PRELIMINARY STUDY OF IMMUNE TOLERANCE INDUCTION IN THE TREATMENT OF PAEDIATRIC HAEMOPHILIA A WITH INHIBITORS

By,

DR AZMANIRA BINTI AZIZ

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DECLARATION

I hereby declare that this thesis represents my own work and all the sources had been quoted and acknowledged by means of complete references. This thesis has been sent to Universiti Sains Malaysia for the degree of Masters of Medicine in Transfusion Medicine and it is not to be sent to any other universities. With that, this research might be used for consultation and will be photocopied as reference.

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Dr Azmanira binti Aziz

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ABBREVIATIONS

AMDI	Advanced Medical and Dental Institute
aPCC	Prothrombin complex concentrates
aPTT	and activated partial thromboplastin time
BBIS	Blood Bank Information System
BIA	Bethasda Inhibitor Assay
BU	Bethesda Unit
CDC	Centers for Disease Control and Prevention
CNS	Central nervous system
CVAD	Central venous access device
ED	Exposure day
FEIBA	Factor eight inhibitor bypassing activity
FIX	Factor IX
FIXa	factor IX activated
FVIII	Factor VIII
FVIIIa	VIII activated
GITR	Germany Immune Tolerance Registry
HLA	Human leukocyte antigen
HREC	Human Research Ethics Committee
IITR	International Immune Tolerance Registry
IQR	Interquartile range

ITI	Immune tolerance induction
IU	International Unit
ml	millilitres
NAITR	North American Immune Tolerance Registry
NBCKL	National Blood Centre Kuala Lumpur
pdFVIII	Plasma derived FVIII
PROFIT	Prognostic Factors in Immune Tolerance
PT	prothrombin time
rFVIIa,	Recombinant activated factor VII
rFVIII	Recombinant Factor VIII
SIPPET	Survey of Inhibitors in Plasma-Products Exposed Toddlers
US	United State
USM	Universiti Sains Malaysia
vWF	von Willebrand Factor
WHO	World Health Organisation

ABSTRAK

Kajian awal Induksi Toleransi Imun (ITI) dalam Rawatan

Pediatrik Hemofilia A dengan Inhibitor

Pengenalan

Pembentukan antibodi terhadap Factor VIII konsentrat atau lebih dikenali sebagai inhibitor adalah salah satu komplikasi utama dalam perawatan untuk pesakit hemofilia A. Sejak 30 tahun yang lalu, Induksi Toleransi Imun (ITI) digunakan untuk menyahkan inhibitor dan memulihkan farmakokinetik factor VIII (FVIII) kepada normal. ITI adalah pilihan terbaik jangka panjang untuk menyahkan inhibitor, dengan kadar kejayaan yang tinggi dicapai antara 60% hingga 90%. Kajian ini bertujuan mengkaji demografi pesakit, ciri-ciri klinikal dan hasil toleransi terapi ITI di Pusat Darah Negara.

Kaedah

Kajian keratan rentas retrospektif telah dijalankan dengan mengumpulkan data sejumlah 18 pesakit kanak-kanak hemofilia A yang teruk dengan inhibitor yang menjalani ITI di Pusat Darah Negara daripada tahun 2002 sehingga 30 Jun 2016. Data – data berkaitan pesakit diambil daripada fail pesakit dan dimasukkan ke dalam borang kaji selidik yang telah diselaraskan. Antara data yang diambil termasuk demografi pesakit, jenis gen mutasi FVII, jenis FVIII konsentrat, dos FVIII, sejarah paras puncak inhibitor pesakit, paras inhibitor pesakit sebelum, pada permulaan dan semasa perawatan ITI. ITI yang berjaya ditakrifkan apabila paras inhibitor telah negatif, aras

pemulihan FVIII kembali normal dan ketiadaan kesan amnestik apabila terdedah kepada rawatan FVIII berikutnya.

Keputusan.

Sejumlah 12 daripada 18 pesakit (66.6%) telah berjaya menyahkan inhibitor. Kajian ini juga menunjukkan terdapat statistik yang signifikan dengan sejarah paras inhibitor puncak sebelum memulakan terapi ITI ($p = 0.015$) , paras inhibitor sebelum ITI ($p = 0.036$), paras inhibitor ketika permulaan ITI ($p = 0.010$), paras puncak inhibitor semasa ITI ($p = 0.018$), dan tempoh antara pengesanan inhibitor sehingga ITI diberi dengan hasil akhir rawatan.

Kesimpulan.

Kajian ini menunjukkan bahawa terapi ITI boleh menjadi rawatan yang berkesan untuk menghapuskan inhibitor. Antara faktor-faktor kejayaan ITI termasuk sejarah paras puncak inhibitor yang rendah sebelum ITI (<200BU / ml), paras inhibitor yang rendah sebelum dan permulaan ITI(<10BU/ml). Tempoh yang lebih singkat untuk memulakan terapi ITI selepas pengesanan inhibitor juga mempengaruhi hasil rawatan ITI dengan kejayaan yang lebih tinggi. Oleh yang demikian, kajian ini merupakan kajian awal yang baik untuk memberi kefahaman dalam pemilihan pesakit mendapatkan rawatan ITI di Malaysiaagar hasil yang sukses dapat diperolehi.

Kata kunci: hemofilia, inhibitor, factor VIII, induksi toleransi imun, paras inhibitor

ABSTRACT

Preliminary Study of Immune Tolerance Induction in the Treatment of Paediatric Haemophilia A with Inhibitor

Introduction

One of the primary complications in the treatment of haemophilia A patient is the development of inhibitor. Over the last 30 years, Immune Tolerance Induction (ITI) has been used to eliminate inhibitor as well as to restore FVIII pharmacokinetics. ITI is the best long term device in eliminating inhibitors with its success rate varying from 60% to 90%. The purpose of this study is to investigate the patient's demography, clinical characteristics and the result from ITI therapy at the National Blood Centre.

Method

This cross sectional retrospective study is undertaken by reviewing the files of 18 severe haemophilia A paediatric patients with inhibitors who underwent ITI therapy at the National Blood Centre from 2002 till June 2016. All information had been obtained from the patient's file and entered into a standardized research proforma. The data were included patient's demographics, type of FVIII gene mutation, type of FVIII product, FVIII dosing, inhibitor history, inhibitor level during ITI, duration of ITI therapy and the outcomes. The successful outcome was defined as negative titre inhibitors, FVIII level were normalized and no trace of amnestic upon subsequent FVIII exposure.

Result

Twelve (66.6%) patients out of 18 from this study successfully eliminated inhibitors. This study also demonstrated that statistically there was a significant association with the historical peak inhibitor before start ITI ($p = 0.015$), peak of inhibitor during ITI ($p = 0.018$), inhibitor titre before ITI ($p = 0.036$), inhibitor at the start of ITI ($p = 0.011$) and the duration between inhibitor detection and the start of ITI ($p = 0.046$) with the final outcome of ITI therapy.

Conclusion

This study has demonstrated that ITI therapy can be an effective treatment and can be accepted as inhibitor eliminator. The successful ITI factors includes the history of low inhibitor titre peak prior to therapy (< 200BU/ml), low inhibitor titre peak during ITI, low inhibitor titre before and at the beginning of ITI (<10BU/ml). The shorter duration of starting ITI therapy after inhibitor detection could influence the ITI outcome with higher success rate. This is a good preliminary study that provides an understanding and helping the physicians in patient selection to start ITI therapy for a better success rate.

Key words: haemophilia, factor VIII, inhibitor, immune tolerance induction, inhibitor titre

CHAPTER 1

INTRODUCTION

1.1 Overview of Haemophilia A

Haemophilia A is an inherited X-linked genetic disorder, which is commonly reported as 1 in 5,000 of male live births worldwide (Mannucci and Franchini, 2013). The World Federation of Haemophilia annual global surveys showed approximately 400,000 people had haemophilia across the world with 80% to 85% of them categorised as haemophilia A. The prevalence of haemophilia A in Malaysia was reported as 6.6 in 100,000 males (Stonebraker *et al.*, 2010). In 2015, there were 2,677 cases of bleeding disorders registered in Haemostasis Laboratory in the National Blood Centre. Out of this number, a total of 1,157 cases (43.2%) were diagnosed as haemophilia A (unpublished data report on Haemorrhagic Disorder Registered with Haemostasis Laboratory, National Blood Centre, 2015).

1.1.1 Pathophysiology of Haemophilia A

Haemophilia is a rare bleeding disorder caused by lack or absence of coagulation factor that leads to improper blood clotting. Coagulation factor is a protein needed for normal blood clotting whereby in patients with haemophilia A, their factor VIII (FVIII) level is low as a result from the mutation of the particular clotting factor genes. The defect leads to disruption of the intrinsic coagulation cascade due to insufficient formation of the thrombin by the factor IX activated (FIXa) and factor VIII activated (FVIIIa) complex. As a result, patients are presented with spontaneous bleeding and/or excessive haemorrhage following to trauma (Kabel,

2014). The gene for FVIII is located on the long arm of the X chromosome. Males from the maternal side are commonly affected as it inherited in an X-linked recessive pattern. However, about 30% of the cases show that an individual can have the disease without any family history of haemophilia which occurs due to spontaneous mutation (Mannucci and Tuddenham, 2001).

1.1.2 Clinical Presentations of Haemophilia A

Bleeding tendency is one of the most prominent clinical features in a haemophilia patient. They either present with spontaneous bleeding or following any trauma or surgery. The severity of bleeding manifestation in haemophilia is usually related to the FVIII levels in the patient's plasma, as shown in Table 1.1.

Table 1.1 Relationship of Bleeding Severity to Clotting Factor Level (Berntorp *et al.*, 2011).

Severity	Clotting Factor Level	Bleeding Episodes
Severe	< 1 IU/dl (< 0.01 IU/ml) or < 1 % of normal	Spontaneous bleeding into joints or muscles, predominantly in the absence of any haemostatic causes.
Moderate	1-5 IU/dl (0.01-0.05 IU/ml) or 1-5% of normal	Occasional spontaneous bleeding; prolonged bleeding with minor trauma or surgery
Mild	> 5-40 IU/dl (0.05-0.40 IU/ml) or >5 to <40% of normal	Severe bleeding with major trauma or surgery. Spontaneous bleeding is rare.

Most of the bleeding occurs in the joint and the muscle as shown in Table 1.2 and Table 1.3. Repeated joint bleed to the target joint will lead to chronic haemophilic arthropathy and cause morbidity to the patient. In severe cases of haemophilia A, the patient can also suffer from spontaneous life threatening bleeding tendencies such as intracranial bleed where immediate treatment is needed (Farrugia *et al.*, 2012).

Table1.2 Sites of Bleeding in Haemophilia (Hay *et al.*, 2006).

Serious	• Joints (haemarthrosis) • Muscles, especially deep compartments (iliopsoas, calf, and forearm) • Mucous membranes in the mouth, gums, nose, and genitourinary tract
Life threatening	• Intracranial • Neck/throat • Gastrointestinal

Table 1.3 Approximate Bleeding Frequency at Different Sites (Srivastava *et al.*, 2013).

Sites	Frequency (%)
Haemarthrosis	70%–80%
■ more common into hinged joints: ankles, knees, and elbows	
■ less common into multi-axial joints: shoulders, wrists, hips	
Muscle	10% - 20%
Other major bleeds	5% - 10%
Central nervous system (CNS)	< 5%

1.1.3 Diagnosis of Haemophilia A

The principles to diagnose haemophilia A includes the understanding of clinical presentation followed by confirmation through laboratory findings (Srivastava *et al.*, 2013) . Complete blood count and coagulation profile which consist of prothrombin time (PT), and activated partial thromboplastin time (aPTT) are needed as a baseline in the patient with suggestive of haemophilia or other bleeding disorders. The diagnosis of haemophilia is usually suspected when the patient had isolated prolonged activated partial thromboplastin time (aPTT) and the confirmation of diagnosis is done by factor assays (Chalmers, 2004).

1.1.4 Treatment of Haemophilia A

The treatment of bleeding episodes for haemophilia depends on the sites of bleeding. The patient should be treated with relevant factor concentrate to the specific factor deficiency. Adequate replacement therapy includes adequate dose and adequate duration of factor coverage necessary to ensure the bleeding is well controlled, if possible within the two hours of the onset of symptoms (Srivastava *et al.*, 2013). In the 1950s other than whole blood, fresh plasma was the only blood product available to treat haemophilia. However, a large volume was required in order to get an adequate dose. A decade later, cryoprecipitate became available before plasma derived FVIII was discovered (Franchini and Mannucci, 2012).

The patients also can recognize early symptoms of bleeding by a tingling sensation within the joint or known as ‘aura’ prior to the manifestation of overt signs of an acute

haemarthrosis (Alhaosawi, 2014). Table 1.4 shows the standard dosage of an on demand therapy for haemophilia A based on the types of bleeding.

Table 1.4 Suggested Plasma Factor Peak Level and Duration of Administration (When there is no significant resource Constraint) for On Demand Therapy (Srivastava *et al.*, 2013)

Type of bleeding	Haemophilia A	
	Desired Level (IU/kg)	Duration (Days)
Joint	40-60	1-2 , may longer if response is inadequate
Superficial muscle / no neurovascular compromise (except iliopsoas)	40-60	2-3 , sometimes longer if response is inadequate
Iliopsoas and deep muscle with neurovascular injury or substantial blood loss	80-100 (initial) 30-60 (maintenance)	1-2 3-5, sometimes longer as secondary prophylaxis during physiotherapy.
CNS / Head	80-100 (initial) 50 (maintenance)	1 -7 8 -21
Throat and neck	80-100 (initial) 50 (maintenance)	1 -7 8 -14
Gastrointestinal	80-100 (initial) 50 (maintenance)	1 -14
Renal	50	3 -5
Deep laceration	50	5 -7
Surgery (Major)	Pre-op (80 – 100) Post op (60-80) (40-60) (30-50)	 1 -3 4 -6 7-14
Surgery (Minor)	Pre-op (50 -80) Post-op (30-80)	 1-5 , depending on types of procedure

1.1.5 Complication of Replacement Therapy

There are a few complications that may occur relating to the replacement therapy that includes antibody formation toward FVIII concentrate or known as inhibitor, which causes the bleeding to become more difficult to control and does not respond to the standard therapy(DiMichele *et al.*, 2007a). In the past, there was also a risk of getting transfusion transmitted infection such as the hepatitis B virus, hepatitis C virus and Human Immunodeficiency virus from the blood products such as fresh frozen plasma, cryoprecipitate and the product from plasma derived FVIII concentrate (Franchini and Mannucci, 2012). However, the safety of the blood products has been improved in the last 25 years as all the blood donors were carefully screened, in addition to the plasma derived FVIII concentrate going through a serial viral inactivation during manufactured and the availability of the recombinant FVIII concentrate(Marwaha, 2013). Other than that, morbidity to the muscles, joints or other parts of the body due to delay in treatment (Fischer *et al.*, 2002).

1.2 Overview of Inhibitor Development

Factor replacement therapy with FVIII concentrate is used to treat or prevent the bleeding episodes successfully in patients with haemophilia A. However, it can also cause severe complications associated with this replacement therapy which is the development of inhibitor towards FVIII (Coppola *et al.*, 2010b). Approximately 30% of patients with severe haemophilia A who received FVIII concentrate will have an inhibitor as the body recognizes it as a foreign antigen and stimulates the antibody

production (Berntorp *et al.*, 2006). In Malaysia, about 10% of patients with haemophilia A had developed inhibitors in 2015 (Unpublished data report on Haemorrhagic Disorder Registered with Haemostasis Laboratory, National Blood Centre,2015).

The patients with inhibitors mostly occurred in children (median age, 1.7 – 3.3 years), whose risk was higher in the first 50 days after the treatment doses are administered(DiMichele *et al.*, 2007a). Other patient related factors that have been associated with the risk of inhibitor formation included ethnicity where the numbers are higher among the black compared to white patients (Viel *et al.*, 2009). Patients with a family history of inhibitor also had threefold higher risk of development to this situation (Astermark *et al.*, 2001). The factor VIII gene mutation type, human leukocyte antigen (HLA) complex genotype and polymorphism in genes involved in the regulation of the immune system also contributed to inhibitor formation (Astermark *et al.*, 2006b). Presence of large gene defects such as inversion, insertion and deletion in multiple domains will caused reduction endogenous FVIII protein synthesis, and once the exogenous FVIII infused, the body will recognize as foreign protein and lead to production of alloantibodies or known as a FVIII inhibitor (Oldenburg and Schwaab, 2001). Environmental factors such as the age of first exposure, intensive FVIII usage during infancy, immunologic challenges such as infection, surgery and immunizations or types of factor concentrate used (recombinant or plasma derived) also play a role in inhibitor development but the aetiology process is still debatable (Gouw *et al.*, 2007).

1.2.1 Method of Analysis for Inhibitor Development

The presence of inhibitor was suspected when the prolonged aPTT was not fully corrected by the mixing test (Srivastava *et al.*, 2013). The Bethesda assay is used to determine the inhibitor levels and the titres are measured in Bethesda Unit (BU). Inhibitors are classified into a high titre when the titre is equal to or more than 5 Bethesda Unit, whereas a low titre is defined when the titre is less than 5 Bethesda Unit (Kempton and White, 2009).

1.3 Overview Management of Inhibitor

The presence of inhibitors does not increase mortality, yet it will complicate the treatment outcome as it does not respond to the standard therapy. Consequently, the bleeding is difficult to control, increase the morbidity and increase the factor usage in treatment (Hay *et al.*, 2000). To overcome this problem, patients with transient or low titre inhibitors can be managed by giving them higher doses of FVIII concentrate (Peerlinck and Hermans, 2006). However, for those with high titre inhibitor the standard FVIII replacement therapy was found to be ineffective and bypassing agents is required to treat the bleeding episodes such as recombinant activated factor VII (rFVIIa) or an activated prothrombin complex concentrates (aPCC) (Paisley *et al.*, 2003). The patient is considered as a low responding inhibitor when the antibody titre never exceeded 5BU even after being repeatedly exposed to the FVIII. A high responding inhibitor is considered when the patients has an inhibitor titre that exceeds ≥ 5BU at least once and after being repeatedly exposed to FVIII as the immune systems will rapidly trigger new inhibitor formation (Leissinger, 2004).

For the past 30 years, the immune tolerance induction (ITI) therapy has been used to eliminate inhibitors particularly for high titres antibodies. Immune tolerance induction (ITI) therapy is generally comprised of factor concentrate which is given regularly over a period of time until the immune system is tolerant to the antigen and preventing further antibodies production. Once the ITI is successful, the inhibitors are eliminated and the patient's response to factor concentrates is reverted back to normal (Ho *et al.*, 2000).

ITI remains the best long term option to eradicate inhibitors, with success rate varying between 60% to 80% (Wight *et al.*, 2003). The first ITI protocol, known as the Bonn protocol was described in 1977 by Brackmann and Gormsen which required administering high doses of FVIII ≥200IU/kg on a daily basis (Brackmann and Gormsen, 1977). There are also other protocol that evolved based on low doses of FVIII and administration of non-specific therapies such as steroid, cyclophosphamide, high doses of intravenous immunoglobulins or extra corporeal immunoadsorption (Wight *et al.*, 2003) . However, there are still variations in the outcomes and time required to achieve tolerance in the various studies as shown in the table 1.5.

Table 1.5 Characteristics and Outcome of Reported ITI Protocol (Coppola *et al.*, 2010a)

ITI protocol	FVIII dose and associated treatment	Success rate (%)	Median time to success, months	Comments
Bonn protocol (high-dose regimen)*	FVIII 100–150 iu/kg every 12 h until inhibitor <1 BU, then FVIII 150 iu/kg until normalization of FVIII recovery and half-life	92–100	14	Very demanding for patients. High cost
Malmo protocol (high-dose regimen & immune modulation)**	FVIII continuous infusion targeting plasma levels >30 iu/dl until negative inhibitor titre, then 60–90 iu/kg weekly + cyclophosphamide (i.v. 12–15 mg/kg days 1–2, 2–3 mg/kg orally days 3–10) + i.v. immunoglobulins 2.5–5 g/kg day 1, 0.4 g/kg days 4–8. Preliminary protein A sepharose immunoadsorption if initial inhibitor titre >10 BU.	59-82	1	Rapid response and cost-saving but need for hospitalization and concerns regarding the use of cyclophosphamide in children
Dutch protocol (low-dose regimen)***	Neutralizing dose (25–50 iu/kg twice daily, 1–2 weeks), then tolerizing dose (50–75 iu/kg weekly)	61-88	1-12	Less demanding for patients and cost-saving
Other low or intermediate dose protocols	Ewing et *al*, 1988: 50 iu/kg/d	67	2	Developed for improving cost-effectiveness of treatment
	Kucharski et *al*, 1996: 50 iu/kg/week	45	10	
	Unuvar et *al*, 2000: 50–100 iu/kg/d	57	6	
	Rocino et *al*, 2001: 100 iu/kg/d	75	8	

*(Brackmann *et al.*, 1996) and (Oldenburg *et al.*, 1999); activated prothrombin complex concentrates (aPCC) 40–60 iu/kg every 12 h was included until 1996.
**(Nilsson *et al.*, 1988) and (Freiburghaus *et al.*, 1999)
***(Mauser-Bunschoten *et al.*, 1995)

Most of the current practice is determined by the clinician choice, expert view, experience, recommendation from the published guidelines and local setting at each haemophilia centre (Robertson *et al.*, 2014). Thus, it is important to understand the factor that is associated with the successful immune tolerance therapy because ITI

therapy is very expensive and not many patients have the opportunity to be offered with this treatment.

In Malaysia, there is no standard ITI protocol used nationwide. Furthermore, due to cost constraints, it causes the ITI therapy inaccessible to many patients. Hopefully, by understanding the factors that may influence the likelihood of a successful ITI, the selection of patients who will or will not benefit from ITI can be determined and provide a better success rate. This can be determined by knowing the character of FVIII inhibitors in patients with ITI therapy and the treatment related factors that are associated with the duration and outcome of the therapy.

1.4 Justification and Benefits of Research Study

The risk of inhibitor formation is higher after 10 to 15 exposure days of FVIII concentrate thus, the children are more prone to develop an inhibitor (Wight and Paisley, 2003). The inhibitor development also more common in patients with haemophilia A compared to haemophilia B(Carcao and Lambert, 2010) and those who had classified as severe (FVIII level less than 1 %) haemophilia compared to moderate or mild type.(Mannucci and Tuddenham, 2001).

A patient with severe haemophilia A with inhibitor is at risk of uncontrolled bleeding, extensive joint disease and joint disability (Morfini *et al.*, 2007). Immune tolerance induction (ITI) is the best approach to eliminate inhibitors. This may reduce the risk of bleeding and improved the quality of life. However, up to the best of our knowledge,

there was no standard ITI protocol in Malaysia. Most of the data on the ITI protocol for haemophilia A available are from the international studies. Based on the published data several predictors of ITI can be used to guide in the selection of inhibitor patients for better outcome and reduce wastage of resources.

ITI therapy was started in NBCKL since 2002 and the protocol is used based on the international ITI recommendation. However, the main hindrance for patients to receive ITI therapy was included the cost, availability of factor concentrate, good venous access and the commitment from the patients and the family members. Till December 2015 only 18 out of 25 paediatric patients with inhibitor had undergone ITI therapy. Thus, this study is needed to look at the current ITI protocol and to identify factors that can influence the treatment outcomes. Based on the findings from this study, a comparable data can be made in the future. Furthermore, with a higher success rate achieved with the current ITI protocol a standard ITI protocol can be recommended as guidance for other treatment centre, especially in Malaysia.

Therefore, from this preliminary study, it provides an evidence and understanding for selection of patients to start ITI therapy in Malaysia. This could help the clinicians to decide when is the best time to start immune tolerization, the appropriate dosing and choice of FVIII product for a better outcome. By getting the best and optimal ITI therapy with a good patient selection better outcome will be achieved and reduce waste of resources.

1.5 Objectives of Research Study

1.5.1 General Objective

To study the factors associated with outcomes of immune tolerance induction therapy among paediatric severe haemophilia A with inhibitor in National Blood Center, Kuala Lumpur.

1.5.2 Specific Objectives

a) To describe the demographics and clinical characteristics in patients on ITI therapy.
b) To describe the proportion of treatment outcomes among patient who had undergone the immune tolerance induction therapy.
c) To determine the factors associated with success, partial success or failed of immune tolerance induction therapy.

1.6 Hypothesis of Research Study

- There are associations between patient with low titre inhibitor prior and at start of ITI with successful outcome of immune tolerance induction therapy.
- There is an association between younger age group that recently develop inhibitor with successful outcome of immune tolerance induction therapy.
- There is an association between patients who had received higher doses of FVIII with successful outcome of immune tolerance induction therapy.

1.7 Conceptual Framework

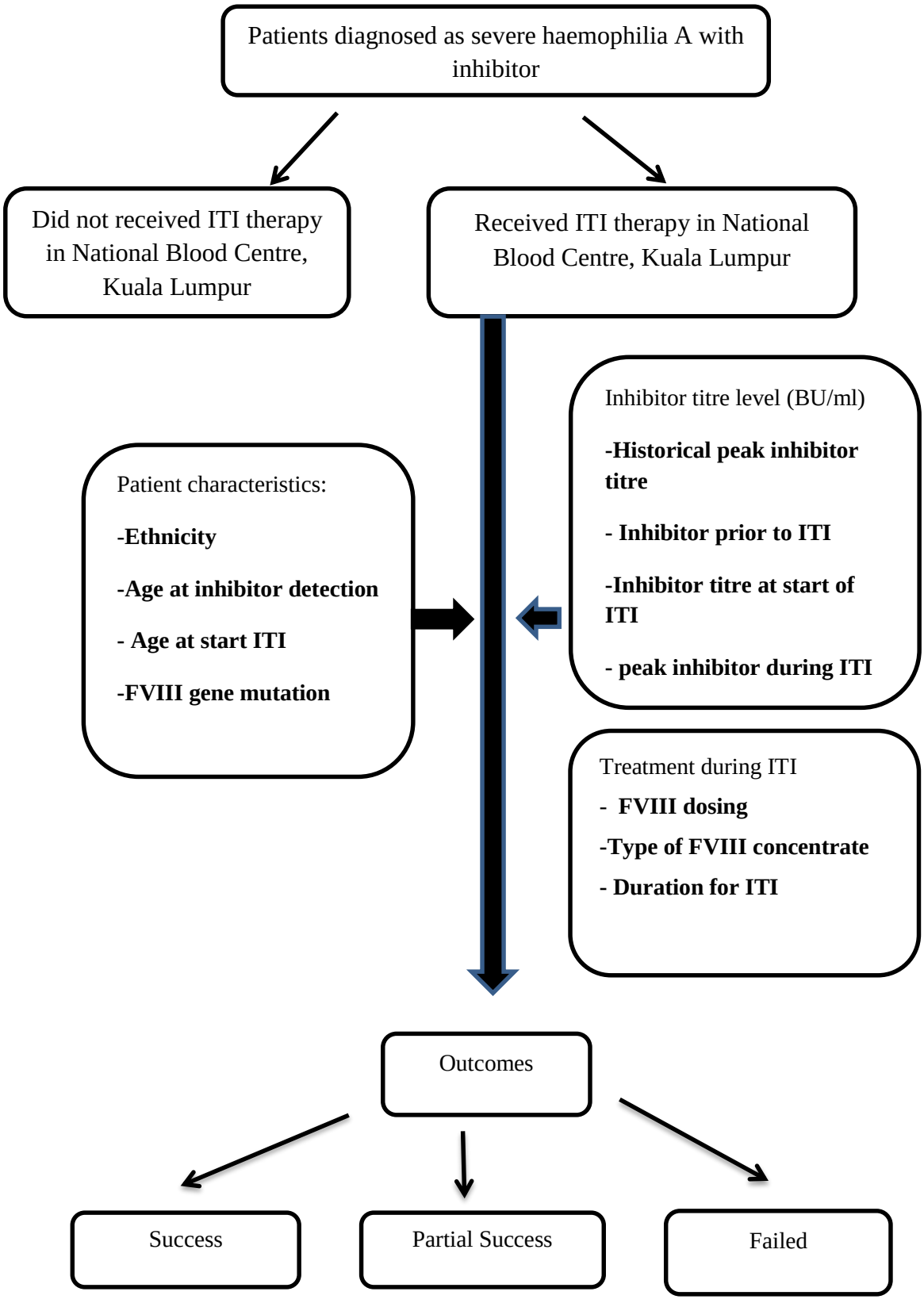

Figure 1.1 Conceptual Framework for the Study

CHAPTER 2

Literature Review

2.1 Treatment for Haemophilia A

Congenital haemophilia A is an X-linked and rare bleeding disorder due to a deficiency of factor VIII (FVIII) (Mannucci and Tuddenham, 2001). FVIII is one of the coagulation protein needed for blood to clot properly. In severe haemophilia cases (factor VIII level is a situation when a patient's plasma is lees than 1%) , the patient can have bleeding episodes as frequently as 20 to 30 times per year and life threatening bleeding like intracranial bleeds also may occur (Farrugia *et al.*, 2012). Haemophilia arthropathy is the major morbidity in patients with haemophilia as a result of recurrent bleeding episodes into joint space (Leslie and Catherine, 2007). The patients will be presented with synovial hypertrophy, narrow joint space, cartilage damage and bony destruction (Pipe and Valentino, 2007).

In 1950s, haemophilia A patients were treated with only whole blood or fresh plasma. However, the inadequate FVIII coagulation factor in these blood products makes the bleeding difficult to stop especially in major bleeds. Therefore, most of the severe haemophilia A patients had lower life expectancy and died during childhood or early adulthood (Franchini and Mannucci, 2012). The treatment evolution started from the 1960s when factor concentrate was discovered, which contained a large amount of factor VIII (Franchini and Mannucci, 2012). Since then, factor replacement has become the basis of treatment for bleeding episodes. Adequate replacement therapy with

specific factor is important to ensure that the bleeding episodes and joint deformities can be prevented (Liesner *et al.*, 1996).

Haemophilia A patients usually requires lifelong FVIII replacement therapy to prevent and treat the bleeding episodes. A survey done in 2006 consist of 147 haemophilia treatment centres from United States, Canada, Australia, Sweden, Belgium, Brazil, Finland, Hungary, Iceland, Israel, Japan, Malaysia, Mexico, Holland, New Zealand, South Africa, Spain and Taiwan, which involved 16115 patients with haemophilia A. This survey showed about 54% of patients were on demand therapy compared to 37% of patient who had received prophylaxis therapy(Geraghty *et al.*, 2006). These data shows that most of the patients with severe haemophilia A in the countries surveyed are still treated as on demand therapy, where the factor replacement is only given during the presence of acute bleeding although it is recommended by the World Health Organisation (WHO) to administer prophylaxis instead(Geraghty *et al.*, 2006). Other than that, some haemophilia patients especially in developing countries like Malaysia will be underdiagnosed due to limited and less optimal treatment available. Only 25% of patients had an access to the treatments (Ayob, 2008).

The concept of prophylaxis in haemophilia A using FVIII concentrate to prevent and reduce the frequency of bleeding was first introduced in Sweden at the Malmo Centre in 1958 by Nilsson and a team of researchers (Ljung, 2009). However, the factor concentrate was not easily available and the dosage given at that time was relatively small. FVIII concentrate was administered once in 14 days when it was first introduced. Over the years with sufficient amount of factor concentrate available,

prophylaxis was given every 2 to 4 weeks which was then increased to three times a week (Carcao, 2014). Most of the studies done showed that prophylaxis treatment had a better short and long term outcome either by primary or secondary prophylaxis. Based on two randomized controlled trial (Manco-Johnson, 2007; Gringeri *et al.*, 2011) their study's outcome showed there is a significant reduction in the frequency of bleeding and had improved quality of life, although it did not reverse the joint that have already been damaged in a patient who was treated with prophylaxis compared to those on demand therapy.

Even though most of the published studies showed prophylaxis was an ideal therapy as recommended by the World Federation of Haemophilia and World Health Organization, unfortunately in many countries it is still not widely applied (Manco-Johnson, 2003). Several barriers related to this problem in starting an early and long term prophylaxis treatment especially in children included the availability of factor concentrate, the cost, the understanding about the need of prophylaxis and insecurities pertaining to the possible complications related to the treatment (Petrini *et al.*, 2004).

The management of haemophilia is complex and the treatment depends on clinical diagnosis, good laboratory facilities and the availability of factor replacement therapy. A comprehensive care clinic is the best setting to manage haemophilia patients, which involves a multidisciplinary team including doctors, nurses, physiotherapist, dentist and also psychologist (Ayob, 2008). A study conducted by US Centres for Disease Control and Prevention (CDC) had shown that patients who had been managed in comprehensive care centres had lower mortality (1:1.6) and less hospitalisation

episodes compared to patients who had been managed by individual physicians or haematologist (Poon and Card, 2012).

2.2 Treatment of Haemophilia A with Inhibitor.

The availability of FVIII concentrate has led to a greater impact in the health status and joint outcomes in patient that suffered from haemophilia A. However, the development of antibody towards exogenous FVIII known as an inhibitor caused the most severe complications of replacement therapy in patients with haemophilia A. Inhibitors was commonly seen in patient with severe haemophilia A compared to haemophilia B or mild/moderate haemophilia patients(Mannucci and Tuddenham, 2001). The risk of inhibitor is also higher in children with severe haemophilia especially after 10 to 15 exposure days to FVIII concentrate (Wight and Paisley, 2003). The other factors that had increased the risk of inhibitor development included a patient with a family history of inhibitor or African- American descent (Astermark *et al.,* 2001; Mannucci and Tuddenham, 2001).

Inhibitor formations cause the replacement therapy to become ineffective, more challenging to achieve haemostasis that leads the bleeding becoming difficult to control. The presence of inhibitors also have an impact on patient's physical activity, morbidity thus decreasing the quality of life as patients had missed from school or work due to bleeding episodes (Brown *et al.*, 2009). Approximately, 20% to 30% of patients with severe Haemophilia A had developed inhibitors compared to haemophilia B (less than 5%) (Astermark *et al.*, 2006b).

The inhibitor formation should be suspected when there is a poor clinical response during any bleeding episodes or following any surgery despite a standard dose of FVIII infusion. Once an inhibitor is suspected, the laboratory test using a Bethasda Inhibitor Assay (BIA) should be carried out. Most of the screening test for inhibitors is done prior to any surgical procedure or monitored within the first 50 days of exposure (Kempton and White, 2009). The inhibitor will bind to the FVIII and prevent its haemostatic action and resulting in the bleeding becoming difficult to control and makes the surgery more hazardous (Darby *et al.*, 2004).

Low titre inhibitor is defined when the titre reading is ≤ 5BU and it can usually can be treated with larger doses of factor VIII concentrate. However, those with high titre inhibitor (>5BU) will usually render the replacement therapy ineffective in treating acute bleeding episodes and requires a bypassing therapy (Hay *et al.*, 2000). Currently, there are two bypassing agents available worldwide that include the recombinant factor VIIa (rFVIIa), NovoSeven or activated prothrombin complex concentrate (aPCC) and factor eight inhibitor bypassing activity (FEIBA). Generally, both products had similar efficacy which ranges from 80% to 90%. Since the agents will promote thrombin formation by a different mechanism, thus it believed that the action of both agents might be variations in individual cases (Coppola *et al.*, 2010a). The rfVIIa acts directly on the platelet surface by activating factor X, thus bypassing the tenase complex (Roberts *et al.*, 2004). FEIBA is a plasma derived of vitamin K-dependent clotting factors (factor II, factor VII, factor IX, factor X and others) that acts by boosting activity of the prothrombinase complex (Turecek *et al.*, 1999). rFVIIa is a recombinant product and does not have FVIII, thus, rFVIIa has no tendency to anamnesis to FVIII

and is usually used as the initial therapy in treating acute bleeding episodes in patients with inhibitors that do not respond to FVIII anymore. The half-life for rFVIIa is 2.3 hours in adult and even shorter in children and it is commonly given in standard doses of 90 to 120 mcg/kg every 2 to 3 hours for the usual joint bleed (Kempton and White, 2009). A study done by Iorio et al , from two RCTs of rFVIIa and aPCC showed that both products can be given as a single bolus infusion and had a low level of thromboembolic risk (Iorio *et al.*, 2010).

There were three (3) prospective studies carried out using bypassing agents as prophylaxis in an inhibitor patient and the result showed that those on rFVIIa and aPCC prophylaxis had significant reduction in bleeding frequency and improved quality of life compared with on demand therapy (Leissinger *et al.*, 2015). The inhibitor patients also had fewer hospital admissions, days missing from school or work and no thromboembolic event reported while patients are on prophylaxis therapy. Even though prophylaxis using bypassing agents is the treatment option to reduce morbidity in patients who had persistently high titres inhibitors or planned for immune tolerance induction therapy, the major barrier in choosing this the treatment is the cost and lack of accessibility (Kempton and Meeks, 2014). The other factor that needs to be considered when starting prophylaxis using bypassing agents is to either use rFVIIa or FEIBA that includes; the frequency of infusion, volume of infusion and anamnestic response (Kempton and White, 2009).