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INHIBITOR EPIDEMIOLOGY IN A COHORT OF SOUTH AFRICAN PEOPLE WITH HAEMOPHILIA-A

Ziphozonke Mafika

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfillment of the requirements for the degree of Master of Medicine in the branch of Haematology,

Johannesburg, 2016

DECLARATION

I, Ziphozonke Mafika declare that this thesis is my own unaided work. It is being submitted for the degree of Master of Medicine in Haematology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

_____

(Signature)

_____ day of _____,2016.

DEDICATION

In memory of my late grandfather

Horatius Sizinzo Mafika

1927-1992

PRESENTATION ARISING

Ziphozonke Mafika,H.Marijke van den berg, Rosemary Schwyzer,Johnny Mahlangu Inhibitor epidemiology in a cohort of South African people with severe haemophilia A. Abstract submitted to World Federation of Haemophilia Congress, Orlando, 2016

ABSTRACT

Background

There are currently no published data about inhibitor epidemiology in the South African haemophilia population. International published studies suggest that people of African descent may have a higher risk of developing inhibitors than other ethnic groups. The uncertainty about an associated risk to and inhibitor risk in the South African patient population motivated the research question in this study. The majority of patients with haemophilia in South Africa are black. We set out to examine the relationship between inhibitor development and the presence of risk factors in our patient population.

Materials and Methods:

We conducted asingle center retrospective study at the Haemophilia Comprehensive Care Center of the Charlotte Maxeke Johannesburg Academic Hospital, South Africa, between January 1989 and January 2010. The study was approved by the University of the Witwatersrand Human Research Ethics Committee. People with haemophilia (PWH) born in the study period were identified in the centre database. All previously untreated patients (PUPs) with severe haemophilia A born betweenJanuary 1st1989 and January 1st 2010 were included. Data on treatment upto and ≥ 50 exposure days(ED) or inhibitor development were collected. All data on every reason for treatment and the clotting factor (CFC) used were

collected. Demographic information, family history of inhibitors, exposure days, type of treatment given, inhibitor test results, inhibitor risk factors, genetic analyses were extracted from the hospital patient records, clinical laboratory information system and genetic counselling files. Data was anonymized and coded prior to analysis.

Results:

Of the 117 files screened, 85 met the eligibility criteria. The race breakdown/ethnicity of the 85 was 67% black, 29% white and 3% mixed race. The mean number of EDs was 143for the entire group while the mean number of EDs was 114 for the inhibitor patients with a range of 30 to 498. Just over 17.6 %(*15/85)* developed inhibitors of which 80% were black, 13% white and 7% mixed race. From the 85 patients 15/85 developed an inhibitor of which 80% were low titre and 20% were high titre inhibitors. A positive family history of haemophilia was present in 60% of inhibitor patients. In the 37/85 patients with mutation results, 16 had Inversion 22 and 21 had other mutations. In this PUP cohort, none of the PWH with inversion 22 developed inhibitors. None of the cohort patients were on CFC prophylaxis, used recombinant CFCs or switched CFCs during the study period.

Conclusion

The inhibitor incidence of 17.6% in ourblack patient cohort is similar to other studies and does not support the suggestion that patients from black ancestry have a higher inhibitor incidence when compared to other ethnic groups.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

BU/mL	Bethesda units per milliliter
CANAL STUDY	Concerted Action on Neutralizing Antibodies insevere hemophilia A
CFC	Clotting factor concentrate
CMJAH-HCCC	Charlotte Maxeke Johannesburg Academic Hospital-Haemophilia Comprehensive Care Center
CRF	Clinical record form
ED	Exposure days
FIX	Factor IX protein
FVIII	Factor VIII protein
F8	Factor VIII genes
F9	Factor IX genes
IgG	Immunoglobulin G
MIBS	Malmo International Brother Study
MHC II	Major HistoCompatibilty class II
PAPCs	Professional Antigen Presenting Cells
PTPs	Previously treated patients
PUPs	Previously untreated patients

CHAPTER 1

1.0 BACKGROUND

Haemophilia is an inherited X-linked lifelong bleeding disorder which is due to a deficiency of coagulation factor VIII(FVIII) in haemophilia A and a deficiency of factor IX (FIX) in haemophilia B. The factor deficiency is a result of a number of genetic mutations which are well described in the literature. Owing to these mutations, the inheritance pattern is X linked indicating that haemophilia almost exclusively affects males. Rare cases of girls with haemophilia have been described. In particular, this has been seen in the setting of non-random inactivation of the normal X chromosome, Turner Syndrome and homozygosity(1). Haemophilia affects 1 in 5000 boys in Haemophilia A while for Haemophilia B, the incidence is somewhat lower with 1 in 30000 boys affected(1).

Although novel therapeutic proteins have been introduced to the market, it is likely that these advances will take a few years before they reach resource constrained populations. In the interim, the standard of care of haemophilia continues to be the replacement of the deficient coagulation factor in order to stop or prevent bleeding. Both plasma derived and recombinant clotting factor concentrates (CFC) are reported to be safe and efficacious(2). The main complication of this therapeutic strategy is the development of neutralizing antibodies against the infused CFC. This is described in 25-30% of patients(3).

Clinically, the presence of an inhibitor is suspected when the administration of a coagulation factor concentrate fails to trigger a haemostatic response. The diagnosis has to be made in conjunction with demonstrable laboratory evidence. In the laboratory, a clinically significant inhibitor is defined as that above 0.6 Bethesda Units(BU)(4). In comparison to FIX and other inherited coagulation factor deficiencies, inhibitors against FVIII are most commonly seen.

Risk factors for inhibitor development are multifactorial and include patient and non-patient related factors. Clinically significant patient related factors include ethnicity, the presence of genetic mutations, family history of inhibitor development and disease severity(5). Non-patient related factors include the role played by the environment in inhibitor development.

The development of inhibitors is known to occur during the first 50 exposure days in previously untreated patients (PUPs) in > 95% of the patients. The incidence of inhibitors has been reported up to 25-30% of patients with severe disease (<1% of factor levels)(2). After 50 exposure days (ED) inhibitor development becomes rare, even more so after 150-200 ED(5, 6).

Studies that included patients from North America have shown that haemophilia patients of African descent may have a higher risk of developing inhibitors than others(7, 8). This was confirmed in an individual patient data meta-analysis that included patients both from the US and Europe and in the CANAL study(9, 10).Based on these findings, the risk of inhibitors among people of African descent is at least twice the risk of people of European descent.

Experiences in South Africa, however, suggest that the incidence of inhibitors among people from African descent may not be higher than the incidence among other populations(11). Observed differences between incidences of inhibitors among the different races may be explained both by true genetic differences, but also by differences in treatment strategies or other non-genetic risk factors.

1.1 PROBLEM STATEMENT

There are currently no published data about inhibitor epidemiology in the South African haemophilia population.

1.2 RESEARCH QUESTION AND OBJECTIVES

1.2.1 Research Question

What is the epidemiology of inhibitor development in patients with severe haemophilia A seen at Charlotte Maxeke Johannesburg Academic Hospital Haemophilia Comprehensive Care Center(CMJAH – HCCC).

1.2.2 Specific Objectives

- To determine the number of new severe haemophilia A factor VIII <1% patients born from January 1st 1989 to January 1st 2010.
- To document inhibitor diagnoses made during the study period.
- To document risk factors for inhibitor development in patients.
- To examine the association between inhibitor risk factors and the development of inhibitor.

CHAPTER 2

2.0 INTRODUCTION AND PATHOGENESIS OF INHIBITOR DEVELOPMENT

The treatment of bleeding episodes with CFC in patients with severe haemophilia is associated with the development of neutralizing antibodies against the infused CFC. See figure 1.This complication is seen in up to ~33% of patients with severe haemophilia A and ~13% of those with non-severe haemophilia A(12). Studies show that many risk factors for inhibitor development are found in previously untreated patients (PUPs) when compared to previously treated patients (PTPs)(9, 13).Patients who have been exposed to CFC for less than 50 days are known as PUPs while those exposed for longer are known as PTPs(13). Particularly, the risk for inhibitor development is increased in the first 20 ED.

These risk factors are multifactorial in origin and include those that are patient related and those that non-patient related and involve a complex interplay between cellular components, cytokines and immune regulatory components(14).

Additionally, the infused CFC is regarded as the initial inducer of an immune response owing to its antigenic potential. In PUPs, dendritic cells are the professional antigen presenting cells (PAPCs) responsible for the presentation of the infused FVIII to the CD4 T cell lymphocytes. The process of presentation is carried through the major histocompatibility class(MHC) II molecules(14). In previously treated patients, this process is carried out by B-cell lymphocytes. Prior to this presentation

to the CD4 T cell lymphocytes, the infused FVIII protein is processed via endocytosis into a PAPC, a process facilitated via mannose-specific receptors.

Following the presentation of the infused FVIII CFC by PAPCs, an immune response ensues and this is evidenced by the production of neutralizing antibodies against the infused CFC. Predominantly, these are polyclonal high affinity immunoglobulins (Ig) belonging to the IgG1 and IgG4 subtypes. Their epitopes are located on both heavy and light chains of FVIII with a preference for the A2 and C2 domains(15).

Steric hindrance is described as a process which results in the cessation of a chemical reaction which might be caused by a molecule's structure. This process is the main mechanism through which the infused FVIII CFC is neutralized. Other mechanisms described as possible contributors include the formation of immune complexes as well as enhanced catabolism and hydrolysis(16).

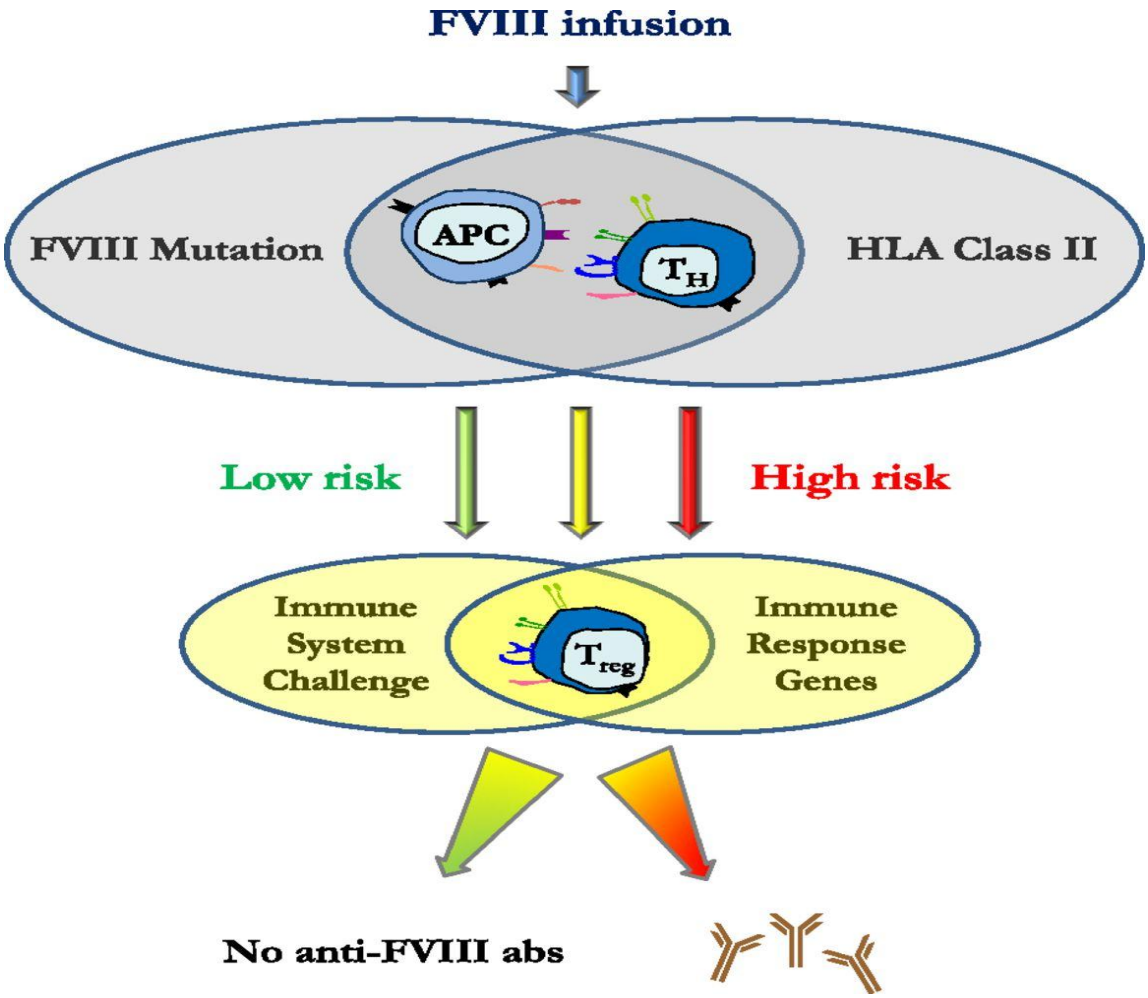


Figure.1 Model of the formation of anti-FVIII neutralizing antibodies. The causative FVIII mutation and HLA class II will be the main contributors to the risk of development of antibodies; from very low risk (green) unlikely to experience any antibodies with commercially available FVIII concentrates to very high risk (red). The final immune response and outcome will then be fine-tuned by T-regulatory cells and a variety of immune regulatory molecules, the activity of which will be defined genetically by therapy-related factors and immune system challenges. Adapted from Astermark 2015

These processes result in the blockage of functional epitopes which include coagulation FIX, phospholipid membrane and Von Willebrand factor interaction sites. Further, based on the kinetics of the inhibition, these inhibitors are broadly classified into two groups namely; type I inhibitors which follow a dose-dependent linear inhibition, which is common in severe haemophilia. The second group is type II inhibitors which have a more complex kinetic profile and completely inactivate

FVIII. Type II inhibitors are seen in the milder form of haemophilia or in those with acquired haemophilia(17).

2.1 RISK FACTORS

2.1.1 ETHNIC BACKGROUND

Ethnicity is reported to be an independent risk factor for inhibitor development. Indications by a number of studies suggest that when compared to Caucasians, African-Americans, Hispanic and Latinos are noted to have a higher propensity to develop neutralizing antibodies against the infused CFC(18). In the South African population setting, black subjects, which included both adults and children, were found to have a statistically and significantly higher prevalence of inhibitor development than white patients, irrespective of the FVIII gene mutation status(19).In this cohort, which is comprised of a majority of black subjects, inhibitor incidence is not higher than what is reported elsewhere.

2.1.2 GENETIC FACTORS

The field of genetics remains a significant contender in inhibitor development. See Fig 2. There are genetic variables concerned with the outcome of replacement therapy with CFC and the subsequent development of a clinically significant inhibitor. These include the presence of a causative gene mutation, the MHC class I and II phenotype, T cell receptor repertoire and polymorphisms of the genes coding for cytokines and immune regulatory molecules(20).

Relating to the type of mutation, in patients with nonsense and missense mutations, the risk of inhibitor development is higher when the mutation is located in the light chain of the FVIII protein than when it is located outside(21). Causative gene mutations may be further subdivided into high risk mutations as well as low risk mutations. High risk mutations are those that result in the complete absence of FVIII protein synthesis. Typically these would include large gene deletions, inversions and nonsense mutations(22). See Fig 3. These are associated with the absence of central tolerance and result in higher risk of inhibitor development than those that result in partial FVIII synthesis(21). Low risk mutations are likely to manifest as missense mutations, small insertions or deletions and splice site mutations(20).

To date, studies support that central to the development of inhibitors, MHC molecules may be contributing in part. Studies of patients with haemophilia A and the intron 22 inversion, seem to suggest that specific alleles signatures were more frequently seen in patients with inhibitors(22). However, this finding was not reproducible in the Malmo International Brother Study (MIBS) (23).FVIII haplotype mismatches are other variables associated with genetic risk factors(24).

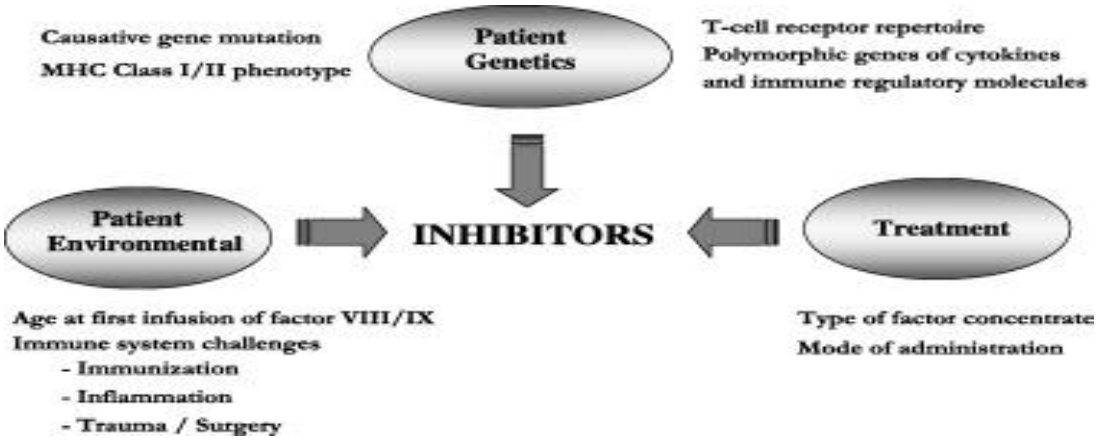


Figure 2. Genetics and inhibitor development. Summary of factors that may influence the risk of inhibitor formation in patients with haemophilia**. Adapted from Astermark et al 2006**

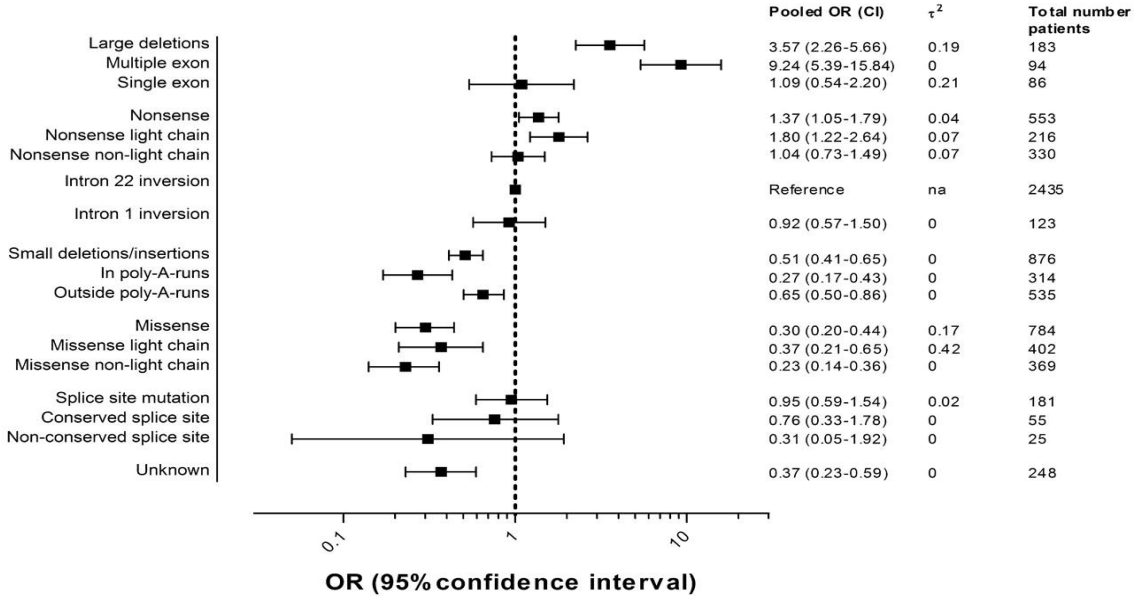


Figure 3. Risk of inhibitor development according to F8 genotype. This is a summary of a meta-analysis and systematic review of 30 studies, 5383 patients of which 1029 had inhibitors. This figure highlights the F8 genotype against an Odds Ratio.**Adapted from Gouw SC et al 2012**

2.1.3 FAMILY HISTORY

There is evidence to suggest that the risk of inhibitor development is higher in families where a positive family history is present than in those with a negative family history.See Table 1. Gill et al found inhibitors to be more prevalent in siblings (~50%) than in extended relatives (~9%)(25).This was also confirmed in the MIBS cohort where it was shown that the rate of inhibitor concordance between siblings was even higher (~78%)(26).

Family history of inhibitors

	Positive inhibitor history			Negative inhibitor history		
	% (N)	RR	aRR*	% (N)	RR	aRR*
MIBS study	48 (96)			15 (292)		
CANAL study	52 (24)	3.1	2.8	21 (138)	1.0	1.0

* Adjusted for other genetic and environmental factors

Higher risk in families with inhibitor history

Table 1. Family history of inhibitors. Adapted from Astermark et al 2001 and Gouw et al 2007.

2.1.4 ENVIRONMENTAL RELATED FACTORS

Environmental factors described in the literature include age at first exposure to CFC, the intensity of treatment, the presence of danger signs and periods i.e. surgery, vaccinations, infections etc, the type of utilized CFC as well as prophylaxis versus on demand therapy.

Age at first treatment with CFC should be examined carefully as the present data is inconclusive. Earlier studies suggested that exposure before the age of 6 months was

associated with an increased incidence of inhibitor development(27). This finding was reciprocated in a European study where 81 patients were followed up for 16 years. In this study, cumulative incidence of inhibitor development in patients who started factor replacement before the age of 6 months was ~34%(28).

However, studies that followed this era of thinking did not confirm any correlation between the age at first exposure to CFC and development of inhibitors(29).Whilst in the CANAL study, a young age at first exposure to factor VIII was associated with an increased risk of inhibitor development, this association largely disappeared after adjustment for intensity of treatment(9).

For this reason, it is extrapolated that earlier studies were comprised of a few number of patients and thus could not be statistically representative of an entire population of haemophiliacs. Patients who are started on regular prophylaxis at an early age have fewer inhibitors than those treated on demand(20). In fact, it appears that regular prophylaxis at an earlier age reduces the incidence of inhibitors by ~60%(9). Tissue damage during surgical intervention causes inflammation and is likely to result inintensified regimens of CFC utilization. For this reason, it has been found that during these periods, surgical procedures and the associated intensity of CFC may lead to an increased risk of inhibitor development.

There is no relationship between the type of CFC used and the development of inhibitors. Further, switching between plasma derived product to a recombinant product or vice versa is not associated with an increased risk of developing inhibitors. This has been investigated and published in the biggest study to date(9).

Nonetheless, black patients treated with FVIII CFC are twice as likely to develop inhibitors against the infused CFC. This is true in the context of mismatched FVIII CFC replacement therapy. It is known that black patients have a F8 genetic haplotype that is different to that of the infused CFC, resulting in an increased risk of inhibitor development against the infused CFC(30).

CHAPTER 3

3.0 PATIENTS AND METHODS

3.1 Ethical considerations

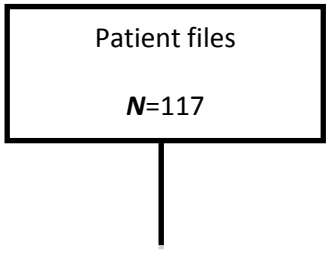
Clearance from the University of the Witwatersrand Human Research Ethics Committee and Post Graduate Committee were obtained prior to study commencement. See appendix A. As this was a retrospective studywith no active therapeutic intervention, individual informed consent was not required.

3.2 Sources of Data

Sources of data included clinical patient files at CMJAH-HCCC, the NHLS clinical laboratory information system as well as the genetic counseling files kept at the CMJAH.

3.3 Study population

The study cases include previously untreated patients with severe haemophilia A seen at the CMJAH-HCCC and were born from January 1st 1989 to January 1st 2010 who meet the eligibility criteria. From this cohort, 117 Files were examined. 85 were eligible while 32 did not meet the eligibility criteria. This is summarized in figure 4 below. Reasons for not meeting the eligibility criteria ranged from patients whose records were incomplete, less than 30 ED and those born outside of the stipulated study period.



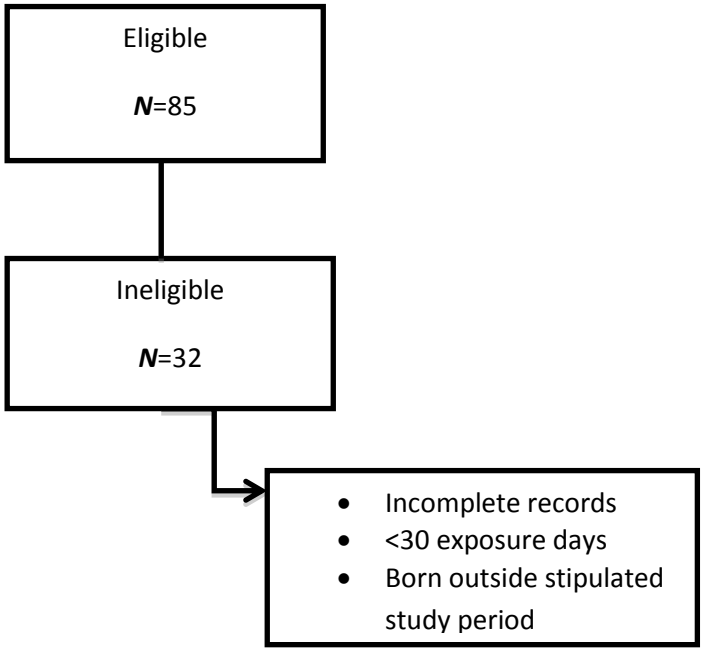


Figure 4 Number of eligible patient files

3.4 Study design

This study was a single centre retrospective cohort studywhich involved retrospective collection of demographic information, family history of inhibitors, inhibitor test results and other risk factors for the development of inhibitors. Only patients with ≥ 50 ED to clotting factor concentrate(CFC) were included. This information was extracted from the hospital patient records, clinical laboratory information system and genetic counselling files. Data was anonymized and coded prior to analysis.

3.5 Inclusion criteria

- Previously untreated severe haemophilia A (FVIII<1%).
- Must have complete clinical records at the clinic especially for first exposure day and peak treatment at first exposure day.

- Follow-up until at least 30 exposure days, but 50 ED is preferred.
- Gene mutation results for factor VIII, although those without will be included.

3.6 Exclusion criteria

- Patients who fall out of the stipulated study period.
- Those with incomplete documentation.
- Those with acquired haemophilia.

Referred patients with an inhibitor or a significant bleed which could represent a potential bias.

3.7 Data Collection

Clinical information including demographic information, age at diagnosis, family history of haemophilia, family history of inhibitors, number of twins with the same deficiency, genetic analyses, number of exposure days, CFC utilized was extracted from the hospital records, genetic counseling files and clinical laboratory information system. This was transferred onto the clinical record form (CRF). See appendix B. This raw data was then coded into an excel spread sheet and summarized into a single data set prior to analysis. This ensured that patient confidentiality was maintained at all times.

3.8 Data Analysis

Statistical analyses werecarried out in SPSS (Statistical Package for Social Sciences) version 13. The analysis involved the description of variables with mean, median, standard deviation, numerical variables and frequency distributions. These were arranged in tables, pie charts or bar graphs.Correlations (associations) between different variables and inhibitor development were examined and results presented in

table formats. The chi square test of independence was carried out to determine whether the associations between variables were statistically significant or not. All the statistical tests were carried out with a 5% significance level for the p-value.

The odds ratio, being a measure of effect size, describing the strength of association or non-independence between two binary data values was calculated.

CHAPTER 4

4.0 RESULTS

4.1 DEMOGRAPHICS AND BASELINE PATIENT INFORMATION

The mean age at diagnosis was +/- 2 years of age with the majority of participants being on on demand CFC.

4.1.1 ETHNIC DISTRIBUTION

The majority of the study participants 67.1%(*n*=57) were black, followed by 29,4% (*n*=25) white and lastly 3,5% (*n*=3) mixed race. See figure 5.

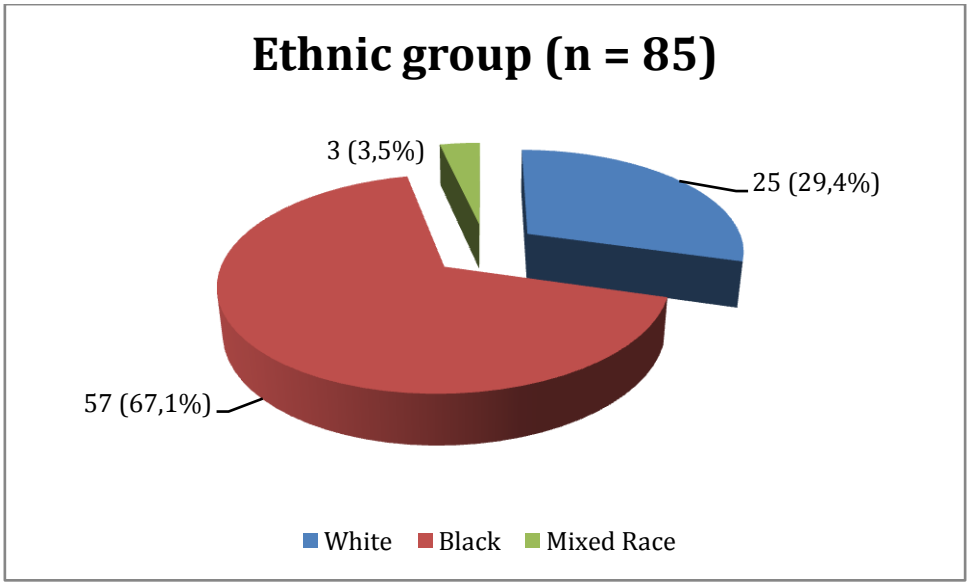


Figure 5. Ethnic Distribution of the study cohort

4.1.2 DIAGNOSIS OF HAEMOPHILIA

Out of the 85 eligible participants, only a single participant was diagnosed prenatally with the parents opting to proceed with the pregnancy to term (**See Table 2**). The majority of the participants were diagnosed postnatally with the majority related to a positive family history (**See table 3**).

Table 2. Prenatal diagnosis

Number of patients diagnosed prenatally *(n)*	Percentage %	Reasons for prenatal diagnosis
1/85	1.2%	Positive family history

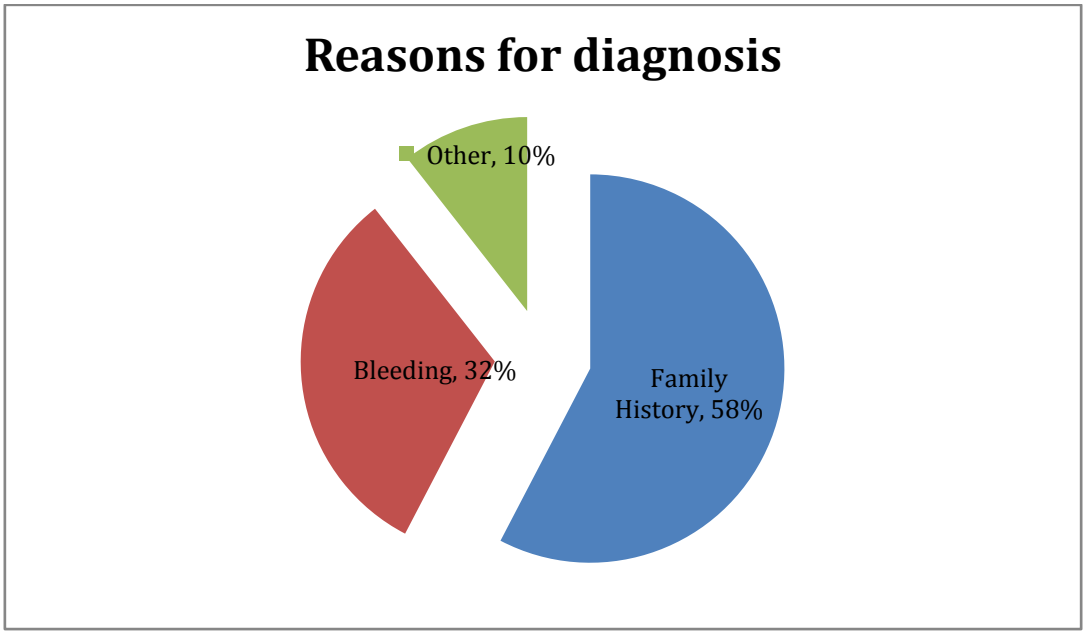


Figure 6. Reasons for diagnosis

Other reasons for the diagnosis comprise ~10%. This is constituted of referrals from other health care providers~7% (*n*=6) and ~3.5% (n=3) diagnosed immediately post surgery. See figure 6.

4.1.3 FAMILY HISTORY

60% (*n*=51) of the participants had a positive family history of haemophilia and 40% (*n*=34) had no prior history of haemophilia in their families nor did they have it in their ancestry.

Table 3. Family history of haemophilia depiction

	Number of patients with a positive family history of haemophilia *(n)*	Percentage %
Yes	51/85	60 %
No	34/85	40%

4.1.4 FAMILY MEMBERS WITH INHIBITORS

Table 4. Family member with inhibitors

	Number of family members with inhibitors *(n)*	Percentage %
Yes	3/85	3.5%
No	82/85	96.5%

4.1.5 PAIR OF TWIN WITH SAME DEFICIENCY

Table 5 Pair of twin with the same deficiency

	Number of a pair of twin with the same deficiency *(n)*	Percentage %
Yes	1/85	1.2%
No	84/85	98.8%

4.1.6 TYPES OF GENETIC MUTATIONS

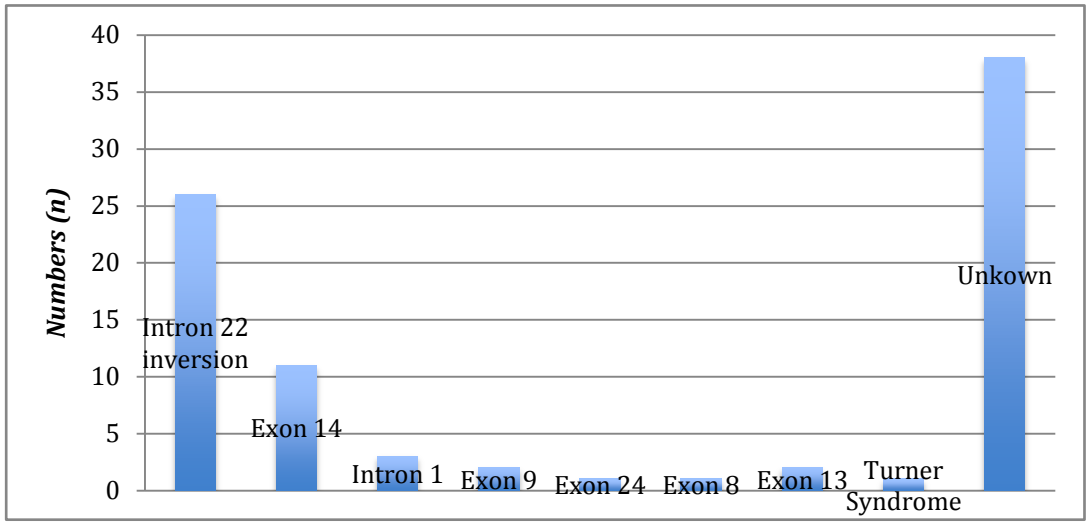


Figure 7. Types of genetic mutations

Just over 30, 5% (*n*=26) tested positive for intron 22 inversion mutation. This was followed by 13% (*n*=11) of participants showing positivity for exon 14 mutation. The majority of the participant's genetic mutational analysis was unknown 44,7% (*n*=38). Of the patients that developed a clinically significant inhibitor (*n*=15/85), none had an inversion of intron 22 mutation. Around 7/15(46.6%) were unknown, 2/15(13.3%) were positive for exon 14, 1/15(6.6%) were positive for exon 4 and exon 9 mutations respectively. The remaining 4/15 patients had no mutations detected.**See table 8**. The single Turner Syndrome patient is a female patient who phenotypically behaved like a severe haemophilia A.

4.2 FIRST 50 EXPOSURE DAYS

The majority of the participants received more than 50 exposure days of CFC on the total group (85 patients) with the average number of days being 142.83 ± 168.67. For the sub-population of patients (15/85) that developed a clinically significant

inhibitor, the average number of ED was 114 with the least number of exposure days being 30 while the highest number of days was 498.

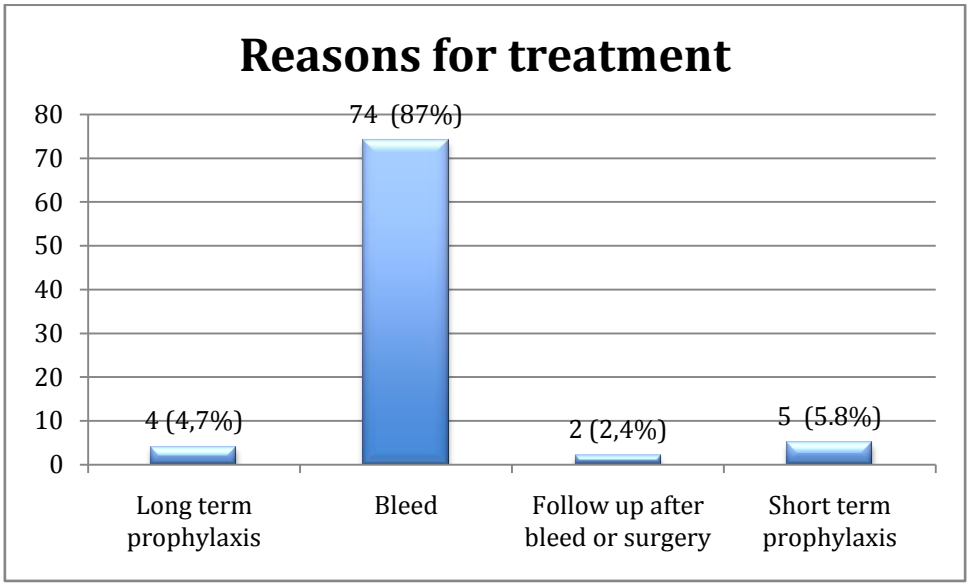


Figure 8. Reasons for treatment

Reasons for treatment in this patient cohort ranged from a few patients on long term prophylaxis (4.7%) and short term prophylaxis (5.8%) to those that required aversion of postoperative bleeding(2.4%). See figure 8. The majority of the patients required treatment for spontaneous bleeding and these were treated on demand (87%).

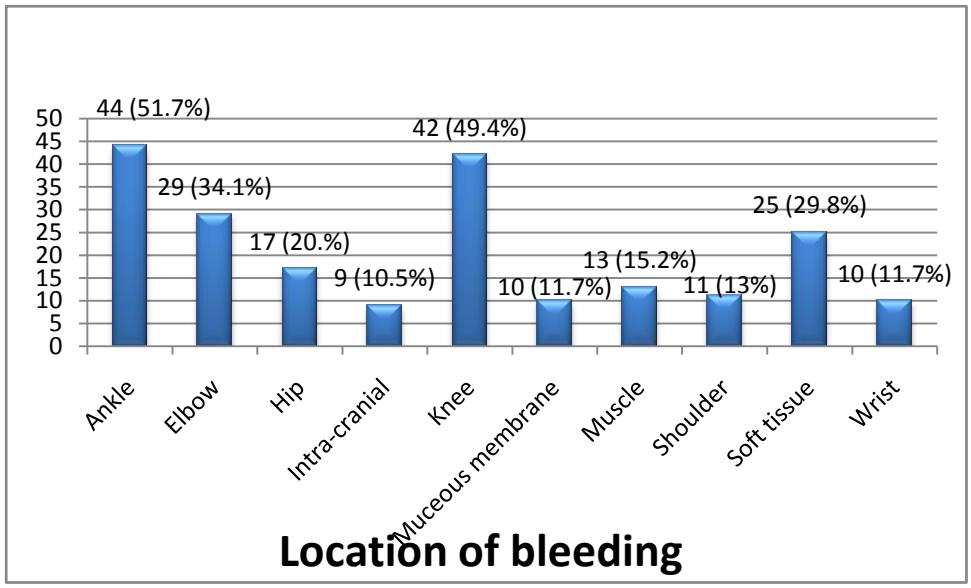


Figure 9. Location of bleeds

In the event of a spontaneous bleed, the affected locations included the knees (49.4%), ankles (51.7%) and elbows (34.1%). The remainder of the bleeding episodes occurred outside these commonly reported joints. Of note is that intracranial bleeding accounted for a small fraction of bleeding episodes in our cohort (10.5%).Bleeding was not confined to a single location only, some patients bled in more than a single area and some had a combination of areas that required CFC replacement at a single clinic visit.

4.3 INHIBITOR DATA RESULTS

4.3.1 INHIBITOR DEVELOPMENT

A clinically significant inhibitor was noted in 17,6% (*n*=15) of the study cohort. See Table 6. 80% of the inhibitor patients had low responding inhibitors while 20% were high responding inhibitors.These participants were on demand CFC replacement having been exposed to replacement therapy for more than 50 days. There was no coincidence of inhibitor development with viral infections, and periods of surgical interventions. The noted inhibitors persisted during the follow up visits and resulted in participants being given a bypassing agent instead of CFC to avert a bleed.

Table 6 Incidence of inhibitor development

How many participants developed a clinically significant inhibitor *(n)*	**Percentage (%)**
15/85	17.6%

4.4 CORRELATION OF INHIBITOR DEVELOPMENT WITH RISK FACTORS

Table 7 Correlation of inhibitor development with risk factors

Results and variables	Ethnicity	Family history of haemophilia	Family member with inhibitor	Twin with the same deficiency	Intron 22 inversion mutation	Other mutations	Type of CFC	Intensity of treatment	Prophylaxis versus on demand
Yes	12/15 (B) 2/15 (W) 1/15 (MC)	9/15 (60%)	1/13 (7.6%)	0	0/8	4/8 (50%)	All on Plasma derived CFC	No Surgery	All on on demand
No	N/A	6/15 (40%)	12/13 (92.3%)	15/15	8/8 (100%)	4/8 (50%)	-	-	-
Odds Ratio	2.792	1	1.466	-	<1	2.667	-	-	-
P value	0.248	1	0.480	0.641	0.119	0.219	-	-	-

- **2 patients had no known family history of inhibitors.**
- **7 patients did not have mutational analysis results.**

Just over 80% (*n*=12, P-value = 0.278) of the patients that developed a clinically significant inhibitor are black patients. This is followed by the white and mixed race patient population with 13,3% (*n*=2) and 6,6% (*n*=1) respectively. A strong family haemophilia is noted in 60% (*n*=9) of the inhibitor population.

Table 8. Genetic mutations in inhibitor subjects

Unknown	Exon 14	Exon 4	Exon 9	No mutation detected
7/15(46.6%)	2/15(13.3%)	1/15(6.6%)	1/15(6.6%)	4/15(26.6%)

CHAPTER 5

5.0 DISCUSSION

5.1 PROFILE OF THE STUDY COHORT

This study cohort comprised of patients from CMJAH-HCCC. Black participants were in excess of 67% relative to the other ethnic populations present in South Africa. A number of published studies in inhibitor development have included a profile of Latinos, African-Americans and Hispanics as the non-Caucasian component of their studies. In these studies, black subjects and Hispanics have a higher propensity to develop inhibitors than white patients(18,26,30). It may thus be argued that results extrapolated from these data may not be entirely representative of purely black patients. In this cohort the black participants are of 100% black ethnicity and do not represent a mixed race. While that may be the case, the findings in this cohort are similar to those published elsewhere.

5.2 INHIBITOR EPIDEMIOLOGY

The reported incidence of inhibitors varies from cohort to cohort and this cohort is not different. Here, I report an incidence of 17.6%. Another cohort within the South African context reported a lower incidence at 13%(19). While these incidences are slightly different in these cohorts, the risk factor profile remains unchanged. When compared to published international data, the South African context inhibitor incidence is lower. In earlier reports, inhibitor incidence was reported at 10-15% of patients receiving CFC (3).

In the CANAL study, the incidence of inhibitor development was reported at 24% (9), while the MIBS study reported higher incidences. Data from across the globe highlights the noted differences in composition and characteristics of study participants. This may explain the wide variation in inhibitor incidences.

5.3 RISK FACTORS AND CORRELATION

5.3.1 DISEASE SEVERITY

Patients in this cohort were all of a severe haemophilia phenotype as dictated by the eligibility criteria. This is inclusive of all the patients that developed a clinically significant inhibitor in this cohort who comprised 17.6% of the study cohort. When compared to a number of published studies showing disease severity as one of the risk factors for inhibitor development in haemophilia, results in this cohort are not different. In particular, patients that have severe haemophilia have a greater propensity of developing inhibitors than those with a moderate and mild phenotype. This is largely related to the underlying gene mutation where large mutations such as deletions lead to a complete halt in the production of FVIII and thus lead to inhibitor formation(31). Owing to some production of endogenous FVIII in the moderate and milder forms of haemophilia, there is less inhibitor formation in these patients. The reported incidence is estimated to be between 3-13%(32-34).

5.3.2 GENETICS

In this cohort, none of the inhibitor positive participants had intron 22 inversion mutation. This is possibly due to the fact that the mutational status of the majority of participants in this cohort is unknown. See **Table 8**. This is in contradiction of what has been reported in the literature where there is a strong correlation between inhibitor development and the presence of intron 22 inversion mutation. Refer back to **figure 3.**In datapublished byLochan et al, another South African cohort, 20% of intron 22 inversion positive participants developed a clinically significant inhibitor, supporting other studies(35, 36).

Causative genetic mutations form the cornerstone for inhibitor development in patients with haemophilia. A recent meta-analysis confirms that large deletions and nonsense mutations have higher inhibitor risk than those in patients with intron 22(21). While that may be the case, intron 22 inversion mutation has been described as the main mutation which when present results in inhibitor development.

5.3.3 ETHNICITY

Although this cohort is comprised of a small number of participants relative to other published studies, there is a reinforcement of the positive association between a strong ethnic background and inhibitor development. Approximately 80% of the participants that have developed a clinically significant inhibitor in this cohort are of the black ethnic background. In Lochan et al, a three-fold increase in inhibitor development was noted in black intron 22 inversion positive participants when compared with the white intron 22 inversion positive counter parts. This may be attributed to differences in genetic haplotypes noted in the different ethnic groups as will be discussed under the section genetic

haplotypes below. Additionally, there is data to suggest the presence of other genetic markers with ethnic variability that may play a role. These include HLA class II molecules and the polymorphic nature of the regulatory genes concerned with the immune system(37).

5.3.4 FAMILY HISTORY

Just over 60% of the participants who developed a clinically significant inhibitor had a positive family history of haemophilia in our cohort. The strong association of inhibitor development in families with a positive family history of inhibitors was examined in the MIBS study. The MIBS study noted inhibitor incidences in families with a positive family history of inhibitors at 48% when compared to those without(26). These findings have also been corroborated in other studies where a higher incidence of inhibitors has been seen in siblings with haemophilia than those without(9, 25). In this cohort, the single participant whose family member had an inhibitor did not develop an inhibitor.

5.3.5 GENETIC HAPLOTYPES

In South Africa, the majority of our patients are on plasma derived products. One may extrapolate that a haplotype mismatch in our patients may not explain the inhibitor risk. Further in another South African cohort, haplotypes 3 and 5 noted in a black population was associated with a 38% inhibitor development in keeping with what has been published before(19).

Current evidence talks to the importance of studying genetic haplotypes amongst the different ethnic groups as part of an attempt to dissect contributors to inhibitor development. Information on genetic haplotypes in this cohort is unknown as their investigation is beyond the scope of this study. Nonetheless, studies report that of the studied haplotypes, haplotype 3,4,5 are found in individuals of the black ethnic group while haplotype 1 and 2 are found in whites and recombinant replacement products(19). It is postulated that mismatches between recombinant products and endogenous haplotypes may be the mechanism for inhibitor development in black patients(19). Published data from the Hemophilia Genetics Study (HIGS) Combined Cohort shows that while genetic haplotypes are likely to contribute to inhibitor development, FVIII gene mutation remains a significant predictor of inhibitor risk(24).

5.3.6 PROPHYLAXIS VERSUS ON DEMAND THERAPY

In this present cohort, all the patients that developed a clinically significant inhibitor were on on demand CFC replacement. In the CANAL cohort, regular prophylaxis was associated with a 60% reduction in inhibitor risk than the on demand arm. In fact, early, regular prophylaxis was suggested to be protective against the development of inhibitors(9).

5.3.7 RECOMBINANT VERSUS PLASMA DERIVED CFC

In a South African population, there are no studies examining plasma derived CFC versus recombinant CFC. In fact, a few of the patients that are on the recombinant product are either sponsored within a trial setting or this product is being supplied by the medical insurance. It is therefore needless to mention that the participants in this cohort were on plasma derived CFC while a few were on a recombinant product. For that reason, these participants could not be compared. In the 17,6% that developed inhibitors in this cohort, none were on recombinant CFC.

5.3.8 OTHER RISK FACTORS

The majority of participants in this cohort had been exposed to CFC for more than 50 EDs.The mean number of EDs was 143 for the total study participants and 114 EDs for inhibitor patients. Patients that developed inhibitors did so within the first 50 exposure days, in keeping with what the literature reports. Data suggests that the first 20 exposure days carry the highest risk of inhibitor development in severe haemophilia PUPs patients(38). None of our patients developed a clinically significant inhibitor within the first 20 days of exposure to CFC. Thus routine surveillance should be carried out in these periods, particularly if intensive therapy is to be instituted. In our setting, routine inhibitor surveillance is carried out every 6 months or at any point in the care of the patient should clinical suspicion arise.

Although a third of the inhibitor patients had various surgeries throughout this period, these were not performed around the time when an inhibitor was detected. This was thus

not a significant finding in this cohort. Further, none of the patients in this cohort were switched.

5.4 LIMITATIONS OF THE STUDY

While this study has clarified a few unanswered questions in our patient population, the following limitations should be noted:

1) This is a retrospective study, therefore gives a historical perspective of what inhibitor epidemiology was at the specific time period. With advances in healthcare systems, this is likely to have changed.

2) This is a single center cohort and thus not fully representative of the entire haemophilia population in South Africa.

3) Some of the mutational analysis data is unknown as some of these patients have never been investigated at initial centers where participants were first seen. Additionally, some of the patients have been counselled but choose not to proceed with testing owing to cultural beliefs.

4) The data is representative of participants on plasma derived products and not inclusive of those on recombinant products.

5) The data represents participants on on-demand CFC therapy and not inclusive of those on prophylaxis.

5.5 RECOMMENDATIONS

Epidemiological data serves to educate the relevant structures in healthcare systems in better planning and providing resources for the care of patients with specific disease types. This data has certainly provided that information as the treatment of this sub-population of patients with bleeding diathesis is complicated and has cost implications.As this is a single centre cohort, a multicenter cohort in a South African setting may assist in better characterizing a more representative sample of the population.The migration of families from our neighbouring African countries implies that the existing data is probably not accurately representative of the on-going changes. For this reason another epidemiological study, prospectively may be advantageous.

5.6 CONCLUSION

The research question in this study was to determine the incidence of inhibitors in a cohort of severe haemophilia A patients that were previously untreated at CMJAH-HCCC over a period of 21 years. 85 patients met the eligibility criteria. For the inhibitor patients, that comprised17.6%(15/85) of the study population,the mean number of EDs was 114. 80% were black, 13% white and 7% mixed race. 80% were low titre and 20% were high titre inhibitors.None of the patients were exposed to periods of intense CFC infusion.The inhibitor incidence in our mainly Black patients is similar to other studies and do not support that patients from Black ancestry have a higher inhibitor incidence.

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APPENDIX A: ETHICS CLEARANCE CERTIFICATE

APPENDIX B

DATA COLLECTION SHEET

A. FORM1 : Baseline Data		
A1	Date of Baseline assessment	
A2	Date of Birth	
A3	Sex *	
	* Rare carrier can have severe hemophilia	
A4	Ethnic origin	
A5	Clotting activity & date of measurement	
A6	Pair of twin with same deficiency	
A7	Type of genetic mutation	

	B. FORM2 : DIAGNOSIS	
B1	Date of diagnosis and weight at diagnosis	
B2	Age at diagnosis/Prenatal diagnosis?	
B3	Reason of diagnosis	
B4	Additional bleeding disorders?	
B5	Any untreated bleeds prior to diagnosis?	
B6	Other diseases that may interfere in prognosis?	

C. FORM3: FAMILY HISTORY		
C1	Family history of haemophilia?	
C2	Brothers with haemophilia?	
C3	Carrier status of sisters?	
C4	Family members with inhibitors?	

D. Form 4 : FIRST 50 EXPOSURE DAYS			
	fill in for every exposure day until number 50 or inhibitor development if earlier		
D1	Date of treatment		
D2	Number of exposure day (chronological)		
D3	Reason of treatment		
D4	In case of a bleed	location	
		side	
		severity	
D5	Total number of units given		
D6	Name of the product		

E. Form 5: Special & inhibitor information			
	fill in from data of first treatment until date ED 50 or inhibitor development		**50 exposure days**
E1	Date first treatment		
E2	Date ED 50 or first positive inhibitor		
E3	Surgery in this period?		
	if yes	date	
		type of surgery performed	
E4	Total number of days in hospital in this period		
E5	Inhibitor?		
	if yes	first titer & date first positive	
		send all inhibitor result (also negative) to study staff	

E6	Recovery measured?		
E7	Prophylaxis started?		
	if yes	give start DATE	
E8	Home treatment?		
	if yes	since when?	
E9	Weight at time E1 (approximate)in kg		