Haemophilia A - In pursuit of optimised outcomes via personalised treatment

Arvanitakis, Alexandros

2024

Document Version:
Publisher's PDF, also known as Version of record

Haemophilia A

In pursuit of optimised outcomes via personalised treatment

ALEXANDROS ARVANITAKIS
DEPARTMENT OF TRANSLATIONAL MEDICINE | FACULTY OF MEDICINE | LUND UNIVERSITY

Haemophilia A
In pursuit of optimised outcomes via personalised treatment

Haemophilia A

In pursuit of optimised outcomes via personalised treatment

Alexandros Arvanitakis

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended publicly on May 3rd, 2024, at 9.00 am
at the Auditorium of the Dept. of Obstetrics and Gynaecology,
Jan Waldenströms gata 47, Malmö, Sweden

Faculty opponent

Professor Catherine Lambert
Cliniques Universitaires Saint-Luc
Brussels, Belgium

Organization: Lund University, SWEDEN. Faculty of Medicine, Department of Translational Medicine, Clinical Coagulation Research Unit

Document name: Doctoral Dissertation **Date of issue**

Author(s): Alexandros Arvanitakis **Sponsoring organisation:**

Title and subtitle: Haemophilia A – In pursuit of optimised outcomes via personalised treatment

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.

Classification system and/or index terms (if any)

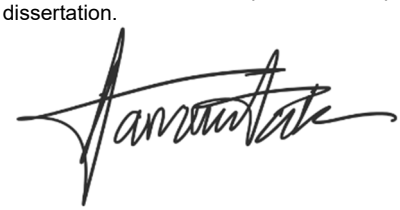
Supplementary bibliographical information

ISSN and key title: 1652-8220 **Language:** English

ISBN: 978-91-8021-549-7 **Number of pages**: 119

Recipient's notes

Price Security classification

Signature  Date 2024-03-14

Haemophilia A

In pursuit of optimised outcomes via personalised treatment

Alexandros Arvanitakis

LUND
UNIVERSITY

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.

Faculty of Medicine, Lund University

Department of Translational Medicine

ISBN 978-91-8021-549-7

ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University

Lund 2024

Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se

MADE IN SWEDEN

“I’m going on an adventure”,

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

Table of Contents

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 ABR 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 HRQoL, 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 genotype.

In Paper IV, the long-term joint outcomes, bleeding phenotype, and treatment patterns during 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.

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]

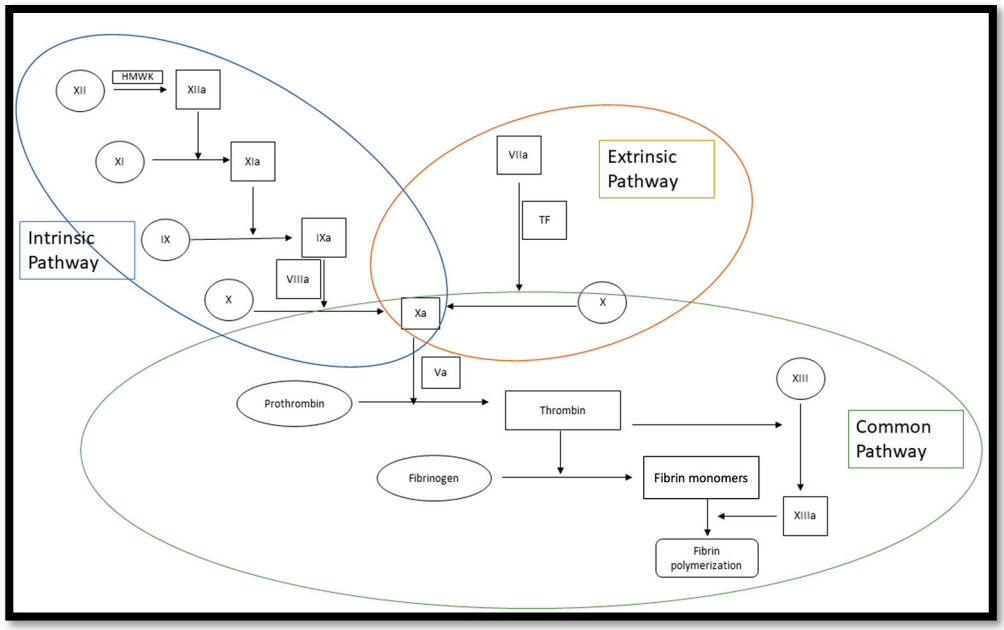


Figure 1. The waterfall/cascade model for haemostasis.

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]

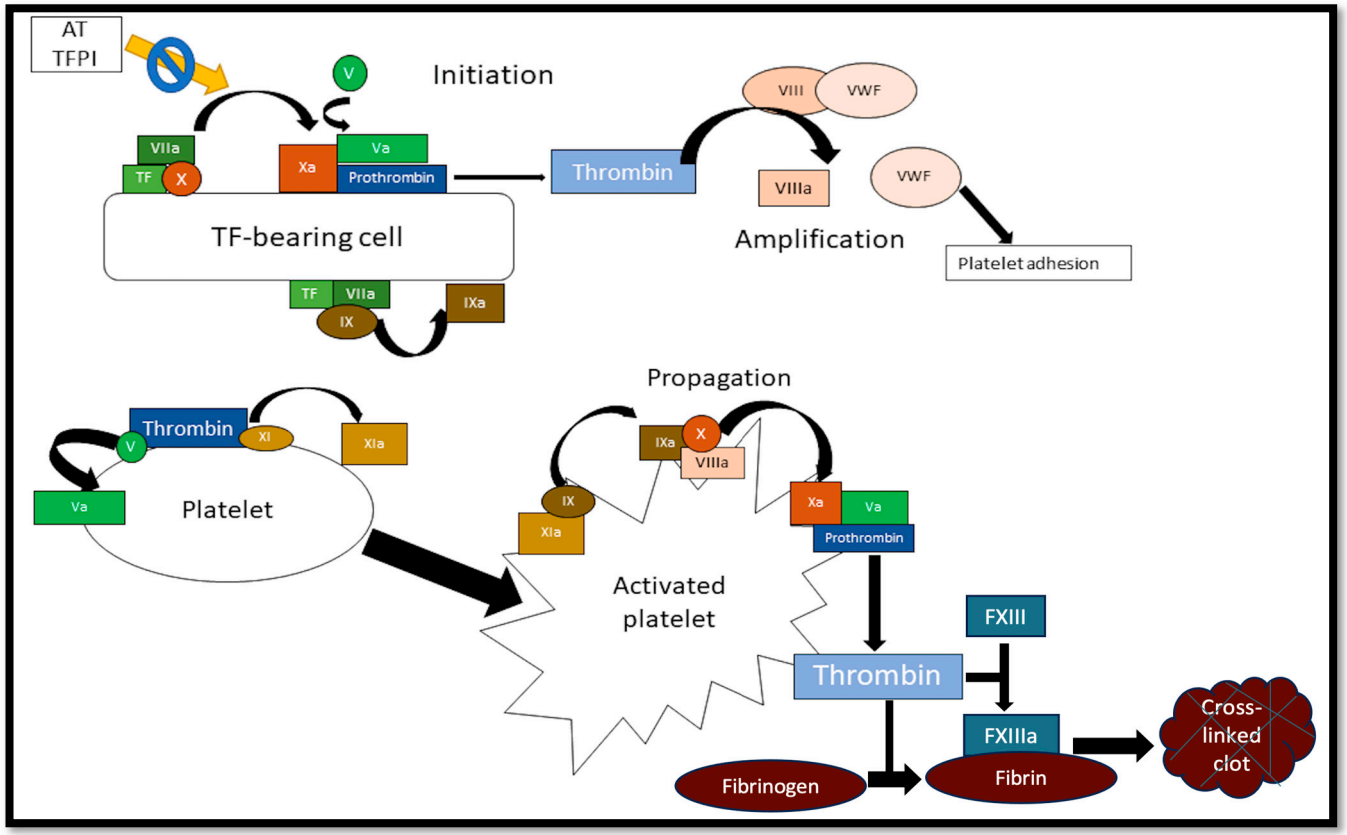


Figure 2. The cell based model of coagulation

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]

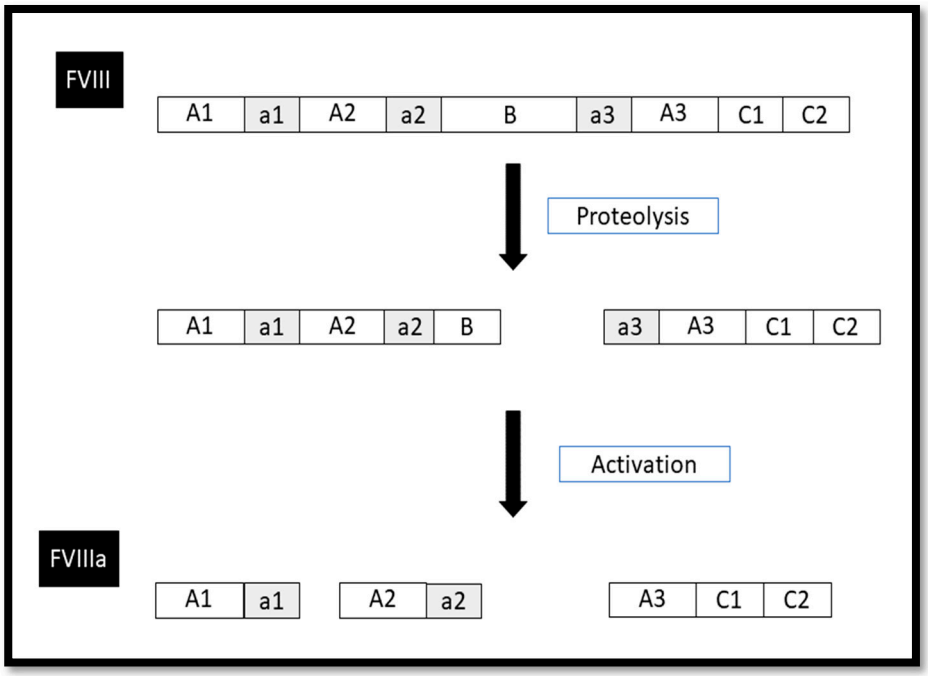


Figure 3. FVIII structure, proteolysis and activation.

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)

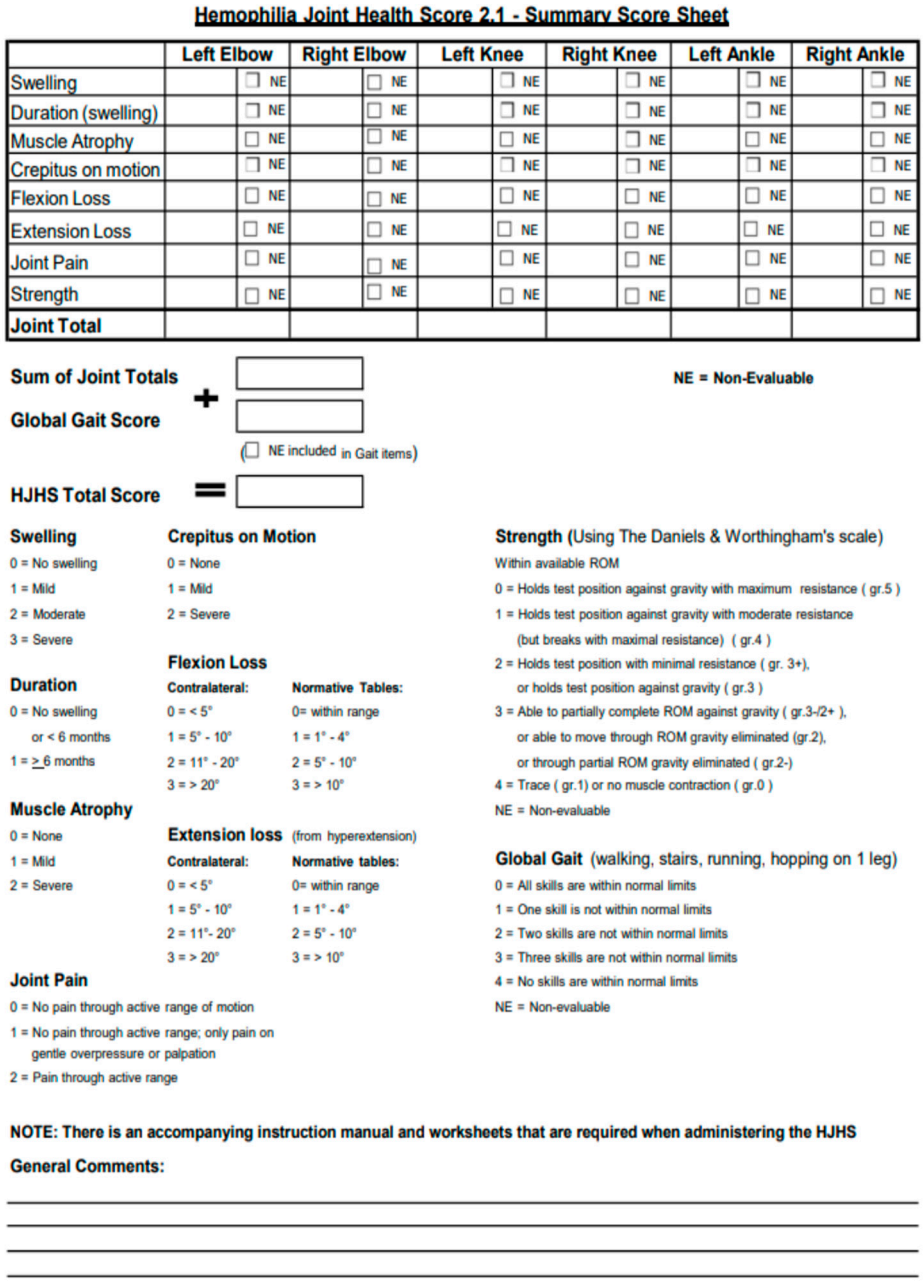
The validated scoring tool HJHS 2.1,[104] performed by a physiotherapist at each participating centre, was used to assess joint impairment in the elbow, knee and ankle joints. The HJHS 2.1 was initially developed for use in paediatric patients, but has since also been validated for use in adult patients.[260] 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 ❑

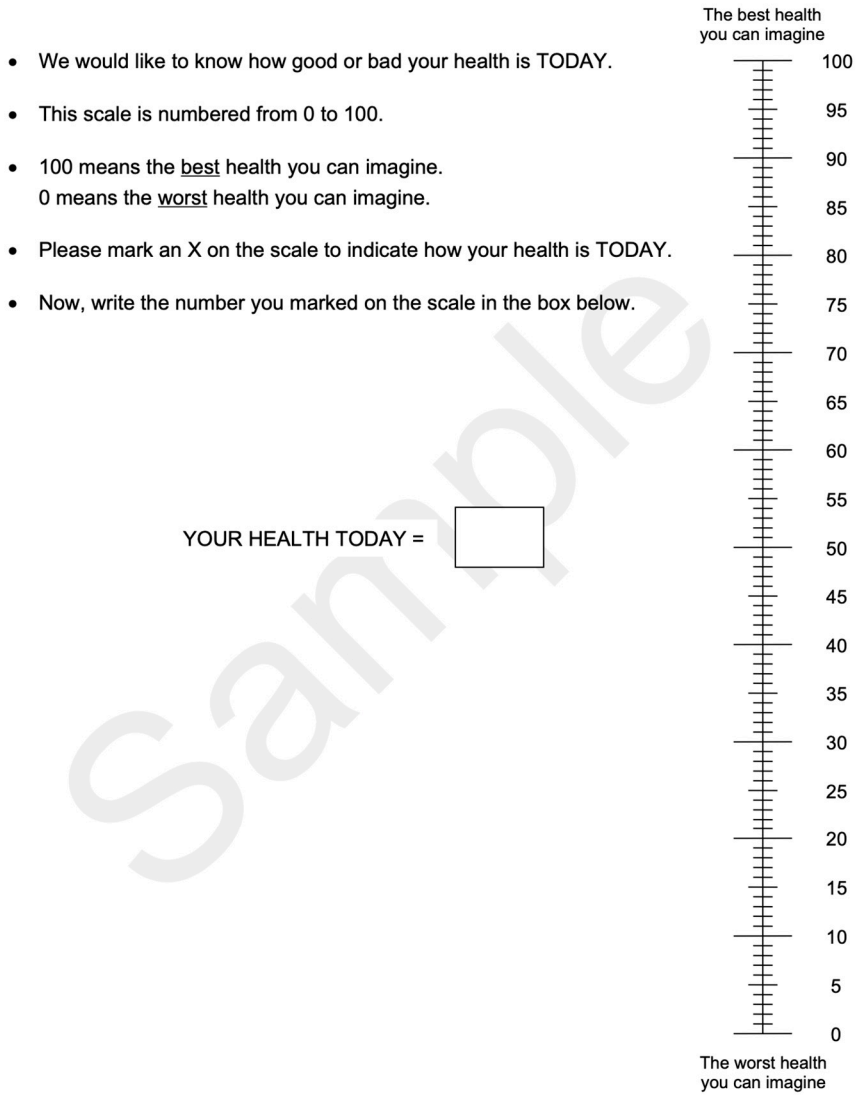
PAIN / DISCOMFORT

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).

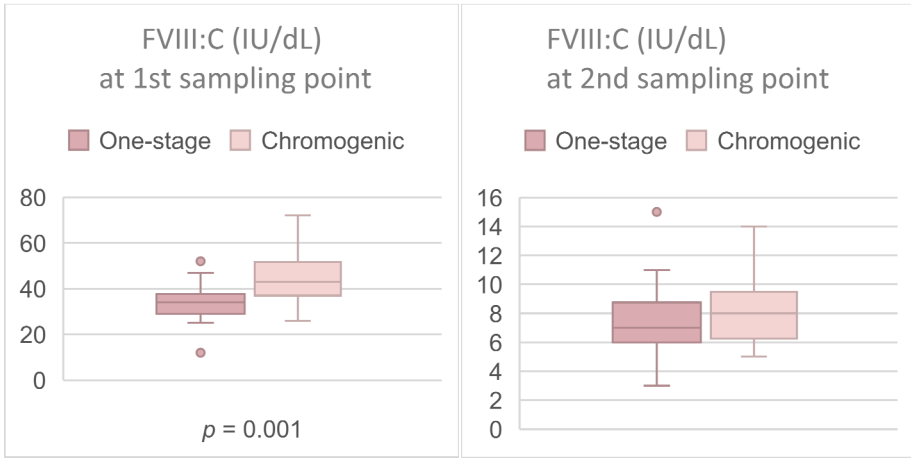


Figure 8. Assay Discrepancy between Chromogenic and One-stage assays.
FVIII:C (IU/dL) at first and second sampling point.

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.

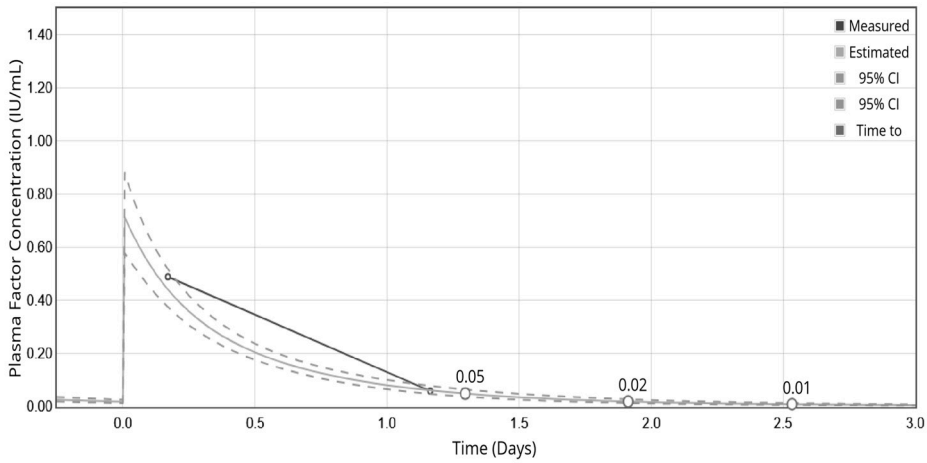


Figure 9A. PK estimation by WAPPS-HEMO, patient #14

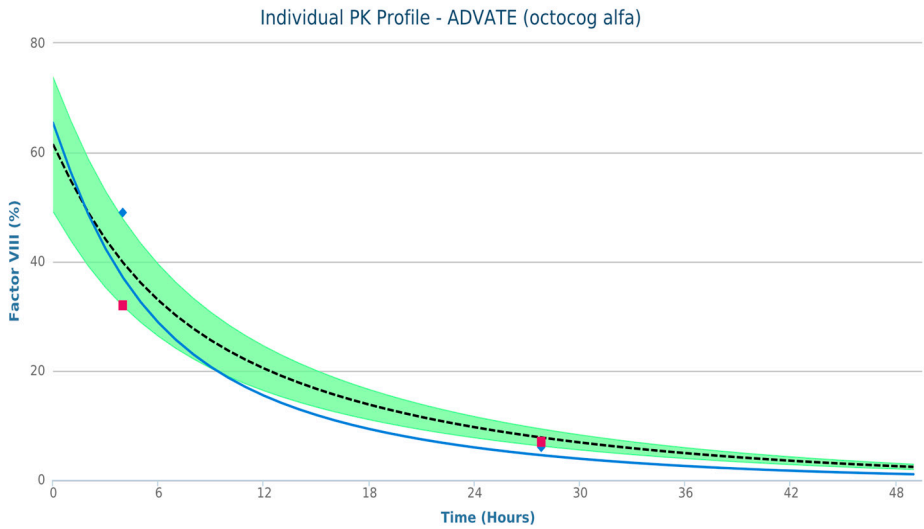


Figure 9B. PK estimation by MyPKFiT, patient #14

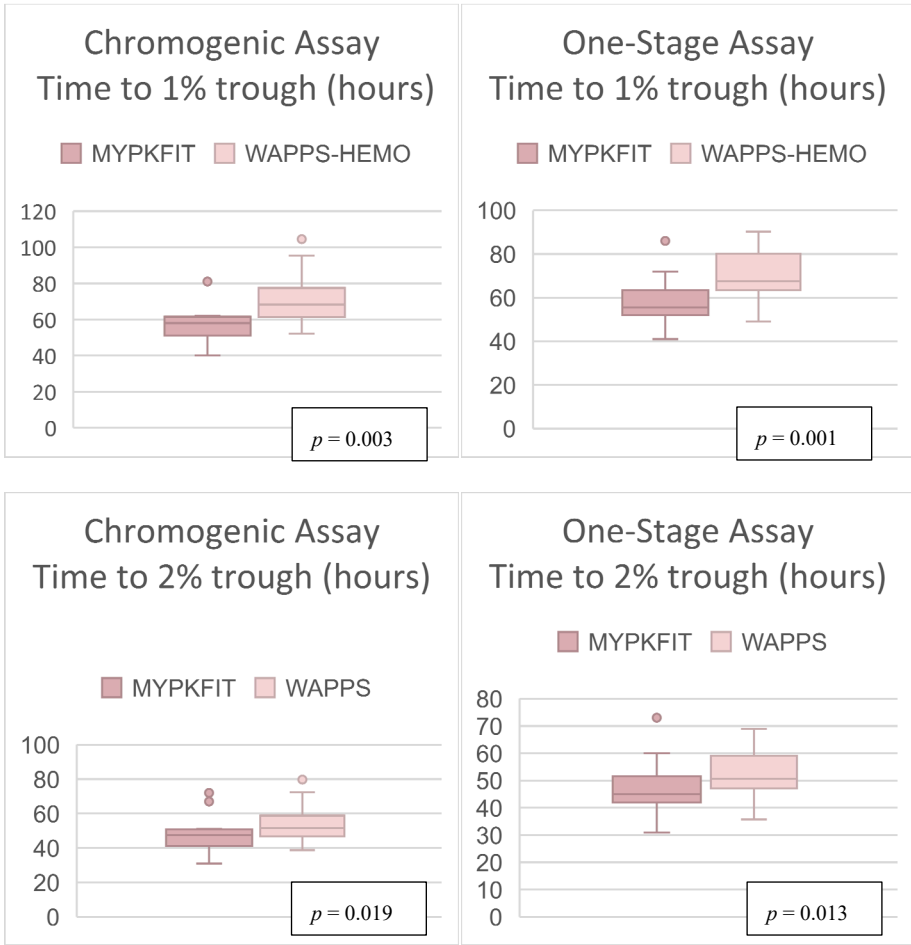


Figure 10A. Differences in PK estimations by MyPKFiT and WAPPS-Hemo (continues on next page).
PK estimations by MyPKFiT and WAPPS-Hemo of time to reach trough 1%,3% and 5% by the chromogenic and one-stage-assays.

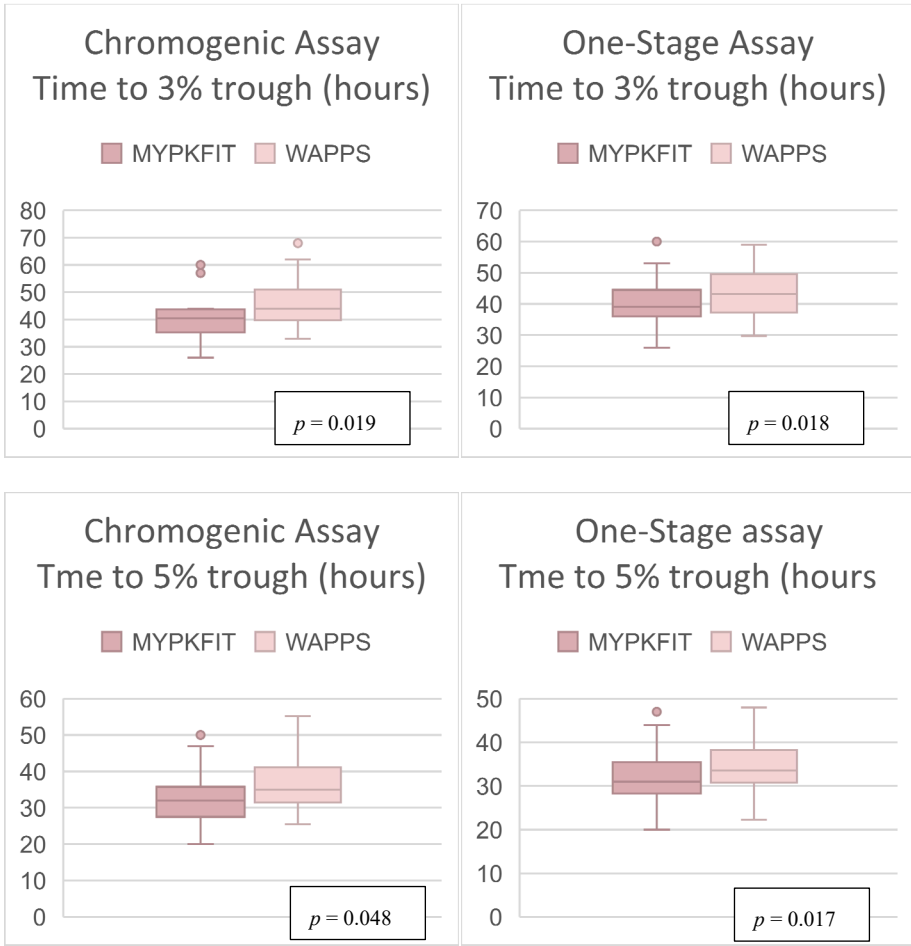


Figure 10B. Differences in PK estimations by MyPKFiT and WAPPS-Hemo (Continued). Pharmacokinetic estimations by MyPKFiT and WAPPS-Hemo of time to reach trough 1%,3% and 5% by the chromogenic and one-stage-assays.

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%.

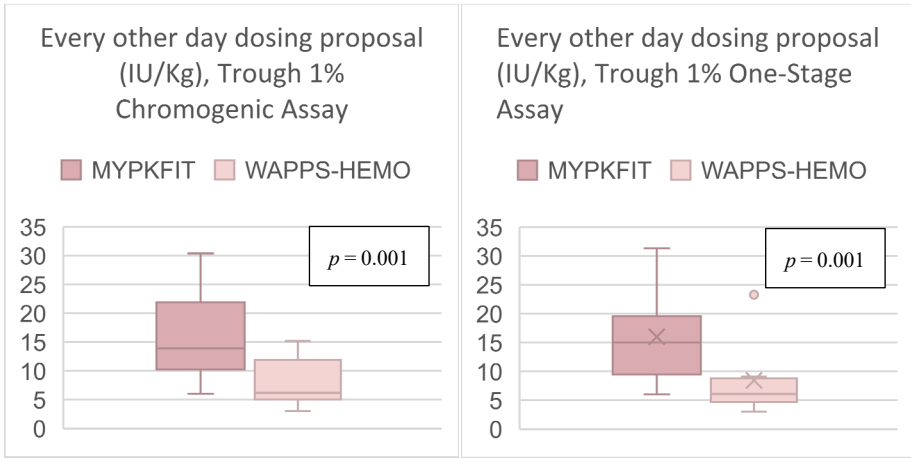


Figure 11. Dosing proposals by MPKiT and WAPPS-Hemo.
Dose proposals by MyPKFiT and WAPPS-Hemo for reaching a trough level of 1% after 48 hours in each patient, i.e. for every other day dosing schedule.

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.

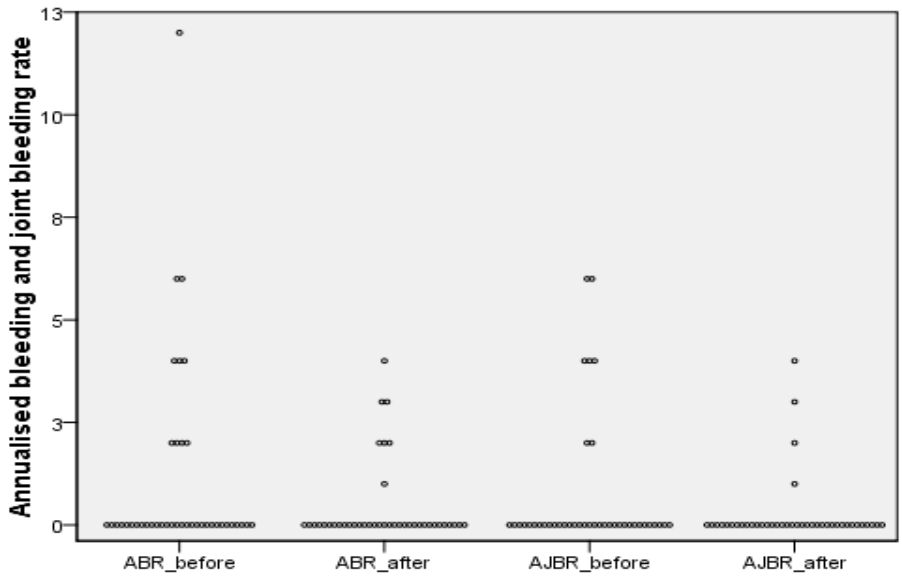


Figure 12. ABR and AJBR before and after the switch to BAY 81-8973.
2D-dot plot showing ABR and AJBR before and after switch to BAY 81-8973, respectively. Each dot symbolises one patient.

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.

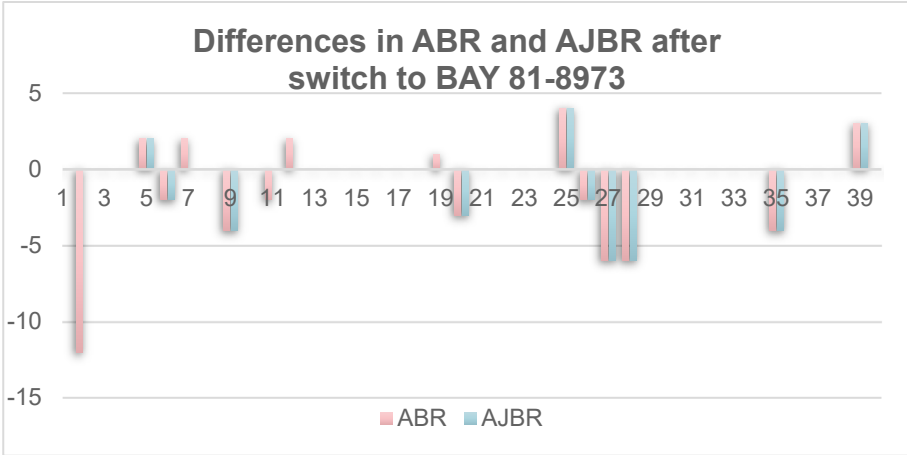


Figure 13. Difference in bleeding phenotype after switch to BAY 81-8973.
Bar chart showing the difference in ABR and AJBR in all 40 patients of the cohort after the switch to BAY 81-8973. Negative values indicate a reduction in ABR and AJBR after the switch, whereas positive values indicate increase, 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]

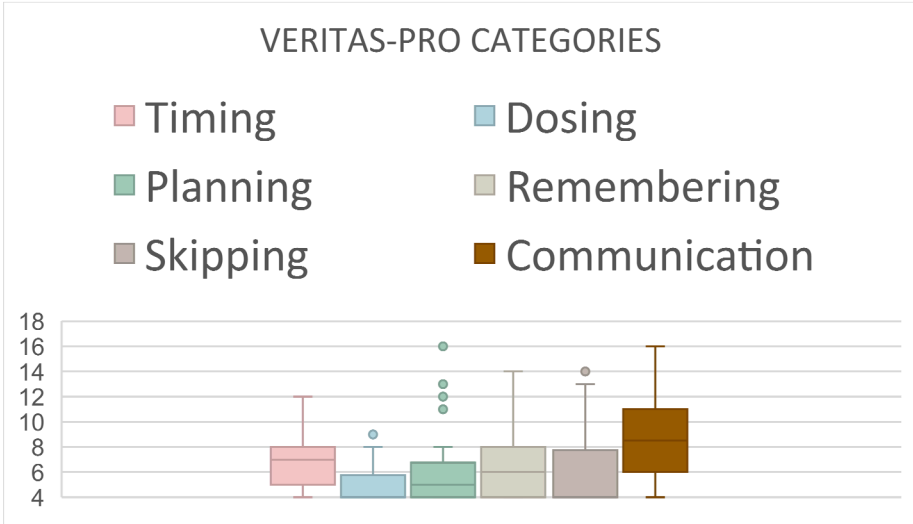


Figure 14. Boxplots showing the results of the VERITAS-PRO categories.

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.

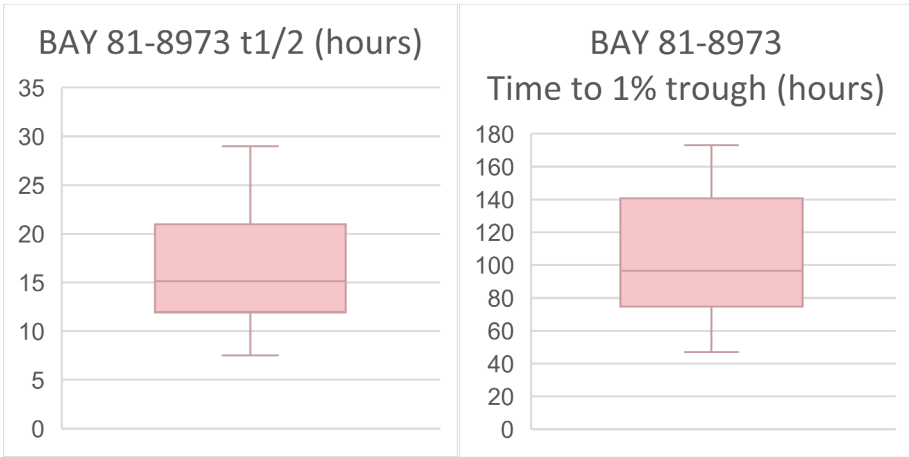


Figure 15. Pharmacokinetic estimations by WAPPS-Hemo.
Boxplots showing WAPPS-Hemo estimations of t½ and time to trough 1%, using the chromogenic assay, in 14 patients treated with BAY 81-8973 at the Malmö Haemophilia Centre.

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.

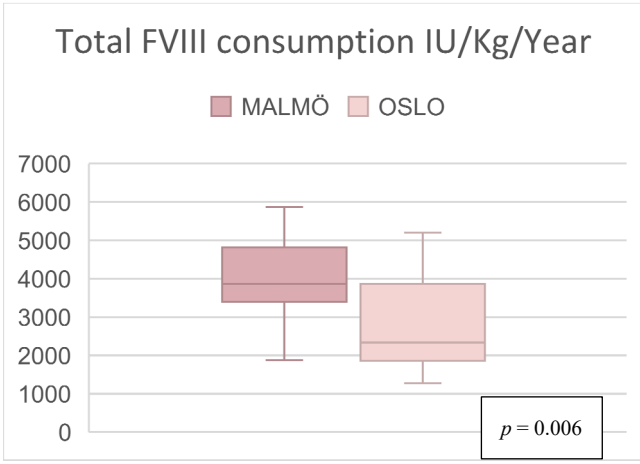


Figure 16. Annual total FVIII consumption (IU/kg/year) in Malmö and Oslo.

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.

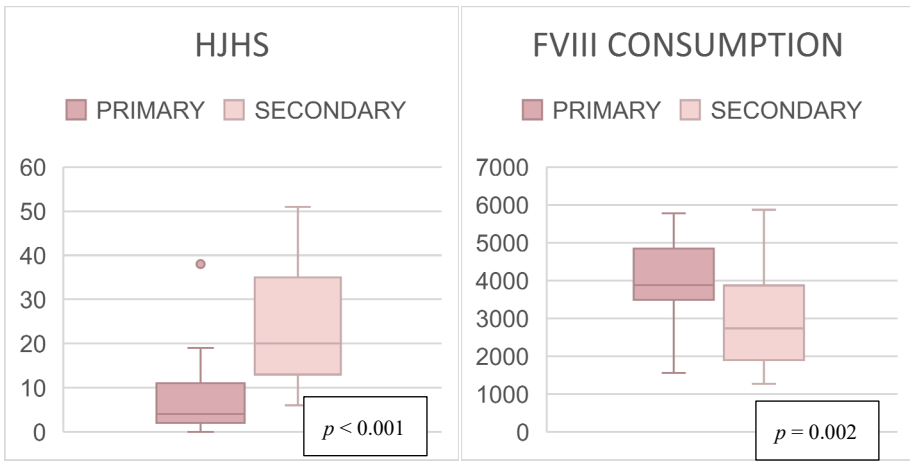


Figure 17. HJHS and FVIII consumption in primary and secondary group.
Boxplots showing difference in HJHS (A) and FVIII consumption (IU/kg/year) (B) between primary and secondary group.

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.

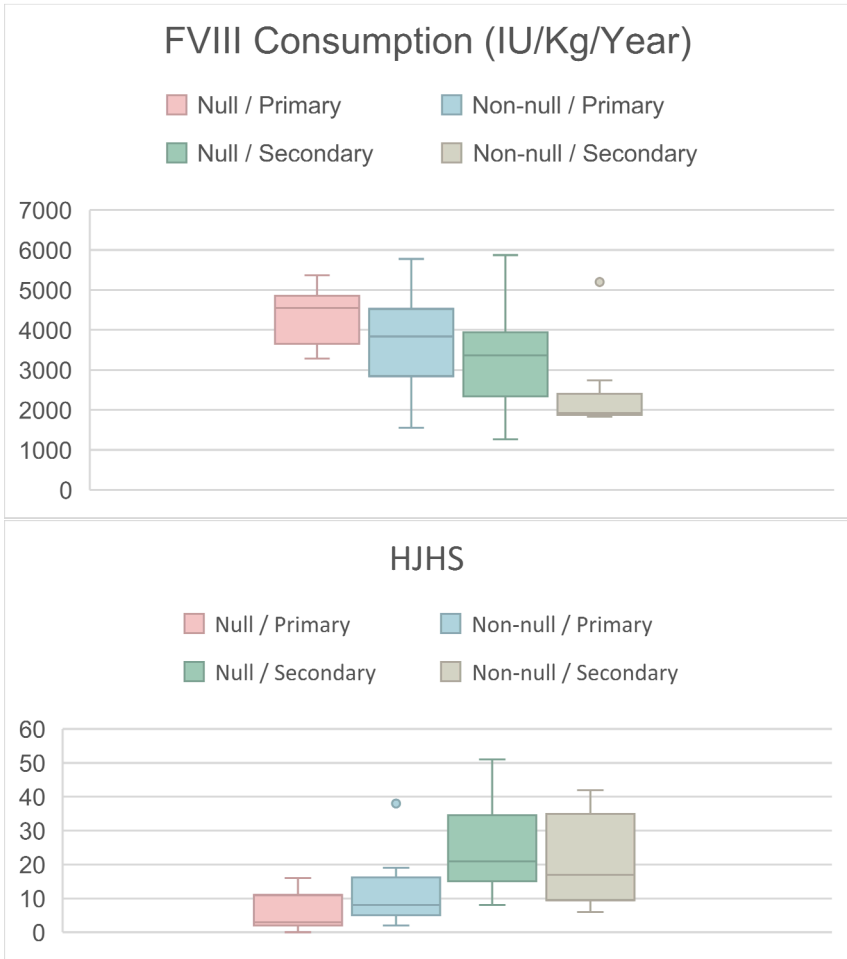


Figure 19. FVIII consumption and HJHS according to genotype and prophylaxis type.
Boxplots showing differences in FVIII consumption (IU/kg/year) and HJHS, according to FVIII variant status (non/non-null) and type of prophylaxis (primary/secondary).

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).

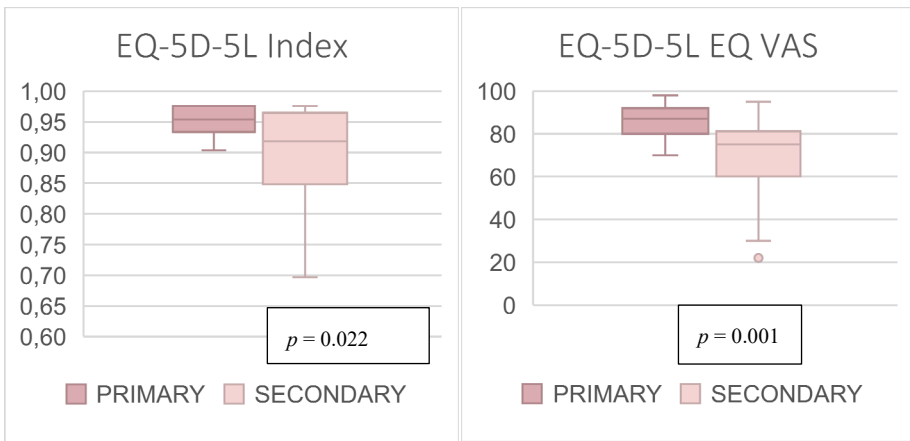


Figure 20. Health-related quality of life results according to prophylaxis type.
Boxplots showing differences in EQ-5D-5L Index value and EQ VAS between the primary and secondary prophylaxis group.

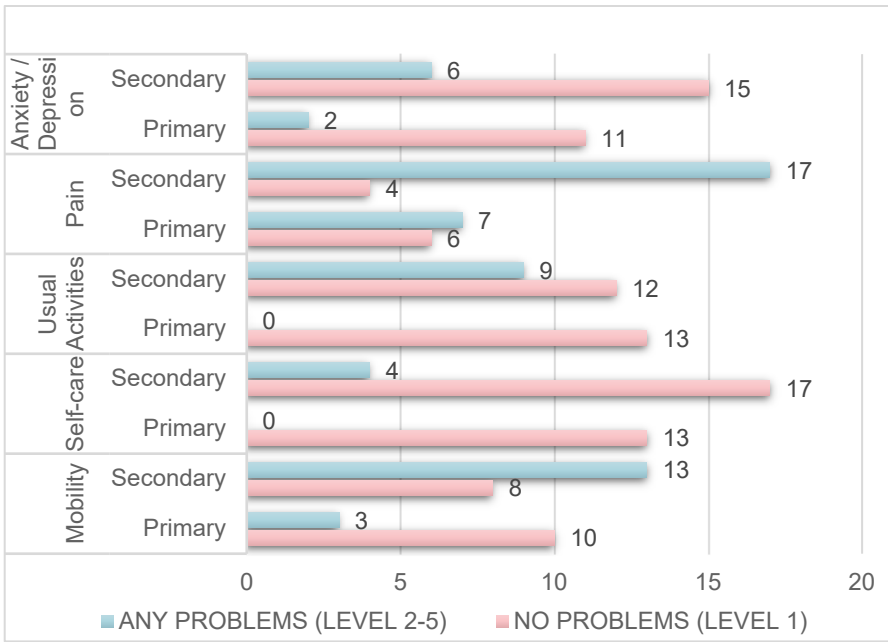


Figure 21. EQ-5D-5L dimension results.
Bar chart showing dimension results of EQ-5D-5L after dichotomisation in "no problems" (level 1) and "any problems" (levels 2-5).

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).

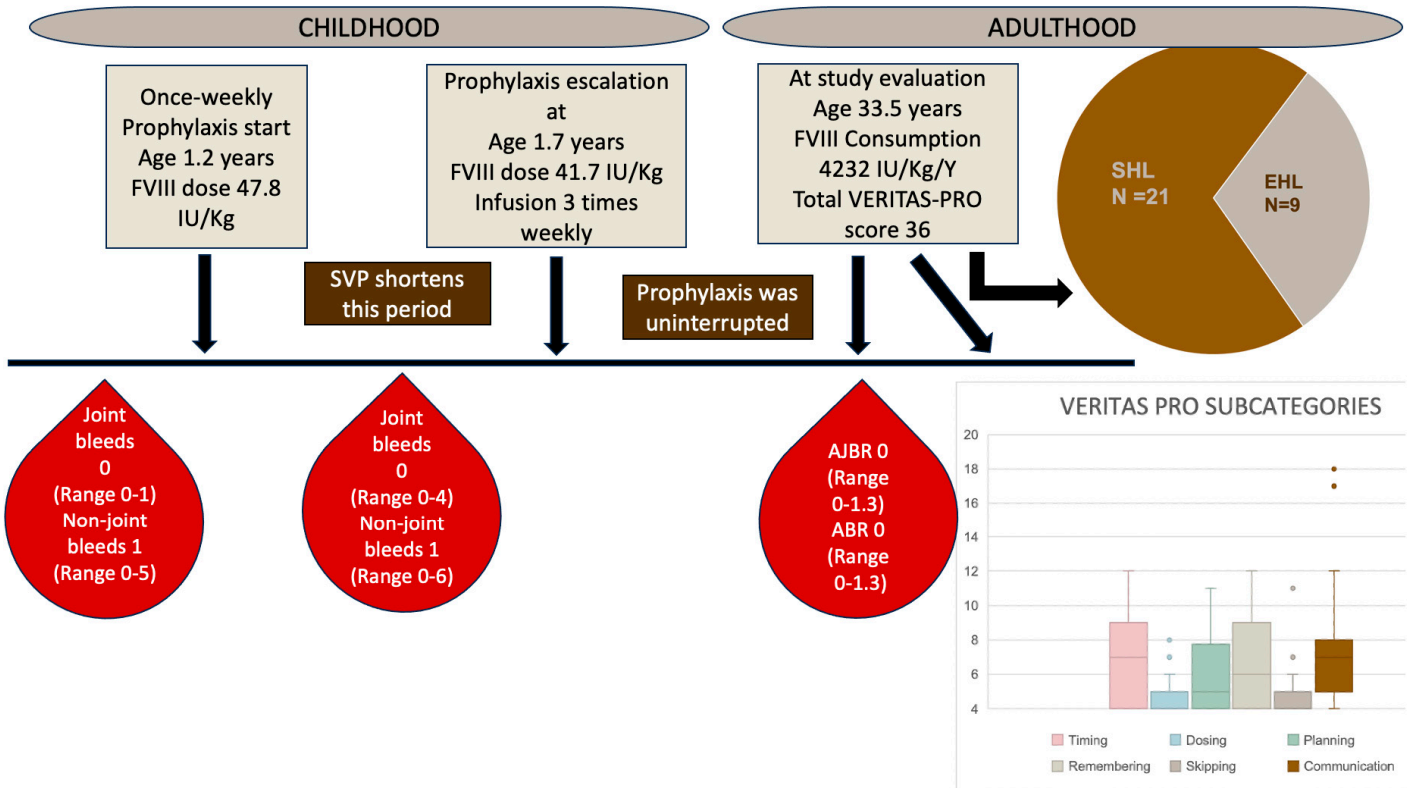


Figure 22. Clinical and treatment characteristics at study inclusion and prophhylaxis start.

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.

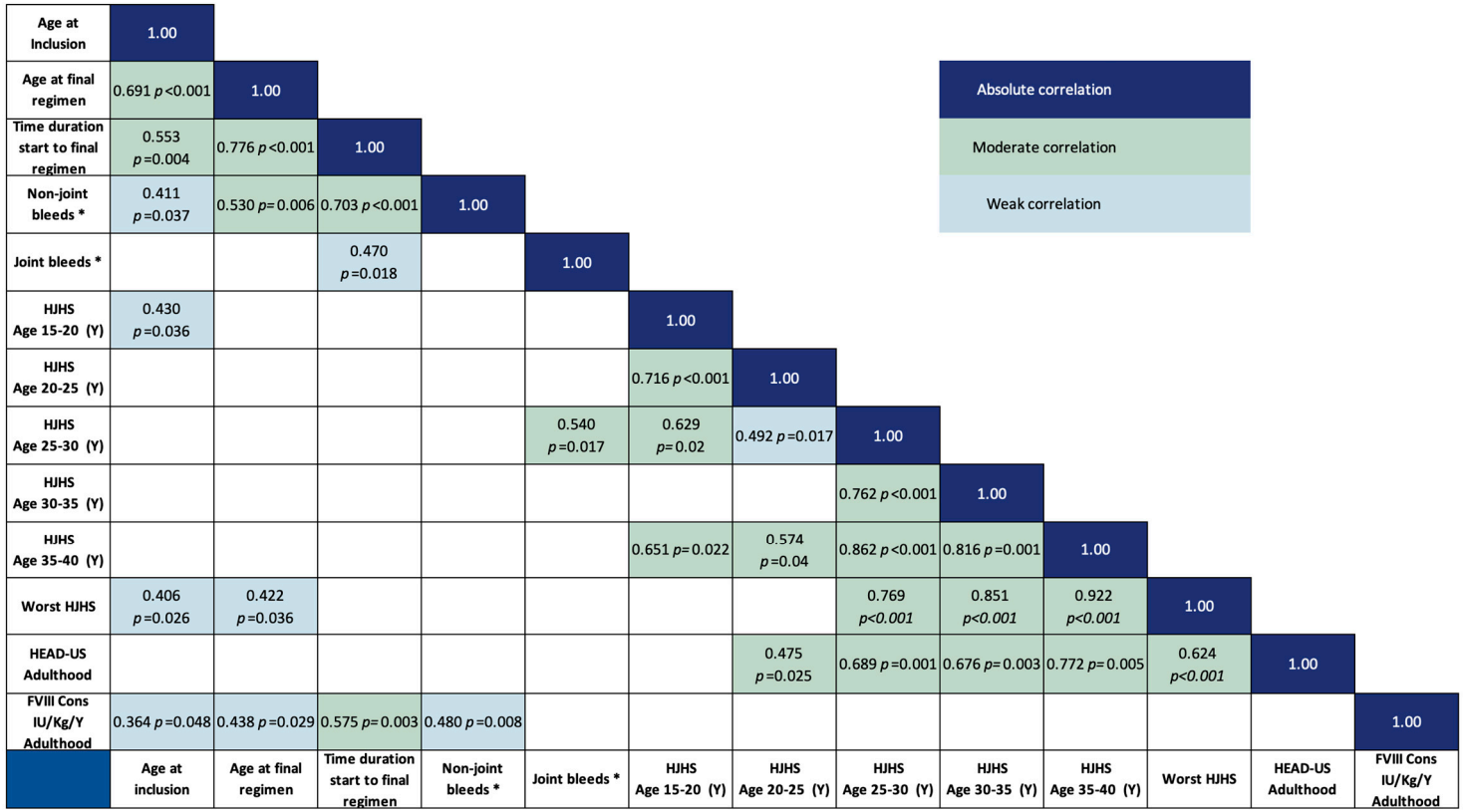
This study's findings thus signify the need for an early start of joint assessments, as they can be indicative of future joint outcomes. In contrast, bleeds prior to prophylaxis did not impact upon joint outcomes, which was probably caused by the sparsity of joint bleeds prior to prophylaxis start in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

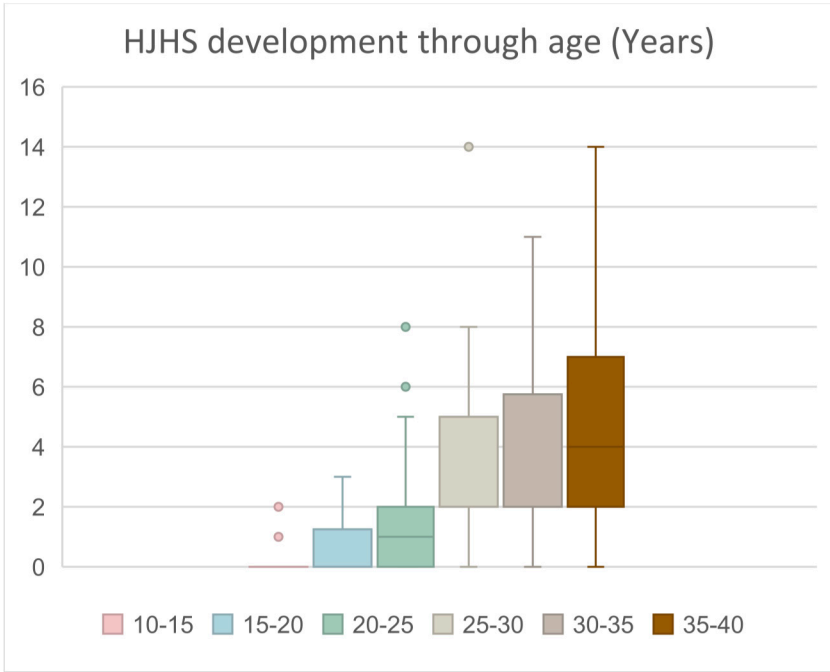
Evaluation of joint health was also performed by ultrasound analysis, according to the HEAD-US (Haemophilia Early Arthropathy Detection with Ultrasound) protocol.[106] Twenty-six patients were evaluated at a median age of 32 years (21.5-36). The median total HEAD-US score was 1 (0-2). The median score 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.

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.

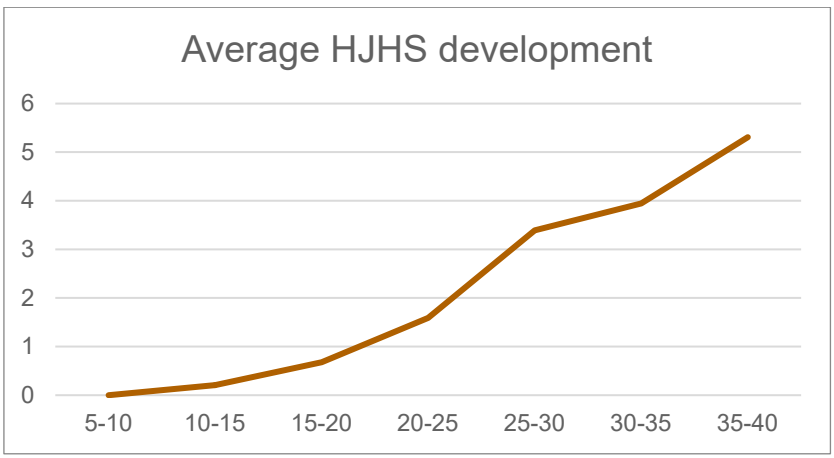
The right ankle joint was the most affected, as 76% of joints (19/25) were classified as pristine, whereas 84% (21/25) of left ankle, and 86.5% (45/52) of all knee joints were pristine. This is consistent with a recent magnetic resonance imaging (MRI) evaluation of a younger (mean age 23.5 years) Swedish 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.

A



B



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.

Denna avhandling har således undersökt kliniska, farmakokinetiska, genetiska och behandlingsrelaterade aspekter av hemofili A som behövs för att individanpassa behandlingen och erbjuda bästa möjliga vård till personerna som lever med denna sjukdom.

Acknowledgements

They say, “it takes a village to raise a child” and Malmö did feel like a village to me, when I first moved here from Athens. I would therefore like to rephrase this quote to say: “it takes a village to help a PhD student through their doctorate studies”. I am so very thankful that I have found my village and my people here in Malmö, and the even smaller village of Lund, in Sweden.

I would like to give special thanks to:

Jan Astermark, my main supervisor. I can safely say that without your unwavering commitment, this thesis would never have seen the light of day. You supported me and this project all these years, shared your knowledge, and had lots of patience (which I sometimes tested). This PhD project has helped me understand the meaning of collaboration and many times humbled my ego. For helping me become not only a better doctor but (I would like to think) a more mature person, I will forever be grateful.

Erik Berntorp, my co-supervisor. Thank you for your great help in starting and completing these projects, and for providing both wise (brutally honest) comments and words of support, when they were needed the most.

Nadine Gretenkort Andersson, my co-supervisor. Thank you for your support through it all, your friendliness, and for teaching me what little paediatric coagulation I know.

Elena Holm, my dear colleague and friend. Thank you for supporting me unconditionally every step of the way, and for working even harder so I can have time to do research. And for all the vodka.

Eva Zetterberg and **Kristina Kihlberg,** thank you for your help enrolling patients in these studies and your support and encouragement.

Malin Axelsson, **Emma Engman Grahn** and all personnel at the Coagulation centre in Malmö. Thank you for your invaluable assistance in making these studies happen.

Johan Theander, head of the Department of Haematology, for providing me with the time to see this project to fruition.

Anders Lindblom, Peter Svensson, Johan Elf, **Joanne Silan, Josefin Roslund, Marcus Fager Ferrari**, **Signe Olin,** and **Albert Sigurdsson**, my colleagues at the Haematology and Coagulation Department in Malmö. Thank you for making work fun! We will always have each other's backs.

All my **colleagues in Lund**, for being great doctors and people. By now, I have had the pleasure of working with many of you for longer than 15 years!

Karin Strandberg, head of the Coagulation laboratory. Thank you for your help with the laboratory aspects, and your comments on the haemostasis chapter.

Anna Letelier, thank you for your work with the *F8* gene variants.

Our **nurses** at the Haematology Department in Malmö, thank you for taking care of the patients when I was away and making my work life easier.

My previously unmentioned co-authors **Pål Andre Holme**, **Fariba Baghaei** and **Caroline Jepsen,** for great collaboration.

The **anonymous reviewers of my papers**. I hated your guts occasionally, but you invested your time and expertise to improve the quality of my papers.

Most importantly, the **haemophilia patients** and their caregivers, who participated in the studies that comprise this thesis.

Konstantina Sargenti and **Evangelia Baimpa**, my dearest friends. You know me so well (the good and the bad). I am so happy that you have been my friends for so many years (but you are as beautiful as when I first met you, of course).

All my **friends**. Thank you for making life fun and keeping me social.

My "**svärfamij**" in Sweden. Thank you for welcoming me into your family.

My mother **Maria,** sister **Dimitra** and my family in Greece. My brothers **Michael** and **Chris** and my family in Australia. We may be scattered at the four corners of the world, but love perseveres.

And finally, my partner **Dennis Karlsson**. You choose to share your life with me. You accept me, support me, and love me despite my flaws. I love you.

References

1. Davie EW, Ratnoff OD. WATERFALL SEQUENCE FOR INTRINSIC BLOOD CLOTTING. *Science.* 1964;145(3638):1310-1312.
2. Macfarlane RG. AN ENZYME CASCADE IN THE BLOOD CLOTTING MECHANISM, AND ITS FUNCTION AS A BIOCHEMICAL AMPLIFIER. *Nature.* 1964;202:498-499.
3. Versteeg HH, Heemskerk JW, Levi M, Reitsma PH. New fundamentals in hemostasis. *Physiol Rev.* 2013;93(1):327-358.
4. McMichael M. New models of hemostasis. *Top Companion Anim Med.* 2012;27(2):40-45.
5. Hoffman M. Remodeling the blood coagulation cascade. *J Thromb Thrombolysis.* 2003;16(1-2):17-20.
6. Monroe DM, Hoffman M. What does it take to make the perfect clot? *Arterioscler Thromb Vasc Biol.* 2006;26(1):41-48.
7. Mann KG, Krishnaswamy S, Lawson JH. Surface-dependent hemostasis. *Semin Hematol.* 1992;29(3):213-226.
8. Smith SA. The cell-based model of coagulation. *J Vet Emerg Crit Care (San Antonio).* 2009;19(1):3-10.
9. Ho KM, Pavey W. Applying the cell-based coagulation model in the management of critical bleeding. *Anaesth Intensive Care.* 2017;45(2):166-176.
10. Price GC, Thompson SA, Kam PC. Tissue factor and tissue factor pathway inhibitor. *Anaesthesia.* 2004;59(5):483-492.
11. Pérez-Gómez F, Bover R. [The new coagulation cascade and its possible influence on the delicate balance between thrombosis and hemorrhage]. *Rev Esp Cardiol.* 2007;60(12):1217-1219.
12. Lane DA, Philippou H, Huntington JA. Directing thrombin. *Blood.* 2005;106(8):2605-2612.
13. Hoffman M. A cell-based model of coagulation and the role of factor VIIa. *Blood Rev.* 2003;17 Suppl 1:S1-5.
14. Bagoly Z, Koncz Z, Hársfalvi J, Muszbek L. Factor XIII, clot structure, thrombosis. *Thromb Res.* 2012;129(3):382-387.
15. Cesarman-Maus G, Hajjar KA. Molecular mechanisms of fibrinolysis. *Br J Haematol.* 2005;129(3):307-321.
16. Chapin JC, Hajjar KA. Fibrinolysis and the control of blood coagulation. *Blood Rev.* 2015;29(1):17-24.

17. Rezaie AR, Giri H. Anticoagulant and signaling functions of antithrombin. *J Thromb Haemost.* 2020;18(12):3142-3153.
18. Singh S, Saleem S, Reed GL. Alpha2-Antiplasmin: The Devil You Don't Know in Cerebrovascular and Cardiovascular Disease. *Front Cardiovasc Med.* 2020;7:608899.
19. Bajzar L, Manuel R, Nesheim ME. Purification and characterization of TAFI, a thrombin-activable fibrinolysis inhibitor. *J Biol Chem.* 1995;270(24):14477-14484.
20. Franchini M, Mannucci PM. Past, present and future of hemophilia: a narrative review. *Orphanet J Rare Dis.* 2012;7:24.
21. Kaadan AAM. Who discovered hemophilia? *Journal of the British Islamic Medical Association* 2022;11 - No.4.
22. Hoyer LW. Hemophilia A. *N Engl J Med.* 1994;330(1):38-47.
23. Rosner F. Hemophilia in the Talmud and rabbinic writings. *Ann Intern Med.* 1969;70(4):833-837.
24. Otto JC. An account of an hemorrhagic disposition existing in certain families. *Clin Orthop Relat Res.* 1996(328):4-6.
25. Schramm W. The history of haemophilia - a short review. *Thromb Res.* 2014;134 Suppl 1:S4-9.
26. Ingram GI. The history of haemophilia. *J Clin Pathol.* 1976;29(6):469-479.
27. Lewis JH, Davidson CS, Minot GR, Soulier JP, Tagnon HJ, Taylor FH. CHEMICAL, CLINICAL AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION: XXXII. THE COAGULATION DEFECT IN HEMOPHILIA. AN IN VITRO AND IN VIVO COMPARISON OF NORMAL AND HEMOPHILIC WHOLE BLOOD, PLASMA AND DERIVED PLASMA PROTEIN FRACTIONS. *J Clin Invest.* 1946;25(6):870-875.
28. Wright IS. The nomenclature of blood clotting factors. *Thromb Diath Haemorrh.* 1962;7:381-388.
29. Rogaev EI, Grigorenko AP, Faskhutdinova G, Kittler EL, Moliaka YK. Genotype analysis identifies the cause of the "royal disease". *Science.* 2009;326(5954):817.
30. Rosendaal FR, Smit C, Briët E. Hemophilia treatment in historical perspective: a review of medical and social developments. *Ann Hematol.* 1991;62(1):5-15.
31. Brinkhous K.M. HH, editors. A short history of hemophilia, with some comments on the word 'hemophilia, . *Handbook of Hemophilia Amsterdam: Excerpta Medica.* 1975:**3-20**.
32. Srivastava A, Santagostino E, Dougall A, et al. WFH Guidelines for the Management of Hemophilia, 3rd edition. *Haemophilia.* 2020;26 Suppl 6:1-158.
33. Soucie JM, Miller CH, Dupervil B, Le B, Buckner TW. Occurrence rates of haemophilia among males in the United States based on surveillance conducted in specialized haemophilia treatment centres. *Haemophilia.* 2020;26(3):487-493.
34. Iorio A, Stonebraker JS, Chambost H, et al. Establishing the Prevalence and Prevalence at Birth of Hemophilia in Males: A Meta-analytic Approach Using National Registries. *Ann Intern Med.* 2019;171(8):540-546.

35. Larsson SA. Life expectancy of Swedish haemophiliacs, 1831-1980. *Br J Haematol.* 1985;59(4):593-602.
36. Mauser-Bunschoten EP, Fransen Van De Putte DE, Schutgens RE. Co-morbidity in the ageing haemophilia patient: the down side of increased life expectancy. *Haemophilia.* 2009;15(4):853-863.
37. Berntorp E, Fischer K, Hart DP, et al. Haemophilia. *Nat Rev Dis Primers.* 2021;7(1):45.
38. Stonebraker JS, Bolton-Maggs PH, Soucie JM, Walker I, Brooker M. A study of variations in the reported haemophilia A prevalence around the world. *Haemophilia.* 2010;16(1):20-32.
39. Blanchette VS, Key NS, Ljung LR, Manco-Johnson MJ, van den Berg HM, Srivastava A. Definitions in hemophilia: communication from the SSC of the ISTH. *J Thromb Haemost.* 2014;12(11):1935-1939.
40. Hemofiliregistret S. Årsrapport 2022. 2022.
41. den Uijl IE, Fischer K, Van Der Bom JG, Grobbee DE, Rosendaal FR, Plug I. Clinical outcome of moderate haemophilia compared with severe and mild haemophilia. *Haemophilia.* 2009;15(1):83-90.
42. Berntorp E, Shapiro AD. Modern haemophilia care. *Lancet.* 2012;379(9824):1447-1456.
43. Mazurkiewicz-Pisarek A, Płucienniczak G, Ciach T, Płucienniczak A. The factor VIII protein and its function. *Acta Biochim Pol.* 2016;63(1):11-16.
44. Fischer K, Ljung R, Platokouki H, et al. Prospective observational cohort studies for studying rare diseases: the European PedNet Haemophilia Registry. *Haemophilia.* 2014;20(4):e280-286.
45. Lannoy N, Hermans C. Genetic mosaicism in haemophilia: A practical review to help evaluate the risk of transmitting the disease. *Haemophilia.* 2020;26(3):375-383.
46. Manderstedt E, Nilsson R, Ljung R, Lind-Halldén C, Astermark J, Halldén C. Detection of mosaics in hemophilia A by deep Ion Torrent sequencing and droplet digital PCR. *Res Pract Thromb Haemost.* 2020;4(7):1121-1130.
47. Andersson NG, Labarque V, Letelier A, et al. Novel F8 and F9 gene variants from the PedNet hemophilia registry classified according to ACMG/AMP guidelines. *Hum Mutat.* 2020;41(12):2058-2072.
48. Oldenburg J, Rost S, El-Maarri O, et al. De novo factor VIII gene intron 22 inversion in a female carrier presents as a somatic mosaicism. *Blood.* 2000;96(8):2905-2906.
49. Labarque V, Mancuso ME, Kartal-Kaess M, Ljung R, Mikkelsen TS, Andersson NG. F8/F9 variants in the population-based PedNet Registry cohort compared with locus-specific genetic databases of the European Association for Haemophilia and Allied Disorders and the Centers for Disease Control and Prevention Hemophilia A or Hemophilia B Mutation Project. *Res Pract Thromb Haemost.* 2023;7(1):100036.
50. Margaglione M, Castaman G, Morfini M, et al. The Italian AICE-Genetics hemophilia A database: results and correlation with clinical phenotype. *Haematologica.* 2008;93(5):722-728.

51. Carcao MD, van den Berg HM, Ljung R, Mancuso ME. Correlation between phenotype and genotype in a large unselected cohort of children with severe hemophilia A. *Blood.* 2013;121(19):3946-3952, s3941.
52. Spena S, Garagiola I, Cannavò A, et al. Prediction of factor VIII inhibitor development in the SIPPET cohort by mutational analysis and factor VIII antigen measurement. *J Thromb Haemost.* 2018;16(4):778-790.
53. Gouw SC, van den Berg HM, Oldenburg J, et al. F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis. *Blood.* 2012;119(12):2922-2934.
54. Peyvandi F, Mannucci PM, Garagiola I, et al. A Randomized Trial of Factor VIII and Neutralizing Antibodies in Hemophilia A. *N Engl J Med.* 2016;374(21):2054-2064.
55. Pipe SW, Montgomery RR, Pratt KP, Lenting PJ, Lillicrap D. Life in the shadow of a dominant partner: the FVIII-VWF association and its clinical implications for hemophilia A. *Blood.* 2016;128(16):2007-2016.
56. Vehar GA, Keyt B, Eaton D, et al. Structure of human factor VIII. *Nature.* 1984;312(5992):337-342.
57. Toole JJ, Knopf JL, Wozney JM, et al. Molecular cloning of a cDNA encoding human antihaemophilic factor. *Nature.* 1984;312(5992):342-347.
58. Wood WI, Capon DJ, Simonsen CC, et al. Expression of active human factor VIII from recombinant DNA clones. *Nature.* 1984;312(5992):330-337.
59. Fay PJ, Jenkins PV. Mutating factor VIII: lessons from structure to function. *Blood Rev.* 2005;19(1):15-27.
60. Turecek PL, Johnsen JM, Pipe SW, O'Donnell JS. Biological mechanisms underlying inter-individual variation in factor VIII clearance in haemophilia. *Haemophilia.* 2020;26(4):575-583.
61. Lenting PJ, Christophe OD, Guéguen P. The disappearing act of factor VIII. *Haemophilia.* 2010;16(102):6-15.
62. Lee C BE, Hoots K. Textbook of Hemophilia. 3rd ed. *WIley-Blackwell.* 2014.
63. Lenting PJ, van Mourik JA, Mertens K. The life cycle of coagulation factor VIII in view of its structure and function. *Blood.* 1998;92(11):3983-3996.
64. Venkateswaran L, Wilimas JA, Jones DJ, Nuss R. Mild hemophilia in children: prevalence, complications, and treatment. *J Pediatr Hematol Oncol.* 1998;20(1):32-35.
65. Matsuda M, Hoshiyama Y, Ogawa K, Emmi M, Terai S, Moriyama M. Performance characteristics of 5 numerical indexes in mixing test interpretation under coexistence of lupus anticoagulant and coagulation factor deficiency. *Res Pract Thromb Haemost.* 2023;7(2):100065.
66. Verbruggen B, Meijer P, Novákova I, Van Heerde W. Diagnosis of factor VIII deficiency. *Haemophilia.* 2008;14 Suppl 3:76-82.
67. Franchini M, Favaloro EJ, Lippi G. Mild hemophilia A. *J Thromb Haemost.* 2010;8(3):421-432.

68. Langdell RD, Wagner RH, Brinkhous KM. Effect of antihemophilic factor on one-stage clotting tests; a presumptive test for hemophilia and a simple one-stage antihemophilic factor assy procedure. *J Lab Clin Med.* 1953;41(4):637-647.
69. Potgieter JJ, Damgaard M, Hillarp A. One-stage vs. chromogenic assays in haemophilia A. *Eur J Haematol.* 2015;94 Suppl 77:38-44.
70. Peyvandi F, Oldenburg J, Friedman KD. A critical appraisal of one-stage and chromogenic assays of factor VIII activity. *J Thromb Haemost.* 2016;14(2):248-261.
71. Armstrong E, Hillarp A. Assay discrepancy in mild haemophilia A. *Eur J Haematol Suppl.* 2014;76:48-50.
72. Oldenburg J, Pavlova A. Discrepancy between one-stage and chromogenic factor VIII activity assay results can lead to misdiagnosis of haemophilia A phenotype. *Hamostaseologie.* 2010;30(4):207-211.
73. Mumford AD, Laffan M, O'Donnell J, et al. A Tyr346-->Cys substitution in the interdomain acidic region a1 of factor VIII in an individual with factor VIII:C assay discrepancy. *Br J Haematol.* 2002;118(2):589-594.
74. Raut S, Heath AB, Barrowcliffe TW. A collaborative study to establish the 6th International Standard for factor VIII concentrate. *Thromb Haemost.* 2001;85(6):1071-1078.
75. Hubbard AR, Bevan SA, Weller LJ. Potency estimation of recombinant factor VIII: effect of assay method and standard. *Br J Haematol.* 2001;113(2):533-536.
76. Lee C, Barrowcliffe T, Bray G, et al. Pharmacokinetic in vivo comparison using 1-stage and chromogenic substrate assays with two formulations of Hemofil-M. *Thromb Haemost.* 1996;76(6):950-956.
77. Ingerslev J, Jankowski MA, Weston SB, Charles LA. Collaborative field study on the utility of a BDD factor VIII concentrate standard in the estimation of BDDr Factor VIII:C activity in hemophilic plasma using one-stage clotting assays. *J Thromb Haemost.* 2004;2(4):623-628.
78. Sarmiento Doncel S, Díaz Mosquera GA, Cortes JM, Agudelo Rico C, Meza Cadavid FJ, Peláez RG. Haemophilia A: A Review of Clinical Manifestations, Treatment, Mutations, and the Development of Inhibitors. *Hematol Rep.* 2023;15(1):130-150.
79. Donaldson J, Goddard N. Compartment syndrome in patients with haemophilia. *J Orthop.* 2015;12(4):237-241.
80. van den Berg HM, De Groot PH, Fischer K. Phenotypic heterogeneity in severe hemophilia. *J Thromb Haemost.* 2007;5 Suppl 1:151-156.
81. van Dijk K, Fischer K, van der Bom JG, Grobbee DE, van den Berg HM. Variability in clinical phenotype of severe haemophilia: the role of the first joint bleed. *Haemophilia.* 2005;11(5):438-443.
82. Onwuzurike N, Warrier I, Lusher JM. Types of bleeding seen during the first 30 months of life in children with severe haemophilia A and B. *Haemophilia.* 1996;2(3):137-140.

83. Måseide RJ, Berntorp E, Astermark J, et al. Joint health and treatment modalities in Nordic patients with moderate haemophilia A and B - The MoHem study. *Haemophilia.* 2020;26(5):891-897.
84. Nichols WC, Amano K, Cacheris PM, et al. Moderation of hemophilia A phenotype by the factor V R506Q mutation. *Blood.* 1996;88(4):1183-1187.
85. Escuriola Ettingshausen C, Halimeh S, Kurnik K, et al. Symptomatic onset of severe hemophilia A in childhood is dependent on the presence of prothrombotic risk factors. *Thromb Haemost.* 2001;85(2):218-220.
86. Elsheikh E, Lavin M, Heck LA, et al. Heterogeneity in the half-life of factor VIII concentrate in patients with hemophilia A is due to variability in the clearance of endogenous von Willebrand factor. *J Thromb Haemost.* 2023.
87. Vlot AJ, Mauser-Bunschoten EP, Zarkova AG, et al. The half-life of infused factor VIII is shorter in hemophiliac patients with blood group O than in those with blood group A. *Thromb Haemost.* 2000;83(1):65-69.
88. Goren R, Pullenayegum E, Blanchette VS, et al. Patterns of joint damage in severe haemophilia A treated with prophylaxis. *Haemophilia.* 2021;27(4):666-673.
89. Gualtierotti R, Solimeno LP, Peyvandi F. Hemophilic arthropathy: Current knowledge and future perspectives. *J Thromb Haemost.* 2021;19(9):2112-2121.
90. Drake TA, Morrissey JH, Edgington TS. Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am J Pathol.* 1989;134(5):1087-1097.
91. Wyseure T, Mosnier LO, von Drygalski A. Advances and challenges in hemophilic arthropathy. *Semin Hematol.* 2016;53(1):10-19.
92. Valentino LA. Blood-induced joint disease: the pathophysiology of hemophilic arthropathy. *J Thromb Haemost.* 2010;8(9):1895-1902.
93. Hilberg T, Czepa D, Freialdenhoven D, Boettger MK. Joint pain in people with hemophilia depends on joint status. *Pain.* 2011;152(9):2029-2035.
94. Kempton CL, Antoniucci DM, Rodriguez-Merchan EC. Bone health in persons with haemophilia. *Haemophilia.* 2015;21(5):568-577.
95. Gerstner G, Damiano ML, Tom A, et al. Prevalence and risk factors associated with decreased bone mineral density in patients with haemophilia. *Haemophilia.* 2009;15(2):559-565.
96. Cazanave C, Dupon M, Lavignolle-Aurillac V, et al. Reduced bone mineral density in HIV-infected patients: prevalence and associated factors. *Aids.* 2008;22(3):395-402.
97. Baud'huin M, Duplomb L, Téletchéa S, et al. Factor VIII-von Willebrand factor complex inhibits osteoclastogenesis and controls cell survival. *J Biol Chem.* 2009;284(46):31704-31713.
98. Schiefke I, Fach A, Wiedmann M, et al. Reduced bone mineral density and altered bone turnover markers in patients with non-cirrhotic chronic hepatitis B or C infection. *World J Gastroenterol.* 2005;11(12):1843-1847.

99. Den Uijl IE, De Schepper AM, Camerlinck M, Grobbee DE, Fischer K. Magnetic resonance imaging in teenagers and young adults with limited haemophilic arthropathy: baseline results from a prospective study. *Haemophilia.* 2011;17(6):926-930.
100. Gringeri A, Ewenstein B, Reininger A. The burden of bleeding in haemophilia: is one bleed too many? *Haemophilia.* 2014;20(4):459-463.
101. van Leeuwen FHP, van Bergen EPD, Timmer MA, et al. Magnetic resonance imaging evidence for subclinical joint bleeding in a Dutch population of people with severe hemophilia on prophylaxis. *J Thromb Haemost.* 2023.
102. Kraft J, Blanchette V, Babyn P, et al. Magnetic resonance imaging and joint outcomes in boys with severe hemophilia A treated with tailored primary prophylaxis in Canada. *J Thromb Haemost.* 2012;10(12):2494-2502.
103. Optimal treatment regimens for patients with bleeding disorders. *Haemophilia.* 2001;7(3):313-320.
104. Feldman BM, Funk SM, Bergstrom BM, et al. Validation of a new pediatric joint scoring system from the International Hemophilia Prophylaxis Study Group: validity of the hemophilia joint health score. *Arthritis Care Res (Hoboken).* 2011;63(2):223-230.
105. Kilcoyne RF, Lundin B, Pettersson H. Evolution of the imaging tests in hemophilia with emphasis on radiography and magnetic resonance imaging. *Acta Radiol.* 2006;47(3):287-296.
106. Martinoli C, Della Casa Alberighi O, Di Minno G, et al. Development and definition of a simplified scanning procedure and scoring method for Haemophilia Early Arthropathy Detection with Ultrasound (HEAD-US). *Thromb Haemost.* 2013;109(6):1170-1179.
107. Chan MW, Leckie A, Xavier F, et al. A systematic review of MR imaging as a tool for evaluating haemophilic arthropathy in children. *Haemophilia.* 2013;19(6):e324-334.
108. Gringeri A, Von Mackensen S. Quality of life in haemophilia. *Haemophilia.* 2008;14 Suppl 3:19-25.
109. Bago M, Butkovic A, Faganel Kotnik B, et al. Health-Related Quality of Life in Patients with Haemophilia and Its Association with Depressive Symptoms: A Study in Croatia and Slovenia. *Psychiatr Danub.* 2021;33(3):334-341.
110. Schiavoni M, Pruneti C, Guidotti S, et al. Health Related Quality of Life and Psychopathological Symptoms in People with Hemophilia, Bloodborne Co-Infections and Comorbidities: An Italian Multicenter Observational Study. *Mediterr J Hematol Infect Dis.* 2023;15(1):e2023005.
111. Cassis FR, Querol F, Forsyth A, Iorio A. Psychosocial aspects of haemophilia: a systematic review of methodologies and findings. *Haemophilia.* 2012;18(3):e101-114.
112. Osooli M, Steen Carlsson K, Baghaei F, et al. The association between health utility and joint status among people with severe haemophilia A: findings from the KAPPA register. *Haemophilia.* 2017;23(3):e180-e187.

113. Fischer K, de Kleijn P, Negrier C, et al. The association of haemophilic arthropathy with Health-Related Quality of Life: a post hoc analysis. *Haemophilia.* 2016;22(6):833-840.
114. Kihlberg K, Baghaei F, Bruzelius M, et al. No difference in quality of life between persons with severe haemophilia A and B. *Haemophilia.* 2023.
115. Steen Carlsson K, Winding B, Astermark J, et al. Pain, depression and anxiety in people with haemophilia from three Nordic countries: Cross-sectional survey data from the MIND study. *Haemophilia.* 2022;28(4):557-567.
116. Lindvall K, Von Mackensen S, Berntorp E. Quality of life in adult patients with haemophilia--a single centre experience from Sweden. *Haemophilia.* 2012;18(4):527-531.
117. Schnohr C, Ekholm O, Poulsen LH, et al. Health and quality of life of patients with haemophilia: A national study of 124 Danish men. *Haemophilia.* 2023.
118. d'Oiron R, O'Brien S, James AH. Women and girls with haemophilia: Lessons learned. *Haemophilia.* 2021;27 Suppl 3:75-81.
119. van Galen KPM, d'Oiron R, James P, et al. A new hemophilia carrier nomenclature to define hemophilia in women and girls: Communication from the SSC of the ISTH. *J Thromb Haemost.* 2021;19(8):1883-1887.
120. Sharathkumar A, Hardesty B, Greist A, et al. Variability in bleeding phenotype in Amish carriers of haemophilia B with the 31008 C-->T mutation. *Haemophilia.* 2009;15(1):91-100.
121. Osooli M, Donfield SM, Carlsson KS, et al. Joint comorbidities among Swedish carriers of haemophilia: A register-based cohort study over 22 years. *Haemophilia.* 2019;25(5):845-850.
122. Gilbert L, Rollins L, Hilmes M, et al. Haemophilia A carriers demonstrate pathological and radiological evidence of structural joint changes. *Haemophilia.* 2014;20(6):e426-429.
123. Mauser Bunschoten EP, van Houwelingen JC, Sjamsoedin Visser EJ, van Dijken PJ, Kok AJ, Sixma JJ. Bleeding symptoms in carriers of hemophilia A and B. *Thromb Haemost.* 1988;59(3):349-352.
124. Greer IA, Lowe GD, Walker JJ, Forbes CD. Haemorrhagic problems in obstetrics and gynaecology in patients with congenital coagulopathies. *Br J Obstet Gynaecol.* 1991;98(9):909-918.
125. Kadir RA, Economides DL, Braithwaite J, Goldman E, Lee CA. The obstetric experience of carriers of haemophilia. *Br J Obstet Gynaecol.* 1997;104(7):803-810.
126. Kulkarni R, Soucie JM, Lusher J, et al. Sites of initial bleeding episodes, mode of delivery and age of diagnosis in babies with haemophilia diagnosed before the age of 2 years: a report from The Centers for Disease Control and Prevention's (CDC) Universal Data Collection (UDC) project. *Haemophilia.* 2009;15(6):1281-1290.
127. Chalmers E, Williams M, Brennand J, Liesner R, Collins P, Richards M. Guideline on the management of haemophilia in the fetus and neonate. *Br J Haematol.* 2011;154(2):208-215.

128. Peyvandi F, Garagiola I, Young G. The past and future of haemophilia: diagnosis, treatments, and its complications. *Lancet.* 2016;388(10040):187-197.
129. Nilsson IM, Blomback M, Von Francken I. On an inherited autosomal hemorrhagic diathesis with antihemophilic globulin (AHG) deficiency and prolonged bleeding time. *Acta Med Scand.* 1957;159(1):35-57.
130. Nilsson IM, Berntorp E, Löfqvist T, Pettersson H. Twenty-five years' experience of prophylactic treatment in severe haemophilia A and B. *J Intern Med.* 1992;232(1):25-32.
131. Ahlberg A. Haemophilia in Sweden. VII. Incidence, treatment and prophylaxis of arthropathy and other musculo-skeletal manifestations of haemophilia A and B. *Acta Orthop Scand Suppl.* 1965:Suppl 77:73-132.
132. Astermark J, Petrini P, Tengborn L, Schulman S, Ljung R, Berntorp E. Primary prophylaxis in severe haemophilia should be started at an early age but can be individualized. *Br J Haematol.* 1999;105(4):1109-1113.
133. Collins PW, Blanchette VS, Fischer K, et al. Break-through bleeding in relation to predicted factor VIII levels in patients receiving prophylactic treatment for severe hemophilia A. *J Thromb Haemost.* 2009;7(3):413-420.
134. Manco-Johnson MJ, Abshire TC, Shapiro AD, et al. Prophylaxis versus episodic treatment to prevent joint disease in boys with severe hemophilia. *N Engl J Med.* 2007;357(6):535-544.
135. Gringeri A, Lundin B, von Mackensen S, Mantovani L, Mannucci PM. A randomized clinical trial of prophylaxis in children with hemophilia A (the ESPRIT Study). *J Thromb Haemost.* 2011;9(4):700-710.
136. Manco-Johnson MJ, Soucie JM, Gill JC. Prophylaxis usage, bleeding rates, and joint outcomes of hemophilia, 1999 to 2010: a surveillance project. *Blood.* 2017;129(17):2368-2374.
137. Kavakli K, Yang R, Rusen L, Beckmann H, Tseneklidou-Stoeter D, Maas Enriquez M. Prophylaxis vs. on-demand treatment with BAY 81-8973, a full-length plasma protein-free recombinant factor VIII product: results from a randomized trial (LEOPOLD II). *J Thromb Haemost.* 2015;13(3):360-369.
138. Warren BB, Thornhill D, Stein J, et al. Young adult outcomes of childhood prophylaxis for severe hemophilia A: results of the Joint Outcome Continuation Study. *Blood Adv.* 2020;4(11):2451-2459.
139. Valentino LA. Secondary prophylaxis therapy: what are the benefits, limitations and unknowns? *Haemophilia.* 2004;10(2):147-157.
140. Fischer K, Steen Carlsson K, Petrini P, et al. Intermediate-dose versus high-dose prophylaxis for severe hemophilia: comparing outcome and costs since the 1970s. *Blood.* 2013;122(7):1129-1136.
141. Feldman BM, Pai M, Rivard GE, et al. Tailored prophylaxis in severe hemophilia A: interim results from the first 5 years of the Canadian Hemophilia Primary Prophylaxis Study. *J Thromb Haemost.* 2006;4(6):1228-1236.
142. Delavenne X, Dargaud Y. Pharmacokinetics for haemophilia treaters: Meaning of PK parameters, interpretation pitfalls, and use in the clinic. *Thromb Res.* 2020;192:52-60.

143. Collins PW, Fischer K, Morfini M, Blanchette VS, Björkman S. Implications of coagulation factor VIII and IX pharmacokinetics in the prophylactic treatment of haemophilia. *Haemophilia.* 2011;17(1):2-10.
144. Hermans C, Dolan G. Pharmacokinetics in routine haemophilia clinical practice: rationale and modalities-a practical review. *Ther Adv Hematol.* 2020;11:2040620720966888.
145. Björkman S, Folkesson A, Jönsson S. Pharmacokinetics and dose requirements of factor VIII over the age range 3-74 years: a population analysis based on 50 patients with long-term prophylactic treatment for haemophilia A. *Eur J Clin Pharmacol.* 2009;65(10):989-998.
146. Björkman S, Berntorp E. Pharmacokinetics of coagulation factors: clinical relevance for patients with haemophilia. *Clin Pharmacokinet.* 2001;40(11):815-832.
147. Iorio A, Edginton AN, Blanchette V, et al. Performing and interpreting individual pharmacokinetic profiles in patients with Hemophilia A or B: Rationale and general considerations. *Res Pract Thromb Haemost.* 2018;2(3):535-548.
148. Barnes C. Importance of pharmacokinetics in the management of hemophilia. *Pediatr Blood Cancer.* 2013;60 Suppl 1:S27-29.
149. Collins PW, Björkman S, Fischer K, et al. Factor VIII requirement to maintain a target plasma level in the prophylactic treatment of severe hemophilia A: influences of variance in pharmacokinetics and treatment regimens. *J Thromb Haemost.* 2010;8(2):269-275.
150. Blanchette VS, Shapiro AD, Liesner RJ, et al. Plasma and albumin-free recombinant factor VIII: pharmacokinetics, efficacy and safety in previously treated pediatric patients. *J Thromb Haemost.* 2008;6(8):1319-1326.
151. Carlsson M, Berntorp E, Björkman S, Lindvall K. Pharmacokinetic dosing in prophylactic treatment of hemophilia A. *Eur J Haematol.* 1993;51(4):247-252.
152. Carlsson M, Berntorp E, Björkman S, Lethagen S, Ljung R. Improved cost-effectiveness by pharmacokinetic dosing of factor VIII in prophylactic treatment of haemophilia A. *Haemophilia.* 1997;3(2):96-101.
153. Lee M, Morfini M, Negrier C, Chamouard V. The pharmacokinetics of coagulation factors. *Haemophilia.* 2006;12 Suppl 3:1-7.
154. Thomson AH, Whiting B. Bayesian parameter estimation and population pharmacokinetics. *Clin Pharmacokinet.* 1992;22(6):447-467.
155. Björkman S. Limited blood sampling for pharmacokinetic dose tailoring of FVIII in the prophylactic treatment of haemophilia A. *Haemophilia.* 2010;16(4):597-605.
156. Westbury CF. Bayes' rule for clinicians: an introduction. *Front Psychol.* 2010;1:192.
157. Björkman S, Oh M, Spotts G, et al. Population pharmacokinetics of recombinant factor VIII: the relationships of pharmacokinetics to age and body weight. *Blood.* 2012;119(2):612-618.
158. Björkman S, Collins P. Measurement of factor VIII pharmacokinetics in routine clinical practice. *J Thromb Haemost.* 2013;11(1):180-182.
159. Iorio A. Using pharmacokinetics to individualize hemophilia therapy. *Hematology Am Soc Hematol Educ Program.* 2017;2017(1):595-604.

160. Dunn A. The long and short of it: using the new factor products. *Hematology Am Soc Hematol Educ Program.* 2015;2015:26-32.
161. Mannucci PM. AIDS, hepatitis and hemophilia in the 1980s: memoirs from an insider. *J Thromb Haemost.* 2003;1(10):2065-2069.
162. Morfini M. Innovative approach for improved rFVIII concentrate. *Eur J Haematol.* 2014;93(5):361-368.
163. Josephson CD, Abshire T. The new albumin-free recombinant factor VIII concentrates for treatment of hemophilia: do they represent an actual incremental improvement? *Clin Adv Hematol Oncol.* 2004;2(7):441-446.
164. Schmidbauer S, Witzel R, Robbel L, et al. Physicochemical characterisation of rVIII-SingleChain, a novel recombinant single-chain factor VIII. *Thromb Res.* 2015;136(2):388-395.
165. Mei B, Pan C, Jiang H, et al. Rational design of a fully active, long-acting PEGylated factor VIII for hemophilia A treatment. *Blood.* 2010;116(2):270-279.
166. Coyle TE, Reding MT, Lin JC, Michaels LA, Shah A, Powell J. Phase I study of BAY 94-9027, a PEGylated B-domain-deleted recombinant factor VIII with an extended half-life, in subjects with hemophilia A. *J Thromb Haemost.* 2014;12(4):488-496.
167. Mahlangu J, Powell JS, Ragni MV, et al. Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. *Blood.* 2014;123(3):317-325.
168. Santagostino E, Negrier C, Klamroth R, et al. Safety and pharmacokinetics of a novel recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) in hemophilia B patients. *Blood.* 2012;120(12):2405-2411.
169. von Drygalski A, Chowdary P, Kulkarni R, et al. Efanesoctocog Alfa Prophylaxis for Patients with Severe Hemophilia A. *N Engl J Med.* 2023;388(4):310-318.
170. Brod M, Bushnell DM, Neergaard JS, Waldman LT, Busk AK. Understanding treatment burden in hemophilia: development and validation of the Hemophilia Treatment Experience Measure (Hemo-TEM). *J Patient Rep Outcomes.* 2023;7(1):17.
171. Schrijvers LH, Uitslager N, Schuurmans MJ, Fischer K. Barriers and motivators of adherence to prophylactic treatment in haemophilia: a systematic review. *Haemophilia.* 2013;19(3):355-361.
172. Hacker MR, Geraghty S, Manco-Johnson M. Barriers to compliance with prophylaxis therapy in haemophilia. *Haemophilia.* 2001;7(4):392-396.
173. Schrijvers LH, Kars MC, Beijlevelt-van der Zande M, Peters M, Schuurmans MJ, Fischer K. Unravelling adherence to prophylaxis in haemophilia: a patients' perspective. *Haemophilia.* 2015;21(5):612-621.
174. Krishnan S, Vietri J, Furlan R, Duncan N. Adherence to prophylaxis is associated with better outcomes in moderate and severe haemophilia: results of a patient survey. *Haemophilia.* 2015;21(1):64-70.
175. Van den Berg HM, Dunn A, Fischer K, Blanchette VS. Prevention and treatment of musculoskeletal disease in the haemophilia population: role of prophylaxis and synovectomy. *Haemophilia.* 2006;12 Suppl 3:159-168.

176. Di Minno A, Spadarella G, Nardone A, et al. Attempting to remedy sub-optimal medication adherence in haemophilia: The rationale for repeated ultrasound visualisations of the patient's joint status. *Blood Rev.* 2019;33:106-116.
177. Torres-Ortuño A. Adherence to prophylactic treatment. *Blood Coagul Fibrinolysis.* 2019;30(1S Suppl 1):S19-s21.
178. Gouw SC, van den Berg HM, Fischer K, et al. Intensity of factor VIII treatment and inhibitor development in children with severe hemophilia A: the RODIN study. *Blood.* 2013;121(20):4046-4055.
179. d'Oiron R, Pipe SW, Jacquemin M. Mild/moderate haemophilia A: new insights into molecular mechanisms and inhibitor development. *Haemophilia.* 2008;14 Suppl 3:138-146.
180. Leissinger CA, Singleton T, Kruse-Jarres R. How I use bypassing therapy for prophylaxis in patients with hemophilia A and inhibitors. *Blood.* 2015;126(2):153-159.
181. Lillicrap D, Fijnvandraat K, Santagostino E. Inhibitors - genetic and environmental factors. *Haemophilia.* 2014;20 Suppl 4:87-93.
182. Astermark J, Donfield SM, Gomperts ED, et al. The polygenic nature of inhibitors in hemophilia A: results from the Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort. *Blood.* 2013;121(8):1446-1454.
183. Volkers P, Hanschmann KM, Calvez T, et al. Recombinant factor VIII products and inhibitor development in previously untreated patients with severe haemophilia A: Combined analysis of three studies. *Haemophilia.* 2019;25(3):398-407.
184. Cormier M, Batty P, Tarrant J, Lillicrap D. Advances in knowledge of inhibitor formation in severe haemophilia A. *Br J Haematol.* 2020;189(1):39-53.
185. Astermark J. FVIII inhibitors: pathogenesis and avoidance. *Blood.* 2015;125(13):2045-2051.
186. Aledort LM, Dimichele DM. Inhibitors occur more frequently in African-American and Latino haemophiliacs. *Haemophilia.* 1998;4(1):68.
187. Gouw SC, van der Bom JG, Marijke van den Berg H. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL cohort study. *Blood.* 2007;109(11):4648-4654.
188. Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. *Thromb Haemost.* 1995;73(2):247-251.
189. Lassila R. Management of coagulation factor VIII (FVIII) inhibitors. *Thromb Res.* 2019;181 Suppl 1:S60-s61.
190. Verbruggen B. Diagnosis and quantification of factor VIII inhibitors. *Haemophilia.* 2010;16(102):20-24.
191. Leissinger CA. Prevention of bleeds in hemophilia patients with inhibitors: emerging data and clinical direction. *Am J Hematol.* 2004;77(2):187-193.

192. Tjønnfjord GE, Holme PA. Factor eight inhibitor bypass activity (FEIBA) in the management of bleeds in hemophilia patients with high-titer inhibitors. *Vasc Health Risk Manag.* 2007;3(4):527-531.
193. Astermark J, Donfield SM, DiMichele DM, et al. A randomized comparison of bypassing agents in hemophilia complicated by an inhibitor: the FEIBA NovoSeven Comparative (FENOC) Study. *Blood.* 2007;109(2):546-551.
194. Benson G, Auerswald G, Elezović I, et al. Immune tolerance induction in patients with severe hemophilia with inhibitors: expert panel views and recommendations for clinical practice. *Eur J Haematol.* 2012;88(5):371-379.
195. Ljung R, Auerswald G, Benson G, et al. Inhibitors in haemophilia A and B: Management of bleeds, inhibitor eradication and strategies for difficult-to-treat patients. *Eur J Haematol.* 2019;102(2):111-122.
196. DiMichele DM, Hoots WK, Pipe SW, Rivard GE, Santagostino E. International workshop on immune tolerance induction: consensus recommendations. *Haemophilia.* 2007;13 Suppl 1:1-22.
197. Hay CR, DiMichele DM. The principal results of the International Immune Tolerance Study: a randomized dose comparison. *Blood.* 2012;119(6):1335-1344.
198. Collins PW, Chalmers E, Hart DP, et al. Diagnosis and treatment of factor VIII and IX inhibitors in congenital haemophilia: (4th edition). UK Haemophilia Centre Doctors Organization. *Br J Haematol.* 2013;160(2):153-170.
199. Oldenburg J, Mahlangu JN, Kim B, et al. Emicizumab Prophylaxis in Hemophilia A with Inhibitors. *N Engl J Med.* 2017;377(9):809-818.
200. Nogami K, Shima M. Current and future therapies for haemophilia-Beyond factor replacement therapies. *Br J Haematol.* 2023;200(1):23-34.
201. Carcao M, Mancuso ME, Young G, Jiménez-Yuste V. Key questions in the new hemophilia era: update on concomitant use of FVIII and emicizumab in hemophilia A patients with inhibitors. *Expert Rev Hematol.* 2021;14(2):143-148.
202. Warrier AI, Lusher JM. DDAVP: a useful alternative to blood components in moderate hemophilia A and von Willebrand disease. *J Pediatr.* 1983;102(2):228-233.
203. Mannucci PM. Desmopressin (DDAVP) in the treatment of bleeding disorders: the first 20 years. *Blood.* 1997;90(7):2515-2521.
204. Hews-Girard J, Rydz N, Lee A, Goodyear MD, Poon MC. Desmopressin in non-severe haemophilia A: Test-response and clinical outcomes in a single Canadian centre review. *Haemophilia.* 2018;24(5):720-725.
205. Castaman G. Desmopressin for the treatment of haemophilia. *Haemophilia.* 2008;14 Suppl 1:15-20.
206. Mannucci PM, Bettega D, Cattaneo M. Patterns of development of tachyphylaxis in patients with haemophilia and von Willebrand disease after repeated doses of desmopressin (DDAVP). *Br J Haematol.* 1992;82(1):87-93.
207. Neff AT. Current controversies in the diagnosis and management of von Willebrand disease. *Ther Adv Hematol.* 2015;6(4):209-216.
208. Neff AT, Sidonio RF, Jr. Management of VWD. *Hematology Am Soc Hematol Educ Program.* 2014;2014(1):536-541.

209. Mannucci PM. Hemostatic drugs. *N Engl J Med.* 1998;339(4):245-253.
210. Frachon X, Pommereuil M, Berthier AM, et al. Management options for dental extraction in hemophiliacs: a study of 55 extractions (2000-2002). *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2005;99(3):270-275.
211. Kouides PA, Byams VR, Philipp CS, et al. Multisite management study of menorrhagia with abnormal laboratory haemostasis: a prospective crossover study of intranasal desmopressin and oral tranexamic acid. *Br J Haematol.* 2009;145(2):212-220.
212. Young G, Liesner R, Chang T, et al. A multicenter, open-label phase 3 study of emicizumab prophylaxis in children with hemophilia A with inhibitors. *Blood.* 2019;134(24):2127-2138.
213. Mahlangu J, Oldenburg J, Paz-Priel I, et al. Emicizumab Prophylaxis in Patients Who Have Hemophilia A without Inhibitors. *N Engl J Med.* 2018;379(9):811-822.
214. Shima M, Nogami K, Nagami S, et al. A multicentre, open-label study of emicizumab given every 2 or 4 weeks in children with severe haemophilia A without inhibitors. *Haemophilia.* 2019;25(6):979-987.
215. Pipe SW, Trzaskoma B, Minhas M, et al. Efficacy of emicizumab is maintained throughout dosing intervals for bleed prophylaxis. *Res Pract Thromb Haemost.* 2023;7(2):100077.
216. Pipe SW, Shima M, Lehle M, et al. Efficacy, safety, and pharmacokinetics of emicizumab prophylaxis given every 4 weeks in people with haemophilia A (HAVEN 4): a multicentre, open-label, non-randomised phase 3 study. *Lancet Haematol.* 2019;6(6):e295-e305.
217. Lenting PJ. Laboratory monitoring of hemophilia A treatments: new challenges. *Blood Adv.* 2020;4(9):2111-2118.
218. Ferrière S, Peyron I, Christophe OD, et al. A hemophilia A mouse model for the in vivo assessment of emicizumab function. *Blood.* 2020;136(6):740-748.
219. Mancuso ME, Male C, Kenet G, et al. Prophylaxis in children with haemophilia in an evolving treatment landscape. *Haemophilia.* 2021;27(6):889-896.
220. Hassan E, Motwani J. Breakthrough bleeding episodes in pediatric severe hemophilia a patients with and without inhibitors receiving emicizumab prophylaxis: a single-center retrospective review. *Pediatr Hematol Oncol.* 2022;39(5):418-426.
221. Collins PW, Liesner R, Makris M, et al. Treatment of bleeding episodes in haemophilia A complicated by a factor VIII inhibitor in patients receiving Emicizumab. Interim guidance from UKHCDO Inhibitor Working Party and Executive Committee. *Haemophilia.* 2018;24(3):344-347.
222. Barg AA, Budnik I, Avishai E, et al. Emicizumab prophylaxis: Prospective longitudinal real-world follow-up and monitoring. *Haemophilia.* 2021;27(3):383-391.
223. Samuelson Bannow B, Recht M, Négrier C, et al. Factor VIII: Long-established role in haemophilia A and emerging evidence beyond haemostasis. *Blood Rev.* 2019;35:43-50.

224. Gay ND, Lee SC, Liel MS, Sochacki P, Recht M, Taylor JA. Increased fracture rates in people with haemophilia: a 10-year single institution retrospective analysis. *Br J Haematol.* 2015;170(4):584-586.
225. Iorio A, Fabbriciani G, Marcucci M, Brozzetti M, Filipponi P. Bone mineral density in haemophilia patients. A meta-analysis. *Thromb Haemost.* 2010;103(3):596-603.
226. Machin N, Ragni MV. An investigational RNAi therapeutic targeting antithrombin for the treatment of hemophilia A and B. *J Blood Med.* 2018;9:135-140.
227. Chowdary P, Lethagen S, Friedrich U, et al. Safety and pharmacokinetics of anti-TFPI antibody (concizumab) in healthy volunteers and patients with hemophilia: a randomized first human dose trial. *J Thromb Haemost.* 2015;13(5):743-754.
228. Ahnström J. The potential of serpins for future treatment for haemophilia. *J Thromb Haemost.* 2019;17(10):1629-1631.
229. Négrier C PK, Ragni MV, et al. Fitusiran, an siRNA therapeutic targeting antithrombin for the treatment of haemophilia: proposed revisions to dose and regimen as a risk mitigation for vascular thrombosis. 14th Annual Congress of the European Association for Haemophilia and Allied Disorders, Feb 3–5, 2021; virtual congress.
230. Young G, Srivastava A, Kavakli K, et al. Efficacy and safety of fitusiran prophylaxis in people with haemophilia A or haemophilia B with inhibitors (ATLAS-INH): a multicentre, open-label, randomised phase 3 trial. *Lancet.* 2023;401(10386):1427-1437.
231. Matsushita T, Shapiro A, Abraham A, et al. Phase 3 Trial of Concizumab in Hemophilia with Inhibitors. *N Engl J Med.* 2023;389(9):783-794.
232. Stephanie Valer Seremetis M, Katarina Cepo, MD, Josephine Skovgaard Rasmussen, DVM, Trine Høyer Rose, PhD, Søren Tamer, MSc, Thomas Porstmann, PhD, Jesper Haaning, PhD. Risk Mitigation Strategy for Concizumab Clinical Trials after Pause Due to Non-Fatal Thrombotic Events. *Blood* 2020;Volume 136, Supplement 1, 5 November 2020, Page 40.
233. Trevor Baglin AK, Irina Mocanu, Levani Makhaldiani, James A. Huntington. Serpinpc in Persons with Severe Hemophilia (PwH): Updated Results from a Multi-Center, Multi-Part, First-in-Human Study. *Blood (2022) 140 (Supplement 1): 460–461.*
234. Swan D, Mahlangu J, Thachil J. Non-factor therapies for bleeding disorders: A primer for the general haematologist. *EJHaem.* 2022;3(3):584-595.
235. Nathwani AC. Gene therapy for hemophilia. *Hematology Am Soc Hematol Educ Program.* 2022;2022(1):569-578.
236. Mingozzi F, High KA. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. *Blood.* 2013;122(1):23-36.
237. Erles K, Sebökovà P, Schlehofer JR. Update on the prevalence of serum antibodies (IgG and IgM) to adeno-associated virus (AAV). *J Med Virol.* 1999;59(3):406-411.
238. Batty P, Lillicrap D. Gene therapy for hemophilia: Current status and laboratory consequences. *Int J Lab Hematol.* 2021;43 Suppl 1:117-123.

239. Ozelo MC, Mahlangu J, Pasi KJ, et al. Valoctocogene Roxaparvovec Gene Therapy for Hemophilia A. *N Engl J Med.* 2022;386(11):1013-1025.
240. George LA, Monahan PE, Eyster ME, et al. Multiyear Factor VIII Expression after AAV Gene Transfer for Hemophilia A. *N Engl J Med.* 2021;385(21):1961-1973.
241. Kaczmarek R. Gene therapy - are we ready now? *Haemophilia.* 2022;28 Suppl 4(Suppl 4):35-43.
242. Pasi KJ, Rangarajan S, Mitchell N, et al. Multiyear Follow-up of AAV5-hFVIII-SQ Gene Therapy for Hemophilia A. *N Engl J Med.* 2020;382(1):29-40.
243. Mahlangu J, Kaczmarek R, von Drygalski A, et al. Two-Year Outcomes of Valoctocogene Roxaparvovec Therapy for Hemophilia A. *N Engl J Med.* 2023;388(8):694-705.
244. Leebeek FWG, Miesbach W. Gene therapy for hemophilia: a review on clinical benefit, limitations, and remaining issues. *Blood.* 2021;138(11):923-931.
245. Pierce GF, Iorio A. Past, present and future of haemophilia gene therapy: From vectors and transgenes to known and unknown outcomes. *Haemophilia.* 2018;24 Suppl 6:60-67.
246. Hermans C, Gruel Y, Frenzel L, Krumb E. How to translate and implement the current science of gene therapy into haemophilia care? *Ther Adv Hematol.* 2023;14:20406207221145627.
247. Rodriguez-Merchan EC. Musculoskeletal complications of hemophilia. *Hss j.* 2010;6(1):37-42.
248. Roosendaal G, Jansen NW, Schutgens R, Lafeber FP. Haemophilic arthropathy: the importance of the earliest haemarthroses and consequences for treatment. *Haemophilia.* 2008;14 Suppl 6:4-10.
249. Holstein K, Klamroth R, Richards M, Carvalho M, Pérez-Garrido R, Gringeri A. Pain management in patients with haemophilia: a European survey. *Haemophilia.* 2012;18(5):743-752.
250. Mannucci PM, Schutgens RE, Santagostino E, Mauser-Bunschoten EP. How I treat age-related morbidities in elderly persons with hemophilia. *Blood.* 2009;114(26):5256-5263.
251. Auerswald G, Dolan G, Duffy A, et al. Pain and pain management in haemophilia. *Blood Coagul Fibrinolysis.* 2016;27(8):845-854.
252. Rodriguez-Merchan EC, De la Corte-Rodriguez H, Alvarez-Roman MT, Gomez-Cardero P, Jimenez-Yuste V. Radiosynovectomy for the Treatment of Chronic Hemophilic Synovitis: An Old Technique, but Still Very Effective. *J Clin Med.* 2022;11(24).
253. Anazor FC, Uthraraj N, Southgate C, Dhinsa B. Mid-to long-term postoperative outcomes of ankle joint fusion in patients with haemophilia: A systematic review. *Haemophilia.* 2023.
254. Rodriguez-Merchan EC, De la Corte-Rodriguez H, Alvarez-Roman T, Gomez-Cardero P, Encinas-Ullan CA, Jimenez-Yuste V. Total knee arthroplasty in hemophilia: lessons learned and projections of what's next for hemophilic knee joint health. *Expert Rev Hematol.* 2022;15(1):65-82.

255. Lobet S, Pendeville E, Dalzell R, et al. The role of physiotherapy after total knee arthroplasty in patients with haemophilia. *Haemophilia.* 2008;14(5):989-998.
256. McEneny-King A, Yeung CH, Edginton AN, Iorio A, Croteau SE. Clinical application of Web Accessible Population Pharmacokinetic Service-Hemophilia (WAPPS-Hemo): Patterns of blood sampling and patient characteristics among clinician users. *Haemophilia.* 2020;26(1):56-63.
257. Inc. B. MyPKFiT User Manual, DHF-000951 Revision 4.0
. 2020; https://fr-prd-hema.mypkfit.com/documents/DHF-000951_MYPKFIT3_HCP_USER_MANUAL_en_GB.pdf. Accessed October 2020
258. Hajducek DM, Chelle P, Hermans C, et al. Development and evaluation of the population pharmacokinetic models for FVIII and FIX concentrates of the WAPPS-Hemo project. *Haemophilia.* 2020;26(3):384-400.
259. Chelle P, Hajducek D, Mahdi M, et al. External qualification of the Web-Accessible Population Pharmacokinetic Service-Hemophilia (WAPPS-Hemo) models for octocog alfa using real patient data. *Res Pract Thromb Haemost.* 2021;5(7):e12599.
260. St-Louis J, Abad A, Funk S, et al. The Hemophilia Joint Health Score version 2.1 Validation in Adult Patients Study: A multicenter international study. *Res Pract Thromb Haemost.* 2022;6(2):e12690.
261. Hilliard P, Funk S, Zourikian N, et al. Hemophilia joint health score reliability study. *Haemophilia.* 2006;12(5):518-525.
262. Gilbert MS. Prophylaxis: musculoskeletal evaluation. *Semin Hematol.* 1993;30(3 Suppl 2):3-6.
263. Gouw SC, Timmer MA, Srivastava A, et al. Measurement of joint health in persons with haemophilia: A systematic review of the measurement properties of haemophilia-specific instruments. *Haemophilia.* 2019;25(1):e1-e10.
264. Foppen W, van der Schaaf IC, Fischer K. Value of routine ultrasound in detecting early joint changes in children with haemophilia using the 'Haemophilia Early Arthropathy Detection with UltraSound' protocol. *Haemophilia.* 2016;22(1):121-125.
265. Kandagaddala M, Sundaramoorthy M, Keshava SN, et al. A new and simplified comprehensive ultrasound protocol of haemophilic joints: the Universal Simplified Ultrasound (US-US) protocol. *Clin Radiol.* 2019;74(11):897.e899-897.e816.
266. Duncan N, Kronenberger W, Roberson C, Shapiro A. VERITAS-Pro: a new measure of adherence to prophylactic regimens in haemophilia. *Haemophilia.* 2010;16(2):247-255.
267. Cuesta-Barriuso R, Torres-Ortuño A, Galindo-Piñana P, Nieto-Munuera J, Duncan N, López-Pina JA. Validation of the VERITAS-Pro treatment adherence scale in a Spanish sample population with hemophilia. *Patient Prefer Adherence.* 2017;11:653-660.
268. Miesbach W, Kalnins W. Adherence to prophylactic treatment in patients with haemophilia in Germany. *Haemophilia.* 2016;22(5):e367-374.
269. EuroQol--a new facility for the measurement of health-related quality of life. *Health Policy.* 1990;16(3):199-208.

270. Foundation ER. EQ-5D-5L User Guide. 2019; https://euroqol.org/publications/user-guides.
271. Burström K, Teni FS, Gerdtham UG, et al. Experience-Based Swedish TTO and VAS Value Sets for EQ-5D-5L Health States. *Pharmacoeconomics.* 2020;38(8):839-856.
272. Ljung R, Fischer K, Carcao M, Santagostino E, Manco-Johnson MJ, Mathew P. Practical considerations in choosing a factor VIII prophylaxis regimen: Role of clinical phenotype and trough levels. *Thromb Haemost.* 2016;115(5):913-920.
273. Cid AR, Calabuig M, Cortina V, et al. One-stage and chromogenic FVIII:C assay discrepancy in mild haemophilia A and the relationship with the mutation and bleeding phenotype. *Haemophilia.* 2008;14(5):1049-1054.
274. Trossaërt M, Boisseau P, Quemener A, et al. Prevalence, biological phenotype and genotype in moderate/mild hemophilia A with discrepancy between one-stage and chromogenic factor VIII activity. *J Thromb Haemost.* 2011;9(3):524-530.
275. Marlar RA, Strandberg K, Shima M, Adcock DM. Clinical utility and impact of the use of the chromogenic vs one-stage factor activity assays in haemophilia A and B. *Eur J Haematol.* 2020;104(1):3-14.
276. Mikaelsson M, Oswaldsson U, Sandberg H. Influence of phospholipids on the assessment of factor VIII activity. *Haemophilia.* 1998;4(4):646-650.
277. St Ledger K, Feussner A, Kalina U, et al. International comparative field study evaluating the assay performance of AFSTYLA in plasma samples at clinical hemostasis laboratories. *J Thromb Haemost.* 2018;16(3):555-564.
278. Mikaelsson M, Oswaldsson U. Assaying the circulating factor VIII activity in hemophilia A patients treated with recombinant factor VIII products. *Semin Thromb Hemost.* 2002;28(3):257-264.
279. Preijers T, van Moort I, Fijnvandraat K, Leebeek FWG, Cnossen MH, Mathôt RAA. Cross-evaluation of Pharmacokinetic-Guided Dosing Tools for Factor VIII. *Thromb Haemost.* 2018;118(3):514-525.
280. Bukkems LH, Jönsson S, Cnossen MH, Karlsson MO, Mathôt RAA. Relationship between factor VIII levels and bleeding for rFVIII-SingleChain in severe hemophilia A: A repeated time-to-event analysis. *CPT Pharmacometrics Syst Pharmacol.* 2023.
281. Rayment R, Chalmers E, Forsyth K, et al. Guidelines on the use of prophylactic factor replacement for children and adults with Haemophilia A and B. *Br J Haematol.* 2020;190(5):684-695.
282. van Galen KPM, de Kleijn P, Foppen W, et al. Long-term impact of joint bleeds in von Willebrand disease: a nested case-control study. *Haematologica.* 2017;102(9):1486-1493.
283. Dover S, Blanchette VS, Wrathall D, et al. Hemophilia prophylaxis adherence and bleeding using a tailored, frequency-escalated approach: The Canadian Hemophilia Primary Prophylaxis Study. *Res Pract Thromb Haemost.* 2020;4(2):318-325.
284. Iorio A, Blanchette V, Blatny J, Collins P, Fischer K, Neufeld E. Estimating and interpreting the pharmacokinetic profiles of individual patients with hemophilia A or B using a population pharmacokinetic approach: communication from the SSC of the ISTH. *J Thromb Haemost.* 2017;15(12):2461-2465.

285. Shah A, Delesen H, Garger S, Lalezari S. Pharmacokinetic properties of BAY 81-8973, a full-length recombinant factor VIII. *Haemophilia.* 2015;21(6):766-771.
286. Shah A, Solms A, Garmann D, et al. Improved Pharmacokinetics with BAY 81-8973 Versus Antihemophilic Factor (Recombinant) Plasma/Albumin-Free Method: A Randomized Pharmacokinetic Study in Patients with Severe Hemophilia A. *Clin Pharmacokinet.* 2017;56(9):1045-1055.
287. Di Paola J, Smith MP, Klamroth R, et al. ReFacto and Advate: a single-dose, randomized, two-period crossover pharmacokinetics study in subjects with haemophilia A. *Haemophilia.* 2007;13(2):124-130.
288. Klamroth R, Simpson M, von Depka-Prondzinski M, et al. Comparative pharmacokinetics of rVIII-SingleChain and octocog alfa (Advate(®)) in patients with severe haemophilia A. *Haemophilia.* 2016;22(5):730-738.
289. Tagliaferri A, Di Perna C, Rivolta GF. Secondary prophylaxis in adolescent and adult haemophiliacs. *Blood Transfus.* 2008;6 Suppl 2(Suppl 2):s17-20.
290. Ljung R. Aspects of prophylactic treatment of hemophilia. *Thromb J.* 2016;14(Suppl 1):30.
291. Collins P, Faradji A, Morfini M, Enriquez MM, Schwartz L. Efficacy and safety of secondary prophylactic vs. on-demand sucrose-formulated recombinant factor VIII treatment in adults with severe hemophilia A: results from a 13-month crossover study. *J Thromb Haemost.* 2010;8(1):83-89.
292. Roosendaal G, Tekoppele JM, Vianen ME, van den Berg HM, Lafeber FP, Bijlsma JW. Articular cartilage is more susceptible to blood induced damage at young than at old age. *J Rheumatol.* 2000;27(7):1740-1744.
293. O'Hara J, Martin AP, Nugent D, et al. Evidence of a disability paradox in patient-reported outcomes in haemophilia. *Haemophilia.* 2021;27(2):245-252.
294. Oldenburg J, Tran H, Peyvandi F, et al. Health-related quality of life and health status in adolescent and adult people with haemophilia A without factor VIII inhibitors-A non-interventional study. *Haemophilia.* 2021;27(3):398-407.
295. Cavazza M, Kodra Y, Armeni P, et al. Social/economic costs and quality of life in patients with haemophilia in Europe. *Eur J Health Econ.* 2016;17 Suppl 1:53-65.
296. Carlsson KS, Höjgård S, Lindgren A, et al. Costs of on-demand and prophylactic treatment for severe haemophilia in Norway and Sweden. *Haemophilia.* 2004;10(5):515-526.
297. Witkop M, Lambing A, Divine G, Kachalsky E, Rushlow D, Dinnen J. A national study of pain in the bleeding disorders community: a description of haemophilia pain. *Haemophilia.* 2012;18(3):e115-119.
298. Rodriguez-Merchan EC, Jimenez-Yuste V, Aznar JA, et al. Joint protection in haemophilia. *Haemophilia.* 2011;17 Suppl 2:1-23.
299. Wojdasiewicz P, Poniatowski Ł A, Nauman P, et al. Cytokines in the pathogenesis of hemophilic arthropathy. *Cytokine Growth Factor Rev.* 2018;39:71-91.

300. Liesner RJ, Abashidze M, Aleinikova O, et al. Immunogenicity, efficacy and safety of Nuwiq(®) (human-cl rhFVIII) in previously untreated patients with severe haemophilia A-Interim results from the NuProtect Study. *Haemophilia.* 2018;24(2):211-220.
301. Scott MJ, Xiang H, Hart DP, et al. Treatment regimens and outcomes in severe and moderate haemophilia A in the UK: The THUNDER study. *Haemophilia.* 2019;25(2):205-212.
302. St-Louis J AA, Akins S, Austin S, Chowdary P, Classey S, Funk S, Hernandez G, Hilliard P, Manco-Johnson M, Mangles S, McLaughlin P, Nugent D, Shapiro A, Srivastava A, Tilak M, Wells A, Zourikian N, Blanchette V, Feldman BM. . Reference Ranges of HJHS Scores in Healthy Adult Males without Hemophilia [abstract]. *Res Pract Thromb Haemost 2020; 4 (Suppl 1).* 2020.
303. Schmidt DE, Michalopoulou A, Fischer K, et al. Long-term joint outcomes in adolescents with moderate or severe haemophilia A. *Haemophilia.* 2022;28(6):1054-1061.
304. Lundin B, Baghaei F, Holmström M, et al. Haemophilia A and B - evaluation of the Swedish prophylactic regimen by magnetic resonance imaging. *Haemophilia.* 2023;29(1):193-198.
305. Foppen W, van der Schaaf IC, Beek FJA, Mali W, Fischer K. Diagnostic accuracy of point-of-care ultrasound for evaluation of early blood-induced joint changes: Comparison with MRI. *Haemophilia.* 2018;24(6):971-979.
306. Stephensen D, Classey S, Harbidge H, Patel V, Taylor S, Wells A. Physiotherapist inter-rater reliability of the Haemophilia Early Arthropathy Detection with Ultrasound protocol. *Haemophilia.* 2018;24(3):471-476.
307. Sun J, Hilliard PE, Feldman BM, et al. Chinese Hemophilia Joint Health Score 2.1 reliability study. *Haemophilia.* 2014;20(3):435-440.
308. Hmida J, Hilberg T, Ransmann P, et al. Most subjectively affected joints in patients with haemophilia - what has changed after 20 years in Germany? *Haemophilia.* 2022;28(4):663-670.
309. Papadatou-Pastou M, Ntolka E, Schmitz J, et al. Human handedness: A meta-analysis. *Psychol Bull.* 2020;146(6):481-524.
310. Nijdam A, Bladen M, Hubert N, et al. Using routine Haemophilia Joint Health Score for international comparisons of haemophilia outcome: standardization is needed. *Haemophilia.* 2016;22(1):142-147.
311. van Leeuwen FHP, van Bergen EDP, Timmer MA, et al. Magnetic resonance imaging evidence for subclinical joint bleeding in a Dutch population of people with severe hemophilia on prophylaxis. *J Thromb Haemost.* 2023;21(5):1156-1163.
312. Meijón Ortigueira MDM, Álvarez-Román MT, De La Corte Rodríguez H, Butta Coll N, Jiménez-Yuste V. Long-term impact of primary prophylaxis on joint status in patients with severe hemophilia A. *Res Pract Thromb Haemost.* 2023;7(1):100005.
313. Faber JC, Burnouf T. Bitter progress in the treatment of haemophilia A in low-income countries. *Lancet Haematol.* 2018;5(6):e239.

314. Ndoumba-Mintya A, Diallo YL, Tayou TC, Mbanya DN. Optimizing Haemophilia Care in Resource-Limited Countries: Current Challenges and Future Prospects. *J Blood Med.* 2023;14:141-146.
315. Bolton-Maggs PH. Optimal haemophilia care versus the reality. *Br J Haematol.* 2006;132(6):671-682.
316. Berntorp E, Astermark J, Jurgutis R, Lethagen S, Petersson C. The Malmö-Klaipeda WFH twinning programme: a comparative description of the haemophilia cohorts. *Haemophilia.* 1998;4(2):79-82.
317. Lambert C, Meité N, Sanogo I, et al. Haemophilia in Côte d'Ivoire (the Ivory Coast) in 2017: Extensive data collection as part of the World Federation of Hemophilia's twinning programme. *Haemophilia.* 2019;25(2):236-243.
318. Pierce GF, Adediran M, Diop S, et al. Achieving access to haemophilia care in low-income and lower-middle-income countries: expanded Humanitarian Aid Program of the World Federation of Hemophilia after 5 years. *Lancet Haematol.* 2022;9(9):e689-e697.
319. Lambert C, Meité N, Kouassi GK, et al. Nonreplacement therapy for hemophilia in low-income countries: experience from a prospective study in Ivory Coast. *Res Pract Thromb Haemost.* 2023;7(1):100033.
320. Lambert C, Meité N, Sanogo I, Lobet S, Hermans C. Feasibility and outcomes of low-dose and low-frequency prophylaxis with recombinant extended half-life products (Fc-rFVIII and Fc-rFIX) in Ivorian children with hemophilia: Two-year experience in the setting of World Federation of Haemophilia humanitarian aid programme. *Haemophilia.* 2021;27(1):33-40.
321. Shetty S, Bansal S, Kshirsagar S, Rangarajan S, Hajirnis K, Phadke V. Low-dose prophylaxis and its impact on the health of haemophilia patients. *Vox Sang.* 2022;117(7):900-912.
322. Nwagha TU, Okoye HC, Udo CE, Yuguda S, Korubo KI, Adeyemo TA. Clinical Audit of Low Dose Prophylaxis Programme for Nigerian Children with Haemophilia. *West Afr J Med.* 2022;39(1):11-15.

Printed by Media-Tryck, Lund 2024 NORDIC SWAN ECOLABEL 3041 0903

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.

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Translational Medicine

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2024:56
ISBN 978-91-8021-549-7
ISSN 1652-8220