GHANA COLLEGE OF PHYSICIANS AND SURGEONS

FACULTY OF LABORATORY MEDICINE

DIVISION OF HAEMATOLOGY

FACTOR VIII INHIBITOR STATUS OF HAEMOPHILIA A PATIENTS AT THE KORLE-BU TEACHING HOSPITAL

Submitted to the Faculty of Laboratory Medicine, in partial fulfilment of the requirements for conferment of a *Fellow of the Ghana College of Physicians (FGCP).*

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LM/06/17/ 002/F

March 2023

DECLARATION

I, Nana Agyeiwah Awuku, hereby declare that this research work represents my own work except where specified in reference and has not been previously included in any dissertation submitted to any college or an institution for the award of a degree or other qualification, neither has it been published in any journal.

CERTIFICATION

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DEDICATION

To all persons living with Haemophilia.

ACKNOWLEDGEMENT

I would like to express my deepest appreciation to my supervisors Prof E. Olayemi and Dr Y. Dei-Adomakoh; their insight, suggestions, patience, and guidance helped bring this work to fruition.

My deepest gratitude goes to my husband Prof Yaw Asante Awuku; his belief in me, understanding, and continuous support has led to the successful completion of this work.

I am also grateful to Prof J.K. Acquaye for his push and encouragement.

Special thanks to Prof and Dr (Mrs) Obeng-Adjei for providing the coagulometer to carry out this work.

I would like to extend my sincere thanks to Mr Antwi Amoateng, Mrs Aimee Degbotse, Mrs Diana Dwomoh-Badu, Mr Enoch Alabi Mensah, all doctors, and nurses of the department of Haematology for their immense assistance during this project.

Many thanks to all persons living with Haemophilia A who participated in the research, without you, this would not have been possible.

This endeavour would not have been possible without the generous support from Roche Gh. Ltd.

My eternal thanks to the Almighty God for life and fortitude.

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LIST OF ABBREVIATIONS

ABR	Annual Bleeding Rate
APC	Activated Protein C
aPCC	Activated Prothrombin Complex Concentrate
APTT	Activated Partial Thromboplastin Time
Ca	Calcium
CANAL	Concerted Action on Neutralising Antibodies in Severe Haemophilia A
CBA	Chromogenic Bethesda Assay
ECH	Extracranial Haemorrhage
ED	Exposure Days
ELISA	Enzyme Linked Immunosorbent Assay
FEIBA	Factor Eight Inhibitor Bypassing Activity
FII	Factor Two
FIX	Factor Nine
FIXa;	Activated Factor Nine
FV	Factor Five
FVa	Activated Factor Five
FVIII	Factor Eight
FVIIIa	Activated Factor Eight
FX	Factor Ten
FXa	Activated Factor Ten
FXIII	Factor Thirteen
FXIIIa	Activated Factor Thirteen
GI	Gastrointestinal
HA	Haemophilic Arthropathy
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HTC	Haemophilia Treatment Centre
ICH	Intracranial Haemorrhage
IFNγ-	Interferon gamma
IL	Interleukin

IL-10	Interleukin Ten
ISTH	International Society on Thrombosis and Haemostasis
ITI	Immune Tolerance Induction
IU	International Units
MIBS	Malmo International Brother Study
ml	Millilitres
NBA	Nijmegen-Bethesda Assay
NNA	Non-neutralising Antibodies
NPP	Normal Pooled Plasma
pdFVIII	Plasma Derived Factor Eight
PL	Phospholipids
PPP	Platelet Poor Plasma
PT	Prothrombin Time
PUP	Previously Untreated Patient
rFVIIa	Recombinant Activated Factor Seven
rFVIII	Recombinant Factor Eight
RODIN	Research of Determinants of Inhibitor Development among PUPs with haemophilia
SIPPET	Survey of Inhibitors in Plasma Product Exposed Toddlers
TAFI	Thrombin Activatable Fibrinolysis Inhibitor
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TNF-α	Tumour Necrosis Factor-Alpha
tPA	Tissue Plasminogen Activator
TT	Thrombin Time
UK	United Kingdom
uPA	Urokinase Plasminogen Activator
US	United States
VEGF	Vascular-derived Endothelial Growth Factor
VWF	Von Willebrand Factor

ABSTRACT

Background: Haemophilia is an inherited bleeding disorder resulting from mutations in the Factor VIII (FVIII), Factor IX (FIX) or Factor XI (FXI) gene. These mutations give rise to Haemophilia A, B or C respectively. Haemophilia A and B clinically manifest as bleeding into joints, soft tissues, and muscles spontaneously or after minor trauma whilst Haemophilia C is commonly associated with mucosal bleeding which is usually provoked. Recombinant factor replacement is the mainstay of treatment for Haemophilia A, with inhibitor formation remaining the major challenge in the treatment with factor concentrate.

General Aim: This study determined the factor VIII inhibitor status among patients with Haemophilia A at the Korle-Bu Teaching Hospital

Methods: Cross-sectional study involving all haemophilia A patients attending both adult and paediatric clinics at Korle-Bu Teaching Hospital (KBTH). Patients were recruited after giving informed consent or assent and or parental permission where appropriate. A data extraction form was used to extract clinical data from the patient's medical records/folder.
4.5mls of blood was taken for measuring residual FVIII activity and inhibitor assay using Bethesda Assay. Statistical analysis was done using STATA version 14.

Results: All eighty-one (81) participants were male with 70.4%, 14.8%, 14.8% having severe, moderate, and mild disease respectively. The mean age of participants was 17.1 years (±13.5). Majority (93.8%) of participants had received some form of therapy which included recombinant FVIII concentrate, plasma derived FVIII concentrate, cryoprecipitate and emicizumab. A small number (6.2%) of participants had never been exposed to any form of therapy. Prevalence of inhibitors in this study was found to be 11.1% with 85.7% (n=6) having low titre inhibitors and 14.3% (n=1) having high titre inhibitors. All inhibitor positive participants were on on-demand therapy with majority (85.7%) having severe disease. Inhibitor positive participants had had higher bleeding episodes in the past year 2.14 (p=0.286). Most (57.1%) of the inhibitor positive participants had received only recombinant factor concentrate and 42.9% had received both recombinant and plasma derived concentrates (p=0.211).

Bleeding from the oral cavity was the commonest presentation at diagnosis (32.1%) followed by joint swelling and pain (21.0%). Almost half (46.9%) presented with spontaneous bleeding at diagnosis and 53.1% had provoked bleeding out of which 60% was trauma related and 40% from surgical/medical procedure**.** A small percentage (6.2%) of participants were on prophylactic therapy.

Conclusion: This study reported prevalence of FVIII inhibitor in haemophilia A patients to be 11.1% with majority of participants classified as severe haemophilia A. There is the need for education and increased awareness for early diagnosis and prevention of complications of haemophilia A; especially the development of inhibitors in Ghana to enable institution of appropriate therapy.

CHAPTER 1: INTRODUCTION

1.1. Background

Haemophilia is an inherited bleeding disorder resulting from mutations in the clotting factor VIII (FVIII), factor IX (FIX) or factor XI gene leading to deficiencies in FVIII, FIX, FXI respectively.[1–4] Haemophilia is classified as type A, B or C following deficiencies in FVIII, FIX or FXI respectively.[2–4] Haemophilia A and B are X-linked recessive diseases affecting mainly males in all ethnic groups whilst Haemophilia C is an autosomal recessive disease.[3,4]

Haemophilia is estimated to occur in about one in 10,000 births and over one million expected males worldwide having haemophilia, however most of these individuals are undiagnosed with over 400,000 diagnosed people living with haemophilia globally.[3] Haemophilia A is the commonest, representing 80-85% of all cases with haemophilia B accounting for 15-20%.[3] Based on Ghana's estimated population of 33,475,870 as at July 2022, the projected number of persons living with haemophilia will be over three thousand (3,000).[5] However, the Ghana Society of Haemophilia has three hundred and seventy (370) confirmed registered persons living with haemophilia as of September 2022, with the Korle-Bu Teaching Hospital having about one hundred and ten (110) individuals on its registry at both adult and child haemophilia clinics.

Haemophilia A and B clinically manifests as bleeding into joints, soft tissues and muscles after minor trauma, or spontaneously.[3,6] Haemophilia A and B are clinically indistinguishable, and the disease phenotype correlates with the degree of FVIII or FIX deficiency.[2,3,6] Individuals with severe phenotype usually present with intraarticular bleeds, intramuscular and soft tissue bleed often without significant trauma whilst those with mild disease may only present with a bleeding episode after facing a haemostatic challenge.[3]

Majority of patients with haemophilia A will present with at least one bleeding episode in the first two years of life.[6,7] In early childhood, bleeding usually results from circumcision and as the child grows, haemarthrosis involving weight bearing joints may be apparent.[6] Bleeding can however occur in any tissue such as mucosa, gastrointestinal, genitourinary and central nervous systems depending on the severity of the disease.[6]

Haemophilia C most often presents with bleeding episodes after surgical procedures.[8]

Management of haemophilia A requires multidisciplinary team care to achieve an overall excellent outcome.[3] The specialties include haematology, orthopaedics, dentistry, nursing, physiotherapy, social work, clinical psychology and other related fields depending on the presentation.[3]

Clotting factor replacement is the mainstay of treatment for bleeding episodes.[9] This may be given prophylactically to prevent bleeding episodes or progressive joint disease or given on-demand: where treatment is given at the time of clinically evident bleeding.[9,10] Prophylactic therapy started in early childhood or soon after first joint bleed has been shown to delay arthropathy; it is known to offer better overall outcome compared to on demand therapy.[11,12]

Inhibitor formation remains the major challenge in the management of haemophilia with limited treatment options for individuals with inhibitors.[11,13] Nearly one-third of individuals with severe haemophilia A will develop neutralizing antibodies/ inhibitors to clotting factor concentrates as well as 5% in individuals with moderate and mild disease.[13] Inhibitors are antibodies that are produced by the individual against factor concentrates.[3] Inhibitors render ineffective FVIII concentrate, leading to raised financial and psychological burden on patients as well as adverse consequences on morbidity and mortality through increased utilization of clotting factor concentrate, high hospitalization rate as well as surgeries.[11,13,14]

Risk of inhibitor formation is associated with many factors which include type of FVIII mutation, severity of haemophilia, polymorphism in immune modulatory genes, positive family history of inhibitor, black race, early age of onset of FVIII therapy, type of treatment regime, intensity of therapy and type of FVIII product.[6,14,15]

Individuals with null mutations, stop mutations and large deletions are associated with higher risk of developing inhibitors as opposed to those with non-null mutations or small deletions.[14,15] Severe haemophilia is associated with **a** higher risk of inhibitor formation compared with mild and moderate haemophilia.[15] Several studies have shown a higher risk of development of inhibitors in individuals with a family history of inhibitors compared to individuals without.[15] Individuals of African descent have been found to have higher risk of inhibitor formation compared to Caucasians.[15] Interleukin-10 (IL-10) and Tumour necrosis factor- alpha (TNFα) polymorphisms have been shown to have higher risk of inhibitor formation.[15] Development of inhibitors is at its highest during the initial 20-50 exposure days and reduces significantly after this period.[15]

Patients on standard prophylaxis have lower risk of formation of inhibitors compared to patients who received on-demand therapy.[15] FVIII concentrates are usually given in high doses and for a prolonged period during severe bleeding episodes, trauma and during surgeries. This causes an up-regulation of B and T lymphocytes leading to higher risk of inhibitor development.[15]

Early diagnosis of inhibitor is essential to enable required therapy to be started. The presence of inhibitor can be suspected on clinical grounds when the factor VIII concentrate is inadequate to prevent or stop bleeding in a patient who was earlier responsive to same dose of factor VIII concentrate.[3] Patients with mild or moderate disease who have inhibitors usually present with mucocutaneous bleeding, bleeding in the gastrointestinal system or genitourinary bleeding.[3]

Mixing studies is a screening tool for detecting FVIII inhibitors. More confirmatory inhibitor assays need to be done to confirm the presence of inhibitor. This can be performed by various methods which includes clot based-method, chromogenic methods or enzyme linked immunosorbent assays (ELISA).[16] The Bethesda assay or its Nijmegen modification are the most commonly used assays to quantify inhibitors to FVIII.[16] The Bethesda assay measures the residual FVIII after equal amounts of normal pooled plasma and plasma of suspected inhibitor are mixed.[16]

Treatment for individuals with FVIII inhibitor should be individualised and should depend on the site and severity of bleed, titre of inhibitor and availability of therapeutic agents.[3] High doses of FVIII concentrate are given to individuals with low titre inhibitors during bleeding episodes whilst patients with high titre inhibitors are managed with bypassing agents such as recombinant opj(rFVIIa) or activated prothrombin complex concentrate.[3,17] Immune tolerance induction (ITI) is currently the only way of achieving cure by eliminating FVIII inhibitors.[17] This is achieved by extended exposure to repeated high doses of FVIII concentrates.[17] Individuals can go back on factor concentrates once ITI is successful.[17] Individuals with mild haemophilia with low titre inhibitors who present with minor bleeding episodes can be given desmopressin.[18] Emicizumab, a bispecific monoclonal antibody that mimics the function of FVIII is a relatively new agent used in preventing bleeding episodes haemophilia A with and without inhibitors.[19,20]

1.2. Problem Statement

Haemophilia A is a chronic disease which requires long-term therapy and involves the use of clotting factor concentrate as the mainstay of treatment to prevent bleeding and joint disease. Clotting factor concentrate can be given prophylactically to prevent bleeding which is known as "prophylactic therapy".[3] The use of clotting factor concentrates in the management of Haemophilia A leads to development of inhibitors which is a major complication of treatment. These inhibitors are antibodies that neutralise the clotting factor given thus decreasing the efficacy of the clotting factor in stopping or preventing bleeding episodes leading to increased risk of morbidity and mortality.

The prevalence of clotting factor inhibitors has been reported to be between 20-33% in settings where prophylactic therapy is used which is the practice in the developed countries.[11] Prevalence of inhibitors in countries such as India where on-demand therapy is practiced is between 8.2-13%.[21]

In Ghana, patients only receive clotting factor concentrates on-demand due to unavailability of funds to support the prophylaxis approach. The cost of factor VIII concentrate ranges from $1.19 - $2.85 per international unit (IU) depending on the formulation and brand.[22] This will amount to $571.2/kg -$1,368/kg for a month's treatment for an individual on a 40IU/kg three times a week prophylaxis.

The current calculation of required dosage during on-demand therapy does not consider the presence of inhibitors. There is scanty data on clinical presentation of haemophilia A patients presenting to the Korle-Bu Teaching Hospital.

This study sought to do a clinical characterisation of patients with haemophilia A at the Korle-Bu Teaching Hospital and to establish the prevalence of inhibitor development among patients with Haemophilia A receiving on-demand therapy and help in developing an algorithm or the administration of emicizumab; a bispecific monoclonal antibody to haemophilia A with inhibitor to improve treatment outcomes.

1.3. Justification for the Study

Haemophilia A is an important clinical condition with improved quality of life over the years due to the use of clotting factors in the prevention and treatment of bleeding episodes and other complications.

The downside of clotting factor use in the management of haemophilia A is the development of inhibitors in the clotting factors which has the potential to reduce its efficacy and in the long run increase morbidity and mortality.

This study will provide baseline data on clinical characteristics of patients with haemophilia A and prevalence of factor inhibitors. The knowledge of the prevalence of inhibitors is important to evaluate the burden of inhibitors in patients in our unique setting and identify patterns and or clinical associations in inhibitor occurrence. This study will thus help develop protocols and influence guidelines for the improvement in the care of patients with haemophilia A in Ghana.

1.4. Aim

To determine factor VIII inhibitor status among patients with Haemophilia A.

1.5. Specific Objectives

- Classify patients with haemophilia A based on severity.
- Determine type of therapies received since diagnosis.
- Determine prevalence of inhibitor among patients with haemophilia A.
- Determine clinical factors associated with inhibitor development.

CHAPTER 2: LITERATURE REVIEW

2.1. History

The earliest mention of a condition in which there is excessive bleeding like haemophilia goes as far back as the 2nd century.[23] This is seen in Jewish writings in which the third son of a woman who had lost two sons earlier from circumcision because of excessive bleeding was exempted from the procedure.[23] There is also the description of males in a family who passed away due to bleeding from insignificant cuts by an Arabic physician in the 12th century.[23] The report is also made of a Jewish Rabbi who prevented a boy from being circumcised because sons of the mother's sisters had passed away after being circumcised.[24]

Modern descriptions of haemophilia however are seen much later in the 1800's when American physician, Dr John Conrad Otto described an inherited bleeding disorder in which only males are affected and transmission was through females who were unaffected.[23,24] The first genetic description was made by Nasse in 1820.[25] The word "haemophilia" which means "affinity to blood" was first documented by the German physician Schonlein and his student Hopff in 1828.[23,24] Schonlein and Hopff however preferred the term "haemorraphilia" which means "affinity to bleed" and both terms were used till the 1850's when haemophilia became the preferred choice.[24]

Haemophilia has been termed 'the royal disease' or 'the disease of kings' due to Queen Victoria of England (1837-1901) who was a carrier and passed on the disease to her son.[23,24] He had repeated bleeding episodes and passed away following an intracranial bleed.[23,24] Queen Victoria through her two daughters passed on the disease to other royal families of Europe namely the German, Spanish and Russian royal families.[23,24,26]

Excessive bleeding in haemophilia was initially thought to be as a result of weak blood vessels and later was attributed to defective platelet.[23] It was not until the late 1930's that Taylor and Patek established that they could correct the excessive bleeding with substance taken out of plasma and was known as anti-haemophilic globulin.[23] Pavlosky from Argentina in mid-1940, found that blood from one individual with haemophilia could correct the bleeding defect of another individual with haemophilia eventually leading to the identification of two different deficiencies.[23,24] The more common form was termed haemophilia A whilst the term haemophilia B was given to the

less common form. Haemophilia B was distinguished from haemophilia A in 1952 and named Christmas disease after its first documented patient by Biggs.[23,25]

The management of haemophilia A has advanced over the period from a fatal disease to a chronic disease in most recent times.[27] The treatment of haemophilia has evolved over the years from whole blood to fresh plasma, cryoprecipitate, plasma derived factor concentrates, recombinant factor concentrates and monoclonal antibody.

In the early years individuals living with haemophilia were managed with whole blood and fresh plasma however, these products did not contain adequate amounts of FVIII or FIX to stop severe episodes of bleeding.[23] Lancet in 1940 published the first successful treatment of haemophilia by transfusing whole blood in a boy who bled after strabismus surgery.[27] Transfusion with whole blood which did not contain adequate amounts of required clotting factor resulted in individuals with severe haemophilia passing away in early childhood most especially following trauma or surgery. Judith Pool in 1964 discovered that large amount of FVIII could be found in cryoprecipitate and this afforded the infusion of adequate FVIII in small volumes to stop bleeding episodes.[23,28]

The 1970's saw contemporary management of haemophilia where plasma concentrates were readily available and prophylactic therapy was being used to control bleeding episodes and reduce joint damage from recurrent bleeding as well as desmopressin which is used in mild haemophilia A.

Viral contamination of plasma concentrates in the 1980's globally, led to the development of new viral inactivation methods as well as enhanced ways of screening for viruses in blood donors as plasma concentrates were pooled from numerous donors to improve the safety of these plasma products. About two-thirds of individuals with severe haemophilia A were infected with human immunodeficiency virus (HIV) as well as hepatitis C virus (HCV) during that period mostly in the US.[23,24] This further led to the development of recombinant factor concentrates which have minimal human-derived proteins.[25]

2.2. Haemophilia A and B

Haemophilia A and B are X-linked recessive diseases due to mutations in the FVIII (Haemophilia A) or FIX (Haemophilia B) gene resulting in a deficiency in respective plasma proteins whilst Haemophilia C is an autosomal recessive disease resulting in deficiency in FXI.[2–4] Haemophilia A affects one in 5,000-10,000 live births, haemophilia B affects one in 25,000-30,000 live births and haemophilia C is estimated to affect 1in 1,000,000 births.[2,3,8]

Males are clinically affected in haemophilia A and B, even though females can also be affected in rare cases whilst haemophilia C is inherited in a Mendelian pattern.[29,30] Females who carry a single mutated gene in haemophilia A and B are carriers and generally asymptomatic, however it can occur in homozygous female, a female with lyonization that is inactivation of an X chromosome and in chromosomal abnormalities such as Turner's syndrome where there is only one X-chromosome.[29] Family history of the disease is absent in about 30% of individuals affected due to spontaneous mutations and mothers are usually the carriers of these de novo mutations.[2,9,31]

Haemophilia A and B are indistinguishable clinically and characterised by bleeding into joints, muscle, and other soft tissues spontaneously or after minor trauma.[2,31] The severity and frequency of bleeding correlates with residual activity of either FVIII or FIX.[2,3] Concentration for coagulation factor is expressed in international units (IU), and 1IU is the amount of the factor in 1ml of normal pooled plasma.[32] Haemophilia A or B are classified as severe (<0.01IU/ml; <1% of normal), moderate (0.01-0.05 IU/ml; 1-5% of normal) and mild (>0.05-0.4IU/ml; >5-40% of normal).[3,33] However, there can be discordance between the factor level and clinical phenotype.[2,31]

Bleeding into joints or muscles occur spontaneously in severe disease; bleeding is mostly in the absence of identifiable haemostatic challenge.[3] Individuals with moderate disease experience occasional spontaneous bleeding or prolonged bleeding in the presence of minor trauma or surgical procedure.[3] Spontaneous bleeding is rare in mild disease but they can have severe bleeding with major trauma or surgery.[3]

2.3. Haemophilia A

Haemophilia A represents about 80-85% of all haemophilia cases [3]. Kulkarni et al studied 547 babies who had been registered in Haemophilia Treatment Centres in the United States (US) out of which 82% had Haemophilia A.[6] Shokunbi et al had 93% haemophilia A patients in individuals with haemophilia presenting with acute bleeding episodes in a teaching hospital in Nigeria whilst Shaheen et al had 82.1% patients with haemophilia A in a study conducted to assess orthopaedic complications among persons living with haemophilia in Khartoum, Sudan.[31,34]

Haemophilia A is due to mutations in FVIII gene resulting in absent or reduced amounts of the FVIII protein and the gene which is made up of 26 exons is located on the distal end of the long arm of the X chromosome.[35]

Over 2100 mutations in the FVIII gene have been described with the type of mutation found in haemophilia A being an important determinant of the disease severity which directly correlates with the severity and frequency of bleeding episodes.[14,36] Point mutations, small rearrangements, single nucleotide deletions or splicing error mutations are associated with mild to moderate haemophilia A.[14] However, severe haemophilia A is associated with defects resulting from large deletions, inversions, insertions, non-sense and mis-sense mutations.[14] Inversion of intron 22 and intron 1 has been shown to occur in about 40-50% and 5% respectively in individuals with severe haemophilia A and it is one of the most common mutations found in severe haemophilia A.[35] Large deletions have also be shown to occur in about 5% of individuals.[35] Non-null mutations in the FVIII gene has been described in severe haemophilia A exhibiting mild bleeding episodes.[35]

Identification of mutation in individuals with haemophilia aids in the diagnosis of carriers, prenatal diagnosis, management in the neonatal period as well as to identify individuals with high risk of developing inhibitors to clotting factor concentrates.[36,37] Mutation analysis also helps differentiate mild haemophilia A from other inherited bleeding disorders.[36]

Haemostasis

Haemostasis is a tightly regulated physiological process whereby blood coagulation is started and terminated with the removal of blood clot resulting in the prevention of blood loss in response to vascular injury.[2,38] Haemostasis involves five (5) components namely blood vessels, platelet, coagulation factors, coagulation inhibitors and fibrinolytic system.[39]

The vessel wall is lined by endothelium which allows the various components of blood to pass uninterrupted through the circulatory system.[39,40] In the event of vascular injury, there is immediate vasoconstriction of the involved vessel as well as reflex constriction of nearby arterioles.[39] This is to slow down blood flow to the affected area and allow for activation of platelet and coagulation factors. The vascular endothelium is also responsible for triggering and regulating fibrinolysis.

Together with vessel and adhesive proteins, platelets play an essential role in haemostasis leading to the formation of an initial platelet plug. Platelet initially binds to collagen exposed by damage to the endothelium via glycoprotein receptor complexes and adherence is via GP1b which is mediated by von Willebrand factor.[39]

Collagen exposure as well as thrombin generated at the site of injury causes adhered platelet to release contents of its granules leading to activation of platelet. Activation of platelet results in a change in shape from discoid to spherical increasing platelet-platelet interaction with glycoprotein GPIIb-GPIIIa serving as the main receptor for platelet aggregation.[39] Platelet aggregation leads to the formation of the primary haemostatic/ platelet plug.

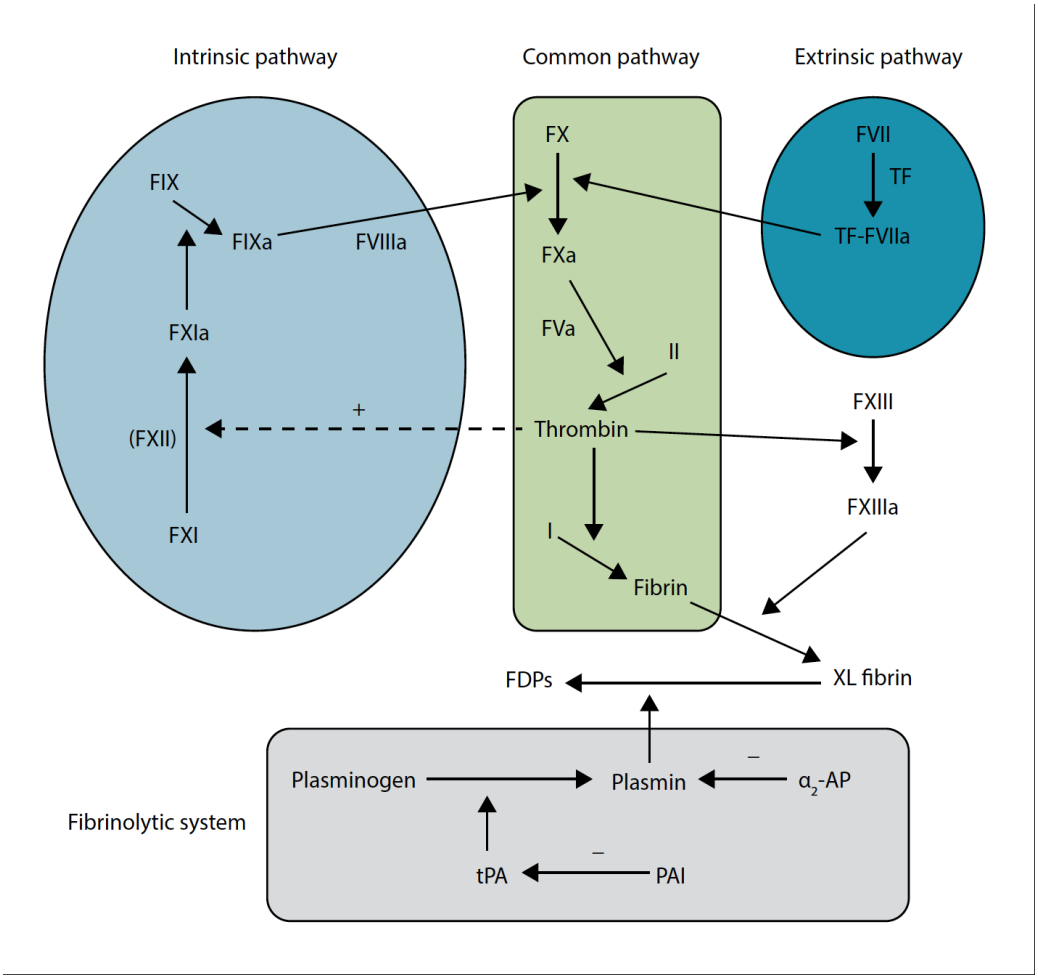


Figure 2.1 Schematic presentation of the coagulation cascade and fibrinolytic system.[2]

The coagulation cascade is an essential part of haemostasis and involves plasma proteins which when activated act as proteolytic enzymes which activate other inactive enzymes leading to the formation of an insoluble fibrin meshwork consolidating the platelet plug.[38,40] Coagulation results in the conversion of soluble fibrinogen to insoluble fibrin strands, which strengthen the aggregated platelets.[40]

Blood is exposed to cells expressing tissue factor (TF) following vascular injury, initiating blood coagulation. Exposure of circulating FVII to TF leads to activation of FVII with the TF-FVIIa complex (extrinsic tenase) activating FX and FIX.[39,40] Activated FX in the absence of FVa forms small amount of thrombin from prothrombin, this thrombin generated is however insufficient for

significant fibrin polymerization.[39] This is known as the initiation phase. Nonetheless, the thrombin generated by extrinsic tenase activates FV, FVIII and FXI.[38,39]

In the amplification phase of the coagulation process, activated FXI in turn activates FIX and forms the FIXa-FVIIIa complex (intrinsic tenase) which activates sufficient FXa.[38] Activated FX in the presence of its co-factor FVa, calcium(Ca) and phospholipids(PL) (prothrombinase) generates larger amounts of thrombin, and this is termed propagation.[39]

The thrombin generated results in the formation of fibrin from fibrinogen and activation of FXIII which covalently links soluble fibrin monomers to form a stable polymer and gives strength and stability to fibrin incorporated in the platelet plug.[39]

In-vivo, this traditional concept of coagulation with separate intrinsic and extrinsic pathways occurs concurrently.[38,40]

Coagulation is regulated and localised by several physiological anticoagulant factors. Antithrombin inhibits mainly thrombin and FXa and forms inactive complexes with factors IXa, Xa, XIa and XIIa.[39]

Thrombin activates protein C through binding to thrombomodulin and this Activated Protein C (APC) interacts with Protein S bound to phospholipid surface of activated platelets and enhance anticoagulant activity of APC against FVa and FVIIIa.[38–40]

Tissue Factor Pathway Inhibitor (TFPI) is mostly found on endothelial cell surface, and it inactivates TF-VIIa complex.[39]

Fibrinolysis is an enzymatic process mediated by plasmin that dissolves the fibrin clot into fibrin degradation products; this ensures excess fibrin deposition is prevented or rapidly removed.[39,40] The inactive precursor of plasmin i.e., plasminogen is activated by tissue plasminogen activator and urokinase released into the blood by the damaged endothelium.[40] Plasmin activity is tightly regulated by its inhibitor, alpha-2 antiplasmin, preventing widespread fibrinolysis.[38,39]

Haemostasis is a tightly regulated process and depends on all components working together well to ensure free circulation of blood.

2.3.1. FVIII

FVIII also known as the Antihaemophilic factor is a cofactor that is an essential component of the coagulation cascade and it is synthesised by the endothelial cells of the liver.[38,41,42] It is a large multidomain glycoprotein with a domain structure of A1-A2-B-A3-C1-C2 which **consists** of a light chain containing A3-C1-C2 domains and a heavy chain containing the domains A1-A2-B.[15,43,44] Some studies have shown the A2, C1 and C2 domains to be particularly immunogenic.[43,44] FVIII circulates in its inactive heterodimer form bound to Von Willebrand Factor (VWF); it is the acidic region of the light chain and the C2 domain that are involved in forming this high affinity binding site for VWF.[15,43] This noncovalent bonding with VWF stabilises FVIII and prevents its breakdown in the bloodstream.[43,45] When there is injury to the vascular endothelium, FVIII is activated from its heterodimer form to a heterotrimer (A1/A2/A3-C1-C2) by thrombin through cleavage of the B domain.[43,46,47] Once activated, FVIII detaches itself from VWF and it is then bound to the surface membrane of activated platelets and interact with FIXa leading to the formation of insoluble fibrin meshwork.[43,47]

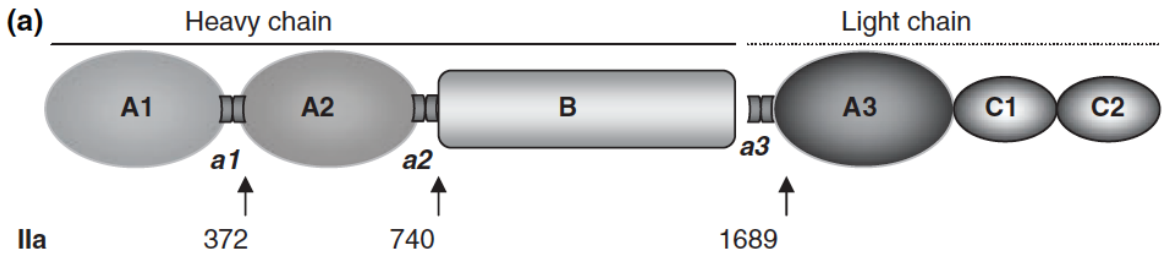


Figure 2.2 Domain structure of FVIII showing arrangement of light and heavy chains.[46]

Pathophysiology

The pathological process in haemophilia A is a complicated interaction between reduced levels of FVIII activity, incapability of the extrinsic tenase to make up for the low levels of thrombin that is generated due to the deficiency in the intrinsic tenase, inhibition of the extrinsic pathway by factors such as TFPI and an increase in fibrinolysis.[33] This leads to a poorly formed clot that is friable, easily displaced, readily broken down and thus results in excessive prolonged bleeding and poor wound healing.

The deficiency in clotting factor FVIII results in less intrinsic tenase being formed; this disturbance in the intrinsic tenase formation causes reduced production of prothrombinase leading to generation of low levels of thrombin.[33] The extrinsic tenase is however unable to generate adequate amounts of thrombin to compensate for the insufficient thrombin generated by the defect in the intrinsic tenase.[33] This low level of thrombin generated has various sequelae all of which form part of the pathogenesis of prolonged bleeding seen in haemophilia A.

The prolonged bleeding in haemophilia A could also be due to reduction of platelet activation and aggregation due to the low levels of thrombin.[33]

Thrombin is important in activating FV to FVa which is an essential part of the prothrombinase complex (FXa, FVa, Ca, PL**)** thus low levels of thrombin lead to insufficient production of the prothrombinase complex resulting in continual low levels of thrombin and thus reduced fibrin formation. Low levels of thrombin in haemophilia A leads to inadequate fibrin formation from fibrinogen.

Thrombin inhibits the breakdown of the fibrin clot, and this involves the activation of FXIII that catalyses reactions that lead to formation of cross-linked fibrin more impervious to breakdown by plasmin.[33] Thrombin also activates Thrombin Activatable Fibrinolysis Inhibitor (TAFI) which prevents fibrinolysis and promotes the stability of the fibrin clot.[33] Therefore, low levels of thrombin lead to inadequate fibrin cross-linkage and increased degradation of the fibrin clot thus increased bleeding.

There is physiological activation of tissue plasminogen activator (tPA) and FXII in the event of vascular injury which promotes fibrinolysis. However, in individuals with haemophilia A, the absence of the thrombin burst causes inadequate activation of TAFI which results in a poorly regulated and increased fibrinolysis thus playing an important role in the bleeding diathesis seen in haemophilia A.

Tissue factor pathway inhibitor (TFPI) is usually produced mainly by the endothelial cells as well as chondrocytes and synovial cells.[33] TFPI inhibits the extrinsic tenase as well as FXa as a means of local physiological regulator mechanism. The increased production of TPFI in the joints, compounded by the low levels of tissue factor in the synovium of joints to initiate coagulation results in higher tendency of bleeding in the joints which is a hallmark of haemophilia.[33,48]

All these abnormalities result in increased tendency to bleeding in haemophilia A. Bleeding episodes are most often directly related to disease severity.[33] In mild and moderate haemophilia, bleeding is typically trauma-induced; due to significant trauma which includes surgery. However, bleeding in severe haemophilia is usually due to insignificant trauma which can occur during regular routine physical activities.[33] This bleeding due to insignificant trauma is conventionally termed ‘spontaneous bleeding’.[33]

Clinical Presentation

The hallmark of bleeding episodes in individuals with severe haemophilia A is spontaneous/ unprovoked bleeding into joints, muscle, and other soft tissue whilst those with mild and moderate disease usually have provoked/trauma-induced bleeding.

In early childhood, bleeding episodes usually involve soft tissue, oral and nasal mucosa as well as bleeding following surgical procedure such as circumcision.[3,6,7] Muscle and joint bleeding become more evident when the child begins to crawl or walk. Kulkarni et al in a study done across Haemophilia Treatment Centres in the US showed that bleeding post circumcision was the most common presenting symptom in the newborn period whilst soft tissue/intramuscular haematomas was commoner as the babies grew older [6]. Similar presenting pattern was observed by Tonbary et al in Mansoura, Egypt.[7]

Haemarthrosis is common in the older age group, and it is the commonest bleeding manifestation in severe haemophilia A in this age group. Haemarthrosis can occur in any joint i.e., knee, ankle, hip, elbow, shoulder, and wrist joints; however, the weight bearing joints are the commonly affected with the knee joint the most affected joint.[6,31,49] Shaheen et al in a study done in Khartoum, Sudan reported the knee joint as the most commonly affected joint in patient presenting with haemarthrosis (51.3%).[31]

Muscle bleeding can occur at any site but commonly presents in the large load-bearing groups of the thigh, posterior abdominal wall and buttocks.[31] Haematoma accounts for between 10-25% of all bleeding episodes in individuals with severe haemophilia A.[50] Individuals will present with pain, inflammation and restriction of movement correlating to the site or muscle group involved. Intramuscular injection is a common cause of muscle haematoma in haemophilia A.

Genitourinary bleeding manifesting as haematuria occurs in haemophilia A with some studies reporting frequencies of about 66%.[51] This is due to increased tissue plasminogen activator and urokinase plasminogen activator (uPA) which both act to prevent clot formation in the urinary tract, thus minor injuries in the tract leads to bleeding as occurs in haemophilia A.[33]

Gastrointestinal (GI) bleeding is not a common clinical presentation in haemophilia A with the incidence of Upper GI bleeding in individuals with haemophilia reported to be about 1.3% yearly.[52] This incidence is however higher than what is reported in general adult population who are not using non-steroidal anti-inflammatory drugs.[52] The cause of GI bleeding is usually due to a pathology in the tract rather than due to the haemophilia A.[52]

Central nervous system bleeding is uncommon especially in adults but can occur after minimal head injury and can have dire outcomes. It is common in children and adolescents due to higher physical activity. Central nervous system bleeding could be intracranial or extracranial. Extracranial haemorrhage (ECH) can be swift and have devastating consequences because of subgaleal bleed.[53] Intracranial haemorrhages (ICH) can be due to delivery/birth trauma.[6] Kulkarni et al reported that 39% of ICH occurring within the first 2 years of life was spontaneous and 30% of ICH was associated with delivery. Majority of spontaneous ICH occurred in babies with severe disease.[6] A meta-analysis by Davies and Kadir reported that babies with haemophilia A were at increased risk of both ICH (44times) and ECH (8times) compared with babies in the general population.[53]

Haemarthrosis, muscle bleeds especially bleeding deep compartments such as forearm, iliopsoas and calf muscles and mucosal bleeding in the mouth, gums, gastrointestinal tract, and epistaxis are termed serious bleeds whilst intracranial bleeding, gastrointestinal bleeding and bleeding in the neck/throat are termed life-threatening bleeds.[3]

Haemarthrosis

Bleeding into joints is one of the hallmarks of haemophilia and can result in profound damage to the joint and increase morbidity as well as reduced quality of life.[54] Vasculature, mechanical stress, and local haemostatic factors are all thought to be contributing factors in the predilection of bleeding into joints in haemophilia.

Low levels of tissue factor in synovial joints leads to restrained initiation of the coagulation cascade.[48,54] This coupled with highly vascularised nature of synovial joints, mechanical stress and the increased fibrinolytic activity in the joints culminates into repeated joint bleeding.[48,54]

Acute haemarthrosis is painful and presents with local swelling and erythema.[31] To avoid pain, the patient may adopt a fixed position, which leads to muscle contractures. Very young children unable to communicate verbally show irritability and lack of movement of the affected joint.

Repeated haemarthroses eventually results in damage to the synovium, cartilage and subchondral bone in a condition known as haemophilic arthropathy (HA).[54] The cells lining the synovium clears blood from the joint with help from macrophages within seven (7) days after an acute bleeding into the joint. Due to recurrent bleeding into the joints, the capacity of the synovium to remove the blood is overwhelmed resulting in excess blood in the joint space. There is a resultant deposition of iron as synovial haemosiderin into the joint space which provokes inflammation in the synovium (synovitis) via cytokines such as interleukin (IL) -6 (IL-6), interferon-gamma (IFNγ) and tumour necrosis factor-alpha (TNFα) as well as causes hypertrophy of the synovium through cell proliferation.[48,54,55] Inflammation and hypertrophy of the synovium stimulates new blood vessel formation in the synovium via growth factors such as vascular-derived endothelial growth factor (VEGF).[54,55] The thickened synovium makes the affected joint vulnerable to mechanical damage as well as bleeding due to vascular remodelling. A vicious cycle is created from the combination of iron deposition, synovitis, synovial hypertrophy, and neovascularisation leading to a "target joint". A target joint is defined as a joint with three (3) or more spontaneous bleeding within a successive six (6) months period.[3,54]

Bleeding into the joints causes formation of a destructive layer of tissue that covers the surface of the cartilage known as Pannus.[54,55] Pannus contains cells that releases enzymes that break down cartilage leading to degeneration of joint cartilage. Blood also has a direct effect through iron deposition on the cartilage by inducing oxidative stress leading to apoptosis of chondrocytes.[48,54,55] Bone changes that occur in haemophilia include osteoporosis due to imbalance between bone formation and bone resorption and formation of subchondral cysts.[31,55]

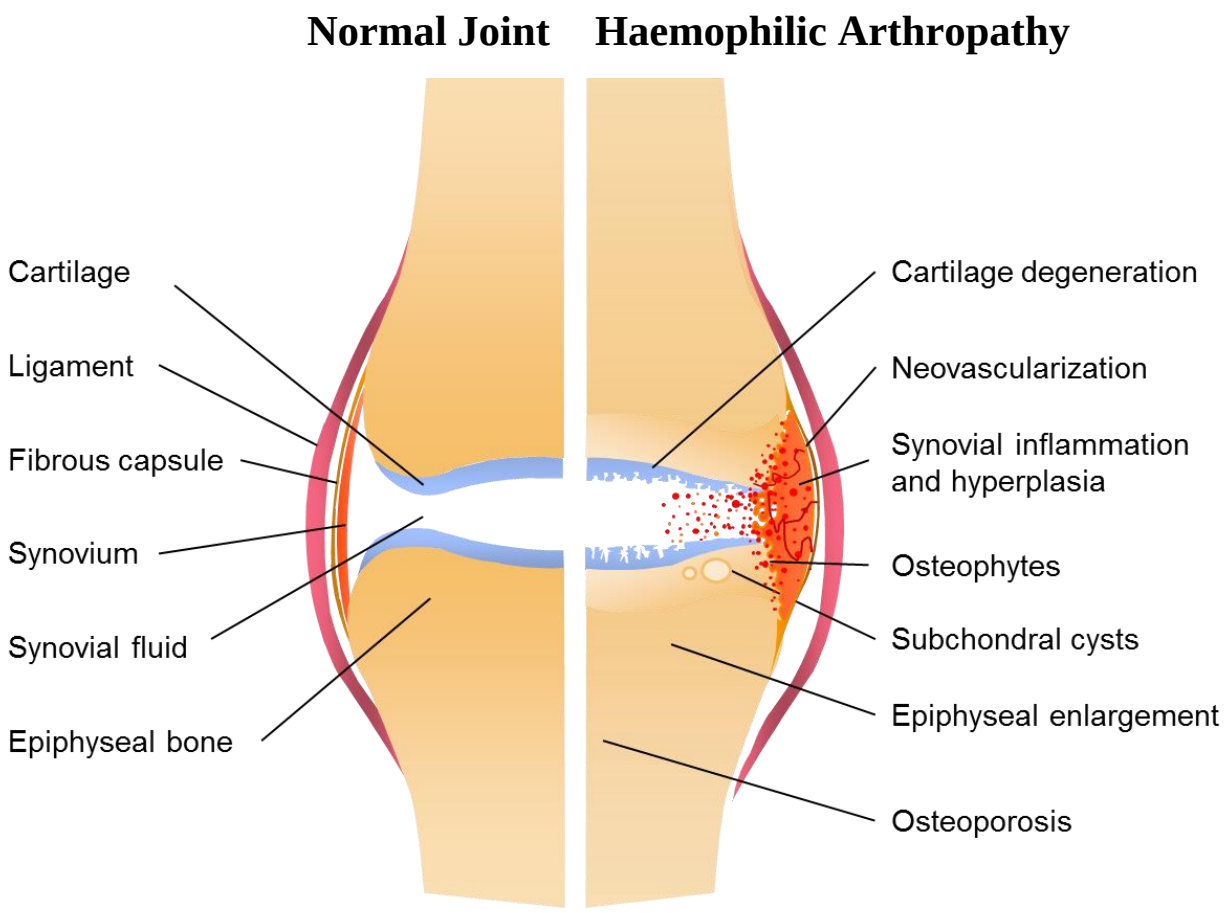


Figure 2.3 Diagram comparing a normal joint and a joint with haemophilic arthropathy.[54]

Diagnosis

An early and correct diagnosis of haemophilia A is important as this ensures that an individual gets appropriate and timely intervention. The diagnosis of haemophilia involves clinical diagnosis, the use of screening tests and finally confirmatory tests to determine the type and severity of haemophilia.

Screening for haemophilia A is indicated in a son of a known carrier female as well as a male child with a positive family history of haemophilia A.[1,32]

Bleeding symptoms such as intracranial haemorrhage after delivery, bruising, haematomas and joint bleeds when a child becomes active, extensive bleeding after venepuncture, vaccination, circumcision as well as post-surgery all prompt investigation which can lead to the diagnosis of haemophilia A being made.[32]

Individuals with mild haemophilia A may however be undiagnosed until they are much older or experience some form of trauma or undergo surgery.

Clotting profile comprising prothrombin time (PT) and activated partial thromboplastin time (APTT), thrombin time (TT) and mixing study are all screening tests that are done on an individual with a clinical suspicion of haemophilia A.

APTT is prolonged in individuals with haemophilia A whilst PT and TT are normal; APTT is usually prolonged with factor levels of less than thirty percent (<30%).[1,50] Mixing study is done when APTT is prolonged to evaluate the cause of the prolongation. Factor assay is done to confirm factor deficiency/levels and can be done using one-stage assay, two-stage or chromogenic assay.[50] Blood samples for these coagulation tests are taken into a trisodium citrate (0.109M solution) bottle with sodium citrate to blood ratio of 1:9 and spun at 1500xg for 15minutes to obtain platelet poor plasma (PPP).[50] Trisodium citrate works by chelating calcium thus prevent the blood from clotting.

APTT measures the time it takes for plasma to clot after contact factor activation and the addition of calcium chloride and phospholipids without the addition of tissue thromboplastin.[56] Thus, APTT gives a measure of how efficient the intrinsic pathway is. When performing APTT, PPP is initially incubated with the APTT reagent which contains contact activating agents such as kaolin, silica, clay, or ellagic acid for 3-10 minutes at a temperature of 37^0 C.[50] The contact activator binds to

FXII and produces FXIIa which activates FXI ; coagulation however ceases at this point due to the absence of calcium.[50,56] The addition of prewarmed calcium leads to the activation of FIX by FXIa. FX is activated through the FIXa/VIIIa complex with the continuation of the coagulation process to the endpoint where fibrin strands are formed.[50] The APTT is the time it takes from the addition of calcium to when the fibrin clot is formed. The normal range for APTT is 26-40seconds.[56] Differences is due to different reagents and manufacturers' recommendation. Each laboratory usually calculates its normal range for APTT. APTT is prolonged in coagulation factor deficiencies FVIII, FIX, FX, FXI,FXII, or its inhibitors, unfractionated heparin anticoagulant, disseminated intravascular coagulation, non-specific anticoagulants such as lupus anticoagulant (LAC).[56]

Mixing/Correction study is performed to evaluate the cause of prolonged APTT. It can help differentiate factor deficiency and the presence of inhibitor. It is carried out by mixing plasma of the patient which is prolonged with normal pooled plasma (NPP) in a 50:50 ratio.[56,57] APTT is then carried out on the mixture; a normalised APTT is indicative of factor deficiency.[56,57]

Factor VIII assay is carried out after clinical history, examination, and screening tests to confirm the diagnosis of Haemophilia A as well as the severity of the disease.
The one-stage assay was initially described in 1953 and it compares the ability of a test plasma and of standard/'normal' plasma to correct the APTT of a substrate plasma which is deficient in factor VIII.[56,58] In performing the one-stage assay, doubling dilutions of 1 in 10, 1in 20 and 1in 40 of the standard plasma and test plasma are made using buffered saline[56] 0.1ml of FVIII deficient plasma is added to 0.1ml of each the dilutions above and then APTT is then performed on each dilution[56] The clotting times of standard and test plasma are plotted against the dilutions and the two lines should be parallel to each other.[56] The difference in potency is measured by the horizontal shift between the line for the standard plasma and that of the test plasma.[56]

The two stage and chromogenic assay are based on the same principle. The first stage involves generating FXa and the second stage measures the amount of generated FXa which is directly proportional to the amount of FVIIIa produced.[59] In the first step, diluted test plasma which has been treated to remove prothrombin is mixed with the reagent which contains serum, FV, calcium and phospholipids and results in the production of FXa.[58] The sample is then mixed with normal

plasma which contains prothrombin and fibrinogen for a clot to form.[58] The FXa is measured using clotting end point in the two-stage assay.[56]

The first step in the chromogenic assay involves mixing test plasma with a reagent that contains FX, thrombin and FIX a.[58] In the chromogenic assay, FXa generated, hydrolyses the chromogenic substrate, and the intensity in colour of the end-product is measured.[56,59] The two stage and chromogenic assays quantifies the capability of FVIII to act as cofactor to FIX in the activation of FX.[59]

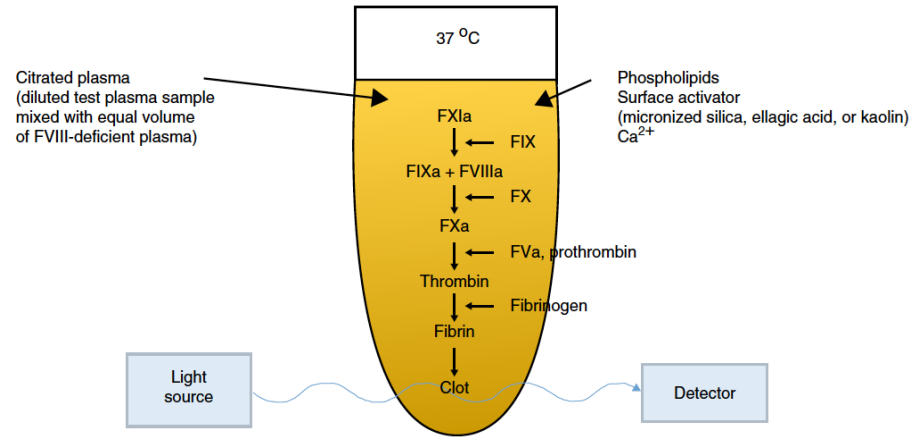


Figure 2.4a Diagram illustrating stages in one-stage assay for FVIII activity.[59]

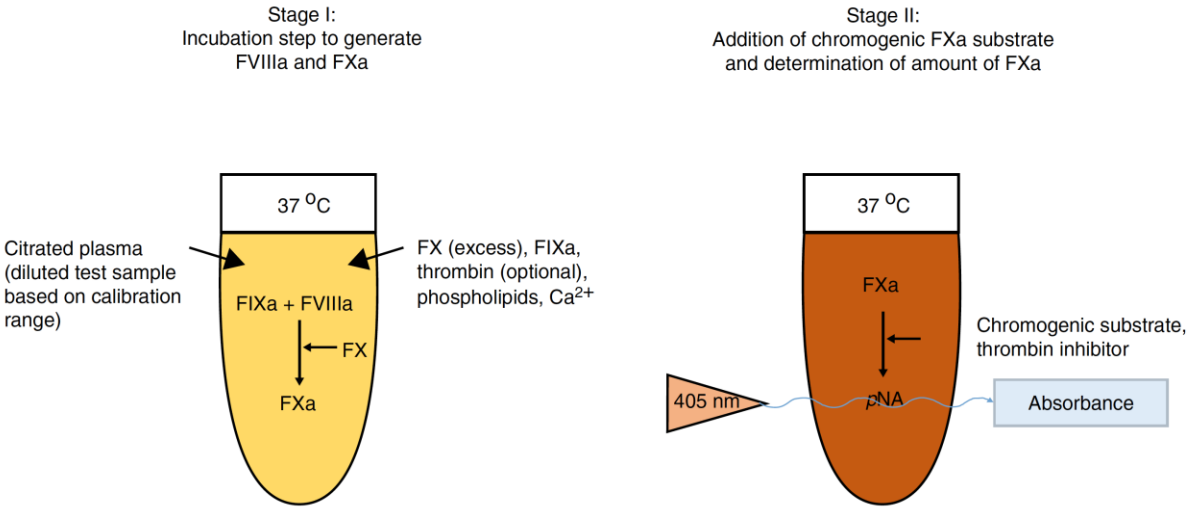


Figure 2.4b Diagram illustrating stages in Chromogenic assay for FVIII activity.[59]

Table 2.1 Table showing advantages and disadvantages of the one stage and chromogenic assays.[59,60]

	One-stage Assay	Chromogenic Assay
Advantages	1. Very simple and fast 2. Inexpensive 3. Easily automated 4. Useful in clinical monitoring of FVIII levels in standard factor concentrate.	1. Used across all FVIII concentrations; more sensitive at low FVIII:C levels. 2. Insensitive to Lupus anticoagulant 3. Has lower interlaboratory variability. 4. Monitoring treatment for patients on modified replacement products
Disadvantages	1. High interlaboratory variability due to wide variety of assay kits, conditions for assay, reagents, and analysers. 2. Sensitive to lupus anticoagulant 3. Less sensitivity to lower concentrations of FVIII	1. Expensive 2. Not widely used compared to one-stage assay especially in diagnosis and monitoring. 3. Technically complex 4. More difficult to automate

The one-stage assay is the most common assay method used in determining FVIII activity with a survey showing 193 out of 217 laboratories across Europe reporting the use of one-stage method compared with 15 laboratories that reported using the chromogenic method.[60] Another survey conducted in seven countries in North America, Europe, and Asia showed 90% of laboratory scientists used a clot based assay in determining FVIII activity with 55% having frequent use whilst 68% reported using chromogenic assay for FVIII activity with 23% having frequent use.[61] World Federation of Haemophilia, British Committee for Standards in Haematology and Nordic

Haemophilia Council however advocate for the use of both one-stage and chromogenic assays for the diagnosis of moderate and mild haemophilia A.[60]

Treatment

Management of haemophilia A is multidisciplinary as it provides less morbidity to patients with better overall outcome.[3] The multidisciplinary approach involves prevention of bleeding and joint damage, expeditious management of bleeding, management of complications including joint and muscle damage, management of inhibitor and attention to psychosocial health.[3] Specialties involved in the care include haematology, orthopaedics, dentistry, surgery, nursing, physiotherapy, social workers, clinical psychology and related allied health professionals.[3] Management involves the use of haemostatic agents and other adjuvant care.

The advent of clotting factor concentrates have led to life expectancy similar to individuals without haemophilia A.[9]

Factor Replacement

Factor replacement is the mainstay for treatment and prevention of bleeding episodes in individuals with severe haemophilia A with the use of clotting factor concentrates. Two types of clotting factor concentrates are available for use in clinical practice. Plasma-derived clotting factor concentrates which are made from human plasma with inactivation of viruses and recombinant clotting factor concentrates which are made from recombinant technology and genetically engineered cells. Clotting factor concentrates initially developed from plasma was accessible in the 1970's whilst recombinant factor concentrates were developed in the 1990's.[32] There are standard half-life and extended half-life FVIII concentrate with a half-life of about 12 hours and 19hours respectively used in the treatment of haemophilia A [3]. These affect the frequency of dosing; with standard half-life FVIII concentrate having more frequent dosing compared to extended half-life FVIII concentrate that have less frequent dosing.

Factor replacement can be on-demand or prophylactic. On-demand therapy also known as episodic therapy is when clotting factor concentrates are given during bleeding episode.[3]

Prophylactic therapy was initiated in individuals with severe haemophilia A after it was observed that individuals with moderate to mild disease hardly had spontaneous bleeding with subsequent healthy joints thus maintaining FVIII levels at more than 1% reduce spontaneous bleeding especially into joints.[3]

Table 2.2 Definitions of Factor Replacement Protocols. [62,63]

Protocol	Definitions
Episodic ("on-demand")	Therapy administered at the time of clinically apparent bleeding
Primary prophylaxis	Regular continuous treatment started: 1. No documented joint disease 2. Prior to 2nd clinically evident joint bleed 3. Before age 3
Secondary prophylaxis	Regular continuous treatment started: 3. After 2 or more clinically evident joint bleeds 4. Prior to the onset of documented joint disease
Tertiary prophylaxis	Regular continuous treatment started: After onset of documented joint disease

Continuous treatment is the intention of giving therapy for 52weeks in a year and with a minimum of at least 45weeks.[62,63]

Dosing for prophylactic therapy is to maintain FVIII trough levels of 3-5% (0.03-0.05 IU) or higher.[3] Different dosing regimen for prophylactic therapy are available for starting patients with haemophilia A. Low-dose prophylaxis with escalation method which starts with low dose with less frequent administration with dose and frequency is increased over time if increased bleeding episodes occurred to allow patients and care givers adapt to starting prophylaxis.[3,63] Dose is 10-15IU/kg given 2-3 days in a week.[3] Then there is the high- dose prophylaxis in which patients are started on high doses from initiation of prophylaxis. Dose is 25-40IU/kg given every 2 days in a week.[3] In Intermediate- dose prophylaxis 15-25IU/kg is given 3 days in a week.[3]

Prophylactic therapy has been shown to have better overall outcome than on-demand therapy: there is reduction of total bleeds resulting in reduced joint disease leading to improved quality of life.[3,9,32] A multicentre study done comparing two prophylaxis regimens and paired comparison of on-demand and prophylaxis treatments in haemophilia A management showed a relative reduction in median annual bleeding rate (ABR) of 99.4% ($P < 0.0001$) for participants treated with prophylaxis compared with those on on-demand therapy.[12]

Prophylactic therapy has also been shown to reduce the incidence of intracranial bleeds.[32,63] A cost utility analysis study done by Colombo et al in Italy comparing prophylactic therapy with on-demand therapy showed that prophylactic therapy is a cost-effective form of treatment when compared with on-demand therapy.[10]

Indefinite prophylaxis as patients transition into adulthood is still unclear as some data have suggested that a proportion of young adults do well off prophylaxis.[62,63]

Individuals with mild or moderate haemophilia A are usually managed on 'on-demand' therapy.[32]

The dose and duration of clotting factor concentrate given during acute bleeding episodes or breakthrough bleeds is determined by the severity and type of bleeding.[32] During acute bleeding episodes especially in the event of serious or life-threatening bleeding, it is important to give clotting factor first before any diagnostic or interventional procedures are performed. Clotting factor concentrates are given repeatedly during these episodes with the aim of maintaining the concentration in plasma above 0.40–0.50 IU/ml which is the haemostatic threshold.[32]

Other plasma products such as fresh frozen plasma and cryoprecipitate are still in use in many parts of the world where clotting factor concentrates are not readily available. Generally, FFP contains 1.0 IU/ml of FVIII whilst 30-40ml bag cryoprecipitate and may have about 70-80IU of FVIII.[3]

Adjuvant therapy

Desmopressin can be used in individuals with mild or moderate haemophilia A with mild bleeding episodes.[32] Desmopressin can be given via intranasal or intravenous routes. The dosage of intravenous route is 0.3ug/kg (should be diluted in about 100ml of normal saline and given slowly over a period of about 30minutes or 150ug or 300ug in children or adults respectively when being given intranasally.[3,32] Desmopressin raises the plasma concentrations of FVIII 3-5times above normal through the release of VWF; however it's effects wanes after a period due to the depletion of stored VWF and usually not given for more than 3 continuous days.[32]

Antifibrinolytic drugs such as tranexamic acid has been found to be useful especially when there is mucocutaneous bleed and can be given orally or intravenously. Antifibrinolytics can be given alongside clotting factor concentrates. Tranexamic acid should however be avoided in haematuria and individuals presenting with throat bleeding as it may cause retention of clots in the bladder or throat.

Pain management is important during acute bleeding episodes as it can be very painful. The use of paracetamol, selective COX-2 inhibitors such as celecoxib, paracetamol with codeine or tramadol or tramadol and slow-release morphine.[3]

An essential part of managing acute bleeding episodes is the PRICE (P-Protect, R-Rest, I-Ice, C-Compression, E-Elevation**)** principle, which is beneficial in stopping the bleed, reducing the swelling as well as the pain. Protect the involved area by reducing movement to prevent worsening the injury; this can be done using splint, slings, brace, or crutches. Rest of the involved area by stopping the usage of that area for between 24-48 hours or sometimes longer depending on the site of injury. Ice applied to the affected part may help reduce pain and swelling; it should be applied 15-20minutes every 2-4 hours for up to 48hours and the ice should be wrapped in cloth, plastic wrap, or paper towel to avoid direct contact with the skin. Compression by wrapping the involved area with an elastic bandage to help reduce swelling as well as help stop the bleed. Compression should be started below the involved area and should not be tight to prevent compartment syndrome. The compression material should be loose enough to accommodate a finger. Elevation: the affected limb should be raised as much as possible especially in the initial 48 hours to aid in reducing the swelling. Joint function can be negatively affected following resting the affected joint

for an extended period as such ‘Optimal Loading” has been suggested to replace “Rest” thus POLICE (P-Protect, OL-Optimal Loading, I-Ice, C-Compression, E-Elevation). Optimum loading concentrates on finding the right balance on resting the affected joint, and mobilising that affected joint early to reduce the risk of complications that come with prolonged rest.[3]

Physical therapy is important in the management of individuals with haemophilia as it helps preserve joint function through functional exercises and should be undertaken under adequate cover with clotting factor concentrates and with a trained therapist.[3]

2.4. Inhibitors

Inhibitor formation is a major complication in the treatment of haemophilia A which results in increased morbidity, poor quality of life and increased healthcare cost.[21,64] Two types of antibodies have been found in individuals with haemophilia A: FVIII neutralising antibodies (inhibitors) and non-neutralising antibodies (NNAs).[65]

NNAs are antibodies derived from B lymphocytes and secrete antibodies against FVIII even before therapy is instituted and they are similar to anti FVIII antibodies secreted in individuals without haemophilia A.[65] NNAs have been found in both individuals without haemophilia A and those with haemophilia A who have not received any kind of treatment with an estimated prevalence of 2-3% and 12-54%respectively.[65,66] These NNAs are thought to bind to the non-functional epitopes of the FVIII molecule and do not have any inhibitory effect on FVIII.[65,66] It has been suggested that the presence of NNA in individuals with haemophilia A could precede the development of neutralising antibodies (inhibitor). A study done in Italy among 237 patients with severe haemophilia A, found the prevalence of NNAs to be 7.6% with those patients having 83% higher incidence of developing an inhibitor later compared with those without NNAs.[65] Lupus anticoagulant, the classic non-specific inhibitor has been found in 21% of individuals with haemophilia A.[67] These inhibitors do not render FVIII activity ineffective but rather inhibit tests that measure coagulation factors.[67]

Inhibitors(neutralising antibodies) are IgG immunoglobulins; polyclonal in nature and the first report of FVIII inhibitors was made in 1941 by Lawrence and Johnson.[28,68] Inhibitors neutralise FVIII leading to increased frequency and severity of bleeding. It also causes ineffective response to factor replacement as such the patient may need higher doses of factor replacement to stop bleeding episodes invariably leading to more intense therapy and ultimately increased cost of treatment.[14,69] Individuals with inhibitors are two times more likely to be hospitalised and have an increased cost of about 10times more compared with those without inhibitors.[70] Individuals with inhibitors are more likely to have more arthropathies and its resultant disabilities.[68] In the Cost of Haemophilia across Europe- a Socioeconomic Survey (CHESS) study, patients with inhibitors showed a more than double mean annualised bleeding rate (ABR) compared to those without inhibitors, increased prevalence in chronic haemophilia-related pain, more frequent visits to the outpatient and more admissions on account of bleeding episodes.[71]

Risk factors for inhibitor formation include genetic factors such as severity of haemophilia, type of mutation, positive family history of inhibitor, ethnicity (black race), and non-genetic factors such as early age of onset of FVIII therapy, intensity of therapy, prophylaxis, number of exposure days (ED), and type of FVIII product.[14,15,21]

Risk Factors for Developing Inhibitors

Prevalence of inhibitors is 25-30% in severe haemophilia world-wide.[21,70] A study of 115 patients at an HTC in Korea showed prevalence of 10.1% in individuals with severe haemophilia A, compared with 3.8% in those with non-severe haemophilia A.[68] Pinto et al in a study on inhibitors across various cities in India which included 1285 patients with haemophilia A showed an inhibitor prevalence of 6.07% of which all had severe haemophilia A.[72]

Inhibitors are most common in patients with mutations that cause lack of the FVIII protein compared with mutations that had some amount of FVIII protein being synthesised.[73] Thus patients with large deletions and stop mutations had a higher risk of developing inhibitors whilst those with non-null mutations carried a lower risk of developing inhibitors.[14,15] A meta-analysis involving 30 studies which included a total of 5383 patients with haemophilia A found that the risk of developing an inhibitor was higher in individuals with nonsense mutations and large deletions 3.6% compared with those with intron 22 inversions 1.4%.[15] The same meta-analysis showed risk of 0.5% and 0.3% for development of inhibitors in patients with small deletions and missense mutations respectively.[15]

Inhibitors can be found in mild to moderate haemophilia in individuals who have missense mutations and those with mutations clustered in A2 as well as C2 domains have been found to have a higher risk of developing inhibitors.[3,28]

A study by Eckhardt et al conducted in 14 HTC across Europe and Australia involving 1112 patients with mild or moderate haemophilia A showed that, all 19 mutations associated with the development of inhibitors were found in the A2 domain as well as C2, C1 and A3 domains and all these mutations were missense mutations.[74]

Inhibitors found in mild to moderate haemophilia in individuals with missense mutations usually

occurs when FVIII is administered in large amounts or in association with inflammatory stimuli [3]. These inhibitors when they occur may decrease endogenous FVIII and reduce the levels of FVIII to <1 IU/dl.[74]

The effect of positive family history on inhibitor development was brought to attention by the Malmo International Brother Study (MIBS), which involved 113 families which had two or more siblings with haemophilia A.[15] The study found that, patient families with a history of inhibitor development were found to have a 48% higher risk of developing inhibitors themselves.[15] Similar results were found in the Concerted Action on Neutralising Antibodies in severe Haemophilia A (CANAL) study in which a 3-fold higher risk was found in families with positive history of inhibitors.[15] Arshad et al in a study involving 300 patients with haemophilia A from five cities in India also found 58.6% of individuals receiving on-demand therapy with inhibitors had a positive family history of inhibitors.[21]

The risk of developing inhibitors is affected by race and ethnicity and a higher risk has been found in individuals of African origin. The MIBS study (done in Sweden) found inhibitors in 56% of patients of African descent studied compared with 27% in Caucasians.[15]

Prophylactic therapy has been associated with reduced risk of developing inhibitors to FVIII. In the RODIN study, individuals who were started on regular prophylaxis had a 32% lower risk for the development of inhibitor compared with patients who received on-demand therapy.[76]

Gouw et al reported that individuals receiving regular prophylaxis had 60% less risk of inhibitor formation as compared with those on on-demand therapy.[77]
The type of prophylaxis also influences the risk of inhibitor development as it has been shown that only 3.8% of individuals on low-dose prophylactic therapy developed inhibitor compared with 47% of individuals on standard prophylaxis developing inhibitor.[15]

It has been postulated that the type of FVIII concentrate received by patient can influence the development of inhibitors; however, there has been conflicting data on this. The Survey of Inhibitors in Plasma-Product Exposed Toddlers (SIPPET), which was a randomised trial that involved 251 children less than 6years with severe haemophilia A who had not been exposed to FVIII concentrates and minimal exposure to blood products were monitored for inhibitors after

exposure to either recombinant or plasma-derived FVIII concentrates.[79] The study found that, the development of inhibitor was highest in the patients who received recombinant FVIII concentrates, inhibitors developed earlier and these inhibitors were long-lasting compared to those who received plasma-derived FVIII concentrates.[79] This occurrence has been suggested to be due to some parts of rFVIII products not binding to VWF and thus reduces its recognition by antigen presenting cells which may increase risk of inhibitors.[79] It has also been suggested that plasma derived FVIII concentrates had increased amount of VWF, provided a cytokine environment that reduced the risk of the development of inhibitors.[79,80] A systematic review involving 2094 patients with haemophilia A with all severity, showed a higher incidence of inhibitor in patients who received rFVIII concentrates 27.4% compared to those with who received pdFVIII 14.3%.[80]

A study by Chalmers et al, found that, inhibitor formation was higher in children treated with recombinant FVIII concentrate compared with the children who received plasma-derived FVIII.[81] The CANAL study however did not find any increased risk of inhibitor formation comparing the type of FVIII concentrate used.[82] There was no increase in inhibitors over the initial 50ED.[15]
A prospective surveillance study by The European Haemophilia Safety Survey(EUHASS) involving 74 HTC showed no significant difference in the risk of inhibitor development in respect to the type of FVIII Concentrate.[15]

Several studies have shown an increased risk of developing inhibitors when treatment was started earlier compared to starting treatment later. Other studies have however shown no difference.[75]
A study conducted in the United Kingdom which involved 348 children with severe haemophilia A showed that there was a higher development of inhibitors 20-26% in children who were started on treatment between 0-18 months compared to 9% in those started on therapy after 18months.[75]
A retrospective study by Goudemand involving 148 patients showed that children who received therapy after 12 months had less inhibitors compared to those started before attaining 6 months.[75]

Increased risk of inhibitor development associated with early start of therapy is however related to the intensity of treatment. Peak treatments have been suggested to trigger inhibitor formation and this has been regarded to be the most important determinant of inhibitor formation.[15]

Intense treatment or peak treatment is usually defined as the treatment with clotting FVIII concentrates for surgical procedure or bleeding episode on at least 3 consecutive days.[76] Surgery, trauma, and severe bleeding episodes (danger signals) lead to the use of increased doses of FVIII concentrates or increased duration of therapy. The Research Of Determinants of Inhibitor development among PUPs with haemophilia (RODIN study) was a multicentre study spanning a 10years period involving 606 individuals with haemophilia A.[76] The study showed a 40% higher risk of development of inhibitors in patients who received intense treatment due to either surgery or bleeding episode compared to those who did not receive intensive therapy.[76]

The risk of developing an inhibitor in haemophilia A is largely brought about depending on the accrued number of exposure days (ED) to FVIII concentrates.[74] Exposure day (ED) is a unit of time (1 day) in which FVIII concentrate is given to an individual with haemophilia A.[78] Patients are most likely to develop inhibitor within the first 20-50 exposures to FVIII concentrates and reduced to fewer than 1% after this period but can develop after years of exposure especially after intense treatment.[15] This is especially true in individuals with severe haemophilia A. Miller et al found that almost 50% of inhibitors developed within the initial 20ED to FVIII concentrates and also a second peak of inhibitor development after 150ED in a prospective screening of 824 patients with haemophilia A in the US.[69]

Eckhardt et al demonstrated that in patients with mild or moderate haemophilia A, risk of developing an inhibitor can go past 100ED.[74] The study found risk of inhibitor to be 6.7% at 50ED and 13.3% at 100ED.[74] This could be explained by the possible age-related dysregulation of the immune system especially in individuals with non-severe haemophilia A.[74]

Pathophysiology of Inhibitors

The pathophysiology and mechanisms involved in the development of inhibitors is complex.[83] Individuals with haemophilia A synthesises small amount of dysfunctional FVIII or no amount of FVIII depending on the severity of the disease. Patients with haemophilia A cannot eradicate high-affinity FVIII reactive T cells.[84] These reactive T lymphocytes invade peripheral tissues and elicit inhibitors when these individuals are given exogenous FVIII concentrates.[84]

The development of inhibitors to exogenous FVIII is a T-cell dependent response.[70] Inhibitors are antibodies usually of IgG subclass, comprising IgG4 and IgG1. IgG1 antibodies do not have any inhibitory effect on FVIII whilst IgG4 antibodies have an inhibitory effect on FVIII.[84]

After exposure to FVIII concentrates, dendritic cells engulf and internalise, cleave and present the peptides of exogenous FVIII to CD4+ T lymphocytes via HLA class II and results in the activation of these T cells.[83,84] It is thought that the antigen presenting cells (i.e. dendritic cells) are activated due to the presence of substances or inflammatory cytokines released as a result of extensive inflammation and tissue damage especially when there are the danger signals.[76] In the situation where the individual does not have any danger signals, it has been suggested that, exogenous FVIII is presented to T lymphocytes by antigen presenting cells which may elicit immune tolerance to the exogenous FVIII.[76] This occurs either by an abnormal response of previously exposed T lymphocytes or the upregulation of T lymphocytes from naïve T cells.[76]

The interaction between these inflammatory cytokines and activated T cells leads to the amplification of the T cells and they move to the spleen to prime the B-lymphocyte in the splenic follicles.[83,84] The primed follicular B cells internalise the exogenous FVIII via B-cell receptor and present the FVIII on to membrane surface.[84] There is differentiation of the B lymphocytes to either memory B lymphocytes or plasma cells through interaction between the B-cells and activated T lymphocytes via T-cell receptor-HLA and cytokines.[84] There is development of a fast and strong immune response against clotting factor concentrate after repeated exposure of FVIII concentrates by memory B cells through the production of neutralising antibodies.[84]

These antibodies (IgG4) bind to functional epitopes most of which are found in the A2, C1, C2 domains of the factor protein. This binding interferes with the function of the infused FVIII rendering it ineffective.[64,85]

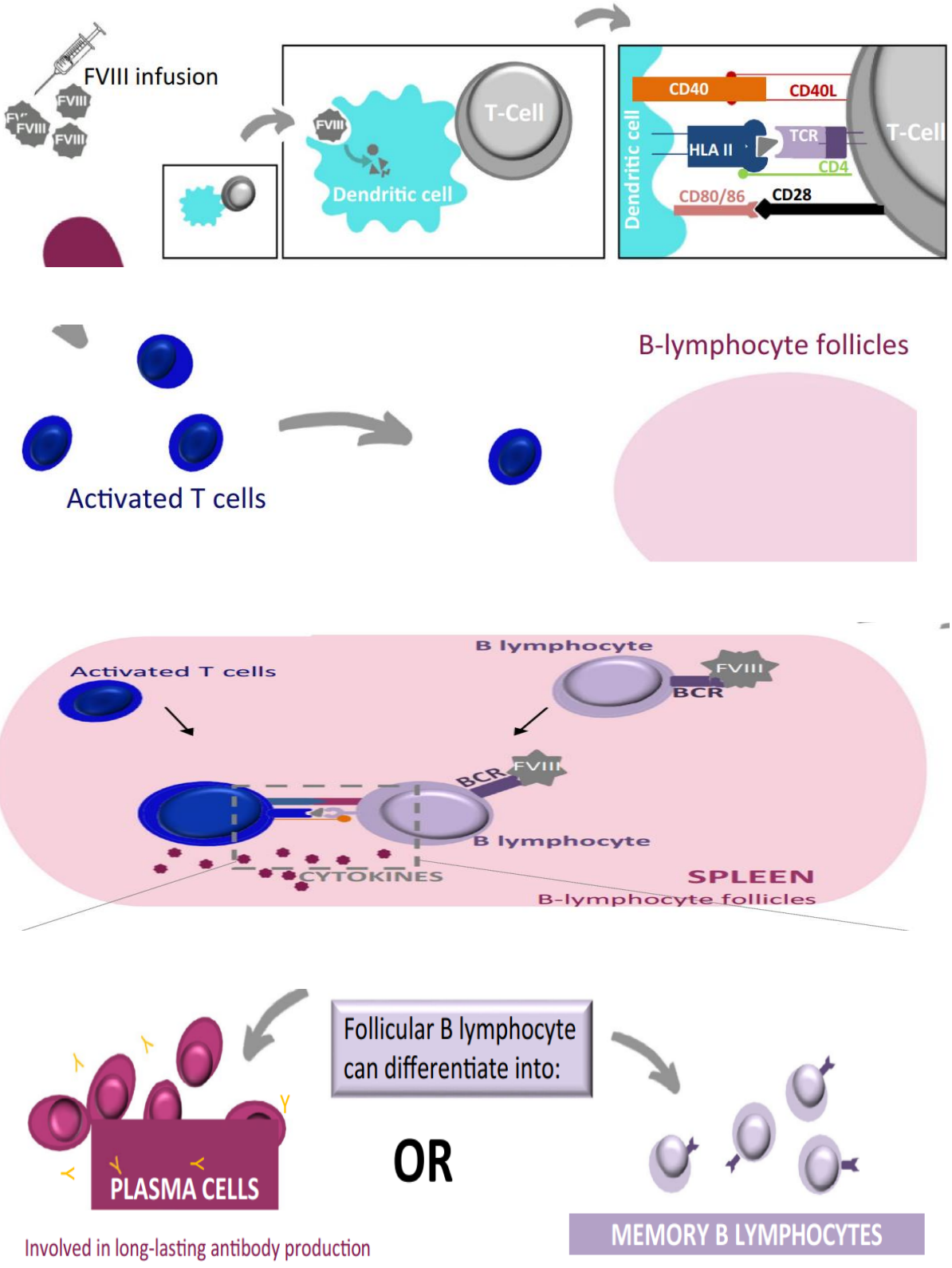


Figure 2.5 Schematic presentation of inhibitor development. [84]

Screening Schedule

It is important to detect inhibitors before being in a situation where a patient fails to respond to treatment, therefore it is important to screen for inhibitor development especially when the individual is at highest risk for developing them.

Screening for children with severe haemophilia should be done every 3ED until 20ED, then every 10ED until 50ED then twice per year till they reach 150ED then annually.[17] In patients with haemophilia irrespective severity or age, it is recommended to screen for inhibitors after intense exposure to factor concentrate (intense exposure is defined as episodes in which FVIII is infused at least once per day for more than 3 consecutive days), suboptimal clinical response to factor concentrate and prior to surgery or other invasive procedures.[3,17] In individuals with mild or moderate haemophilia, it is recommended that screening is done 6 weeks post-surgery or after receiving factor for a major bleeding episode.[17]

Diagnosis of Inhibitors

Early diagnosis of the presence of inhibitors is essential and can be suspected on clinical grounds when a patient does not respond to adequate doses of FVIII concentrate, recurrent bleeding even though the patient is receiving adequate treatment with FVIII concentrate, lower than expected recovery after FVIII concentrate, suboptimal post-operative response.[3]

Early detection of inhibitors is essential to allow for institution of appropriate treatment. Measurement of the concentration of inhibitors is important to ascertain whether the inhibitor is transient or persistent as this affects treatment. Concentration of inhibitors is also used in monitoring inhibitor level as patient receives treatment.

FVIII inhibitors are time and temperature dependent in that, the inhibitors act slowly to inactivate the FVIII and they react best at 37℃.[86] This characteristic aids in differentiating FVIII inhibitors from non-specific inhibitors and lupus anticoagulant.[86]

Mixing study is a screening tool for detection of inhibitors; gives helpful information and aides in conducting further tests. In performing mixing study, NPP is mixed with patient plasma in a 1:1 ratio and APTT performed immediately and APTT repeated after incubating mixture for 1-2 hours at 37℃.[87] Correction of APTT after mixing is indicative of factor deficiency whilst inability to correct immediately indicates the presence of a lupus anticoagulant or an inhibitor and prolonged APPT after incubation at 37℃ suggest FVIII inhibitor.[87] This is as a result of the gradual neutralisation of the FVIII by the time and temperature dependent inhibitors.[56]

Specific diagnosis depends on demonstrating that an appropriate dilution of the patient's plasma when added to normal plasma, specifically neutralises FVIII and no other blood clotting factors that influence the APTT. It differentiates it from other inhibitors of other clotting factors like lupus anticoagulant and nonspecific inhibitors.

Quantification of inhibitors was first described by Biggs and Bidwell in 1959 which employed a two stage assay; however, the test was standardised in 1975 using a one stage assay and known as Bethesda assay.[86] Since then, different methods have been employed in detecting the presence of inhibitors and include Nijmegen Modification of the Bethesda assay, Chromogenic Based Assay and Enzyme Linked Immunosorbent Assay (ELISA). The Bethesda Assay, Nijmegen

Modification as well as the Chromogenic Based Assay mimic conditions occurring in the living being in the detection of FVIII inhibitors whilst ELISA directly detect FVIII inhibitor.[88]

The Bethesda assay is a one-stage clot based functional assay and it's generally the most performed assay used in the quantification of FVIII inhibitors.[16] It is based on the principle that if FVIII is added to plasma containing an inhibitor and the mixture is incubated over a period, then FVIII will be progressively neutralised since FVIII inhibitors are time and temperature dependent.[56,86] In the Bethesda assay, the patient's plasma is diluted such that, when patient plasma is mixed with equal of normal pooled plasma and incubated for 2 hours at 37^0 C, the FVIII activity in the mixture is decreased by 50%. The NPP becomes the source of FVIII with a mixture of buffer and NPP making up the control.[16] Residual FVIII activity for both patient's mixture and control mixture are measured using a one-stage assay.[89] The residual FVIII in the patient's mixture is compared with the residual activity in the control mixture and the percentage residual FVIII in the patient's mixture is determined. The amount of inhibitor in the patient's sample is then calculated from a graph or a chart where the residual FVIII in patient's mixture is interpolated against Bethesda units. Percentage of 25% - 75% for residual FVIII is used in the calculation of inhibitors.[89] This is because test samples with residual FVIII of more than 75% do not have any measurable inhibitor and in those with less than 25% activity, testing has to be redone at appropriate dilutions to ensure accurate quantification.[86,89] A shift in the pH and a decrease in protein concentration as a result of serial dilutions of patient/test plasma compromises the stability of FVIII and this can lead to a false positive inhibitor titre.[16]

The Bethesda Assay was modified to the Nijmegen-Bethesda Assay (NBA) in 1995 and it is the "gold standard"/ recommended assay for detection of inhibitors by the Scientific Standardization Committee of the International Society on Thrombosis and Haemostasis (ISTH).[88] In this assay, the NPP used in the control as well as the test/patient's plasma is buffered with imidazole to pH of 7.4 and FVIII deficient plasma is used in dilutions of the test plasma.[90] These modifications allow for similar protein concentration in the control and test mixture and prevent FVIII inactivation (as high pH can cause FVIII inactivation).[89] The modifications reduce false positive results due to inactivation of FVIII and has been shown to be more dependable at detecting low concentrations of inhibitors.[89] The rest of the test is carried out as it's done for the original Bethesda Assay. The Nijmegen- Bethesda Assay is expensive due to the FVIII deficient plasma used in the

dilutions of the test plasma, as such, some laboratories replace FVIII deficient plasma with 4% bovine-serum albumin buffer.[86,89]

A modification of the Nijmegen-Bethesda Assay was validated in 2012. In this modification, the test samples are pre-heated before testing is done.[86] Pre-heating of test samples is done at 56°C for 10minutes.[89] The sample is then spun at 2000xg for 15minutes and the supernatant subsequently transferred to a new tube.[89] This modification ensures that patients on prophylaxis or those who have recently received FVIII concentrates are accurately tested. Preheating also allows inhibitors to be separated from immune complexes.
The amount of inhibitor assayed is measured in Bethesda units (BU) or Nijmegen Bethesda units (NBU). A positive inhibitor test is accepted at 0.6 BU/ml or more; with titre levels less than 0.6 BU/ml deemed undetectable which is recommended by the ISTH.[16,86]

Non-specific inhibitors such as lupus anticoagulant may produce false positive results in inhibitor assay especially clot-based assay such as the Bethesda and its Nijmegen modification due to interference through its phospholipid binding.[67] A study involving two (2) centres in the UK where parallel testing using both NBA and ELISA on 497 patient samples showed 63 inhibitor positive by NBA; however only 49 samples were positive by ELISA with 14 samples being negative by ELISA.[91] Samples that were positive by NBA but negative by ELISA were all low titre inhibitors.[91]

Proficiency testing by The North American Specialised Coagulation Laboratory Association (NASCOLA) involving between 38-42 laboratories showed that more than 75% of laboratories used Bethesda Assay in quantifying FVIII inhibitors whilst 20% of the laboratories used the Nijmegen modification.[16,92] Proficiency testing of these two (2) assays have shown poor-interlaboratory correlation.[90] Some studies have reported interlaboratory variability of between 13-33% for the Bethesda assay and a 10-15% variability for the Nijmegen modification.[89] These variations can be attributed to differences in the reagents used during the assay such as diluent, NPP and FVIII deficient plasma.

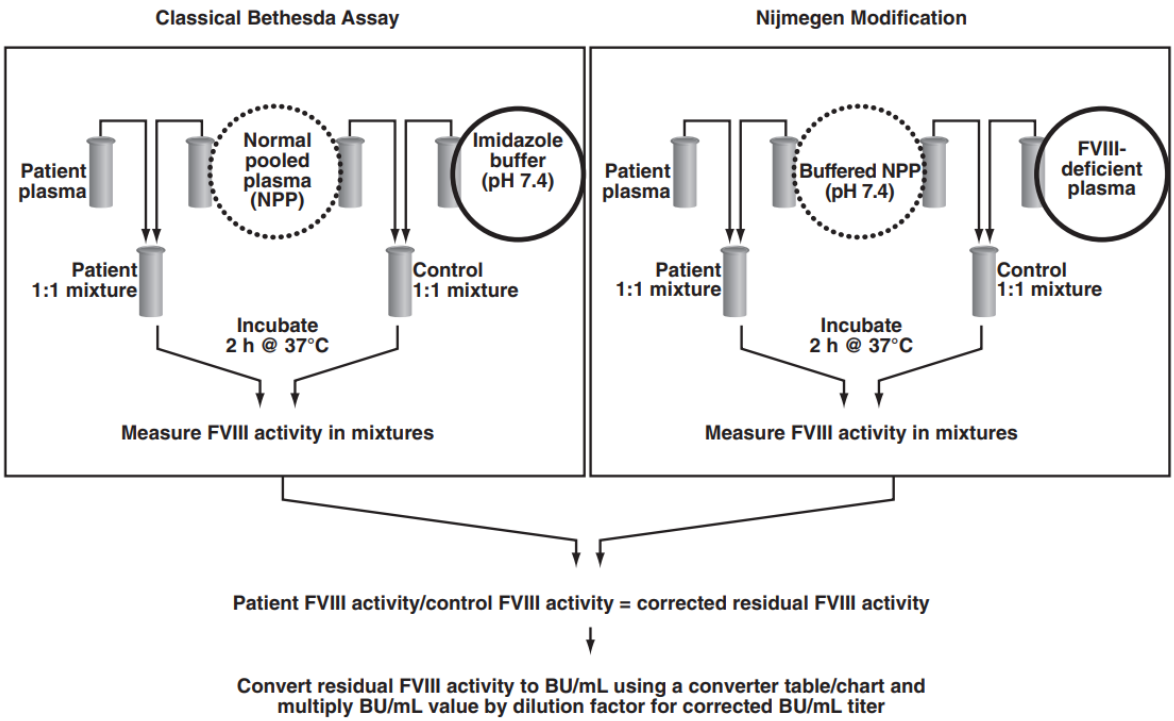


Figure 2.6 Schematic representation of Bethesda Assay and Nijmegen Modification [16]

FVIII endpoint determination via chromogenic means is more specific as such Blanco et al proposed it's use in for inhibitor testing.[86] Chromogenic Bethesda Assay (CBA) is a modification of the NBA in which the endpoint for measuring residual FVIII is determined via chromogenic method.[92] Chromogenic assay is carried out in two steps and is dependent on the activation of FVIII by a standard amount of thrombin and FXa generation and the change in absorbance is read.[93] "One Chromogenic-Bethesda unit is defined as the amount of inhibitor per ml of patient plasma that inactivates 50% of the FVIII activity in 1ml of normal plasma".[93] This method improves sensitivity and specificity of clot-based assays by the removal of confounding effects of the presence of other antibodies such as lupus anticoagulant.[93] Chromogenic assay is thus able to differentiate between specific FVIII inhibitors from lupus anticoagulants, reduce false positive results and it can be used in patients who are on Emicizumab therapy.[94] The chromogenic assay has been shown to produce results similar to when the NBA is used without confounding factors.[92] A study testing 1005 patient samples using both CBA and NBA showed similar results with ≥2 NBU; however, 26 % of samples with inhibitor titres of 0.5-1.0 NBU was negative for CBA indicative of false-positive results.[86]

In using ELISA method to identify antibodies, FVIII is immobilised on a surface usually plastic and the test measures IgG antibodies binding to the FVIII. ELISA measures both non-neutralising antibodies and neutralising antibodies but do not quantify inhibitors.[86] They are sensitive in the detection of low titre inhibitors, as low as 0.03 NBU and are appropriate for screening especially large-scale situations.[86]

Inhibitor levels of >5 BU is termed high titre inhibitor whilst <5 BU is termed low titre inhibitor.[64] Usually, high titre inhibitors neutralise FVIII concentrate quickly compared to the low titre inhibitors which are weaker and act more slowly. Transient inhibitors are low titre inhibitors and can disappear on their own over time; sometimes within a 6-month period.

Inhibitors are also known as high responding and low responding depending on how the immune system responds to infused FVIII based on previous encounter. In the high responders, inhibitor titre has exceeded 5BUs more than once, and repeated exposure will lead to the enhanced formation of new inhibitors whilst low responders have titres less than 5BUs and have weak response to repeated exposure to FVIII concentrates.[95] Majority of patients with high responding inhibitors will have a reduction in their titre levels to undetectable levels after no exposure to FVIII concentrates.[96]

An individual is said to have clinically relevant inhibitors when the individual has a minimum of two (2) positive tests for inhibitors and a reduction in recovery for infused FVIII.[16]

Management of Inhibitors

Treatment of inhibitors is one of the major challenges in the management of haemophilia A; it is expensive, requires expertise and of a long duration. Management of inhibitors involves multidisciplinary team and includes the awareness of the risk and presence of inhibitors in patients, diagnosis, good communication amongst all the team members, specialised nursing care, physiotherapy, psychosocial care and above all haemostatic management.

The aims of haemostatic management are to cease acute bleeds, suppress inhibitors and maintain haemostasis.[64]

Several treatment modalities can be used in the management of inhibitors and includes the use of high dose FVIII in patients with low titre inhibitors, activated prothrombin complex concentrates, activated recombinant FVII, porcine FVIII which has been shown to have low-cross reactivity with FVIII antibody, desensitization, and immune tolerance induction (ITI).

In treating patients with inhibitors, it is important to identify patient's risk profile so that tailored therapy based on the profile can be used to decrease the risk of inhibitor formation.[15] This assessment includes inhibitor titre level, immune response, site and severity of bleed and the intention to start immune tolerance induction therapy.

Management of acute bleeding episodes depends on the inhibitor titre levels. In individuals with low titre inhibitors and or low responders, bleeding episodes can be stopped with higher doses of FVIII concentrates. There is no standardised dosing regimen with different regimen being suggested. The dose to be given should be able to compensate for the level of inhibitor present and the percentage increase required for adequate haemostasis in the particular bleed.[97] A three times higher dose of FVIII concentrate can be used in such instances.[17]

Porcine recombinant FVIII has been used with a dose of 200 IU/ kg as porcine FVIII is able to evade inhibitors but antibodies to porcine FVIII can also develop.[95]

High titre inhibitors tend to be persistent and render the patient completely resistant to factor concentrates and it is important to avoid further FVIII exposure until ITI is initiated. Avoidance of FVIII causes the person's immune system to be less stimulated and produces less inhibitors.

Activated Prothrombin Complex Concentrate (aPCC) and activated recombinant FVII (rFVIIa) are known as bypassing agents and they sidestep the FVIII-dependent step in the clotting cascade

resulting in increased thrombin formation and thus promote haemostasis.[64,98] Both agents have been shown to be effective in retrospective studies with none superior over the other.[97]

Factor Eight Inhibitor Bypassing Activity (FEIBA) is an aPCC that contains FII, FVII, FIX and FX in their zymogen and activated forms in trace amount except FVIIa which is in greater amounts.[98] FEIBA works mainly through prothrombin and FXa to achieve haemostasis.[97] It may contain trace amount of FVIII and FIX which may produce an anamnestic response in inhibitor titre levels in some patients even though the efficacy of therapy is not affected.[97]
rFVIIa sidesteps the formation of the tenase complex through its ability of activating FX on the platelet phospholipid layer with subsequent increase in the generation of thrombin.[97]
Bypass agents can be given either on-demand during a bleeding episode or prophylactically to prevent bleeding. For on-demand therapy, FEIBA is given 50-100IU/ kg every 6-12 hours and rFVIIa; 2-3 doses of 90 ug/kg every 2-3hours or single dose of 270ug/ kg until haemostasis is achieved.[97] For prophylaxis FEIBA is given 70-100 u/kg alternate daily or 3X weekly and rFVIIa is given 90 ug/kg/day.[97] Prophylactic therapy has however been shown to decrease bleeding episodes and thus a reduction in haemarthroses. A retrospective study on individuals with inhibitors who had prophylactic rFVIIa showed a 46-52% reduction in bleeding rates.[15] A meta-analysis involving six (6) studies showed a 64% decrease in the bleeding rate of patients who were put on prophylactic aPCCC.[15] The FEIBA PROOF study also showed a significant reduction in the occurrence of new target joints in individuals receiving prophylactic treatment compared with those who received on-demand treatment.[99] The study was conducted in 17 institutions in the US over a 1 year period showed a 72.5% decrease ABR in the arm that received prophylaxis compared with the arm that was on 'on-demand' therapy.[99]

Patients have different responses to different bypassing agents. In the event of poor response to bleeding episodes; doses of the bypass agent can be increased, or different bypass agent can be used, or a combination of both bypass agents can be used either alternating or at short intervals. There is however an increased risk of thrombosis when combination of bypassing agents is used.
Response to bypassing agents is usually based on clinical assessment such as improved pain, reduction in swelling and improved mobility.[97]

Episodic or on demand treatment for breakthrough musculoskeletal bleeds is usually the treatment regime used in patients with inhibitors as prophylactic therapy is expensive.[64,95]

There are still unresolved issues with treatment of inhibitors in using bypassing agents and this include the uncertainty of response to the bypassing agents, the unavailability of laboratory tests to assess efficacy of therapy and about 10-20% of bleeding episodes are not adequately treated with bypassing agents. There is therefore the demand for eradication of the inhibitors as this allows for therapy with FVIII concentrates; decrease in bleeding rates and thus decrease in damage to the joint.[97]

Immune tolerance therapy results in eradication of inhibitor through the repeated administration of high doses of FVIII over a prolonged period spanning several months.[64] Immune tolerance induction (ITI) suppresses the immune response, restoring tolerance to infused FVIII and allows for replacement FVIII to be used for therapy.[17]
ITI therapy is indicated in patients with high titre inhibitors / high responding inhibitors, as well as patients with low titre inhibitors that are persistent and hinder therapy with standard dose prophylaxis.[97] In a significant number of patients, ITI therapy can take a long time to be achieved, and it is expensive thus limiting its availability especially in low resource areas. Patients with inhibitors lasting more than a year are usually not good candidates for initiation of ITI because they are usually refractory to ITI.[100]

Treatment regime used in ITI involves giving either 200IU/kg FVIII concentrate daily or 50IU/kg three times weekly and both regimens have been found to have similar results.[17,64] The ITI study which was conducted on individuals with inhibitor titres of <10 BU just before the initiation of ITT showed a success rate of 69.7% in both groups (i.e. the group that received high dose and the group that received low-dose treatment regimens).[64] However, individuals on the higher dose regime achieved remission in a shorter time compared with those on the lower dose regime.[17,64] Individuals who received lower dose had a higher risk of experiencing breakthrough bleeding.[17]
International Immune Tolerance Registry found ITI to be successful in 82% of patients with inhibitor titres of <50 Bus.[64] It has been suggested that ITI is more effective at lower titres of inhibitors therefore increasing the need for early detection of inhibitors.[69]

The use of rFVIIa is recommended in individuals with high titre inhibitors before starting ITI as this allows the reduction of the titre levels to less than 10 and enhance the chance of successful ITI.[64] Initial treatment with rFVIIa prevents possible anamnestic response that can be caused by FEIBA which has small amounts of FVIII or FVIII containing blood products.[17,64]

ITI therapy is deemed as successful after three (3) consecutive undetectable inhibitor titres.[16] A less than 20% reduction in the titres in each consecutive 6-months of continuous therapy during ITI has been shown to be a poor prognosis in the eradication of inhibitors.[16]

For patients who experience breakthrough bleed whilst on ITI therapy, should be given bypassing agents in doses as is given in the management of acute bleeds.

ITI also has its challenges as it is costly, efficacy is not always certain, and treatment involves specialised care and over long duration.

Bypassing agents can be used as prophylaxis in failed ITI with FEIBA being given as 85 u/kg on alternate days or rFVIIa 90ug daily when either is used as prophylaxis.[17]

Surgical procedures in individuals with inhibitors has always been a source of concern due to the thought of increased risk of bleeding. A multidisciplinary team should be involved in coordinating such procedures and should include a well laid out plan on preventing increased bleeding as well as post-surgical therapy. Prophylaxis with bypassing agents are given during both minor and major surgical procedures.[17] Antifibrinolytic agents such as tranexamic acid are of immense benefit in helping to reduce the amount of bleeding in dental surgeries.

Due to the challenges in the treatment of inhibitors with both high dose FVIII concentrates and ITI, immunomodulatory methods aimed at eliminating inhibitors have been explored.
One of such is Rituximab; a monoclonal antibody targeted against CD 20 which is expressed on B lymphocytes. Rituximab causes death of B lymphocytes through complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity or stimulation of apoptosis thus remove B lymphocytes that produces inhibitors.[101] Rituximab has been licenced for use in non-

Hodgkin lymphoma, chronic lymphocytic leukaemia, rheumatoid arthritis. It is however used 'off-label' in other conditions such as immune thrombocytopenia, autoimmune haemolytic anaemia, and systemic lupus erythematosus. It is used as second-line therapy in individuals who are resistant to ITI. The Rituximab for the Treatment of Inhibitors in Congenital Haemophilia A (RICH) study involving 16 patients across 13 sites in the US was done to evaluate the effectiveness of rituximab in reducing inhibitor titres. This study showed the usefulness of rituximab in reducing the inhibitor titre levels.[100] Franchini et al in a systematic review and meta-analysis showed a complete remission of 53% in individuals on rituximab.[102] Rituximab is given as intravenous injection 375 mg/m^2 weekly for a month.[101]

Emicizumab a bispecific monoclonal antibody works by bridging FIXa and FX to restore the function of FVIIIa; this is done through the alignment of FIX a and FX in an appropriate spatial distribution.[20] The subsequently activated FX then produces thrombin and it's consequent formation of fibrin clot.[20] Development of the drug started in the 2000s and the concept was based on the fact that the distance between the antigenic sites of FIXa and FX was similar to the distance between two (2) antigen-binding sites of IgG.[20] Unlike FVIII, emicizumab is not activated by thrombin neither is it inactivated by protein C and not affected by the presence of inhibitors to FVIII.[103]

It was first licensed to be used in patients with inhibitors but currently also used in individuals without inhibitors. The four (4) HAVEN trials looked at emicizumab in adult patients with inhibitors in HAVEN-1, HAVEN-2 looked at children < 12 with inhibitors, HAVEN-3 looked at preventing bleed in patients without inhibitors and HAVEN-4 assessed monthly dose for patients with and without inhibitors.[20] The HAVEN-1 study showed a 80% decrease in all bleeding whilst HAVEN 2 showed a reduction of 63% in all bleeds.[20] The HAVEN 3 study showed a substantial reduction in bleeding episodes in individuals who received emicizumab; a 68% reduction in ABR.[104]

The aim of emicizumab prophylaxis is to maintain plasma concentration at 45-50 ug/ml which is equivalent to 13-15% of FVIII activity and has a long half-life of about thirty days (30) due to its IgG structure.[105]

Emicizumab is given as subcutaneous injection 3mg/kg weekly for 4 weeks as loading dose and monthly injections of 6mg/kg.[106]
Adverse reactions associated with emicizumab include reactions at injection site, diarrhoea, bleeding, arthralgia, headache, and fever.[103] Thrombotic events were seen in individuals who received concurrent aPCC and this is due to the provision of substrates for emicizumab by aPCC to form the intrinsic tenase.[103]

Situations such as breakthrough bleeding, increased bleeding rate, and patients undergoing major surgery, calls for the measuring of FVIII activity levels and inhibitor for patients on emicizumab prophylaxis.[107] Emicizumab mimics FVIII, as such APTT is shortened in the presence of emicizumab and this could influence measurements of FVIII activity levels by overestimation and inhibitor levels by underestimation if one-stage APTT based assay is used.[107,108] In order to assess FVIII levels accurately using one-stage APTT based assay, anti-emicizumab monoclonal antibodies is added to patient/test plasma to neutralise emicizumab and then allows for accurate measurement of FVIII activity levels and presence of FVIII inhibitor if any.[107,109] Another method of assessing FVIII activity levels and inhibitors is the use of chromogenic assay with bovine reagents since emicizumab does not attach to bovine FX and FIX.[108]

Specialised nursing care is important in patients with inhibitors as they coordinate and liaise between the various members of the multidisciplinary team, administer factor concentrates or bypassing agents as well as providing support for the patient and family.

Patients with inhibitors are prone to more bleeding episodes and poorer joint health. It is thus important to pay attention to their joint health. There should be a good balance between rest and activity.[17] Physiotherapy should always be done under cover with haemostatic agent.[17]

The provision of psychosocial support in patients with inhibitor is very important as such patients are faced with more bleeding episodes and its associated challenges in terms of school and work, expensive and intense treatment regimen, treatment adherence and a poorer quality of life compared to those without inhibitors. The multidisciplinary team should recognise this and provide the needed support.

Inhibitors render life-saving factor concentrate ineffective leading to poorly controlled bleeding, increased hospitalization and increased cost compounded by unpredictable and suboptimal therapy.[100] It is therefore important to prevent inhibitors before they occur to promote overall better outcomes. This can be achieved by avoidance of excessive treatment for relatively minor bleeding episodes, avoidance of large doses of factor in very young children and encouraging early start of prophylaxis as it has been suggested to have a protective effect against inhibitor formation.[12]

2.5. Haemophilia B

Haemophilia B also known as Christmas disease named after the first person to be diagnosed with the condition; Stephen Christmas in 1952.[110]

FIX gene is found on the long arm of the X chromosome and codes for the glycoprotein FIX.[110] FIX is a vitamin K-dependent factor which is synthesised in the liver, and it's found in the inactive form in circulation. FIX is activated by the TF/FVIIa complex and forms a complex on phospholipid membrane with calcium and FVIIIa (co-factor) which increases its activity.[110]

Clinically, haemophilia B is thought to be less severe than haemophilia A.[110] Haemophilia B has a reported prevalence of 1 in 30,000 male births world-wide.[111] The Ghana Haemophilia Society has twenty-nine individuals with confirmed haemophilia B on their registry.

Haemophilia B is due to deficiency of FIX, is classified according to the factor activity level; severe (<0.01IU/ml; <1% of normal), moderate (0.01-0.05 IU/ml; 1-5% of normal) and mild (>0.05-0.4IU/ml; >5-40% of normal) with bleeding episodes corelating with degree of factor activity level.[3,33] Bleeding into joints or muscles occur spontaneously in severe disease; bleeding is mostly in the absence of identifiable haemostatic challenge.[3] Individuals with moderate disease experience occasional spontaneous bleeding or prolonged bleeding in the presence of minor trauma or surgical procedure.[3] Spontaneous bleeding is rare in mild disease but they can have severe bleeding with major trauma or surgery.[3] There can however be discordance between the factor level and clinical/ bleeding phenotype.[2,31] Clinically, haemophilia B is thought to be less severe than haemophilia A even though the data are conflicting.[110,112]

Diagnosis can be suspected clinically suspicion or male child of known carrier.[3] Screening tests such as APTT and mixing studies are performed in the initial work-up and more confirmatory tests such as the one-stage factor assay and chromogenic assays are used to confirm diagnosis.[3]

Prophylactic therapy with FIX concentrate either plasma derived concentrates or recombinant concentrates is the gold standard for the treatment of haemophilia B even though some countries,

mostly developing countries such as Ghana usually treat patient by on-demand therapy due to unavailability of FIX concentrate.

Inhibitors in haemophilia B is most often found in severe disease and inhibitors is a rarity in mild haemophilia B with incidence of 5% in severe disease.[3] Anaphylactic or allergic reaction to FIX concentrates has been associated with about half of individuals with inhibitors in haemophilia B.[3] The exact mechanism of this anaphylactic reaction is uncertain. Some postulations of causes include activation of complement, possible mast cell activation and hypersensitivity response mediated by IgE. Inhibitors in haemophilia B can also be low titre or high titre.

FIX concentrate can be used as treatment of individuals with low titre inhibitors in the absence of allergic reactions.[3] For individuals with high titre inhibitors, it is recommended that rFVIIa is used in the treatment of acute bleeds as against aPCC since it contains FIX and may worsen or trigger an allergic reaction.

2.6. Haemophilia C

Haemophilia C also known as Rosenthal Syndrome results from a mutation in the factor XI (FXI) gene which is located on chromosome 4.[113,114] It was first described in 1953 in individuals presenting with severe bleeding after surgical procedures.[114] FXI is mostly made in the hepatocytes and released into circulation in the inactivated form (22). It activates FIX which is an important step in the generation of thrombin; thus a deficiency results in reduced thrombin levels.[115] There are more than 190 mutations causing Haemophilia C with majority being missense mutations.[115]

FXI deficiency is classified as either quantitative or qualitative depending on the amount of FXI or activity of FXI respectively.[115]

Individuals with Haemophilia C rarely have spontaneous bleeds, but usually have trauma induced bleeding or bleeding after procedures especially in sites with increased fibrinolytic activity such as the oropharynx and genitourinary.[116]

Diagnosis is through clinical history and an isolated prolonged activated partial thromboplastin time (APTT). Specific FXI assay confirms diagnosis.

Management of haemophilia C is to replace the missing FXI with the required minimum concentration of between 15-30mg/dL required to maintain haemostasis.[116] Fresh frozen plasma (FFP) is often used in the management of haemophilia C as FXI concentrates are difficult to prepare and most often very expensive.[116] Tranexamic acid which is an anti-fibrinolytic is used in the management of individuals with haemophilia C; since it has been shown that bleeding is common in areas of high fibrinolytic activity.[116] Desmopressin has been shown to increase the activity levels of FXI and thus it's used in FXI deficiency.[116]

CHAPTER 3: METHODS

3.1. Study Design

The study design was cross-sectional. All haemophilia A patients who receive care at the Korle-Bu Teaching Hospital (KBTH), both at the adult Haematology Department and Child Health/Paediatric Department were considered for the study.

3.2. Study Site

The study sites were the adult Haematology Department, Child Health/Paediatric Department and Haematology Research Laboratory all at the Korle-Bu Teaching Hospital.

Korle-Bu Teaching Hospital: The KBTH is the first teaching and largest tertiary hospital in Ghana. It is in Accra, the nation's capital which has an estimated population of 4 million. The hospital serves as the major tertiary referral hospital for most of southern Ghana and in some cases for the whole of Ghana.

Haematology Department: The adult Haematology Department until 2016 was one of the two referral points for haematology in the country. The department has over 6000 registered patients since its inception. The department sees an average of 370 out-patients a month at the general out-patient clinic, specialised clinics and the 'Haematology Day Care'.

The department has about thirty-seven registered haemophilia A patients with about 30 active patients (patients who have reported at least once in the past year). Haemophilia A patients are seen on-demand when they have a bleeding episode at the 'Haematology Day Care'. About two (2) patients with haemophilia A are seen on average in a month. Emergency cases are seen at the Accident and Emergency Department and patients needing admission are admitted at the Medical Wards at the KBTH. Patients seen are mostly from the Western, Southern and Eastern parts of the country.

Haematology Research Laboratory: The laboratory at Haematology Department until the early 2000's was running basic haematology laboratory tests for the hospital KBTH. After all laboratory tests in the hospital were moved to the Central Laboratory, the laboratory started running Full Blood Count (FBC) mainly for patients coming to the out-patient clinic of the department. Around

2014, the laboratory was converted to a research laboratory for conducting research in the department by faculty of the department, resident doctors both in and out of the department and MPhil students. The laboratory currently offers tests such as Full Blood Count (FBC), Blood Film Comment, Bone Marrow Aspiration and Cytology and reporting and Hb Electrophoresis to the public. The laboratory processes about 150 FBC and 100 Blood Film Comment and about 5 Hb Electrophoresis averagely in a month. The laboratory is manned by staff of the University of Ghana Medical School. Consultants, Specialists and Resident doctors at the department report blood film comment and bone marrow aspiration. Laboratory equipment such as High-Performance Liquid Chromatography machine, ELISA plate reader, Gazelle machine, Centrifuge and a Chemistry Analyzer are all available in the laboratory for research purposes.

Department of Child Health/Paediatrics: The department of Child Health/Paediatrics receives referral cases from both primary and secondary care facilities. The department has a total bed capacity of 180 and sees about 2200 patients a month on out-patient bases. The department has about 70 confirmed patients with Haemophilia A in their registry with about 60 active patients and runs an outpatient clinic for patients with haemophilia on Tuesdays and Thursdays once a month. The average number of patients seen per month is about two (2). Emergency cases are seen at the Paediatric Emergency Unit.

3.3. Study Period

The study was conducted from August 2021 to February 2022.

3.4. Study Population

The study population included all patients of all age groups who received care at the Departments of Haematology and Child Health/Paediatrics at the Korle-Bu Teaching Hospital. Patients who met the inclusion criteria of the study were recruited into the study. All the selected participants voluntarily provided informed consent for patients 18years and above and parental consent with assent of participants between 8-17 years and parental consent only for participants 7 years and younger.

Eighty-one (81) patients with haemophilia A were recruited in this study.

Inclusion Criteria

Haemophilia A patients confirmed by FVIII assay.

Haemophilia A patients who have Medical Records at the respective departments

Exclusion Criteria

Haemophilia A patients who have incomplete Folders/Medical Records at the respective departments

3.5. Sample Size Determination

To determine the sample size required for the study, the following formula by Yamane was used.

$$n = \frac{N}{1 + Ne^2}$$

n is the required sample

N is the known population being studied i.e., Haemophilia A = 90

(Source of N: Haemophilia Registries at the Departments of Haematology and Child Health/ Paediatrics at the KBTH)

e is the margin of error (5%)

$$n = \frac{90}{1 + 90(0.05^2)}$$

Required sample (n) = 73.469

Addition of 10% Non-Response 7.3469

Required sample (n) = 81

3.6. Ethical Considerations

The study was approved by the Institutional Review Board of the Korle-Bu Teaching Hospital (approval number: KBTH-IRB/0003/2021). Permission was granted by the Departments of Haematology and Child Health/Paediatrics.

The study was fully and clearly explained to all eligible participants in a language understood by them. Acceptance was confirmed by signing or thumb-printing of the consent form by participants 18years and older, parental consent form and assent form by parent/guardian of participant and participant between 8 and 17years respectively and only parental consent form for participants younger than 8years.

The participants and or the parents/guardians were informed of the voluntary nature of their participation and their liberty to withdraw from the study at any point should they wish to do so, without affecting their care subsequently.

All findings from this study were reported in statistical summary form.

Possible Benefit: The participants were made to understand that if inhibitors were detected, they will be contacted so that their treatment plan can be discussed and adjusted accordingly by their clinical team.

Possible Risk: The main risk was discomfort during phlebotomy and the inconvenience of reporting to the hospital outside their scheduled visit.

Participants were not paid for their participation in the study; however, they were given transportation fare because they were reporting to the hospital outside their scheduled visit.

3.7. Study Procedure

All patients on the register of Haemophilia A at both Haematology and Child Health/ Paediatric Departments were called by the principal investigator. Telephone numbers of participants were obtained from the Haemophilia registries and folder/medical records with the help of two (2) study nurses (there are 2 nurses; one in each department who help run the haemophilia clinic at their respective departments).

Each study participant's Medical Record was studied, and information extracted using a data extraction form (appendix vii). Unique study identification numbers were given to all consented participants.

Information extracted included patients' demographics, age at diagnosis, presentation at first visit/diagnosis, residual FVIII level, type of therapy received, number of times a particular therapy has been received by the participants, their guardians/parents, and study personnel adhered strictly to Covid-19 preventive protocols which included:

- Participants and their guardians were given scheduled appointment date and times to prevent over-crowding at the 'Day Care', Day Room and Haematology Research Laboratory for data extraction and sample taking/ phlebotomy respectively and enabled adequate social distancing to be practiced.
- Participants, guardians, and study personnel wore facemasks fully covering nose and mouth. Appropriate facemasks were provided by the researcher for the participants and their guardians/parents for those who did not have any.
- The study provided alcohol-based hand sanitizers for study personnel, participants, and their parents/guardians.

Sampling Methods

Convenient purposive sampling was used for all patients on the Haemophilia registers at both departments. All study procedures were explained to all participants by principal investigator or study nurses in their local language as well as provision of summary of the study procedures in the informed consent.

Study participants and or parent/guardian were given money for transportation to and from the hospital.

Data Collection

The medical records of consenting/assenting study participants as well as consenting parent/guardian were studied, and data extracted using data extraction form (appendix vii)

The data extracted included:

- Demographics: Age, sex, educational level, occupation
- Age at diagnosis
- Residual factor level at diagnosis
- Clinical presentation at diagnosis
- Number of bleeding episodes in the past year
- Type of bleeding episodes in the past year
- Family history of haemophilia A
- Relationship to family member who has haemophilia A if any.
- Type of therapy(ies) received.
- Number of times each therapy has been received.
- Age of first exposure to factor concentrate.
- Factor VIII requirement in a year (iu/kg/year) for the past 3 years
- Type of factor concentrate received.

    Information such as family history was corroborated from participant and or parent/guardian.

Laboratory Tests

***Specimen obtained*:** Venous blood.

***Patient preparation*:** There were no special patient preparations before samples were taken. Patients who were to receive factor concentrate had their samples taken before factor concentrate was given (if there was the need to receive factor concentrate on the day)

Method of sample collection: 4.5mls of venous blood was drawn from each study participant into a trisodium citrated bottle 32g/l (0.109M) solution. (0.5ml of sodium citrate).

Precautions taken during sample collection: The following precautions were adhered to:

- Sample collection was done in a quiet, well-lit area.
- Procedure was explained to the participant before sample collection.
- Sample collection was done under aseptic techniques for both the laboratory personnel and participant.
- Tourniquet was tied for short period during sample collection.
- Vacutainer tubes were filled to appropriate marked level to ensure that the proper 9:1 blood to anticoagulant ratio was achieved.
- The sample was mixed gently by inverting the tube gently and frothing avoided.
- Samples from each participant was duly labelled with unique study identification numbers. This was done systematically to avoid errors.

Handling of sample

- Blood samples were collected and labelled at the Departments of Haematology and Child Health/Paediatrics.
- Samples were visually checked/inspected for adequacy of volume, presence of clot and features of haemolysis before being transported.
- **Sample Transportation**: Blood samples collected were transported in a cold box to the Haematology Research Laboratory within 1 (one) hour of being taken.

- **Receipt of specimen at the Laboratory**: Samples were again visually checked/inspected for adequacy of volume, presence of clot and features of haemolysis.
- Specimen were centrifuged at 4000RPM for 15 minutes.
- This was done within two (2) hours of sample being received at the laboratory.
- The platelet count of every 5^{th} sample was checked to ensure platelet count was less than 10 $X10^{9}$ /L.

Storage of Samples

The resultant Platelet Poor Plasma after centrifugation were put in 0.5ml aliquots and stored at -80^{o}C till run.

Laboratory Analysis

Eighteen (18) participants were already on Emicizumab at the time their samples were being taken and one-stage FVIII assay and Bethesda Assay cannot be used to measure residual FVIII or inhibitors in individuals on Emicizumab.

Efforts to import chromogenic assay which can be used to assess residual FVIII and measure chromogenic Bethesda Assay for inhibitors on all participants (those on Emicizumab and those on factor concentrates) were not successful.

A one stage FVIII assay was performed on sixty-three (63) samples out of the eighty-one (81) samples to confirm the diagnosis of haemophilia A.

A Bethesda Assay was performed on sixty-three (63) samples out of the 81 samples for the presence of inhibitor.

3.7.1. One stage Assay of FVIII

Principle

This assay is based on APTT principle which measures clotting time of plasma after the activation of contact factors but without tissue thromboplastin.

The one stage assay consists of comparing the ability of dilution of patient's plasma and of standard plasma to correct APTT of plasma known to lack FVIII (FVIII deficient plasma) but contains all other factors required for coagulation. The degree of correction is directly proportion to the amount of FVIII in the test plasma.

Reagents and equipment

- Control Plasma N
    - Used for the calibration of coagulation and fibrinolysis tests.
    - It is obtained from pooled citrated plasma collected from selected healthy blood donors and lyophilised.
    - FVIII- WHO Standard – 95%
- Normal Control
    - Control plasma in the normal range
    - Range - 24.5s – 30.8s
    - Lyophilised
- Abnormal Control plasma
    - Control plasma in the therapeutic range and run as "abnormal plasma" for control of accuracy and precision.
    - Range – 35.8s – 45.6s
    - Lyophilised
- FVIII- deficient plasma
    - Lyophilised human plasma with residual FVIII of <1%
- Owren's Verona Buffer
- APTT Reagent
- 0.025mol/L Calcium Chloride ($CaCl_2$)

- Coagulation Analyser – HumaClot Duo Plus
- Water Bath
- Distilled Water

Storage of Reagents

Reagents were stored at 2 to 8^0C according to manufacturer's instructions.

Other Materials Used

- 75 x 10mm test tubes
- Pipettes and pipette tips (100ul and 1000ul)
- Cuvettes for coagulation analyser

Calibration and controls

- The coagulation analyser was serviced and calibrated before use.
- Normal and abnormal controls were run before each batch of samples were run.

Procedure

- The HumaClot Duo Plus is a 2-channel coagulometer that is used for semi-automated photo-optical blood plasma haemostasis analysis. The analyser applies a photometer which generates a quantitative measuring curve of the optical density, and this is shown in seconds. Formation of the fibrin clot increases the turbidity, and this is measured as an increase in scattered light intensity when exposed to light at a wavelength of 660 nanometres (nm).

Figure 3.1 HumaClot Duo Plus with reagents and test tube for FVIII Assay

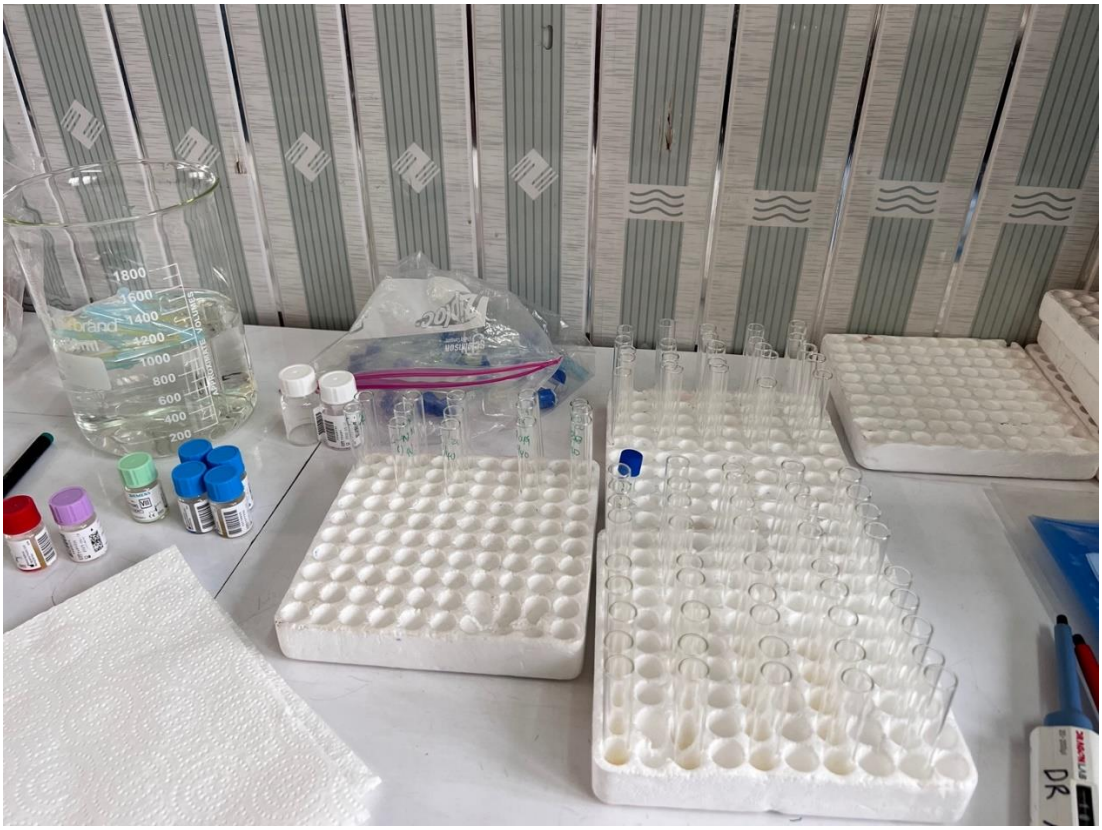


Figure 3.2 Set up for Inhibitor Assay

- Reconstitution of reagents
    - Reagents were brought to room temperature.
    - Normal Control, Abnormal Control, Control Plasma N and FVIII-deficient plasma
        - The contents of these were each dissolved by adding 1ml of distilled water and allowed to stand for a minimum of 15 minutes.
        - They were then gently swivelled to mix the contents well without forming a foam.
        - Each reagent was gently mixed right before use.

    - APTT reagent
        - Reagent was gently mixed by inversion of 5-8 times before being used.
        - Performing APTT
    - APTT reagent and $CaCl_2$ were put in their respective slots in the coagulation analyser and were kept warm at 37 ^{0}C.
    - 100ul of plasma to be tested is added to a cuvette.
    - 100ul of pre-warmed APPT reagent was then added to the plasma in the cuvette.
    - Mixture was incubated for 3 minutes.
    - 100ul of prewarmed $CaCl_2$ was then added after 3 minutes, and time taken for the dilution to clot was determined using the coagulation analyser.
    - Clotting time was recorded in seconds.

- Control- Normal (CON-N)
    - 100ul of CON-N was added to cuvette.
    - APTT was then performed on the control plasma.

- Control- Abnormal (CON-A)
    - 100ul of CON-A was added to cuvette.
    - APTT was then performed on the abnormal control plasma.

The samples were thawed at 37 ^{0}C for 5 minutes and gently mixed.

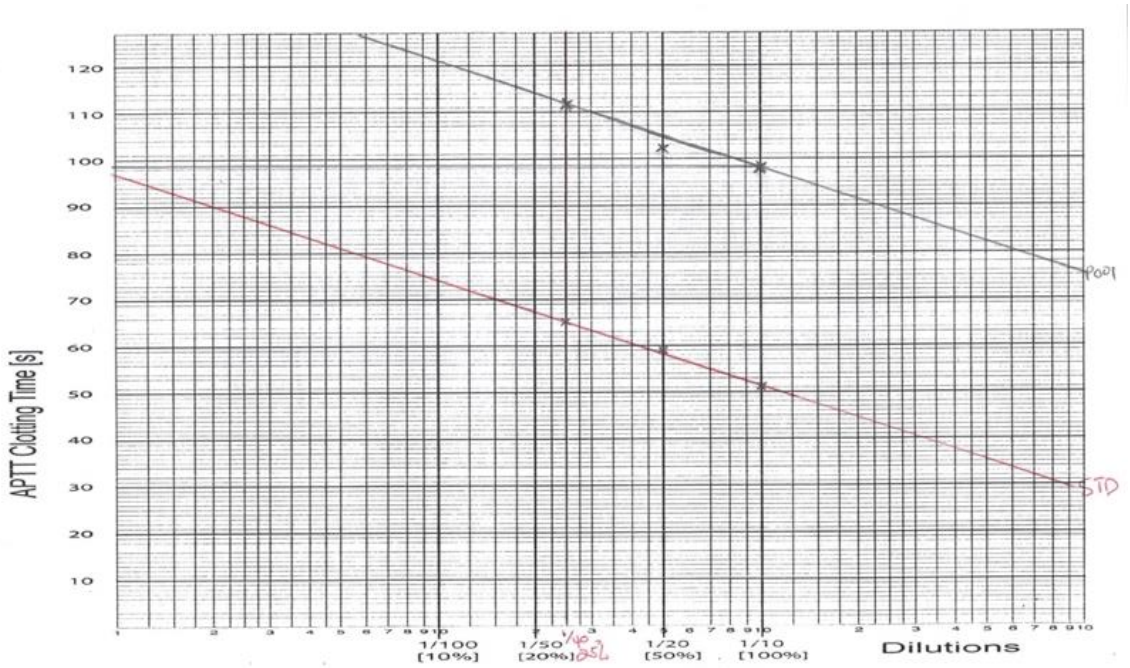
- Calibrator /Standard
    - 3 test tubes were labelled Cal - 1/10, Cal - 1/20, Cal - 1/40
    - 900ul of buffer was added to the 1st test tube labelled Cal – 1/10 and 500ul each was added to the 2nd and 3rd test tubes labelled Cal – 1/20 and Cal – 1/40 respectively.
    - 100ul of Control-N plasma was added to the 1st tube and mixed gently.
    - 500ul from 1st test tube was transferred to the 2nd test tube and gently mixed.
    - 500ul from 2nd test tube was transferred to the 3rd test tube and gently mixed.
    - 100ul each test tube was added separately into separate cuvettes.
    - 100ul of FVIII-deficient plasma was added to each dilution.
    - APTT was performed on each dilution.
    - Clotting time for each dilution was recorded in seconds.
    - Clotting time was done in pairs and average time calculated.

- Sample (Test plasma)
    - 3 test tubes for each test sample were labelled with the unique identity number and I/10, 1/20, 1/40 e.g., P001 – 1/10, P001 – 1/20, P001 – 1/40.
    - 900ul of buffer was added to 1st test tube P001 – 1/10
    - 500ul was added to each of 2nd and 3rd test tubes labelled P001- 1/20, P001-1/40 respectively.
    - 100ul of thawed test plasma (e.g., P001) was added to the 1st test tube and gently mixed.
    - 500ul from 1st test tube was added to the 2nd test tube and gently mixed.
    - 500ul from 2nd test tube transferred to the 3rd test tube and gently mixed.
    - 100ul from each test tube was added separately into separate cuvettes.
    - 100ul of FVIII-deficient plasma was added to each cuvette.
    - APTT was performed on each dilution.
    - Clotting time for each dilution was recorded in seconds.

- Deriving residual FVIII activity
    - A log-linear (semi-log) graph paper was used.
    - Clotting time (y-axis) was plotted against dilution (x-axis)
    - The clotting time for the Calibration/ Standard plasma was plotted against respective dilution on the graph paper and the line of best fit was drawn.
    - The 1/10 dilution was assigned as 100% activity (potency) for FVIII.
    - Clotting times for test plasma was plotted against respective dilutions and line of best fit drawn.
    - A straight line was drawn from the 1/10 point on the y-axis of the test plasma line to the point where it hit the Calibration/Standard plasma line and then dropped down to the x-axis to obtain the % activity.
    - Residual FVIII activity for each plasma was calculated by multiplying % activity with the value of FVIII given by manufacturer for Control-N plasma.

      Residual FVIII Activity = $\frac{\text{FVIII in test plasma}}{\text{FVIII control plasma}}$ X 100%

a



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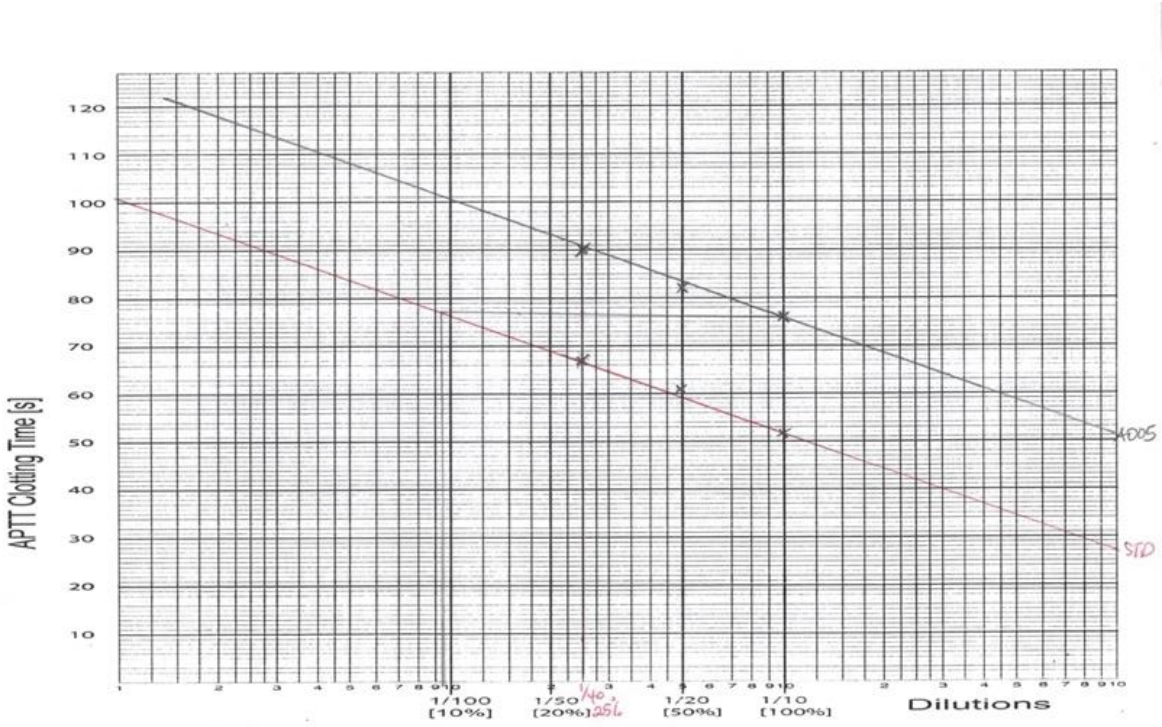

Figure 3.3a & b Semi-Log graph showing the derivation for residual FVIII Activity

Figure 3.3a – The concentration obtained from the graph, fell outside <1%

Figure 3.3b – The concentration obtained from the graph was 9.5.

The concentration of FVIII in Standard plasma according to manufacturer was 95.

$$\text{Residual FVIII activity } \frac{9.5}{95} \times 100\% = 10\%$$

Precautions

- Participants were kept calm to prevent stress as excessive stress could cause an increase in FVIII levels, especially in individuals with moderate or mild disease.
- The tourniquet was tied for a short time (less than 1 minute) to prevent activation of the coagulation cascade.
- It was ensured that the venous blood flowed freely to prevent haemoconcentration and activation of platelet and clotting factors.
- It was ensured that venous blood was filled to the appropriate marked level so that the right blood to anticoagulant ratio was maintained.
- The tube was gently inverted for about 10times to ensure that the venous blood was thoroughly mixed with the anticoagulant.
- Samples for participants who were to receive factor concentrate on the day was taken before they received factor concentrate.
- Sample was thawed at 37 ^{0}C to prevent the formation of cryoprecipitate.
- The pipette tip was changed with each transfer.
- All reagents were visually inspected to ensure there was no contamination.
- Both normal and abnormal controls were used for each batch of samples run to ensure precision.

3.7.2. Bethesda Assay

Principle

FVIII inhibitors are usually time dependent, thus if FVIII is added to plasma containing an inhibitor and the mixture is incubated, FVIII will be progressively neutralised.

One (1) Bethesda unit is defined as the amount of inhibitor that will neutralise 50% of 1 unit of FVIII in test plasma after 2 hours of incubation at 37^{o}C. The normal pool plasma is taken to represent 1 unit of FVIII. An equal volume of normal plasma mixed with buffer is taken to represent the 100% value. At the end of the incubation period, the residual FVIII is assayed, and the inhibitor strength is calculated from a standard graph of residual FVIII activity versus inhibitor units.

Reagents and equipment

- Control Plasma-N
- Normal Control
- Abnormal Control plasma
- FVIII- deficient plasma
- Glyoxaline Buffer
- APTT Reagent
- 0.025mol/L Calcium Chloride ($CaCl_2$)
- Coagulation Analyser – Huma Clot Duo Plus
- Water Bath
- Distilled Water

Storage of Reagents

Reagents were stored at 2 to 8 ^{0}C according to manufacturer's instructions.

Other Materials Used

- 75 x 10mm test tubes
- Pipettes and pipette tips (100ul and 1000ul)
- Cuvettes for coagulation analyser

Calibration and control

- The coagulation analyser was serviced and calibrated before use.
- Normal and abnormal controls were run before each batch of samples were run.

Procedure

- Reconstitution of reagents
    - Reagents were brought to room temperature.
    - Normal Control, Abnormal Control, Control Plasma N and FVIII-deficient plasma
        - The contents of these were each dissolved by adding 1ml of distilled water and allowed to stand for a minimum of 15 minutes.
        - They were then gently swivelled to mix the contents well without forming a foam.
        - Each reagent was gently mixed right before use.
    - APPT reagent
        - Reagent was gently mixed by inversion of 5-8 times before being used.

Control

- The test tube was labelled as Control and add 150ul of buffer to 150ul of NPP.
- The tube was incubated in the water bath at 37^0C for 2 hours.
- FVIII assay at 1:5, 1:10, 1:20 dilutions were performed on the control mixture.

Samples were thawed at 37ºC for 5 minutes and mixed gently.

Making dilutions for the assay

- Test tubes were labelled with unique study ID dilution factor e.g., P001-1:2, P001-1:4, P001-1:8
    - 200ul of buffer was added to each tube.
    - 200ul of corresponding test plasma (e.g., P001) was added to 1st tube 1:2 and mixed well.
    - 200ul from 1st tube was transferred to 2nd tube (1:4) and was mixed well.
    - 200ul from 2nd tube was transferred to 3rd tube (1:8) and mixed well.
- Four Test tubes were labelled and 200ul of NPP added to each tube.
    - Tube 1 (P001) – Neat + NPP – 150ul of test plasma was added.
    - Tube 2 (P001) - 1:2 + NPP – 150ul of 1:2 dilution was added.
    - Tube 3 (P001) - 1:4 + NPP – 150ul of 1:4 dilution was added.
    - Tube 4 (P001) – 1:8 + NPP – 150ul from 1:4 dilution was added.
- All 4 test tubes were incubated in the water bath at 37°C for 2 hours.
- One stage factor assay at 1:5, 1:10, 1:20 dilutions was performed in all 4 tubes

Factor Assay for Residual FVIII Activity

- Control
    - 3 test tubes were labelled C-1/5, C-1/10, C-1/20
    - 800ul, 500ul, 500ul of buffer for 1/5, 1/10, 1/20 dilutions respectively were added to different test tubes.
    - 200ul of control mixture was added to first tube (containing 800ul buffer) and mixed well,
    - 500ul from 1st tube was transferred to 2nd tube and mixed well.
    - 500ul from 2nd tube was transferred to 3rd tube and mixed well.
    - 100ul from 1/5, 1/10, 1/20 dilutions were added to different cuvettes.
    - 100ul of FVIII-Deficient plasma was added to each cuvette.
    - APTT was performed as described earlier.

- Test plasma
    - One stage factor assay at 1/5, 1/10, 1/20 dilutions was performed in all 4 test tubes. One stage factor assay was done as described earlier.

Deriving Residual FVIII Activity

- Log-linear graph paper was used.
    - Control
        - Dilution (concentration) against clotting time was plotted.
        - Line of best fit was drawn.
        - 1/5 dilution was assigned 100% potency.
    - Test plasma
        - Dilution was plotted against concentration for each test plasma and line of best fit drawn.
        - The residual FVIII activity was extrapolated from the control.
        - Residual FVIII = $\frac{\text{FVIII patient mixture}}{\text{FVIII control mixture}} \times 100\%$
        - The dilutions of test plasma that gives residual FVIII within the range of 25%-75% is used to calculate the inhibitor.
        - Inhibitor titre was derived from the conversion table and multiplied by the dilution to give final titre.
        - Average inhibitor titre was found for test plasma that had more than 1 dilution in the range of 25 – 75%.

Table 3.1 Table showing Bethesda Conversion Table for derivation of inhibitor titres from residual FVIII Activity

BETHESDA CONVERSION TABLE

RESIDUAL VIII%	***BU***	***RESIDUAL VIII%***	***BU***	***RESIDUAL VIII%***	***BU***
97	0.05	61	0.7	40	1.35
93	0.1	59	0.75	38	1.4
90	0.15	57	0.8	37	1.45
87	0.2	55	0.85	35	1.5
84	0.25	53	0.9	34	1.55
81	0.3	51	0.95	33	1.6
78	0.35	50	1	32	1.65
75	0.4	48	1.05	30	1.7
73	0.45	46	1.1	29	1.75
70	0.5	45	1.15	28	1.8
68	0.55	43	1.2	27	1.85
66	0.6	42	1.25	26	1.9
64	0.65	41	1.3	25	2

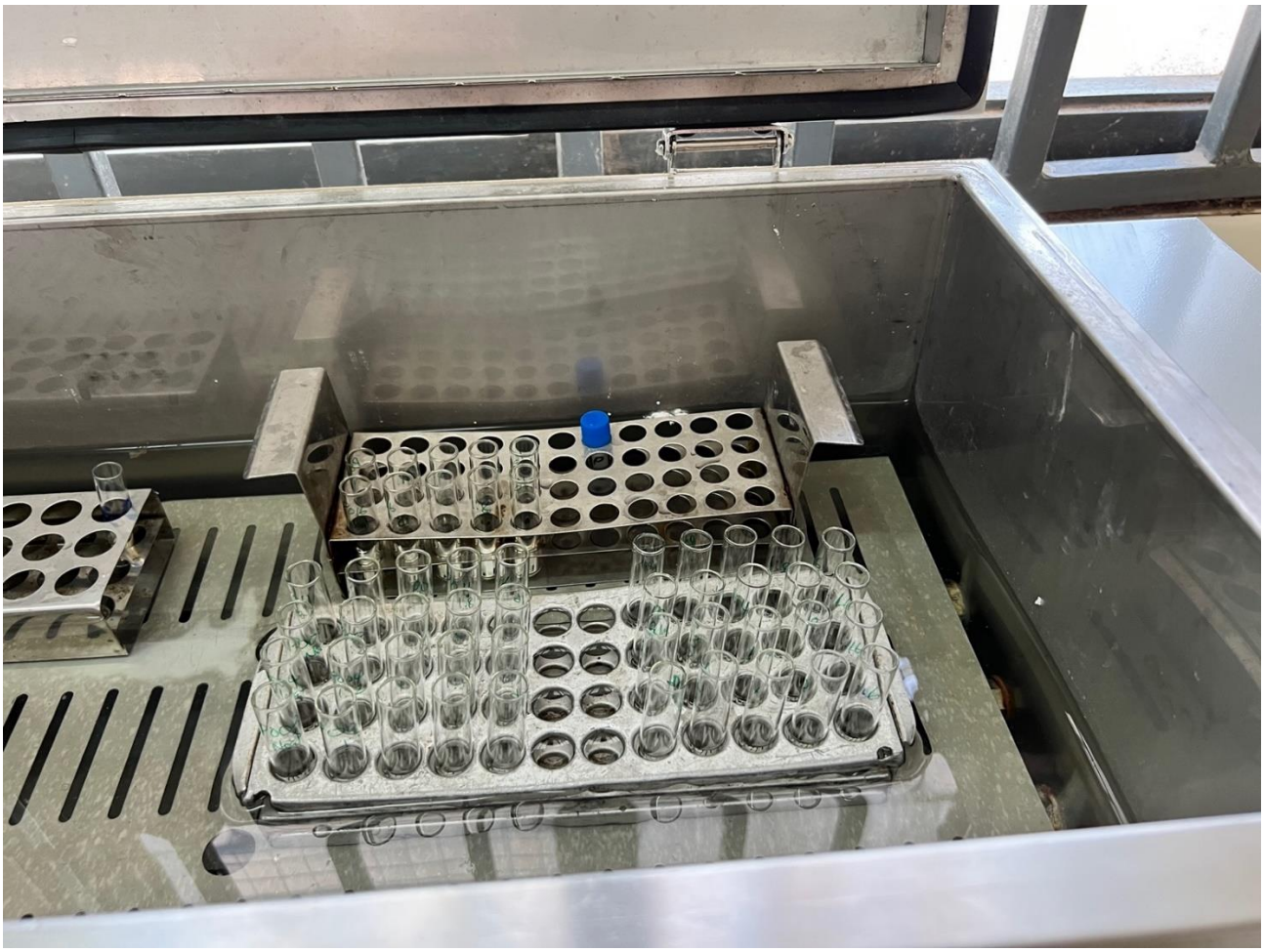

Figure 3.4 Water Bath with test tubes for 2-hour incubation for Bethesda Assay

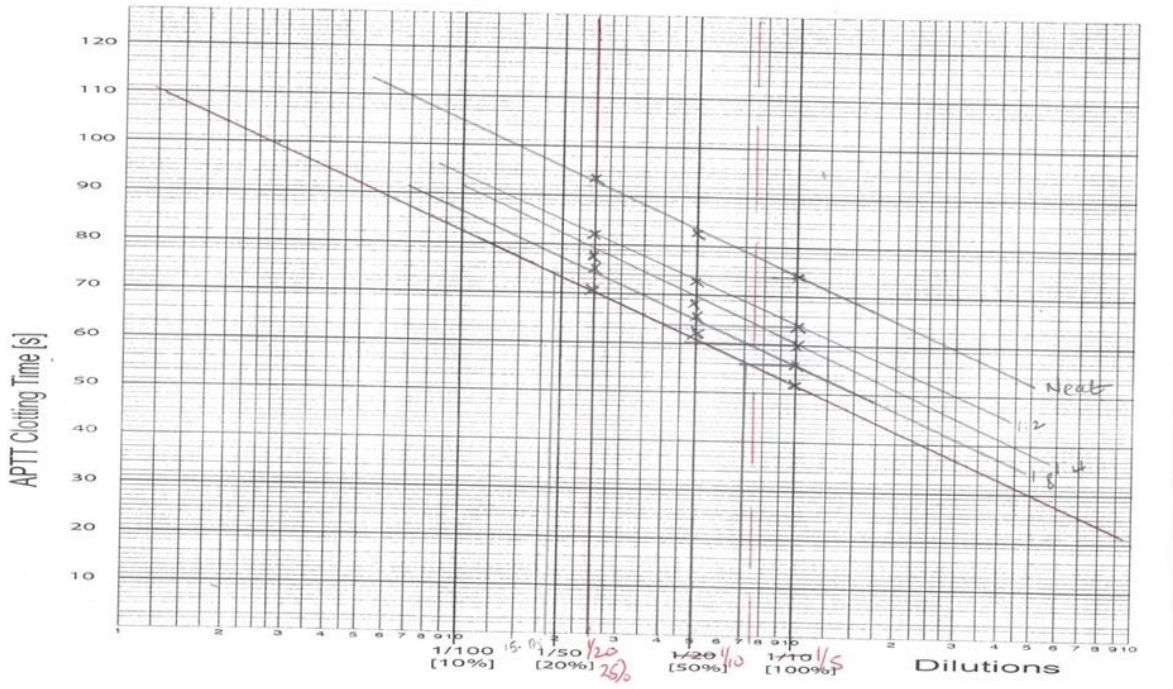

Figure 3.5 Semi-log paper showing residual FVIII Activity of various dilutions after 2-hour incubation in Bethesda Assay

Precautions

- Samples were thawed at 37 ^{0}C to prevent the formation of cryoprecipitate.
- It was ensured that the temperature of the water bath was always at 37 ^{0}C.
- The pipette tip was changed with each transfer.
- All reagents were visually inspected to ensure there were no contamination.
- Both normal and abnormal controls were used for each batch of samples run to ensure precision.

Data Handling

All data collected from the folders/medical record was limited to only what was necessary to carry out the study as outlined. All findings from the study were reported in statistical summary form only. All data were treated with strict confidentiality. Each participant was assigned a unique study code that was used on all data forms and sample and storage bottles.

Data Analysis

The data was captured into a Microsoft Excel Spreadsheet for cleaning and coding. It was then transferred into STATA 14 statistical software for analysis. Descriptive statistics such as frequencies, percentages, mean, and standard deviation were used. Pie chart and bar graphs were also used. Mean and standard deviation were estimated for age of participants, age at diagnosis, number of bleeding episodes, number of times each therapy received, and age of first exposure to factor concentrate. Percentages were used to estimate the type of therapy received and a bivariate analysis was conducted to determine the association between the type of therapies received and disease severity.

The first objective sought to classify patients with haemophilia A based on severity. This was achieved by using the bar graph to display the severity status (mild, moderate, and severe).

The Pearson Chi-square test with an alpha level of 0.05 was used to test for association between the severity status and types of therapies received. The Cohen Kappa analysis was used to compare severity at diagnosis and severity measured during study.

The second objective was to determine the prevalence of inhibitors, and this was done using percentage and types of inhibitors was shown using a pie chart.

The third objective sought to determine the types of therapies received since diagnosis. This was done using percentages.

In terms of objective four, the independent student t-test and proportion test were used to assess the association between inhibitor status and clinical characteristics.

Alpha level of 0.05 was used.

CHAPTER 4: RESULTS

81 participants out of 90 active patients (patients who have reported at least once in the past year) were recruited into the study. 81 participants were used in the analysis for socio-demographic characteristics, type of therapies received, and selected clinical characteristics of patients. 63 participants were used in the analysis for residual FVIII activity, prevalence of inhibitor and clinical factors associated with inhibitor development. This was because the one-stage assay and classical Bethesda Assay cannot be used to measure residual FVIII activity and inhibitor respectively on individuals on Emicizumab.

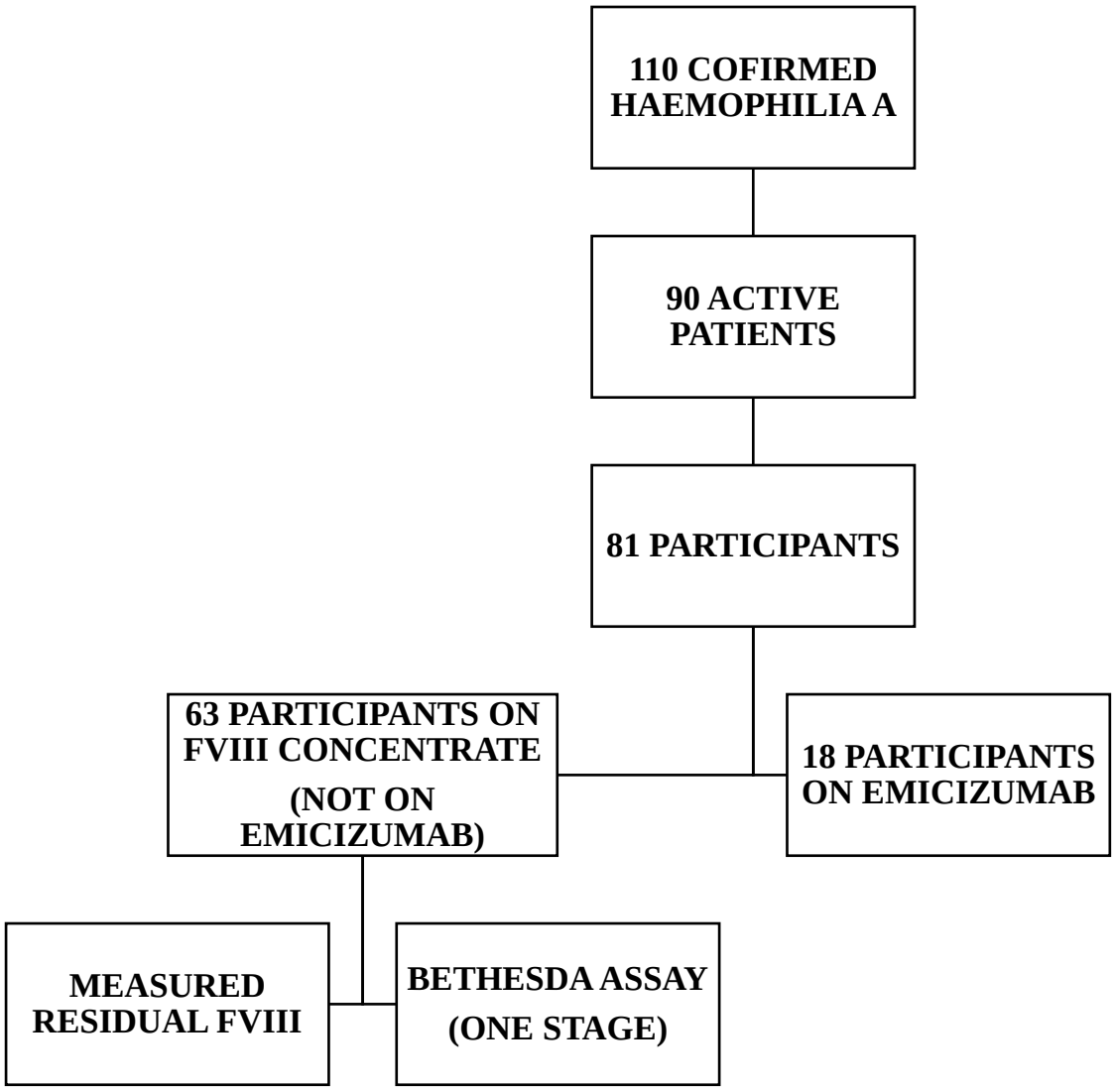

Figure 4.1 Flow chart showing the selection criteria of the study participants.

4.1. Socio-Demographic Characteristics

All participants were males.

The average age of the study participants was 17 years of which 61.7% were children (n=50). The mean age of participants less than 18years was 8.7years whilst that of participants 18years and older was 20.3years.

Approximately 91% (n=74) of participant have formal education with 46% (n=37), 16% (n=13) and 18.5% (n=15) having primary, junior high and tertiary education respectively as their highest level of education

Table 4.1 Age of participants. Age of participants.

Variable	(Mean, SD±)
Age	17.1(±13.5)
<18 (Children)	8.7(±4.5)
≥ 18 (Adults).	20.3(14.4)

Table 4.2 Educational Level of Participants

Variable	Frequency	Percentage (%)
Educational Level		
No Formal Education	7	8.6
Primary	37	45.7
Junior High	13	16.1
Senior High	9	11.1
Tertiary	15	18.5

Table 4.3 Occupation of participants

Variable	Frequency	Percentage (%)
Occupation		
Unemployed/Retired	3	3.7
Employed	17	21.0
Students	55	67.9
Non-Applicable	6	7.4

4.2. Selected Clinical Characteristics of Participants

Participants with severe disease were the youngest (mean age of 13.3 years) with those with moderate disease being the oldest (mean age of 28.1 years)

Majority of participants (61.7%) did not have a family history of haemophilia A.

More participants (54.5%) with severe disease had a family history of haemophilia A.

4.2.1. Bivariate analysis showing association between family history and Factor VIII Severity Status

Table 4.4 Mean age of various severity levels and family history of haemophilia A

Characteristic	Factor Severity Status			P-Value
	Mild n (%)	Moderate n (%)	Severe n (%)	
Age (Mean, SD±)	24.2 ±12.8	28.1±22.3	13.3±9.0	0.002
Family history				0.037
No	4(8)	6(12)	40(80)	
Yes	8(25.8)	6(19.4)	17(54.8)	

4.2.2. Clinical Presentation at diagnosis

Bleeding from the oral cavity was the commonest presentation (32.1%), followed by joint swelling and pain (21.0%) and central nervous system bleeding/haematoma the least common presentation.

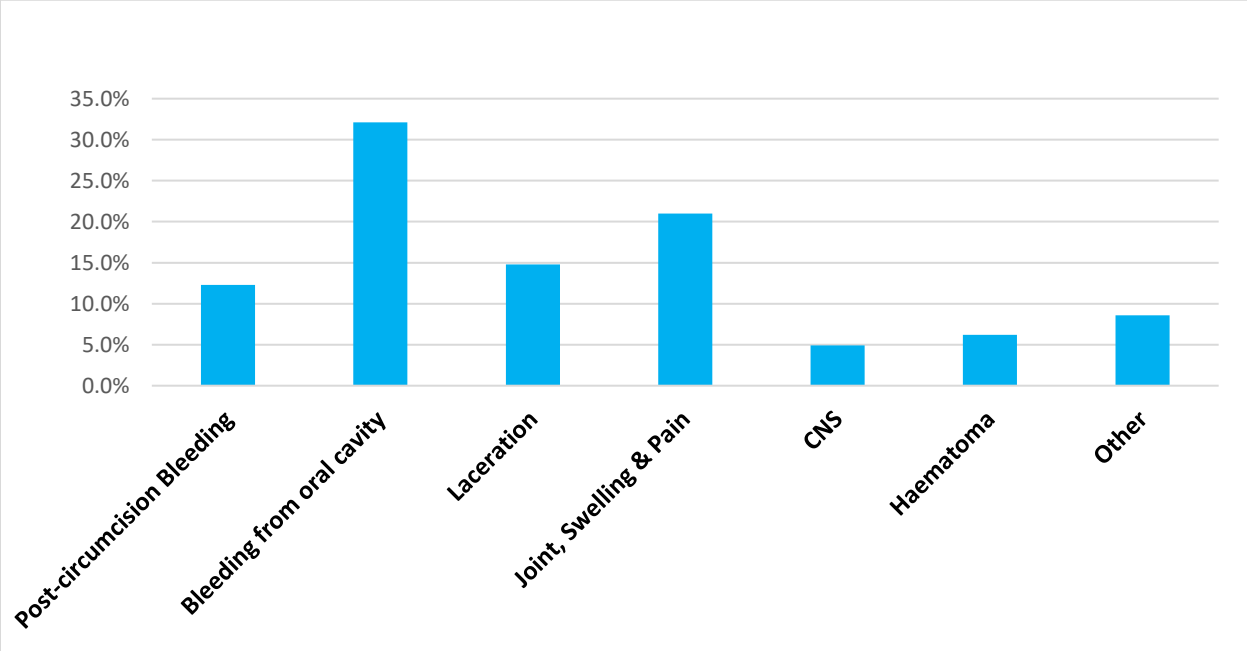

Figure 4.2 Clinical presentation at diagnosis

Table 4.5 Clinical presentation at diagnosis and factor severity

Variable	Factor VIII Severity Status			P-Value
	Mild n (%)	Moderate n (%)	Severity n (%)	
Clinical Presentation at Diagnosis				0.788
Post-circumcision Bleeding	1(10.0)	2(20.0)	7(70.0)	
Bleeding from oral cavity	3(11.5)	4(15.4)	19(73.1)	
Laceration	4(33.3)	1(8.3)	7(58.3)	
Joint Swelling & Pain	2(11.8)	3(17.6)	12(70.6)	
CNS	1(25.0)	0	3(75.0)	
Haematoma	0	2(40.0)	3**(60)**	
Other	1(14.3)	0	6(60.0)	

Spontaneous Bleeding and Factor VIII Severity Status.

53.1% of participants presented with provoked bleeding episode at clinical diagnosis whilst 46.9% presented with spontaneous bleeding.

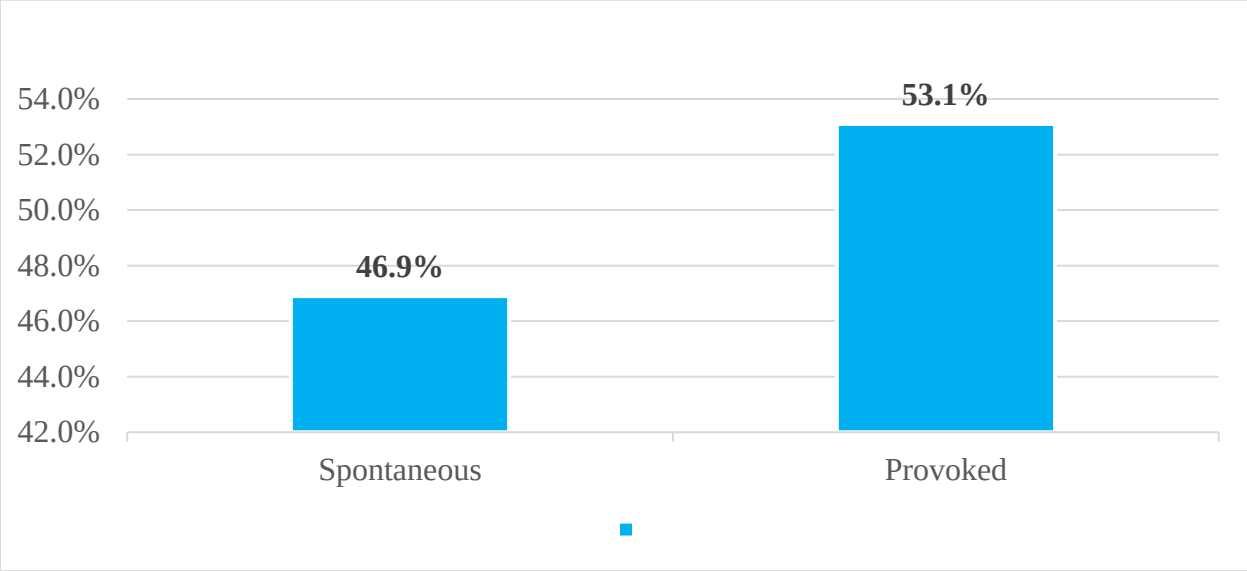

Figure 4.3 Spontaneous bleeding at presentation

Table 4.6 Association between age at clinical diagnosis and age at laboratory diagnosis

Variable	Factor Severity Status			p-Value
	Mild	Moderate	Severity	
Age at diagnosis -Clinical (Mean, SD±)	15.5±0.6	24.1 ± 0.7	7.0 ± 0.4	0.000
Age at diagnosis – Laboratory (Mean, SD±)	15.8± 0.7	24.1 ± 0.8	7.9 ± 0.5	0.001

Age at First Exposure to FVIII concentrate.

Participants with severe disease were exposed to FVIII concentrates at an earlier age (7.3years).

Table 4.7 Age at first exposure to FVIII concentrate and FVIII severity.

Variable	Factor Severity Status			p-Value
	Mild	Moderate	Severity	
Age at First Exposure to Factor Concentrate	18.2±0.5	25.3±0.6	7.3 ± 0.7	0.0001
SD= Standard Deviation				

Prophylaxis and Factor VIII Severity Status.

5 participants are on prophylactic therapy, and all have severe disease.

Table 4.8 Prophylaxis and FVIII severity

Variable	Factor Severity Status			p-value
	Mild (%)	Moderate (%)	Severe (%)	
Prophylaxis				0.466
Yes	0	0	5(100.0)	
No	12(15.8)	12(15.8)	52(68.4)	
Alpha=0.05;	**n=Frequency;**		**%=Percentage**	

The number and types of bleeding episode and FVIII severity status.

Joint bleeding was the commonest bleeding episode.

Table 4.9 Number and types of bleeding episodes and FVIII severity

Value	Factor Severity Status			p-Value
	Mild (%)	**Moderate (%)**	**Severe (%)**	
Number of bleeding episodes in the year (Mean, SD±)	2.1±0.6	1.82± 0.5	2.1±0.5	0.945
Type of Bleeding Episode				0.784
Joint	2(9.5)	6(28.6)	13(61.9)	
Muscle	0	1(50.0)	1(50.0)	
Gum	2(33.3)	0(0)	4(6.7)	
CNS	0	1(25.0)	3(75.0)	
Gastrointestinal	0	0	1(100.0)	
≥2	3(33.3)	3(20.0)	13(68.4)	
Other	1(25.0)	0	3(75.0)	

4.3. Classification of participants based on severity.

About 70.4% (n=57), 14.8% (n=12) were classified as severe and moderate respectively. The remaining 14.8% (n=12) were mild.

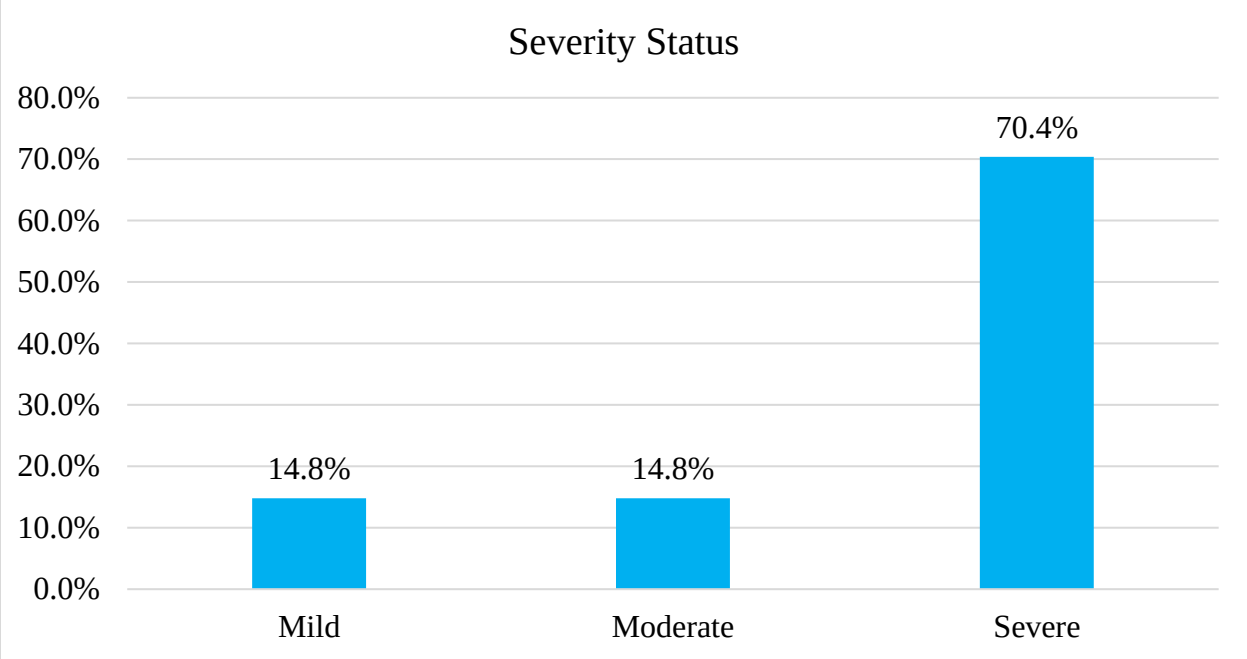

Figure 4.4 Classification of participants based on severity.

4.4. Measured Residual FVIII Activity

60.3% of participants had severe disease, 19% had moderate disease and 12.7% had mild disease.

4.4.1. Analysis of agreement between measured residual activity (severity during study) and severity at Diagnosis

Majority of participants (60.3%) had severe form of the Haemophilia A whereas severity at diagnosis had indicated (66.7%). 17 participants (27%) had moderate disease during the study, but 12 participants (19%) had moderate disease at diagnosis whilst 8 participants (12.7%) had mild disease during study, but 9 participants (14.3%) had mild disease at diagnosis.

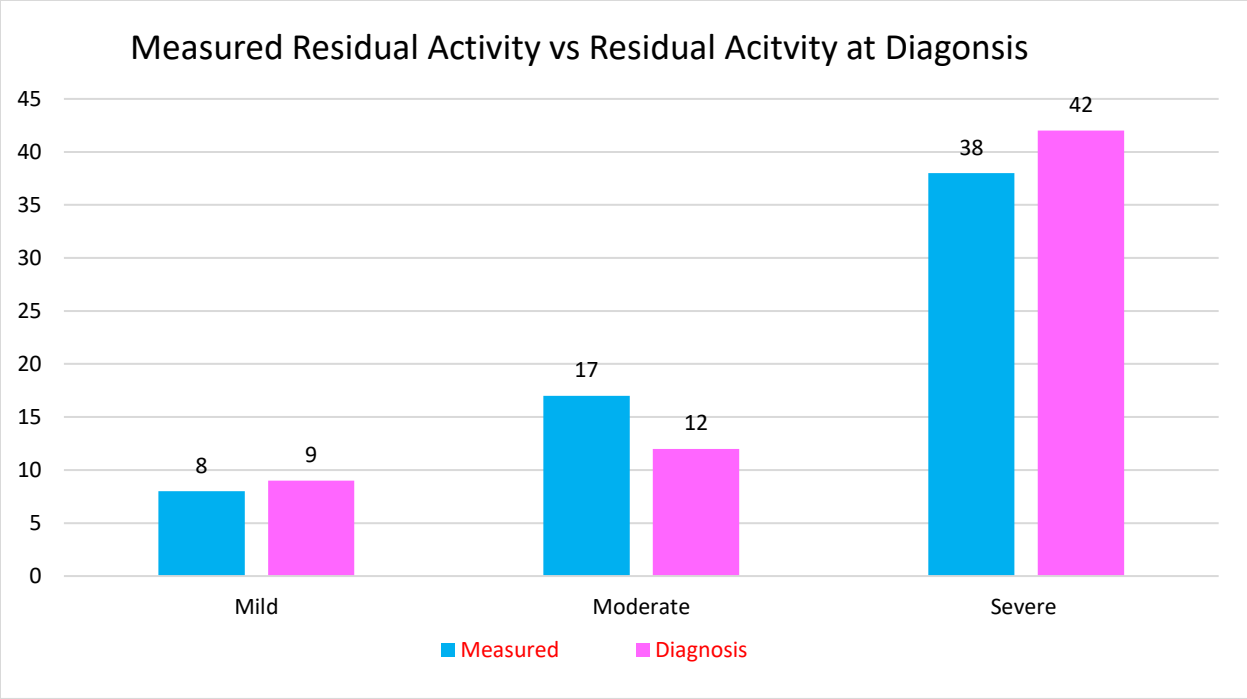

Figure 4.5 Variations in the classification of disease at diagnosis and during study

A Kappa analysis was conducted to assess the agreement between the diagnosis and study severity. The Cohen's kappa results show a moderate (74.6%) agreement between the diagnosis and measured residual activity.

Table 4.10 Agreement between factor activity level at initial diagnosis and during study

Agreement (%)	**Expected Agreement**	**Kappa**
74.6	47.2	0.52

4.5. Type of therapy(ies) received since diagnosis.

About 48% (n=39), 24% (n=19) and 14% (n=11) had received FVIII Concentrate, FVIII Concentrate & Cryoprecipitate, FVIII, Cryoprecipitate & Emicizumab as treatment respectively.

Table 4.11 Type of therapies received.

Types of Treatment	Frequency (N=81)	Percentage
None	5	6.2
FVIII Concentrate (recombinant and or plasma derived)	39	48.2
FVIII Concentrate & Cryoprecipitate	19	23.5
FVIII Concentrate & Emicizumab	7	8.6
FVIII, Cryoprecipitate & Emicizumab	11	13.6

4.5.1. Type of therapy(ies) received and FVIII severity status.

A bivariate analysis was conducted to examine the association between the types of therapies since diagnosis and factor VIII severity status using an alpha level of 0.05.

Table 4.12 Type of therapies received and FVIII severity status.

Variable	Factor Severity Status			p-Value
	Mild (%)	Moderate (%)	Severe (%)	
Types of Treatment				0.139
None	0	3(60.0)	2(40.0)	
FVIII Concentrate **(recombinant and plasma derived)**	4(10.3)	7(17.9)	28(71.8)	
FVIII & Cryoprecipitate	5(26.3)	2(10.5)	12(63.2)	
FVIII & Emicizumab	1(14.3)	0	6(85.7)	
FVIII, Cryo & Emicizumab	2(18.2)	0	9(81.8)	

4.6. Prevalence of inhibitors

11.1% (n=7) of 63 participants had inhibitors.

Approximately 86% (n=6) of them had low titre inhibitor with one participant had a high titre inhibitor.

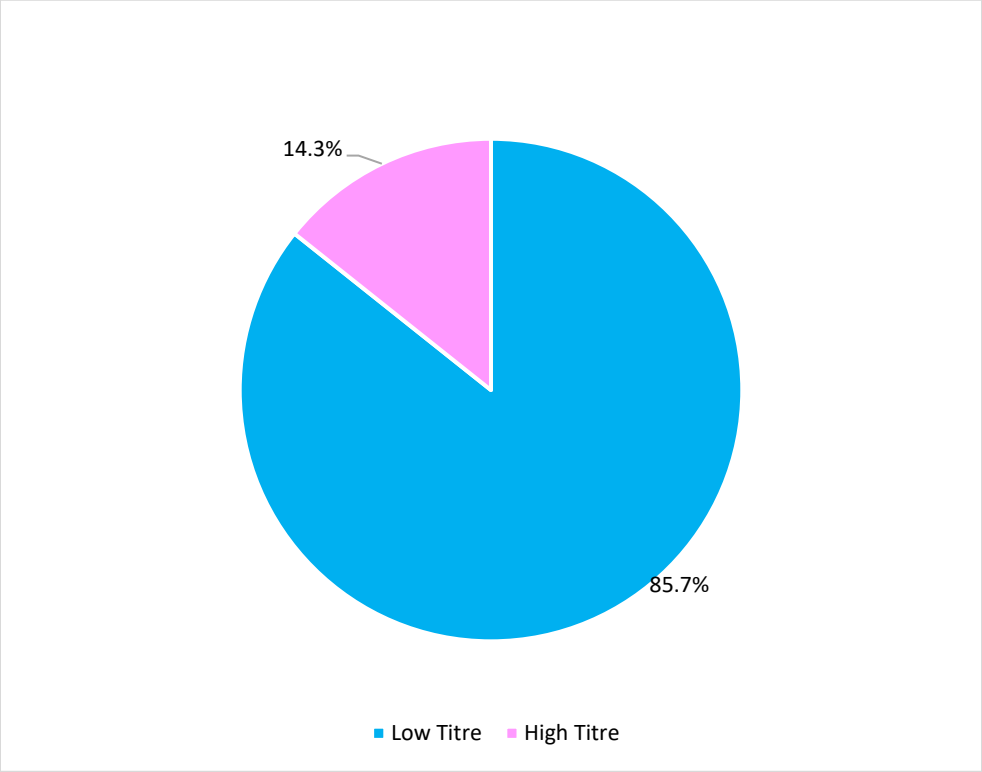

Figure 4.6 Percentage of high titre and low titre inhibitors

4.7. Clinical factors associated with development of inhibitors.

4.7.1. Severity status and Inhibitor Status

A proportion test was carried out for participants who received prophylaxis as explained by severity at diagnosis and severity measured during the study. The results show that most of the participants with inhibitor had the severe (85.7%) form of Haemophilia A (p=0.001).

Table 4.13 FVIII severity status and inhibitor status

	Severity Status			p-Value
	Mild (%)	**Moderate (%)**	**Severe (%)**	
Inhibitor Positive	1(14.3)	0	6(85.7)	0.001

4.7.2. Age at first exposure to FVIII Concentrate

The average age at first exposure to factor concentrate among inhibitor positive participants was 15.6 years. In children less than 18years, average age of inhibitor positive participants was 6.6years whilst that of adults was 38years.

Table 4.14 Age at first exposure to FVIII concentrate and inhibitor positive participants.

Average age at first exposure to factor concentrate	**Mean**	**SD (±)**
Inhibitor Positive (n=7)	15.6	16.1
<18 years(n=5)	6.6	5.6
≥18(n=2)	36.5	4.2

An independent student t-test was conducted to examine the association between the age at first exposure to factor concentrate and Inhibitor Status using an alpha level of 0.05.

Table 4.15 Age at first exposure to FVIII concentrate and inhibitor status.

Characteristic	Inhibitor Status		p-Value
	Negative	Positive	
Age at first exposure to factor concentrate (Mean, SD±)	10.6±13.5	15.6±16.1	0.377

Table 4.16 Number of exposures of inhibitor positive participants

Inhibitor Positive	Exposure (Mean, SD±)
	27.1 (±30)

4.7.3. Number of bleeding episode in the past year

Inhibitor positive participants had on average 2.14 bleeding episodes in the year whilst inhibitor negative participants had less bleeding episode in the year.

Table 4.17 Number of bleeding episodes and inhibitor status

Characteristic	Inhibitor Status		p-value
	Negative (%)	Positive (%)	
Number of bleeding episodes in the year (Mean, SD±)	1.43±1.60	2.14(1.86)	0.286

4.7.4. Type of therapy received and Inhibitor Status

About 86% (n=6) of inhibitor positive participants had received FVIII concentrate only.

The proportion test shows significant differences in proportion according to the types of therapy received (p=0.001).

Table 4.18 Type of therapy received and inhibitor status.

.	Types of therapy received			p-Value
	None (%)	FVIII Concentrate (%)	FVIII Concentrate & Cryoprecipitate (%)	
Inhibitor Positive	0	6(85.7)	1(14.3)	0.001

4.7.5. Type of FVIII concentrate and Inhibitor Status

57.1% inhibitor positive participants had received recombinant factor concentrate whilst 42.9% had received both recombinant and plasma derived factors.

Table 4.19 Type of FVIII concentrate and inhibitor status.

	Types of therapy received			p-Value
	Recombinant Factor (%)	Plasma Derived Factor (%)	Both (%)	
Inhibitor Positive	4(57.1)	0	3(42.9)	0.211

4.7.6. Family History of Haemophilia A and Inhibitor Status

The proportion of participants with inhibitors with a family history of haemophilia A (57.1%) were not significantly lower than those without a family history of haemophilia A (42.9%).

Table 4.20 Family history of Haemophilia A and inhibitor status

	Family History		p-Value
	No (%)	Yes (%)	
Inhibitor	3(42.9)	4(57.1)	0.624

4.7.7. Prophylaxis and Inhibitor status

A bivariate analysis was conducted to ascertain the association between prophylaxis and inhibitor status. **The findings** indicate there is no significant relationship between the variables (p=0.410).

Table 4.21 Prophylaxis and inhibitor status

	Inhibitor Status		**p-Value**
	Negative (%)	Positive (%)	
Prophylaxis			0.410
Yes	5(100)	0	
No	51(81%)	7(11%)	

CHAPTER 5: DISCUSSION

A total of eighty-one (81) patients with haemophilia A were recruited over the period of this study drawn from both the adult and paediatric haemophilia registries at the Korle-Bu Teaching Hospital.

The relatively low numbers of participants in this study are consistent with sample sizes from previous studies in the West African subregion. Ahmed et al (2019) in a study of opioid dependence in persons living with haemophilia in northern Nigeria, reviewed 88 patients between 1996 and 2012 (12 years period) [118] while a study of the epidemiology of inhibitors among persons living with haemophilia A in South West Nigeria by Bolarinwa et al (2020) recruited forty (40) patients.[119] Ghosh and Ghosh reported that only 10-20% of expected persons living with haemophilia in India have been diagnosed.[117] Underdiagnosis of haemophilia in low-income countries is multifaceted. This includes lack of awareness of the disease among general population, health system and policy makers. Poor health care infrastructure, and other compelling priorities in healthcare by most governments in the areas of infectious diseases and recently non-communicable diseases such as hypertension and diabetes all contribute to underdiagnosis. The low numbers of participants can also be explained difficulty in accessing healthcare, low survival rate and high attrition of the patients.[117]

5.1. Socio-Demographic Characteristics

Haemophilia A is predominantly found in males and rare to find females with the disease.[3] The reported number of females worldwide diagnosed with haemophilia is about two hundred and fifty (250), it was therefore not surprising to find all participants in this study to be males.[122]

The average age of the study participants was 17 years was similar to findings by Shaheen and colleagues in 2012 reported a mean age of 17.23years in a study on the orthopaedic complications in haemophilia patients in Sudan [31] and Ashard et al (2018) in a study in India reported mean age to be 18.54years.[21]

The mean age of children (participants less than 18years) was 8.7 years and this was comparable to a study by Abdulaziz and Hassan (2019) in Iran on the nutritional status of children and

adolescents with haemophilia which found mean age to be 10.98years.[123] Gouider et al in 2017 in a study in Tunisia on low dose prophylaxis reported a median age of 9years [124] which is similar to what was reported in the current study.

Timmer et al (2020) in a study in Netherlands reported a median age of 43years [125] whilst the mean age reported in the current study of adults (participants >18 years) was 20.3 years.

According to the 2021 Ghana Population Census, the age structure of the population is mostly individuals within the 15-35years age range and this is consistent with findings from current study.[126] Tagny et al (2014) in Cameroon, reports a 3-fold reduction in life expectancy of persons with haemophilia in poorly resourced countries.[127] However, the life expectancy of persons living with haemophilia A is comparable with that of the general population in developed countries[128,129] The life expectancy of persons with haemophilia in the Netherlands is about 77years compared with 83years of the general male population.[130]

All children in the school going group were in school and this is reassuring as it is suggestive that haemophilia is not limiting them from having formal education. This finding is similar to a study done in Cameroon which reported that there was no difference in level of education between persons with haemophilia and those without haemophilia.[127]

5.1. Selected Clinical Characteristics of Participants

Participants with severe disease were the youngest (mean age 13.3±9 years) and those with moderate and mild disease being older 28.1±22.3 years and 24.2 ±12.8 years respectively. Less adults had severe disease probably due to loss of lives of those with severe disease in childhood due to lack of clotting factor concentrates in the country at the time.

The quality of life of persons living with haemophilia A has significantly improved all over the world with the advent of both factor and non-factor therapy. Life expectancy of persons living in haemophilia has improved with the use of clotting factor concentrates and other improvements in healthcare. Reports suggests life expectancy is comparable to that of an individual who does not have haemophilia A.[9,64]

Majority of participants (61.7%) did not have a family history of haemophilia A. This finding is higher than the expected 30% of individuals not expected to have family history of haemophilia A.[2] The finding is consistent with report by Gouw and colleagues in the CANAL study in which patients who did not have a family history (53%) were more than those without family history of haemophilia A. This could probably mean there were a lot of denovo mutations occurring in our environment or patients reported absence of family history because other relations may have haemophilia A but have not been diagnosed. Again, participants may not be in the know that their family members have haemophilia or symptoms of haemophilia thus will report a negative family history of haemophilia.

The commonest clinical presentation at diagnosis for the cohort was oral cavity bleeding 32.1%, followed by joint swelling and pain 21.0% likely due to pain, with central nervous system bleeding the least common presentation. This finding may suggest participants with severe disease in the present study may have mild bleeding phenotypes as studies have suggested that individuals with severe disease genotypes and mutations may experience different bleeding phenotypes.[135,143]

Ashfaq et al (2022) in a study involving 150 patients in Pakistan found haemarthrosis, bruising and oral bleeds as the commonest presentations at diagnosis.[144] This is similar to study by Arshad et al (2018) who reported haemarthrosis and gum bleeds as the commonest presentation in a study of 300 people with haemophilia in India.[21] Kulkarni and colleagues (2017) in a study in US reported post circumcision bleeding as the as the commonest presentation during the new-born period with haematomas and bleeding from oral cavity common when the children grew older.[6]

Post circumcision bleeding is most often seen as a diagnostic symptom or sign for haemophilia but from the current study, there is the need for more education on other symptoms which could be indicative of haemophilia A. It could also mean parents did not see the need to report post-circumcision bleed as they may see it as a normal complication following a surgical procedure. Saxena and colleagues (2013) found educational barrier as one of the major blockades in the early recognition and diagnosis of haemophilia.[11] Individuals and caregivers are not able to recognise symptoms and report to the facility and may see it as normal or they are not concerned enough to report to the hospital. Lack of trained healthcare workers, distance to treatment centre, cost of diagnosis and treatment and lack of psychosocial support are all barriers to early recognition and

management.[11] Denial and fear of stigmatisation could probably be a barrier preventing parents/caregivers from reporting early.

Participants with severe disease were diagnosed at an earlier age (7.9years) compared with moderate disease 24.1years and mild (15.8 years). It is expected that patients with severe disease are likely to have more frequent bleeding episodes, so they are more likely to present to the hospital earlier than those with mild and moderate disease. Arshad and colleagues in a study in India reported mean age of diagnosis for severe disease to be 1.98years, 5.9years and 9.2years for moderate and mild disease respectively.[21]

Kurlkarni et al (2017) in a study in the US reported that majority (70%) of children were diagnosed within the neonatal period.[6] Kurlkani studied children <2 years whilst the current study included individuals of all age groups, and this could account for the difference in the age of diagnosis. Owaidah (2017) in a study of 7 provinces in Saudi Arabia reported that most patients (74.9%) were diagnosed before their first birthday with majority of the patients having severe disease. [96]

All these studies reported a lower age of diagnosis compared with this study. Financial cost, lack of awareness of symptoms, denial, and fear of stigmatisation all of which are perceived barriers to early diagnosis could account for the late diagnosis in this study.

There was negligible difference between age at clinical diagnosis and laboratory confirmation/diagnosis. This shows that even though financial costs are recognised as a hindrance to early diagnosis. Most patients were able to do the confirmatory test for diagnosis within the time they had a clinical diagnosis.

Only one participant out of the five (5) on prophylaxis experienced one (1) bleeding episode. Valentino et al (2012) in a study comparing on-demand treatment and two different prophylaxis regime showed a median ABR of 43.9 for patients on on-demand therapy in comparison with 1.0 when patients were changed to prophylactic therapy with a reduction rate of 99.4%.[12]

The mean number of bleeding episodes/ annual bleeding rate was the same in participants with severe and mild disease (2.1). This is an unexpected finding as individuals with severe disease are expected to have more bleeding episodes compared with mild and moderate disease. This finding in the current study can be explained by the presence of inhibitors in one participant who had mild

disease. The presence of inhibitors potentially turns the phenotype of those with mild disease to severe disease and thus could account for the finding.

Joint bleeding was the most common bleeding episode with participants with severe disease having the most (61.9%). This finding is expected as haemarthrosis is the hallmark of haemophilia A.

5.2. Classification of participants based on severity.

It has been shown across different studies that severe disease is the most common phenotype of haemophilia A, and this has been attributed to the high prevalence of intron 22 and intron 1 mutation in individuals with haemophilia A as this directly correlates with severe disease. The findings from this study were consistent with this observation as a significant proportion of study participants (70.4%) had severe disease. This finding is similar to what was found in a study by Poongavanam et al (2017) in India which showed patients with severe disease to be 78.0% with moderate and mild severity having 10.0% and 12% respectively (n=48).[131] Ahmed and colleagues (2015) in a study in northern Nigeria found patients with severe disease to be 61.5% (n=39).[132] Miller et al (2015) in a study involving seventeen (17) haemophilia sites across the US (n=824) found 60% of participants to have severe disease and 16% and 23% moderate and mild disease respectively.[69] The finding of majority of participants having severe disease could be because individuals with moderate and mild disease do not present to healthcare facilities due to, their experiencing mild symptoms (bleeding episodes) and thus are undiagnosed.

5.1. Measured Residual FVIII Activity

It was observed that the measured residual FVIII activity had some differences when compared with the FVIII activity at initial diagnosis of haemophilia A. A kappa analysis conducted to assess the agreement between the FVIII activity at diagnosis and measured FVIII activity in this study showed a moderate agreement (74.6%). This can be explained by inter laboratory variability due to the different sensitivities of APTT reagents and the absence of standardisation of these reagents [60]. As such different laboratories may have different assay results for the same patient within considerable limit.

Another factor that could explain the discrepancy was that five (5) participants were on prophylactic therapy with FVIII concentrates at the time of the study. Four (4) of these patients were reclassified as moderate whilst one (1) was reclassified as having mild disease. This shows the efficacy of prophylactic therapy as the goal of prophylaxis is to maintain FVIII levels above 1% and this was demonstrated in this study.

5.2. Type of therapy(ies) received since diagnosis.

In the current study (n=81), majority of participants (93.8%) had received FVIII concentrate (recombinant and or plasma derived) in their lifetime and 22.2 % of participants who had received some form of therapy had benefitted from emicizumab therapy. These findings of access to FVIII concentrate albeit on-demand for majority of patients, availability of emicizumab to selected patients and minimal usage of blood products suggest the improvement in haemophilia A care at the Korle-Bu Teaching Hospital.

Minority of participants (6.2%) of which two (2) had severe disease had never been exposed to FVIII concentrate or any form of therapy at all since diagnosis. This can be explained by the observance of disparity between severity and bleeding phenotype, and this is due to the type of mutation present in the individual. Santagostino et al (2010) in a study in Italy found that individuals with severe haemophilia A and with heterogenous non-null mutations had mild bleeding phenotype.[135] About one-tenth of individuals with severe haemophilia may experience clinically mild disease with a reduction in spontaneous bleeds and thus damage to joints.[136]

5.3. Prevalence of Inhibitors.

The prevalence of inhibitors in this study was found to be 11.1% which is comparable to that of a study by Lambert et al in Cote D'Ivoire (2020) in patients on on-demand therapy where they found prevalence of inhibitors to be 12% (n=54) as well as Arshad et al (2018) in India on patients who receive on-demand therapy where they found prevalence of inhibitors to be 9.6% (n=300) and study by Kim and You (2019) in Korea who reported prevalence of inhibitor to be 8.6% (n=115).[21,68,137]

The reported prevalence in this study is much lower compared with the reported prevalence of inhibitors in haemophilia A reported to be 25-30%.[3] Owaidah and colleagues (2016) in a study in Saudi Arabia reported a prevalence of 29.3% in a study involving 148 patients with haemophilia A of which 76.8% were on on-demand therapy.[96] The difference can be explained by the method used in quantifying inhibitors as Owaidah et al (2016) used the NBA which is more sensitive in detecting low titre inhibitors compared with the classical Bethesda assay used in the current study. This means individuals with low titre inhibitors may not have been picked up hence a lower prevalence recorded in this study. The lower prevalence could also be due to limited exposure of the patients to FVIII concentrates as such there is not adequate stimulation of the immune system to cause the development of inhibitors. Again, the lower prevalence of inhibitors reported in these studies could also be explained by the disappearance of neutralising antibodies over a period of time especially low titre inhibitors without any form of therapy.[96] Patients may have developed inhibitors which have disappeared over time and since the study was cross-sectional, these patients may have been missed. The later age at exposure (15.6years) to factor concentrates could account for the lower prevalence of inhibitors found in this study as some studies have associated early start of treatment to increased risk of inhibitors.[75]

Rocha et al (2015) in a study in Portugal found prevalence of inhibitors to be 4.9% which was lower than what is reported in the current study.[9] Miller and colleagues in 2015 reported a much lower prevalence of 2.8% in a study involving 17 haemophilia treatment centres in the US.[69] The patients in these studies were on prophylactic therapy compared to the patients in the current study who were on on-demand therapy. The finding of higher prevalence of inhibitors in patients on on-demand in the current study supports finding from other studies that reports that patients on on-demand therapy have a higher risk of developing inhibitors compared to those on prophylactic

therapy.[76,138] Intense treatment which has been suggested to trigger inhibitor formation has also been regarded as the most important determinant of inhibitor formation.[15]

The presence of high titre inhibitor was found in one (1) participant, and this was found in a child (15years). Even though the numbers are small in this study, reports from other studies show that high titre inhibitors are found in the younger age group and low titres are common in individuals with mild disease.[69]

5.4. Clinical Factors associated with development of inhibitors.

Majority (85.7%) of study participants with inhibitors had severe disease; and this finding is consistent with global haemophilia data where the risk of inhibitor formation has been identified to be highest in individuals with severe haemophilia A.[3,68] Pinto et al (2014) in a study in India involving 1,285 haemophilia A patients reported all inhibitor positive patients had severe disease.[72] Bolarinwa and colleagues (2020) in their study in South West Nigeria which looked at the epidemiology of inhibitor among persons with haemophilia A also found all patients with inhibitor had severe disease (n=40).[119] This is because inhibitors are most common in patients with mutations that cause lack of the FVIII protein compared with mutations that had some amount of FVIII protein being synthesised.

It has been suggested that plasma derived concentrates with VWF have protective effect as the VWF conceals some epitopes and prevents invagination by antigen presenting cells and blocks immune response to the concentrate.[21] Thus, patients on recombinant factor concentrates were at higher risk of developing inhibitors compared with those on plasma derived concentrates. The findings from the current study were consistent with this observation as a majority (57.1%) of inhibitor positive participants had received only recombinant FVIII and the rest had received both recombinant and plasma derived products. This finding was however not statistically significant. Owaidah et al (2017) , in a study in Saudi Arabia reported that the development of inhibitor was common in patients who had received recombinant concentrates compared with those who received plasma derived products (n=202).[96] Iorio et al (2010) in a systematic review involving 2,094 patients from about twenty-four (24) studies reported a higher inhibitor risk in individuals who used recombinant FVIII concentrates compared with plasma derived FVIII concentrates

(n=2094).[80] An update of this systematic review and meta-analysis by Kohar et al (2022) involving two thousand five hundred and thirty-one patients reported an increased incidence of inhibitor formation in the patients who received recombinant factor concentrates.[140]

The risk of development of inhibitor has been reported to be at its highest within 20-50ED; the finding of mean exposure days of 27.1days in inhibitor positive participants in this study was consistent with this.[69] Similarly, Jonker et al (2019) in a study in Netherlands reported the development of inhibitors within 20 exposure days by 23% of patients and 36.6% developing inhibitors within 50 exposure days.[141] Batorova et al (2016) in a prospective study in Slovakia reported that all patients who developed inhibitors, developed inhibitors within 35 ED.[142] Treating clinicians should therefore be aware of this increased risk at the start of treatment and monitor patients closely to enable them pick up patients who develop inhibitor so that the appropriate and optimal treatment plan is instituted.

Inhibitors were found in only participants who received on-demand (7.3% and n=51) treatment and none in the participants who were on prophylactic therapy (0% and n=5) though there was no statistically significant relationship due to the very small numbers in the study. Similarly, Owaidah et al (2017) reported a higher inhibitor prevalence (31.5%) in patients who received on-demand therapy as opposed to 24.2% in patients who were on prophylactic therapy (n=202).[96] These findings corroborate other studies that reports increased risk of developing inhibitors in patients who receive on-demand therapy.[75]

Even though participants with inhibitors and family history of haemophilia were slightly higher 57.1% compared with participants without family history (42.9%). This finding was not statistically significant, thus positive family history was not a predictor for inhibitor development in the current study though this could be due to the small population of patients with inhibitor in this study. Gouw and colleagues reported similar inhibitor risks for participants with and without family history of haemophilia.[82]

Though not statistically significant due to the relatively small numbers of participants in the study and in the subgroup; the study demonstrated a trend that those with inhibitors had more bleeding episodes (2.14) in the past year compared to the inhibitor negative participants (1.43). This is expected since the presence of inhibitor makes clotting factor concentrates ineffective thus more

bleeding episodes are anticipated. Oladapo et al (2018) in a study done across Europe involving 1,285 participants found higher bleeding rate in patients with inhibitors (8.29) compared with those without (3.72).[71]

5.5. Limitation

- Haemophilia is an uncommon disease and as such difficult to collect data on a large cohort of patients.
- Data was collected from one hospital, hence relatively small numbers.
- Unavailability of equipment and reagents for chromogenic assay for more sensitive test and to evaluate inhibitors in patients on emicizumab.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusion

This study is the first step to understand and characterise persons living with haemophilia A, their phenotypes, use of factor concentrates, prevalence of inhibitor and clinical associations in Ghana.

A total of eighty-One (81) participants from adult and paediatric/ Child Health registries were involved in the study with all participants being males.

Majority of participants (85.7%) had severe haemophilia A (FVIII activity <1%)

The majority of participants (93.8%) had received some form of therapy in their lifetime. Therapy received included FVIII clotting concentrate (both recombinant and plasma-derived types) and cryoprecipitate with 22% of participants being on emicizumab therapy.

Prevalence of inhibitors was found to be 11.1% of which 85.7% were low titre inhibitors and 14.3% high titre inhibitor.

The majority (85.7%) of inhibitor positive participants had severe disease and all were on on-demand therapy.

Inhibitor positive participants had higher bleeding episodes and majority (57.1%) had received recombinant FVIII concentrates only.

6.2. Recommendations

Increased awareness and education amongst health care workers and general populace on inherited bleeding disorders to ensure early diagnosis and treatment.

With increased use of clotting factor concentrates increases in the country, clinicians need to be aware of the development of inhibitors and screening protocols should be developed to ensure their early diagnosis so that appropriate management can be instituted.

Equipping regional hospitals with equipment and reagents to allow for diagnosis of haemophilia and tertiary hospitals to diagnose haemophilia and presence of inhibitors as well as training of laboratory personnel in testing for haemophilia.

Prospective study on individuals with low titre inhibitors who currently have no clinical effects.

Wider study to include haemophilia A patients reporting in other hospitals as well as study of their genotypes.

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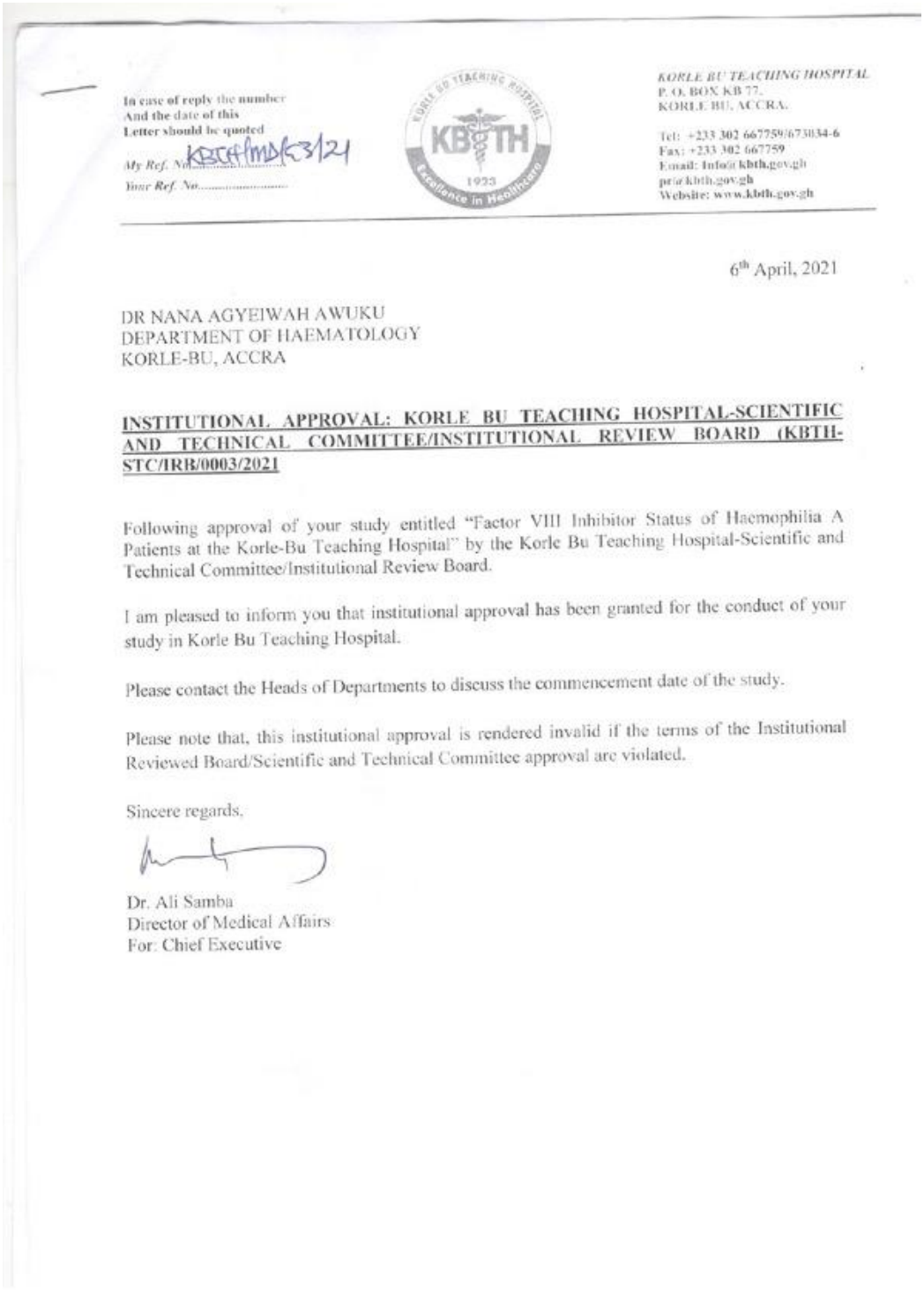
APPENDIX I

INSTITUTIONAL APPROVAL



In case of reply the number
And the date of this
Letter should be quoted
My Ref. No. KBTH/MD/C3/21
Your Ref. No.

KORLE BU TEACHING HOSPITAL
P. O. BOX KB 77,
KORLE BU, ACCRA.

Tel: +233 302 667759/673034-6
Fax: +233 302 667759
Email: Info@kbth.gov.gh
pro@kbth.gov.gh
Website: www.kbth.gov.gh

6th April, 2021

DR NANA AGYEIWAH AWUKU
DEPARTMENT OF HAEMATOLOGY
KORLE-BU, ACCRA

INSTITUTIONAL APPROVAL: KORLE BU TEACHING HOSPITAL-SCIENTIFIC AND TECHNICAL COMMITTEE/INSTITUTIONAL REVIEW BOARD (KBTH-STC/IRB/0003/2021

Following approval of your study entitled "Factor VIII Inhibitor Status of Haemophilia A Patients at the Korle-Bu Teaching Hospital" by the Korle Bu Teaching Hospital-Scientific and Technical Committee/Institutional Review Board.

I am pleased to inform you that institutional approval has been granted for the conduct of your study in Korle Bu Teaching Hospital.

Please contact the Heads of Departments to discuss the commencement date of the study.

Please note that, this institutional approval is rendered invalid if the terms of the Institutional Reviewed Board/Scientific and Technical Committee approval are violated.

Sincere regards.

Dr. Ali Samba
Director of Medical Affairs
For: Chief Executive

APPENDIX II

ETHICAL APPROVAL

In case of reply the number
And the date of this
Letter should be quoted

My Ref. No. KBTHIRB/0003/21

Your Ref. No......

KORLE BU TEACHING HOSPITAL
P. O. BOX KB 77,
KORLE BU, ACCRA.

Tel: +233 302 667759/673034-6
Fax: +233 302 667759
Email: Info@kbth.gov.gh
pr@kbth.gov.gh
Website: www.kbth.gov.gh

1st April, 2021

DR NANA AGYEIWAH AWUKU
DEPARTMENT OF HAEMATOLOGY
KORLE-BU, ACCRA

FACTOR VIII INHIBITOR STATUS OF HAEMOPHILIA A PATIENTS AT THE KORLE-BU TEACHING HOSPITAL

KBTH-IRB /0003/2021

Investigator: Dr Nana Agyeiwah Awuku

The Korle Bu Teaching Hospital Institutional Review Board (KBTH IRB) reviewed and granted approval to the study entitled: "Factor VIII Inhibitor Status of Haemophilia A Patients at the Korle-Bu Teaching Hospital"

Please note that the Board requires you to submit a final review report on completion of this study to the KBTH-IRB.

Kindly, note that, any modification/amendment to the approved study protocol without approval from KBTH-IRB renders this certificate invalid.

Please report all serious adverse events related to this study to KBTH-IRB within seven days verbally and fourteen days in writing.

This IRB approval is valid till 29th February, 2022. You are to submit annual report for continuing review.

Sincere regards,

DR. DANIEL ANKRAH
VICE CHAIR (KBTH-IRB)
FOR: CHAIR (KBTH-IRB

Cc: The Chief Executive Officer, KBTH
The Director of Medical Affairs, KBTH

APPENDIX III

SCIENTIFIC AND TECHINICAL COMMITTEE APPROVAL

In case of reply the number
And the date of this
Letter should be quoted
My Ref. No. KBTH/hms/GBP1
Your Ref. No......

KORLE BU TEACHING HOSI
P. O. BOX KB 77,
KORLE BU, ACCRA.

Tel: +233 302 667759/673034-(
Fax: +233 302 667759
Email: Info@kbth.gov.gh
pr@kbth.gov.gh
Website: www.kbth.gov.gh

3rd March, 2021

DR NANA AGYEIWAH AWUKU
DEPARTMENT OF HAEMATOLOGY
KORLE-BU TEACHING HOSPITAL
P.O. BOX KB 77, KORLE-BU, ACCRA

SCIENTIFIC AND TECHNICAL COMMITTEE APPROVAL
PROTOCOL IDENTIFICATION NUMBER: KBTH-STC 0003/2021

The Korle Bu Teaching Hospital Scientific and Technical Committee (KBTH-STC), on 3rd March, 2021 approved your submitted study protocol.

TITLE OF PROTOCOL: "Factor VIII Inhibitor Status of Haemophilia A Patients at the Korle-Bu Teaching Hospital"

PRINCIPAL INVESTIGATOR: Dr Nana Agyeiwah Awuku

This approval requires that you **forward your approved document to Korle Bu Teaching Hospital –Institutional Review Board (KBTH-IRB) for the ethical aspect of the proposal to be assessed before the project can be initiated.**

This STC approval is valid till 30th December, 2021
You may, however, request extension of the approval period, or renewal as the case may be, should the study extend beyond the stated period.

Upon completion, you are required to submit a final report on the study to the STC. This is to enable the STC ensure among others that, the project has been implemented as per the approved protocol. You are also required to inform the KBTH-STC and Research Directorate of any publications that may emanate from the research findings.

Kindly note that, should the need arise, the KBTH-STC or IRB may institute appropriate measures to satisfy itself that study is being conducted according to the highest scientific and ethical standards.

Please note that any modification to the study protocol without Scientific Technical Committee (STC) approval renders this approval invalid.

Sincere regards,

Prof. G. Obeng Adjei
Chairman, KBTH-STC

Cc: The Chairman, KBTH-IRB

APPENDIX IV

LETTER OF INTRODUCTION

MEDICAL DIRECTORATE
KORLE BU TEACHING HOSPITAL

6th April, 2021

LETTER OF INTRODUCTION – DR. NANA AGYEIWAH AWUKU
"FACTOR VIII INHIBITOR STATUS OF HAEMOPHILIA A PATIENTS AT THE KORLE-BU TEACHING HOSPITAL"

I have the pleasure to introduce to you the above named Investigator from Dept. of Haematology, Korle Bu. Dr. Nana Agyeiwah Awuku sought and has been granted approval to conduct a study entitled "Factor VIII Inhibitor Status of Haemophilia A Patients at the Korle-Bu Teaching Hospital".

She is to contact you to discuss the commencement date of the study.

Please verify her identity with a Government issued National ID card and accord her the needed assistance.

Attached is the Scientific and Technical Committee and Institutional Review Board approval, which specifies the terms.

Sincere regards,

Dr. Ali Samba
Director of Medical Affairs
For: Chief Executive

DISTRIBUTION

1. The Head, Dept of Child Health, Korle Bu
2. The Head, Dept of Haematology, Korle Bu

APPENDIX V

CONSENT/ASSENT FORM FOR PARTICIPANT

Title: Factor VIII Inhibitor Status of Haemophilia A Patients at the Korle-Bu Teaching Hospital

Principal Investigator: Dr Nana Agyeiwah Awuku. Department of Haematology, Korle-Bu Teaching Hospital, P.O. Box KB 77, Korle-Bu, Accra.

Information: (To be read or translated to patients and Parents/ Guardians in their mother tongue by study personnel (Principal Investigator or Research Nurse).

Dear Volunteer,

This consent form contains information about the research titled **'FACTOR VIII INHIBITOR STATUS OF HAEMOPHILIA A PATIENTS AT THE KORLE-BU TEACHING HOSPITAL**

In order to be sure that you are duly informed about your participation in this research, I am asking you to read (or have read to you) this Consent/Assent Form. You will be asked to sign it (or thumbprint) in front of a witness. I will give you a copy of this form. This consent form might contain some words that are unfamiliar to you. Kindly ask me to explain anything you may not understand.

Purpose of Study

You are being asked to participate in the above study to enable us look at the clinical characteristics and the presence of inhibitor to factor concentrates. This study also seeks to establish the relationship between the type and number of therapies received as well as severity and the development of inhibitor in our patients with Haemophilia A.

Description of the Study Procedure

If you agree to be in this study, 4.5mls of blood will be taken from you and will be used to run laboratory tests. Clinical information from your folder/medical record will be taken.

Possible Benefits

The direct benefit to you from this study will be that if inhibitor to factor concentrate is detected, then treatment can be adjusted accordingly. Additionally, your participation may help develop better guidelines in management of haemophilia. You will not be paid for your participation in this study and you are also not expected to pay anything. Cost of your transportation to and from the hospital for this study will however be covered by the Principal Investigator.

Possible Risks

This research presents minimal risk to you. The main risks are discomfort during phlebotomy and the inconvenience of reporting to the hospital outside your scheduled review date.

Withdrawal from study

I would like to stress that this study is strictly voluntary. Should you decide not to participate in the study, it will have no consequence for you. Your decision will not affect the health care you normally receive.

Confidentiality

All efforts, within reason, will be made to keep your protected health information (PHI) private. PHI is your health information that is or has been gathered or kept by Korle-Bu Teaching Hospital (KBTH), as a result of your healthcare. This includes data gathered for research purposes than can be traced back to you. Using or sharing such data will follow ethical privacy rules by research personnel. By signing the consent for this study, you are agreeing to the use and likely sharing of your PHI. As part of the study, I may share the results of the study as well as your medical record with personnel from KBTH. Unless told otherwise, your consent to use or share PHI does not expire. If you change your mind, I ask that you kindly contact me (Dr Nana Agyeiwah Awuku) and let me know that you withdraw your consent to participate in the study. My phone number is 0244690229 and mailing address is Department of Haematology, P.O. Box KB77, Korle-Bu, Accra. However, the health data stored before you withdrew your consent may still be used for reporting and research. At any time, you may ask to have your data or samples destroyed. You should contact me (Dr Nana Agyeiwah Awuku) at the Department of Haematology, P. O. Box KB77, Korle-Bu, Accra (Telephone number 0244690229) to have your sample destroyed and no

longer used for research. I will not be able to destroy research data that has already been gathered using your sample. Also, if your identity was removed from the samples, I will not be able to locate and destroy them. There will be no costs to you for any of the tests done on your samples. You will not be paid for the use of your samples.

Contacts

If you ever have any questions about the research study or study-related problems, you may contact me (Dr Nana Agyeiwah Awuku), at the Department of Haematology, P. O. Box KB77, Korle-Bu Teaching Hospital (Telephone number 0244690229). For questions about the ethical aspects of this study or your rights as a volunteer, **you may contact the secretariat Korle-Bu Teaching Hospital Institutional Review Board (0302739510, email; rdo@kbth.gov.gh).**

Your Rights as a Participants

This research has been reviewed and approved by the Ethical and Protocol Review Board, Korle-Bu Teaching Hospital. An Ethical Review Board or Ethical Committee is a committee that ONLY reviews research studies in order to help protect participants. If you have any questions about your rights as a research participant, you may contact the **secretariat Korle-Bu Teaching Hospital Institutional Review Board (0302739510, email; rdo@kbth.gov.gh).**

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

---------- ----------

Date (Day/Month/Year) Name of Volunteer/Study Participant

Signature or Thumbprint of Volunteer/Study Participant

If volunteer/study participant cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered, and the volunteer has agreed to take part in the research.

---------- ----------

Date (Day/Month/Year) Name of Witness

Signature or Thumbprint of Witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

---------- ---------- ----------

Date Time of Consent Name & Signature of Study Personnel

APPENDIX VI

PARENTAL/GUARDIAN CONSENT FORM

Title: Factor VIII Inhibitor Status of Haemophilia A Patients at the Korle-Bu Teaching Hospital.

Principal Investigator: Dr Nana Agyeiwah Awuku. Department of Haematology, Korle-Bu Teaching Hospital, P.O. Box KB 77, Korle-Bu, Accra.

Information: (To be read or translated to patients and Parents/ Guardians in their mother tongue by study personnel (Principal Investigator or Research Nurse).

Dear Parent/Guardian,

This consent form contains information about the research titled "**FACTOR VIII INHIBITOR STATUS OF HAEMOPHILIA A PATIENTS AT THE KORLE-BU TEACHING HOSPITAL"**

The purpose of this form is to provide you (as the parent/ guardian of a prospective research study participant) information that may affect your decision as to whether to let your child/ward participate in this research study. Read the information below and ask any questions you might have before deciding whether to give your permission for your child/ward to take part. If you decide to let your child/ward be involved in this study, this form will be used to record your permission**.**

Purpose of Study

Your child/ward is being asked to participate in the above study to enable us look at the clinical characteristics and the presence of inhibitor to factor concentrates in individuals with Haemophilia A. This study also seeks to establish the relationship between the type and number of therapies received as well as severity and the development of inhibitor in our patients with Haemophilia A.

Description of the Study Procedure

If you agree to have your child/ward in this study, 4.5mls of blood will be taken from your child/ward and will be used to run laboratory tests. Clinical information from your wards folder/medical record will be taken. There will be minimum of 81 people in this study

Possible Benefits

The direct benefit to your child/ward from this study will be that if inhibitor to factor concentrate is detected, then treatment can be adjusted accordingly. Additionally, his/her participation may help develop better guidelines in management of haemophilia. You or your child/ward will not be paid for their participation in this study and you are also not expected to pay anything. Cost of your transportation to and from the hospital for this study will however be covered by the Principal Investigator.

Possible Risks

This research presents minimal risk to your child/ward. The main risks are discomfort during phlebotomy and the inconvenience of reporting to the hospital outside his/her scheduled review date.

Withdrawal from study

I would like to stress that this study is strictly voluntary. Should you decide not to let your child/ward to participate in the study, it will have no consequence for your child/ward. Your decision will not affect the health care your child/ward normally receive.

Confidentiality

All efforts, within reason, will be made to keep your child/ward's protected health information (PHI) private. PHI is your child/ward's health information that is or has been gathered or kept by Korle-Bu Teaching Hospital (KBTH), as a result of your child/ward's healthcare. This includes data gathered for research purposes than can be traced back to your child/ward. Using or sharing such data will follow ethical privacy rules by research personnel. By signing the consent for this study, you are agreeing to the use and likely sharing of your child/ward's PHI. As part of the study, I may share the results of the study as well as your child/ward's medical record with personnel from KBTH. Unless told otherwise, your consent to use or share your child/ward's PHI does not expire. If you change your mind, I ask that you kindly contact me (Dr Nana Agyeiwah Awuku) and let me know that you withdraw your consent to participate in the study. My phone number is 02244690229 and mailing address is Department of Haematology, P.O. Box KB77, Korle-Bu, Accra. However, the health data stored before you withdrew your consent may still be used for

reporting and research. At any time, you may ask to have your child/ward's data or samples destroyed. You should contact me (Dr Nana Agyeiwah Awuku) at the Department of Haematology, P. O. Box KB77, Korle-Bu, Accra (Telephone number 0244690229) to have your child/ward's sample destroyed and no longer used for research. I will not be able to destroy research data that has already been gathered using your child/ward's sample. Also, if your child/ward's identity was removed from the samples, I will not be able to locate and destroy them. There will be no costs to you for any of the tests done on your child/ward's samples. You will not be paid for the use of your child/ward's samples.

Contacts

If you ever have any questions about the research study or study-related problems, you may contact me (Dr Nana Agyeiwah Awuku), at the Department of Haematology, P. O. Box KB77, Korle-Bu Teaching Hospital (Telephone number 0244690229). For questions about the ethical aspects of this study or your child/ward's rights as a volunteer, you may contact **the secretariat Korle-Bu Teaching Hospital Institutional Review Board (0302739510, email; rdo@kbth.gov.gh).**

Your Child/Ward's Rights as a Participants

This research has been reviewed and approved by the Ethical and Protocol Review Board, Korle-Bu Teaching Hospital. An Ethical Review Board or Ethical Committee is a committee that ONLY reviews research studies in order to help protect participants. If you have any questions about your child/ward's rights as a research participant, you may contact the **secretariat Korle-Bu Teaching Hospital Institutional Review Board (0302739510, email; rdo@kbth.gov.gh).**

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to have my child/ward participate as a volunteer.

---------- ----------

Date (Day/Month/Year) Name of Parent/Guardian (PRINT)

---------- ----------

Signature or Thumbprint of Parent/Guardian Name of Child/Ward

If Parent/Guardian cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered, and the volunteer has agreed to take part in the research.

---------- ----------

Date (Day/Month/Year) Name of Witness (PRINT)

Signature or Thumbprint of Witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

---------- ---------- ----------

Date Time of Consent Name &Signature of Study Personnel

APPENDIX VII

DATA EXTRACTION FORM

FACTOR VIII INHIBITOR STATUS OF HAEMOPHILIA A PATIENTS AT THE KORLE-BU TEACHING HOSPITAL

Study ID Number:

1. Age *(years)* ----------
2. Sex (*please tick)* ☐ Male ☐ Female.
3. Educational Level (*please tick)* ☐ Primary ☐ Junior High
   ☐ Senior High ☐ post-Secondary ☐ Tertiary
   ☐ No formal Education
4. Occupation (*please state*) ----------
5. Age at diagnosis (*years*) ----------
6. Residual Factor VIII Level ----------
7. Clinical presentation at diagnosis ----------

   ----
8. Number of Bleeding Episodes in the past year ----------
9. Type of Bleeding Episodes (*please tick as many as apply)*
   ☐ Joint ☐ Muscle ☐ Skin
   ☐ Gum ☐ Epistaxis ☐ Genitourinary
   ☐ Gastrointestinal ☐ Central Nervous System
   ☐ Other (*please state)* ----------
10. Family History of Haemophilia A (*please tick*) ☐ No ☐ Yes.
11. Relationship of Affected Family Member ----------
    ----
12. Type of therapy(ies) received (*please tick as many as apply*)
    ☐ Factor Concentrate ☐ Cryoprecipitate ☐ None.

13. Age at first exposure to factor concentrate (*please state*) ----------

14. Number of times each therapy has been received (*please state)*

    Factor Concentrate ----------

    Cryoprecipitate ----------

15. Factor VIII requirement in the past 3 years (iu/kg/year) (*please state*) ----------

16. Type of factor concentrate received *(please tick)*

    ☐ Recombinant factor ☐ Plasma derived factor

APPENDIX VIII

WORKSHEET – BETHESDA ASSAY

SAMPLE	1/5	1/10	1/20	RESIDUAL FVIII	TITRE X DILUTION	FINAL INHIBITOR TITRE (BU)

APPENDIX IX

WORKSHEET – FACTOR VIII ASSAY

SAMPLE	1/10	1/20	1/40	RESIDUAL FVIII