
	7	Inversion 22			Null	
	8	Small deletion outside poly-A-run/ Frameshift	c.954_955delCT	p.(Leu319Aspfs*18)	Null	Exon 7 (A1)
	9	Duplication / Frameshift	c.6360dupT	p.(Ile2121Tyrfs*5)	Null	Exon 22 (C1)
	10	Substitution / Missense	c.1795G>C	p.(Asp599His)	Non-null	Exon 12 (A2)
	11	Small deletion outside poly-A-run/ Frameshift	c.954_955delCT	p.(Leu319Aspfs*18)	Null	Exon 7 (A1)
	12	Inversion 1			Null	
	13	Inversion 22			Null	
	14	Substitution / Missense	c.6563G>A	p.(Cys2188Tyr)	Non-null	Exon 23 (C1)
	15	Small deletion outside poly-A-run / Nonsense	c.1599delA	p.(Val534*)	Null	Exon 11 (A2)
	16	Inversion 22			Null	
	17	Small deletion inside poly-A run/ Frameshift	c.3637delA	p.(Ile1213Phefs*5)	Non-null	Exon 14 (B)
	18	Substitution / Missense	c.902G>A	p.(Arg301His)	Non-null	Exon 7 (A1)
OSLO	19	Inversion 22			Null	
	20	Inversion 22			Null	
	21	Inversion 22			Null	
	22	Inversion 22			Null	
	23	Small deletion outside poly-A-run/ Frameshift	c.205_206delCT	p.(Leu69Valfs*13)	Null	Exon 2 (A1)

	24	Inversion 22			Null	
	25	Substitution / Nonsense	c.3175A>T	p.(Lys1059*)	Null	Exon 14 (B)
	26	Substitution / Missense	c.5825G>A	p.(Gly1942Asp)	Non-null	Exon 18 (A3)
	27	Substitution / Nonsense	c.5883G>A	p.(Trp1961*)	Null	Exon 18 (A3)
	28	Substitution /Missense	c.6273G>C	p.(Lys2091Asn)	Non-null	Exon 21 (C1)
	29	Substitution / Missense	c.6278A>T	p.(Asp2093Val)	Non-null	Exon 22 (C1)
	30	Substitution / Missense	c.5624T>G	p.(Leu1875Arg)	Non-null	Exon 17 (A3)
	31	Inversion 22			Null	
	32	Inversion 22			Null	
	33	Inversion 22			Null	
	34	Substitution / Missense	c.5825G>A	p.(Gly1942Asp)	Non-null	Exon 18 (A3)
	35	Inversion 22			Null	
	36	Substitution / Splice-cite change within conserved region	c.6115+5G>A		Null	Intron 19
	37	Substitution / Nonsense	c.2440C>T	p.(Arg814*)	Null	Exon 14 (B)
	38	Substitution / Missense	c.6545G>T	p.(Arg2182Leu)	Non-null	Exon 23 (C1)

Table S3. EQ-5D-5L Dimension levels.
EQ-5D-5L Profile, Index Value and EQ VAS.

CENTER	PAT-ID	Mobility	Self-Care	Usual Activities	Pain/ Discomfort	Anxiety/ Depression	EQ-5D-5L profile	EQ-5D-5L index value	EQ-5D-5L VAS
MALMÖ	1	3	2	2	2	1	32221	0.8498	80
	2	3	2	3	3	2	32332	0.7259	22
	3	1	1	1	1	1	11111	0.9755	90
	4	1	1	1	1	2	11112	0.943	80
	5	1	1	1	2	1	11121	0.9647	70
	6	1	1	1	1	1	11111	0.9755	98
	7	1	1	1	3	1	11131	0.9327	80
	8	2	1	1	3	1	21131	0.904	75
	9	1	1	1	2	1	11121	0.9647	90
	10	2	1	1	2	1	21121	0.936	80
	11	1	1	1	2	1	11121	0.9647	87
	12	3	1	1	1	1	31111	0.9409	80
	13	2	1	2	2	2	21222	0.8486	60
	14	MD	MD	MD	MD	MD	MD	MD	MD
	15	1	1	1	1	1	11111	0.9755	95
	16	5	2	3	2	1	52321	0.7704	30
	17	1	1	1	1	1	11111	0.9755	85
	18	1	1	1	1	1	11111	0.9755	95
OSLO	19	MD	MD	MD	MD	MD	MD	MD	MD
	20	3	1	3	3	3	31333	0.697	50
	21	3	1	1	3	1	31131	0.8981	70
	22	MD	MD	MD	MD	MD	MD	MD	MD
	23	1	1	1	2	1	11121	0.9647	95
	24	2	1	1	2	1	21121	0.936	92
	25	1	1	1	1	1	11111	0.9755	95
	26	1	1	1	2	1	11121	0.9647	80
	27	3	2	2	3	1	32231	0.8178	70

	28	1	1	1	1	1	11111	0.9755	90
	29	2	1	1	3	1	21131	0.904	80
	30	1	1	1	2	2	11122	0.9322	60
	31	2	1	2	3	1	21231	0.8491	60
	32	2	1	2	3	2	21232	0.8166	45
	33	2	1	1	2	1	21121	0.936	75
	34	1	1	1	1	1	11111	0.9755	95
	35	2	1	2	2	2	21222	0.8486	85
	36	1	1	1	2	1	11121	0.9647	75
	37	MD	MD	MD	MD	MD	MD	MD	MD
	38	2	1	1	2	2	21122	0.9035	70

Paper IV

Primary prophylaxis implementation and long-term joint outcomes in Swedish haemophilia A patients

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Abstract

Introduction

Primary prophylaxis is the gold standard in severe haemophilia A (SHA) but time to escalate the prophylaxis regimen varies.

Aim

Assess prophylaxis implementation and long-term joint health outcomes in SHA with primary prophylaxis.

Methods

Adult male patients born after 1980, with SHA on primary prophylaxis, started before the age of three years and second joint bleed, and no history of FVIII inhibitors, were enrolled. Repeated joint-health examinations were performed with HJHS or HEAD-US; VERITAS-PRO assessed adherence.

Results

Thirty patients were enrolled with, at inclusion, median age 33.5 years, annualized bleed rate and joint bleed rate 0, and FVIII consumption 4232 IU/kg/year, respectively. The median age was 1.2 years, at prophylaxis start once weekly with a median FVIII dose of 47.7 IU/Kg, and 1.7 years, by the time escalation to a final regimen had occurred, with a median infusion frequency of thrice weekly and FVIII dose 41.7 IU/Kg, respectively. Older age correlated with later transition to escalated prophylaxis ($p<0.001$). Longer time to escalated prophylaxis correlated to more bleeds ($p<0.001$). Median HJHS increased slowly, reaching 4 at 35-40 years. HJHS at 15-20 years correlated with higher HJHS afterwards. Median total HEAD-US score was 1 and correlated with HJHS ($p<0.001$). Median VERITAS-PRO score was 36, indicating good treatment adherence.

Conclusions

Primary prophylaxis is effective but does not completely prevent the gradual development of arthropathy in SHA. Joint assessments with HJHS should start at an early age, as they correlate with arthropathy in later life. Prophylaxis escalation should proceed expeditiously to prevent bleeds.

1. Introduction

The efficacy of prophylactic factor replacement in decreasing the frequency of joint haemorrhages and the development of arthropathy in patients with severe haemophilia A (SHA) has been established in randomised clinical studies.[1-3] Therefore, for many decades, primary prophylaxis has been considered to be the gold standard for treatment. Importantly, once established, arthropathy will mainly be irreversible and in most cases worsen, despite the use of preventive therapies.[4,5] The use of primary prophylaxis, i.e. the start of prophylaxis before the second joint bleed and age of three years,[6] is therefore crucial. However, the initial phase of prophylaxis implementation will differ. Data on the potential impact of this period on long-term outcomes are limited.

The practice in Sweden during the last decades has consisted of starting primary prophylaxis once weekly at about 1 year of age and as soon as possible transition to a dose escalated "final" implemented regimen, at least twice weekly. Nevertheless, interpersonal variation in early bleeding patterns and pharmacokinetics, as well as in the propensity for the development of arthropathy, need to be taken into account.[7-9] The exact time point for starting prophylaxis and the time to prophylaxis escalation may vary with an associated risk for bleeds.[10] Earlier onset of joint bleeds can be a predictor of more severe arthropathy and higher factor product consumption later in life.[11] Importantly, adherence to treatment should always be considered, i.e. the active choice by patients to engage in following their treatment and take responsibility for their well-being,[12] since this may significantly impact upon treatment outcome, regardless of the prophylactic regimen used.[13]

The aim of this study was to evaluate long-term joint-health outcomes with the Swedish primary prophylaxis strategy and correlate this to how prophylaxis was implemented in childhood, i.e., the initial treatment provided, time until prophylaxis was escalated and the final regimen's intensity, the bleeding phenotype, and adherence to treatment.

2. Methods

2.1. Study Design

This is a retrospective double-centre study, enrolling adult male patients with SHA (baseline FVIII:C < 1 IU/dL), born after the year 1980 and treated at the haemophilia comprehensive care centres (HCCC) in Malmö and Gothenburg, Sweden. All enrolled patients had received primary prophylaxis, i.e., prophylaxis that started before the second clinically evident joint bleed and age of three years. Patients with a history of inhibitor to factor VIII (FVIII) were excluded.

2.2. Clinical data collection

Data from the patient's last regular visit regarding age, height, weight, bleeds, and joint bleeds were collected and used to calculate the annualized joint bleeding rate (AJBR) and annualized bleeding rate (ABR). Additionally, data regarding current treatment with standard half-life (SHL) or extended half-life (EHL) recombinant FVIII (rFVIII) product, the current infusion dose (IU/kg), history of orthopaedic interventions, and presence of chronic pain and pain medication, were collected. A target joint was defined as the patient having ≥ three bleeds in that joint during a six-month period. Knowledge of previous family members with HA was considered positive heredity. The pathogenetic *F8* gene variants were characterized using routine methods at the genetic haemophilia laboratory associated with the Malmö HCCC. The *F8* genotype was classified as null (i.e. inversions, nonsense variants, small deletions/insertions outside poly-A-runs, splice site variants within conserved regions, large deletions and deletions of the promoter) or non-null (i.e. missense variants, small deletions/insertions inside poly-A-runs and splice site mutations of non-conserved nucleotides), with no predicted measurable levels of FVIII antigen in the null genotype.[14] Adherence to treatment was assessed by the haemophilia-specific questionnaire Validated Hemophilia Regimen Treatment Adherence Scale–Prophylaxis (VERITAS-Pro),[15] which examines outcomes in six subcategories (Time, Dose, Plan, Remember, Skip and Communicate) and provides a total score. A cut-off level of 57 defines non-adherence.[15,16]

Data on the bleeding phenotype and treatment characteristics during prophylaxis implementation in childhood were collected from patient medical records. The time point and FVIII product dosing at weekly prophylaxis start and when escalation to the final implemented regimen had occurred, the regimens' intensity, impact of central venous access, and bleeds requiring FVIII infusion, both prior to and during prophylaxis implementation, were documented.

Joint status was assessed retrospectively from medical records and the Swedish haemophilia registry, for a period of up to 35 years with a 3 to 5 years interval. At least three assessments per patient were performed. Joint health of the elbow, knee, and ankle joints was assessed at both centres by physicians and/or physiotherapists with experience in haemophilia. The Haemophilia Joint Health Score (HJHS)[17] assessed joint health up to the latest follow-up visit. The World Federation of Haemophilia (WFH) Orthopaedic Joint Score[18] was used prior to 2006, and then converted to corresponding HJH scores by a physiotherapist or physician. The selected assessments were all performed in a non-bleeding state, i.e., without recent bleeding or inflammation. Ultrasound examinations of joint health were performed with the HEAD-US (Haemophilia Early Arthropathy Detection with Ultrasound) protocol,[19] by physiotherapists or physicians with HEAD-US experience, at earliest 5 years prior to inclusion and after the patients' 18th year. HEAD-US assesses synovial hypertrophy, cartilage and bone damage at a point-of-care setting. A joint with previous arthroplasty was assessed as missing data and inability to examine the

joint because of arthropathy-related reduced range of motion resulted in a maximum (worst) score of 8.

2.3. Statistics

Descriptive statistics in the form of median and interquartile ranges (IQR 25^{th} -75^{th} percentile) described continuous variables. Mann-Whitney U test for independent non-parametric variables and Spearman's ρ for correlation analysis, were performed using SPSS software, version 25 (IBM, Chicago, IL). A p-value <0.05 was statistically significant.

3. Results

3.1. Patient and treatment characteristics at study inclusion

Thirty adult male patients (out of 35 eligible) with severe HA on primary prophylaxis, born after 1980 and with no history of inhibitors, were enrolled (clinical characteristics shown in supplementary Table S1). Median age at study inclusion was 33.5 years (24.3-38) and median BMI was 24.8 (22.9-28.9). All had uninterrupted prophylaxis. Eleven patients had a known family history of haemophilia. Twenty-six patients had null *F8* genotype and four had non-null genotype (Table S2).

3.2. Early bleeding phenotype and prophylaxis implementation in childhood

At prophylaxis start in childhood, with a median of once-weekly infusions (range once weekly to every other day) and a median FVIII dose of 47.8 (33.9-54.2) IU/kg, age was a median of 1.2 (1-1.3) years. At the time prophylaxis had been escalated to a final regimen, with median infusion frequency 3 times per week (ranging from twice weekly to every day) and a median FVIII dose of 41.7 IU/kg (37.2-45.6), age was a median of 1.7 (1.3-1.8) years (Figure S1). Before prophylaxis began, there were a median of 0 joint (0-0) and 1 non-joint (1-3) bleeds. Median 0 (0-0) joint and 0 (0-0) non-joint bleeds occurred at the period from start to escalated prophylaxis (Table S2).

There was significant correlation between the time from start to escalated prophylaxis and the incidence of joint (ρ=0.470, p<0.018) and non-joint (ρ=0.703, p<0.001) bleeds (Figure 1). Older age at inclusion correlated with older age to escalated prophylaxis (ρ=0.691, p< 0.001). The insertion of a subcutaneous venous port (SVP) (n=14) did not impact upon the age for prophylaxis start (median 1.2 years with SVP vs 1.3 years without) but correlated with a significantly shorter time between start and escalated prophylaxis (median 0.3 vs 0.7 years, p=0.024) and fewer non-joint bleeds (ρ=0.542, p=0.004) prior to escalated prophylaxis. The *F8* genotype (null vs. non-null) and knowledge of heredity did not impact upon early bleeds or prophylaxis (not shown).

Figure 1. Significant correlations between clinical variables.
All correlations were performed with Spearman's ρ. Only statistically significant correlations (p <0.05) after sensitivity analysis are shown. *Refers to bleeds that occurred in the period between start and escalated prophylaxis Abbreviations: HJHS: Haemophilia Joint Health Score, HEAD-US: Haemophilia Early Arthropathy Detection with Ultrasound, Proph: prophylaxis, Y:years

3.3. Treatment characteristics in adulthood

Median yearly FVIII consumption was 4232 (3631- 4672) IU/kg/year. ABR and AJBR were calculated for the period (median 12 months) between the patients' two latest regular visits. Median AJBR was 0 (0-0) and ABR was 0 (0-0). There were no reported target joints. Median VERITAS-PRO score was 36 (30-42). A score < 56 was seen in all patients, signifying an adherence rate of 100%. Best results were observed in sub-categories of "dosing" and "skipping"; worse results were observed in "timing", "remembering" and "communication" (Figure S1).

At their last visit, 21 patients were treated with SHL products and 9 with EHL products (Table S1). The median age was 34 (31.5-39) vs. 25 (21-35) years (p=0.05), the median FVIII consumption was 4386 (3622-4672) vs. 4056 (3441-4056) IU/kg/year, and the median HJHS was 2 (2-8) vs 2 (2-2) for the SHL vs. EHL groups, respectively. The ABR was 0 (0-0) for both SHL and EHL groups. The EHL group switched a median of 5 (range 2-6) years before inclusion. The choice of product type did not impact upon adherence (not shown).

3.4. Joint health from childhood to adulthood

The patients' HJHS increased slowly through the decades, being a median of 0 until the 20-25 years period, when it became a median of 1, and reaching a median of 4 at 35-40 years (Figure 2). The median HJHS of both elbows was 0 at all assessments. The median HJHS of both knees was 0 up to 25-30 years, when it became a median of 1 and remained at 1 afterwards. The median HJHS of the left ankle was 0 at all time periods. In the right ankle, median HJHS was 0 until it became 1 at the 35-40 years period (Table 1).

Multiple significant correlations between HJHS in youths (15-20 years) and later in life, i.e., at 20-25 years (ρ=0.716, p<0.001), 25-30 years (ρ=0.629, p=0.02) and 35-40 years (ρ=0.651, p=0.022), were identified. The worst HJHS value for each patient correlated to their age (ρ=0.498, p=0.007) at transition to the final prophylaxis regimen (Figure 1).

Five of the six patients who reported chronic pain used paracetamol or anti-inflammatory agents, except patient #24, who also used short-acting opioids (Table S1). Three patients underwent orthopaedical interventions, i.e., two synovectomies with Yttrium-90 (patient #1 in the right knee at age 20 years and #24 in the right elbow at age 25 years) and one right elbow arthroscopy with synovectomy (#27 at age 34 years).

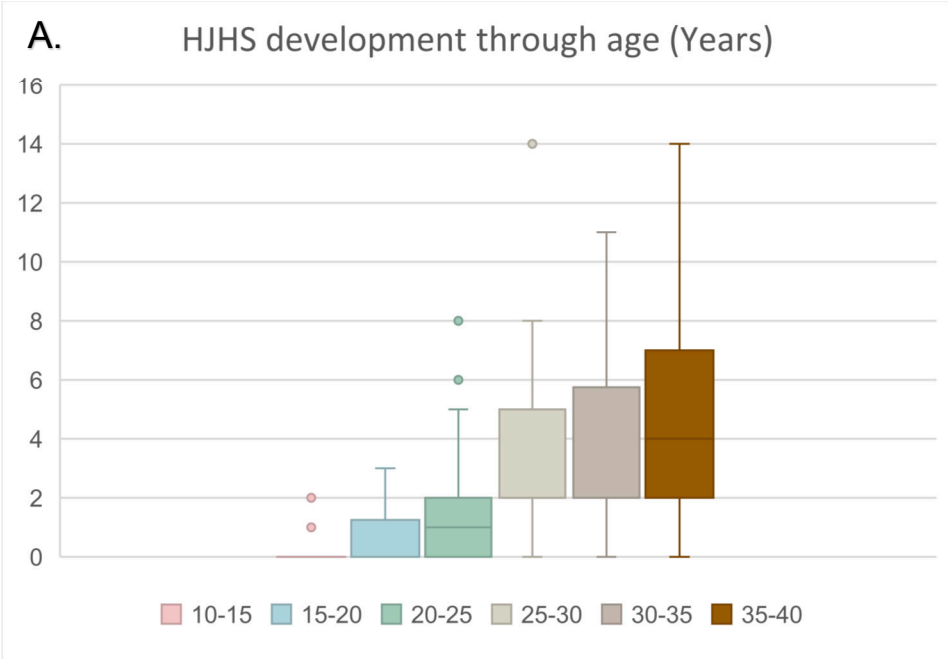
HEAD-US evaluation was performed in 26 patients, at a median age of 32 years (21.5-36). The median total HEAD-US score was 1 (0-2). The median score was 0 (0-0) for elbow, knee, and ankle joints (Table 1). Bone or cartilage changes were identified in six right (23.1%) and five left (19.2%) ankle joints, respectively (Table

S3). In some cases, the assessor could not with certainty distinguish between bone and cartilage damage, but the scoring was documented as bone damage. Significant correlations were found between HEAD-US and HJHS in multiple periods in adulthood (Figure 1).

A pristine joint was defined as an evaluated joint with both HJHS < 4 and HEAD-US without any signs of bone or cartilage damage, as published previously.[20] Joints with HJHS ≥ 4 as the sole finding were defined as non-pristine even in the absence of HEAD-US evaluation. The right ankle joint was the most affected, as 76% of joints (19/25) were classified as pristine, whereas 84% (21/25) of left ankle, and 86.5% (45/52) of all knee joints were pristine. All left elbow joints (28/28) and 84.6% (22/26) of right elbow joints were classified as pristine (Table 1).

Table 1.
Joint health development through progressive age periods. Median (IQR) values.

Joint	HJHS in different age periods (years)							HEAD-US	Pristine joints n/N (%)
	5-10	10-15	15-20	20-25	25-30	30-35	35-40		
Right elbow	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-1)	0 (0-2.5)	0 (0-0)	22/26 (84.6%)
Left elbow	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	25/25 (100%)
Right knee	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	1 (0-1)	1 (0-1)	1 (0-1)	0 (0-0)	23/26 (88.4%)
Left knee	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	1 (0-1)	1 (0-1)	1 (0-1)	0 (0-0)	22/26 (84.6%)
Right ankle	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-1)	1 (0-2)	0 (0-0)	19/25 (76%)
Left ankle	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-0)	21/25 (84%)
Total HJHS	0 (0-0)	0 (0-0)	0 (0-1)	1 (0-2)	2 (2-5)	2 (2-6)	4 (2-7)	1 (0-2)	132/153 (86.3,%)



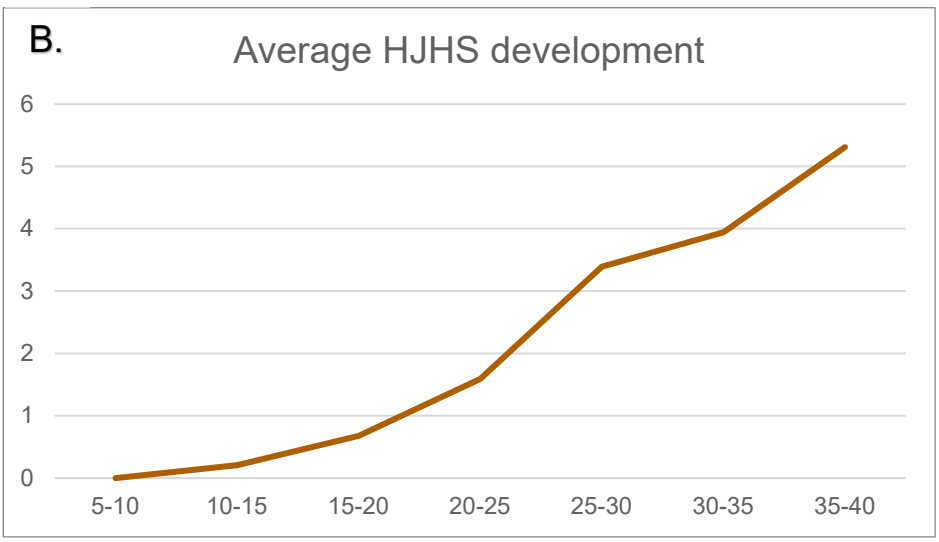


Figure 2.
A. Median HJHS (Haemophilia Joint Health Score) development at progressive time periods of the patients' lives. B. Development of average value of cumulative HJHS through the years.

4. Discussion

Freedom from joint bleeds and the prevention of joint damage have been the paramount goals of primary prophylaxis in SHA.[21] However, the time to reach the final escalated regimen during prophylaxis implementation will differ between patients. The aim of this study was to assess the long-term joint outcomes with primary prophylaxis in SHA and to evaluate the impact of the bleeding phenotype and implementation patterns in childhood in a Swedish cohort of adult males with uninterrupted primary prophylaxis. Joint health was assessed by HJHS at multiple time periods in the patients' lives and by HEAD-US at adulthood. Patients with present or previous FVIII inhibitors were excluded, as the presence of inhibitors increases the bleeding tendency.[22,23]

Our cohort had overall very good joint health, with the median cumulative HJHS remaining at 0 during youth and adolescence stages up to the 20-25 years period. The median HJHS 0 in adolescence is consistent with recent studies.[20,24] Afterwards, HJHS rose slowly, reaching a median of 4 at 35-40 years, but with an upward trajectory (Table 1 and Figure 2), similarly to previous studies showing that early prophylaxis cannot completely prevent joint damage.[25] The impact of ageing on HJHS and HEAD-us scores needs to be considered, and HJHS increases with age despite prophylaxis,[26] but the observed median HJHS of 4 at 35-40 years is higher than seen in age-matched non-haemophiliacs,[27] and likely representative of arthropathy. Nonetheless, our findings indicate that high-intensity primary prophylaxis delays arthropathy and 40% (10 of 26) of patients had pristine joints. Higher HJHS in adolescence and early adulthood correlated to worse joint outcomes later in life, emphasising the importance of close follow-up and early joint assessments. Only six patients (20%) reported pain, even though 60% (16/26) had at least one non-pristine joint, and three had undergone synovectomies, which signals the subjective nature of pain and raises the possibility of subclinical bleeds affecting the cohort.

In this study we also assessed the bleeding phenotype and treatment escalation patterns at prophylaxis implementation. The period from the start regimen to the escalated prophylaxis regimen was shorter in patients born more recently and in those with an SVP, which correlated to fewer bleeds and possibly reflects changing treatment practice. Insertion of an SVP should thus be considered if there is difficulty reaching the injection frequency required for full prophylaxis.

In this primary prophylaxis cohort, bleeds prior to prophylaxis start did not significantly impact upon later outcomes. However, the length of time it took to escalate the prophylaxis regimen correlated with bleeding episodes and worse HJHS in adulthood, indicating the need for rapid transition to more intensive regimens. All patients had uninterrupted prophylaxis and after their prophylaxis regimen had been escalated, most patients were treated with high-dose prophylaxis, defined as FVIII consumption above 4000 IU/kg/year.[28]

The finding that 133 of 153 (86.3%) assessed joints were classified as pristine based on an HJHS < 4 and HEAD-US without bone or cartilage damage, is also indicative of the generally favourable observed joint outcomes. However, even though median HJHS for ankle joints was 0 at almost all time points (becoming 1 at 35-40 years in the right ankle), HEAD-US showed bone or cartilage changes in 10 of 50 assessed ankles (80%) and only 76% of right ankle joints were deemed to be pristine. This is consistent with a recent magnetic resonance imaging (MRI) evaluation of a younger (mean age 23.5 years) Swedish moderate and severe haemophilia cohort that showed osteochondral changes in the ankle but not the knee joints.[29] HJHS and HEAD-US have shown good inter-rater reliability and correlation with MRI findings of synovial hypertrophy and osteochondral damage, even when performed by non-radiologists.[30-32]

Both elbow joints had a median HJHS of 0 at all assessments. However, the right elbow joint was somewhat more affected, with 84.6% pristine joints (Table 1), whereas all left elbows were deemed to be pristine. Furthermore, two of the three performed synovectomies in this cohort were in the right elbow. A recent study evaluating subjectively affected joints in a German haemophilia cohort also showed a higher degree of problems in the right elbow joint.[33] This may be a consequence of the higher percentage of right-handed persons[34], but needs to be further evaluated. Median HJHS of both knee joints became 1 at the 25-30 years period (and remained 1 afterwards) and 45 of 52 assessed knees (86.5%) were classified as pristine, suggesting that primary prophylaxis is highly efficient in preserving knee joint health, which is in unison with MRI findings in a Swedish primary prophylaxis cohort,[29] but in slight contrast to an adolescent HA cohort, in which knees were marginally more affected than ankles, but the overall joint health was very good.[20]

In adulthood, approximately two-thirds of the patients were treated with SHL products. The EHL cohort was younger and the switch to EHL has not significantly influenced outcomes, as measured by bleeding, FVIII consumption, and HJHS. The previous pandemic leading to reluctancy for regimen changes and participation in clinical studies possibly contributed to the continuous use of SHL products. The very low bleeding rates in adulthood may also be related to the cohort's high adherence rate of 100%.[35]

Our study has limitations, such as a retrospective design and that assessment of joint health was performed with HJHS and HEAD-US, which are dependent on operator skill.[36] MRI can show haemosiderin deposits and detect subclinical joint bleeds, occurring in approximately 16% of severe HA patients despite prophylaxis[37], which our study could not assess. In the HEAD-US analysis, some underdiagnosis of cartilage damage may have occurred, possibly implying worse joint outcomes. As a result of the small sample size, our study was most likely underpowered to discover all significant correlations between the examined clinical variables and, as in a previous study,[24] no mathematical correction was applied for multiple comparisons. Moreover, we cannot be certain that treatment patterns remained

unchanged for all patients during their lives, which may have impacted upon joint outcomes. Our study has nonetheless strengths, mainly the well-documented assessment of joint health trough over a great length of time and thorough characterization of the clinical phenotype and treatment practice, both in childhood and adulthood. The risk of selection bias is small, as 85.7% of eligible patients at the two centres were included. Finally, this study is one of the few to examine long-term outcomes in an adult population with primary prophylaxis.

In conclusion, this study shows that the primary prophylaxis regimen used in Sweden for decades is effective but does not completely prevent the gradual development of arthropathy in SHA. Furthermore, prophylaxis escalation should occur as early as possible in order to prevent bleeds and when needed, an SVP or switch to non-factor replacement may be considered. Higher HJHS in adolescence and early adulthood correlated with worse joint outcomes later in life, which illustrates the importance of close follow-up and early joint assessments.

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Author contributions

AA and JA designed the study, acquired, analyzed, and interpreted the data, drafted, and finalized the manuscript. FB and CJ acquired and interpreted the data and finalized the manuscript. NGA interpreted the data and finalized the manuscript.

Conflict of interest disclosures

AA has received honoraria as a consultant and/or speaker and has received research grants from Takeda/Shire and Bayer, as well as speaker's fee and consultant for Sobi, Sanofi, Amgen, Grifols. CJ has served as a speaker for Sobi. FB has received honoraria as a member of an advisory board and/or speaker from Sobi, BioMarin, Bayer, Takeda, Novo Nordisk, CSL Behring, Roche, Octapharma and Pfizer and has been an investigator in clinical trials sponsored by Sobi, Roche, Novo Nordisk, Bayer, Takeda (payments to the institution). NGA has served as a speaker and/or on advisory boards for Bayer, Sobi/Sanofi, CSL Behring and Octapharma.JA has received honoraria as a member of an advisory board and/or speaker from Sobi, BioMarin, Takeda, Novo Nordisk, Bayer, Roche, Octapharma, Pfizer, and CSL Behring, and research support to the institution from Bayer, Octapharma, Takeda, Pfizer, CSL Behring and Sobi/Biogen.

Ethics

The study was approved by the Regional Ethics Review Board of Lund University, Lund. The study subjects provided written informed consent before entering the study.

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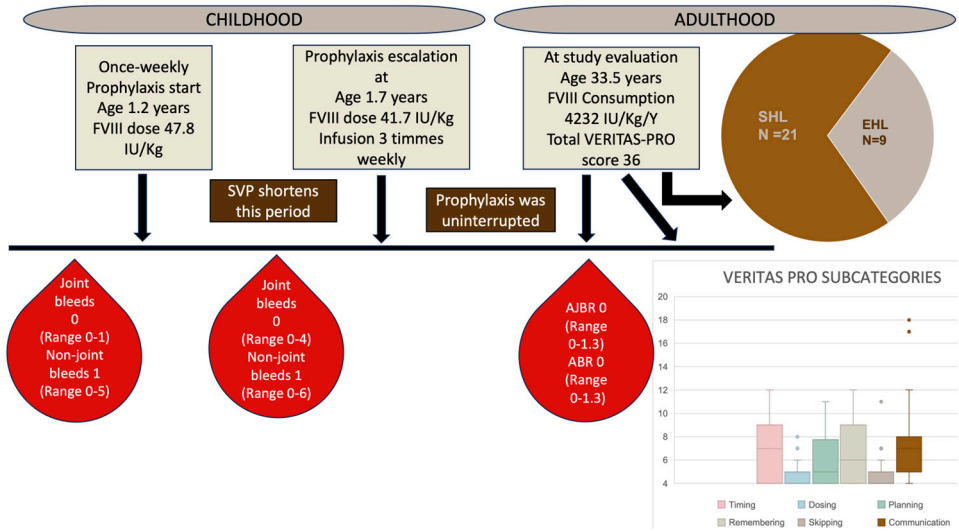

Figure S1. Clinical and treatment characteristics at prophylaxis start and study inclusion.
Age, FVIII dose and annual consumption, joint and non-joint bleeds, ABR and AJBR refer to median values. ABR: annualized bleeding rate, AJBR: annualized joint bleeding rate, SVP: subcutaneous venous port, SHL: standard half-life FVIII product, EHL: extended half-life FVIII product

Table S1. Evaluation at study inclusion.
HJHS: haemophilia joint health score. Worst: highest HJHS measurement and the age period it was first documented BMI: Body Mass Index, Y: years, Proph: prophylaxis, IU: international units, Kg: kilograms, AJBR: Annualized Joint Bleeding Rate, ABR: Annualized Bleeding Rate, SHL: standard half-life, EHL: extended half-life, †paracetamol ‡COX-2 inhibitor, §NSAID: non-steroidal anti-inflammatory drug, *short-acting opiate.

Patient-ID	Age at inclusion (Y)	BMI	FVIII product type	Yearly FVIII consumption (IU/Kg/Year)	AJBR	ABR	Worst HJHS (Period in Yrs)	Total VERITAS-PRO	Chronic pain
1	36	29.8	SHL	4659	0	0	14 (30-35)	35	No ‡
2	34	29.5	SHL	3640	1.3	1.3	2 (25-30)	42	No
3	37	23.6	SHL	4672	0	1.2	4 (30-35)	30	No
4	29	23.5	SHL	4672	0	0	8 (25-30)	32	No
5	41	38.5	SHL	3604	0	0	2 (35-40)	26	Yes †
6	33	22.2	SHL	4672	0	0	2 (25-30)	42	No
7	31	38.5	SHL	5658	0	0	2 (25-30)	29	No
8	34	27.6	SHL	4859	0	0	6 (30-35)	45	No

9	19	23.3	SHL	4277	0	1	0 (15-20)	41	No
10	26	29.6	EHL	2662	0	0	2 (25-30)	39	No
11	38	25.1	SHL	4186	0	0	7 (35-40)	35	No
12	40	25.5	SHL	5438	0	0	2 (25-30)	24	Yes † ‡
13	22	24.9	EHL	4056	0	0	2 (15-20)	37	No
14	35	22.0	SHL	3731	0	0	2 (20-25)	38	No
15	40	28.1	SHL	3385	0	0	3 (35-40)	38	No
16	38	33.7	SHL	3185	0	1	9 (30-35)	40	Yes §
17	34	22.1	SHL	4386	0	0.8	11 (30-35)	51	No †
18	20	23.7	EHL	4807	0	0	2 (15-20)	55	No
19	26	24.6	EHL	3840	0	0	2 (25-30)	26	No
20	38	28.7	SHL	4131	0	0	4 (35-40)	44	No
21	40	23.6	EHL	3952	0	0	8 (35-40)	36	No
22	22	24.7	EHL	4386	0	0	3 (15-20)	31	Yes
23	22	26.9	SHL	7921	0	0	0 (20-25)	26	No
24	41	21.4	SHL	2808	0	0	12 (35-40)	34	Yes *
25	32	22.6	SHL	4659	0	0	2 (25-30)	48	No
26	32	22.2	EHL	3043	0	0.3	2 (20-25)	56	No
27	38	28.7	EHL	4150	0	0	6 (20-25)	34	No †
28	42	31.0	SHL	3214	0	0	0 (35-40)	32	Yes † ‡
29	18	23	SHL	4490	0	0	1 (10-15)	26	No
30	18	20.8	EHL	4597	0	0	3 (15-20)	30	No

Table S2. Early bleeding phenotype and prophylaxis data.

* All patients were treated with standard half-life product at full prophylaxis, †Null genotype, ‡Non-null genotype, §Small deletion/insertion within poly-A-run, x3/W: three times weekly prophylaxis, x2/W: twice weekly prophylaxis, ED: every day prophylaxis, EOD: every other day prpophylaxis MD: missing data. DirectFP: full prophylaxis was started directly, Y: years, IU: international units Kg: kilograms, SVP: subcutenous venous port, proph: prophylaxis, cons: consumption.

	Genetic aspects		Prior to prophylaxis		Start regimen		Time to escalated prophylaxis			Escalated prophylaxis regimen			
PAT-ID	Family history of haemophilia	Genotype	joint bleeds	Non-joint bleeds	Age at start of prophylaxis (Y)	Start regimen dose (IU/Kg)	Time from start to final regimen (Y)	Joint bleeds	Non-joint bleeds	Age at final regimen (Y)	Escalated final regimen frequency	Dose (IU/kg)*	FVIII Cons IU/kg/Y
1	No	Inv Intron 22†	0	4	1.2	35.7	0.6	0	0	1.8	x3/W	35.7	5569
2	Yes	Small deletion or insertion <50bp†	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD
3	No	Inv Intron 22†	1	0	1	55.6	0.8	1	0	1.8	x3/W	41,7	6505
4	Yes	Small deletion or insertion <50bp§‡	0	3	1.3	Direct Escalation	0	0	0	1.3	x3/W	45,4	7082
5	No	Inv Intron 22†	0	1	1.3	50	4.7	4	1	6	x3/W	58,8	9173
6	No	Inv Intron 22†	0	4	1.3	55.6	0.9	0	3	2.2	x3/W	36,7	5725
7	Yes	Inv Intron 22†	0	0	0.8	Direct Escalation	0	0	0	0.8	x3/W	50	7800
8	No	Small deletion or insertion <50bp§‡	0	1	2	10	0.2	0	0	2.2	x3/W	41.7	6505

9	Yes	Small deletion or insertion <50bp†	0	1	1.2	50	0.1	0	0	1.3	x3/W	45.6	7114
10	No	Small deletion or insertion <50bp†	0	0	1.4	45.5	0.3	0	0	1.7	x3/W	45.5	7098
11	No	Small deletion or insertion <50bp†	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD
12	Yes	Inv Intron 22†	0	1	1.3	Direct Escalation	0	0	0	1.3	x3/W	41.7	6505
13	No	Nonsense mutation †	0	1	1	50	0.7	1	2	1.7	x3/W	41.7	6505
14	No	Inv Intron 1†	1	2	1.1	33.3	0.6	0	0	1.7	x3/W	33.3	5195
15	No	Small deletion or insertion <50bp†	0	1	1.7	33.3	3.5	0	6	5.2	x3/W	41.6	6490
16	No	Inv Intron 1†	0	1	0.9	50	0.6	0	1	1.5	x3/W	45.5	7098
17	No	Nonsense mutation †	0	2	1.3	41.7	0.5	0	0	1.8	x3/W	33.3	5195
18	No	Missense mutation ‡	0	2	1.2	Direct Escalation	0	0	0	1.2	EOD	40	7280
19	Yes	Small deletion or insertion <50bp†	0	2	1	31.25	0.6	0	0	1.6	x3/W	23	3588

20	No	Missense mutation ‡	MD	MD	1.3	MD	MD	MD	MD	MD	MD	MD	MD
21	Yes	Inv Intron 22†	0	1	2.2	83.3	MD	0	0	MD	x2/W	71.4	7426
22	No	Small deletion or insertion <50bp†	0	4	1.2	55.55	0.3	0	0	1.5	x3/W	52.6	8206
23	No	Inv Intron 22†	1	0	1.3	Direct Escalation	0	0	0	1.3	x3/W	38.5	6006
24	Yes	Inv Intron 22†	0	1	0.5	58.8	1.8	0	5	2.3	x2/W	33.3	3463
25	No	Inv Intron 22†	0	5	1.2	12	0.5	0	0	1.7	x2/W	MD	MD
26	Yes	Inv Intron 22†	1	3	1.5	50	0.3	0	0	1.8	x2/W	47.6	4950
27	No	Small deletion or insertion <50bp†	1	3	1.4	43.5	0.4	0	0	1.8	x3/W	43.5	6786
28	No	Inv Intron 22†	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD
29	Yes	Small deletion or insertion <50bp†	1	2	0.9	MD	0.3	0	0	1.2	x3/W	MD	MD
30	Yes	Inv Intron 22†	0	1	1.1	41.7	0.2	0	0	1.3	ED	41.7	7589

Table S3. HEAD-US assessment at adulthood.
ROM: Range of motion. B: bone changes. C: cartilage changes S: synovial hypertrophy.

Patient ID	Age at analysis (Years)	Right elbow	Left elbow	Right knee	Left knee	Right ankle	Left ankle	Total Score
1	32	0	0	0	0	8 (no ROM)	0	8
2	31	0	0	1B	0	0	0	1
3	32	0	0	1B	1B	0	0	2
4	25	0	0	0	1C	0	0	1
5	37	0	0	0	0	0	0	0
6	29	0	0	0	0	0	1B	1
7								
8	31	0	0	0	0	0	1B	1
9	18	0	0	0	0	0	0	0
10	22	0	0	0	0	0	0	0
11	34	0	0	0	0	0	0	0
12	37	0	0	0	0	1B	0	0
13	18	0	0	0	0	1B	0	1
14	30	0	0	1B	1B	0	0	2
15	35	0	0	0	1B	0	0	1
16	38	0	0	0	1S	0	1S	2
17	34	1C	0	0	0	4BC	5BCS	13
18				0	0			
19								
20								
21	40	7BCS	0	0	0	1C	0	8
22	21	0	0	1S	0	0	0	1
23	19	0	0	0	0	2C	1C	3
24								
25	32	0	0	0	0	0	0	0
26	32	0	0	0	0	0	0	0
27	38	4BC	0	0	0	0	0	4
28	42	0	0	0	0	0	0	0
29	18	0	0	0	0	0	0	0
30	18	0	0	0	0	0	0	0

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Haemophilia A

IN PURSUIT OF OPTIMISED OUTCOMES VIA PERSONALISED TREATMENT

Haemophilia A is a hereditary bleeding disorder caused by deficiency of coagulation factor VIII. This thesis investigates aspects of the pathogenesis, clinical phenotype and personalised management of haemophilia A, with the aim of promoting favourable outcomes.

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