UNIVERSITI PUTRA MALAYSIA

CYTOTOXIC T-LYMPHOTYCES ANTIGEN 4 GENE POLYMORPHISM AND CYTOKINE LEVELS AMONG SEVERE HAEMOPHILIA A PATIENTS WITH AND WITHOUT INHIBITORS

ANANTHA KUMMAR A/L NADARAJAN

FPSK(m) 2021 43

CYTOTOXIC T-LYMPHOTYCES ANTIGEN 4 GENE POLYMORPHISM AND CYTOKINE LEVELS AMONG SEVERE HAEMOPHILIA A PATIENTS WITH AND WITHOUT INHIBITORS

By

ANANTHA KUMMAR A/L NADARAJAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2020

Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

CYTOTOXIC T-LYMPHOTYCES ANTIGEN 4 GENE POLYMORPHISM AND CYTOKINE LEVELS AMONG SEVERE HAEMOPHILIA A PATIENTS WITH AND WITHOUT INHIBITORS

By

ANANTHA KUMMAR S/O NADARAJAN

June 2020

Chair : Sabariah Md Noor, MD, MPath
Faculty : Medicine and Health Sciences

Introduction: Haemophilia A (HA) is an inherited X-chromosome recessive disorder characterized by factor VIII (FVIII) deficiency. High risk of spontaneous bleeding is present among patients with severe HA (FVIII < 1%). FVIII concentrate infusion is the preferred treatment for patients with severe HA however, the formation of neutralizing antibodies known as inhibitor is one of the most significant complications. The neutralizing antibodies inhibits the coagulation activity against the infused concentrated FVIII. The aim of this study is to characterize the gene polymorphism encoding for -49 (A/G) and -318 (C/T) alleles of Cytotoxic T-Lymphocytes Antigen 4 (CTLA-4) which relate to the inhibitors development. Cytokines level of interleukin 4 (IL4), interleukin 10 (IL10) and tumor necrosis factor alpha (TNFα) were measured to link with inhibitors development. Materials/Methodology: Whole blood and DNA of 64 severe HA respondents with and without inhibitor collected from PDN were analysed for gene polymorphisms using the Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) and cytokines level were measured using the Enzyme-linked Immunosorbent Assay (ELISA). Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 23.0. Results: There were 32 respondents with inhibitors and 32 respondents without inhibitors. Among the respondents, almost half (46.9%) of which had high titre inhibitors (≥5 BU) and another half of the respondents (53.1 %) had low titre inhibitors. Cytokines measurement expressed mixed pattern among respondents. Higher expression of IL4 was noted in repondents with inhibitors especially those with inhibitors of ≥5 BU/ml but this was statistically not significant. Statistical comparison of CTLA-4 -49 (A/G) polymorphisms with IL4 showed homozygous G/G genotypes patients with inhibitors had higher level of IL4 concentration but statistically not significant. Patients with inhibitors titre of ≥ 5 BU/ml expressed higher level of IL10 concentration but TNFα concentration was expressed at a lower level. These findings were statistically significant

($p<0.05$). Discussion/Conclusion: This study found gene polymorphisms of CTLA-4 -49 (A/G) and CTLA-4 -318 (C/T) were not significantly different among severe HA with inhibitors and without inhibitors. However, the findings of the mixed pattern of cytokine profiles of IL4, TNFα and IL10 in severe haemophilia A patients seemed to be associated with the presence of FVIII inhibitors which requires further verification with a study using a larger sample size.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

GEN POLIMORFISME PADA ANTIGEN 4 SITOTOKSIK T LIMFOSIT DAN TAHAP SITOKIN DIKALANGAN PESAKIT HEMOPHILIA A PARAH

Oleh

ANANTHA KUMMAR A/L NADARAJAN

Jun 2020

Pengerusi : Sabariah Md Noor, MD, MPath
Fakulti : Perubatan dan Sains Kesihatan

Pengenalan: Hemofilia A merupakan sejenis penyakit keturunan herisian X-kromosomal yang dicirikan oleh kekurangan faktor VIII (FVIII). Pesakit hemofilia parah (FVIII < 1%) berisiko tinggi untuk mengalami pendarahan spontan. Infusi '*FVIII concentrate*' merupakan rawatan pilihan bagi pesakit hemofilia parah namun penghasilan antibodi peneutralan ataupun lebih dikenali sebagai perencat menjadi satu komplikasi berat yang bererti. Antibodi peneutralan merencatkan aktiviti kogulasi dari infusi '*FVIII concentrate*'. Penyelidikan ini bertujuan untuk mencirikan gen polimorfisme yang mengekodkan alel -49 (A/G) dan -318 (C/T) pada *'a*ntigen 4 sitotoksik T limfosit *(CTLA-4)'* dalam penghasilan antibodi perencat. Tahap sitokin termasuk IL4, IL 10 dan TNFA diukur untuk menilai kemungkinan ada hubungkait dengan penghasilan perencat. Bahan/Metodologi: Darah keseluruhan dan sampel DNA dari 64 responden hemophilia A parah dengan perencat dan tanpa perencat telah di ambil dari PDN dan dianalisa untuk gen polimorfisme menggunakan teknik *'Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR RFLP)'.* Tahap sitokin pula telah diukur menggunakan teknik '*Enzyme-linked Immunosorbent Assay (ELISA)'.* Data terkumpul telah dianalisa menggunakan '*Statistical Package for the Social Sciences (SPSS)'* versi 23.0. Penemuan: Seramai 32 responden adalah hemophilia A parah dengan perencat dan 32 darinya tanpa perencat. Daripada jumlah responden dengan perencat, hampir separuh (46.9%) mempunyai titer antibodi yang tinggi dan separuh lagi (53.1%) mempunyai titer antibodi yang rendah. Pengukuran tahap sitokin dikalangan responden menunjukkan corak yang bercampur. Paras IL4 yang lebih tinggi dikalangan responden dengan perencat terutamanya mereka yang mempunyai perencat titre ≥ 5 BU/ml dinama ianya tidak bererti secara statistic. Perbandingan statistik diantara polimorfisme CTLA-4 -49 (A/G) and IL4 menunjukkan pesakit genotip G/G '*homozygous'* yang menghasilkan perencat mempunyai tahap IL4 lebih tinggi namun tidak bererti secara statistic. Pesakit dengan titre perencat melebihi 5 BU/ml menunjukkan paras IL10 yang tinggi dan tahap TNFalpha yang

rendah. Analisa menunjukkan penemuan ini tidak bererti secara statistik. Diskusi/Kesimpulan: Hasil kajian menunjukkan polimorfirme gen CTLA-4 -49 (A/G) dan CTLA-4 - 318 (C/T) tidak mempunyai perbezaan yang ketara dikalangan responden HA parah dengan perencat dan tanpa perencat. Walau bagaimanapun, corak bercampur profil sitokin IL4, TNFα dan IL10 dikalangan responden HA parah menunjukkan perhubungan dengan kehadiran perencat FVIII yang memerlukan pengenalpastian melalui penyelidikan yang menggunakan saiz sampel yang lebih besar.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude to my project supervisor, Associate Professor Dr. Sabariah Md Noor from the Faculty of Medicine, UPM for her continuous knowledge sharing, guidance and support given during my entire term of study. Among her key contribution in this study include provision of facts and ideas, review of findings, review of write-ups, workflow guidance, motivation and many more whom without which would not have made this project possible.

I would also like to thank my internal project co-supervisor, Dr. Suhaili Abu Bakar @ Jamaludin from the Faculty of Medicine and Health Sciences, UPM for her assistance rendered during my laboratory work especially in the molecular areas of this project. A word of thanks also to my external co-supervisor, Dato' Dr. Faraizah Binti Abd Karim from the National Blood Centre Malaysia for her assistance rendered especially in facilitating the specimen collection and sample procurement for this project.

My sincere gratitude to my family members, friends, colleagues and acquaintances whom has continuously supported and assisted me in all ways possible making this research project a success. Last but not the least, I would like to thank the entire Faculty of Medicine and Health Sceinces, UPM for giving me the opportunity to pursue my post-graduate studies here in this renowned institution. The knowledge and experience garnered here during my post-graduate studies will always be valuable to me.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Sabariah binti Md Noor, MD, MPath
Associate Professor (Medical)
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia
(Chairman)

Suhaili binti Abu Bakar @Jamaludin, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia
(Member)

Faraizah Binti Dato' Abdul Karim, MD
Senior Consultant Pathologist (Haematology/Transfusion)
Pathology Unit
Ampang Hospital
(Member)

_____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 June 2021

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of
Supervisory Committee: Associate Professor Dr. Sabariah Md Noor

Signature: _____
Name of Member of
Supervisory Committee: Dr. Suhaili Abu Bakar @Jamaludin

Signature: _____
Name of Member of
Supervisory Committee: Dato' Dr. Faraizah Binti Dato' Abdul Karim

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A/A	Adenine / Adenine
A/G	Adenine / Guanine
APC	Antigen Presenting Cell
APTT	Activated Partial Thromboplastin Time
BT	Bleeding Time
BU	Bethesda Unit
C/C	Cytosine / Cytosine
C/T	cytosine / Thymine
CI	Confidence Interval
CTLA-4	Cytotoxic T Lymphocyte Antigen
DNA	Deoxyribonucleic Acid
DNPs	Double nucleotide polymorphisms
ELISA	Enzyme Linked Immunosorbent Assay
FFP	Fresh Frozen Plasma
FI	Factor 1 @ Fibrinogen
FII	Factor 2 @ Prothrombin
FIIa	Factor 2a @ Thrombin
FIX	Factor 9
FIXa	Factor 9a
FV	Factor 5
FVIII	Factor 8
FVIIIa	Factor 8a
FX	Factor 10
FXa	Factor 10a
FXI	Factor 11
FXII	Factor 12
FXIII	Factor 13
G/G	Guanine / Guanine
HA	Haemophilia A
HLA	Human Leucocyte Antigen
IL10	Interleukin 10
IL4	Interleukin 4
IQR	Inter Quatile Range
ITI	Immune Tolerance Induction
MHC	Major Histocompatibilty Complex
MNPs	Multiple nucleotide polymorphisms
OR	Odd Ratio
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction - Restriction Fragment Length Polymorphism
pdFVIII	Plasma Derived Factor 8
PT	Prothrombin Time
RFLP	Restriction Fragment Length Polymorphism
rFVIII	Recombinant Factor 8
SD	Standard Deviation
SNPs	Single nucleotide polymorphisms
T/T	Thymine / Thymine
TF	Tissue Factor

TF-FVIIa	Tissue Factor - Activated Factor 7a
TNFα	Tumor Necrosis Factor Alpha
TNPs	Triple nucleotide polymorphisms
TT	Thrombin Time
vWF	von Willebrand Factor

CHAPTER 1

INTRODUCTION

1.1. Introduction

Haemophilia A (HA) is a type of inherited bleeding disorder in which a person has low levels of clotting factors activities which results in coagulation disorder. Haemophilia A patient suffers from deficient levels of factor VIII (FVIII) in their blood which results in poor coagulation process. This X-linked disorder represents a high majority of hereditary deficiencies pertaining to blood clotting protein that occurs nearly one per five thousand male birth (Rohan *et al*., 2019).

An individuals with an FVIII amount of < 1 IU / dL in their blood are generally classified as severe haemophilia and comprise roughly half of the cases diagnosed. Moderate and mild haemophilia is diagnosed if a patient's FVIII levels between 1-5 IU/dL and > 5 IU/dL respectively. The World Federation of Haemophilia (2006) reported that the prevalence of haemophilia A and haemophilia B noticeably differs among nations or countries. Statistical data reported by Management of Haemophilia, HTA Report, (2012) stated that "the prevalence of haemophilia A cases in Malaysia has increased from 5.6 per 100,000 males in 1998 to 6.6 per 100,000 males in 2006; where the mean was 5.9 ± 0.4 per 100,000 males". The recent HA epidemiology in Malaysia still under reported.

Plasma fraction containing FVIII product was first introduced in1970's as a treatment for haemophilia A. Although plasma fractions have been a choice of treatment, patients begin to develop complications resulting from the infusion of plasma fractions. These patients have a risk of developing inhibitors towards FVIII. These inhibitors are generally alloantibodies that binds to an epitope of FVIII to form a complex which is then recognized by the immune system as a foreign peptide. This was known as the most serious complication in haemophilia A patient with respect to the replacement therapy where the treatment becomes more challenging for the patients that develops inhibitors (De Alencar *et al*., 2013).

Several studies revealed that immune genes and Human Leukocytes Antigen (HLA) genes are highly link to the production of haemophilia A inhibitors. TNFα gene polymosphisms is associated with inhibitors development among haemophilia A patients (Jan Astermark et al., 2008). Recent studies showed that the polymorphisms of TNFα, CTLA-4 and IL10 have been involved in inhibitor risk among haemophilia patients (Pavlova *et al*., 2009). In addition, TNFα gene could be useful marker which also has the potential as a modulator of the

immune response in replacement therapy for haemophilia A patient (Zhang *et al*., 2011).

CTLA-4 protein is a receptor which is found on activated T cells. It function in down regulation of T-cell activity by binding with B7 molecules in competing with CD28 molecules (Astermark, 2010). Polymorphism of this particular gene is suspected to increase the inhibitors production but a study conducted in India could not provide significant evidence (Pinto *et al*., 2012). A study conducted in Sweden also did not show any significant statistical difference between the polymorphisms of CTLA-4 and inhibitor development (Pavlova *et al*., 2009). Finding among the Chinese population found a lower frequency pattern for the -318 C/T polymorphism T allele in all haemophilia A patients (Lu *et al*., 2012).

1.2 Problem Statement

Inhibitors development in severe HA patients is the most challenging issue in the treatment of the disorder using factors replacement therapy. The inhibitors development leads to ineffective treatment outcomes which causes serious morbidity and mortality in HA patients. There were studies which suggested that the polymorphism of CTLA-4 gene and immune cytokines level are associated with inhibitors development in severe HA patients that were being treated with factor replacement but this finding is not clearly understood and requires further investigation. The findings from this research hope to facilitate a better understanding of the influence of immune genes particularly the CTLA-4 genes on the pathophysiology of inhibitors development. The findings hope to provide some additional alternative treatment strategies for severe HA patients particularly patients with inhibitors.

1.3 Significance of the Study

The polymorphism of CTLA-4 genes have not been investigated among severe haemophilia A patients in Malaysia. This will be a case and control study looking into the association of these immune genes in different ethnic groups along with the risk and severity of inhibitor among severe haemophilia A in Malaysia. The findings of this study will be a foundation for future studies looking into the same aspect as well as investigating the choice of treatment approach in this group of patients.

1.4 Study objective

1.4.1 General Objective

To characterise the Cytotoxic T Lymphocyte Antigen 4 (CTLA-4) genes polymorphism and selected cytokines levels on inhibitor development in severe haemophilia A patients.

1.4.2 Specific Objectives

1) To determine the demographic factors (age, race) and clinical factors (inhibitor status, inhibitors level) distribution and frequency of severe haemophilia A patients in Malaysia.

2) To determine the prevalence of CTLA-4 gene polymorphism among severe haemophilia A patients with and without inhibitors development.

3) To determine the distribution and frequency of interleukin 4 (IL4), interleukin 10 (IL10) and TNFα cytokines levels according to patients' demographic and clinical factors (age, race, inhibitors status and inhibitor level)

4) To determine the association of patients' demographic and clinical factors (age, race, inhibitor status, inhibitors level) with CTLA-4 genes polymorphism among Malaysian severe haemophilia A patients with and without inhibitors.

5) To determine the association of cytokines levels according to patients' demographic and clinical factors (age, race, inhibitor level, inhibitors status) with CTLA-4 genes polymorphism of severe haemophilia A patients in Malaysia.

1.5 Hypothesis

CTLA-4 gene polymorphisms and the increase in cytokines level is associated with the development of inhibitors among Malaysian patients with severe Haemophilia A.

REFERENCES

Astermark, J. (2010). Inhibitor development: Patient-determined risk factors. *Haemophilia*, *16*(102), 66–70. https://doi.org/10.1111/j.1365-2516.2008.01923.x

Astermark, J., Wang, X., Oldenburg, J., Berntorp, E., Lefvert, A. K., & Group, M. S. (2007). Polymorphisms in the CTLA-4 gene and inhibitor development in patients with severe hemophilia A. *J Thromb Haemost*, *5*(2), 263–265. https://doi.org/10.1111/j.1538-7836.2007.02290.x

Astermark, J. (2017). Inherited Bleeding Disorders FVIII inhibitors : Pathogenesis and Avoidance. *Blood*, *125*(13), 2045–2052. https://doi.org/10.1182/blood-2014-08-535328.

Astermark, J. (2006). Why do inhibitors develop? Principles of and factors influencing the risk for inhibitor development in haemophilia. *Haemophilia*, *12*(SUPPL. 3), 52–60. https://doi.org/10.1111/j.1365-2516.2006.01261.x

Astermark, J., Oldenburg, J., Carlson, J., Pavlova, A., Kavakli, K., & Berntorp, E. (2008). *Polymorphisms in the TNFA gene and the risk of inhibitor development in patients with hemophilia A*. *108*(12), 3739–3745. https://doi.org/10.1182/blood-2006-05-024711

Astermark, J., Oldenburg, J., Escobar, M., White, G. C., & Berntorp, E. (2005). The Malm?? International Brother Study (MIBS). Genetic defects and inhibitor development in siblings with severe hemophilia A. *Haematologica*, *90*(7), 924–930.

Astermark, J., Oldenburg, J., Pavlova, A., Berntorp, E., & Lefvert, A.-K. (2006). Polymorphisms in the IL10 but not n the IL1beta and IL4 genes are associated with inhibitor development in patients with hemophilia A. *Blood*, *107*(8), 3167 LP – 3172. https://doi.org/10.1182/blood-2005-09-3918.A

Berg, M. van den. (2007). Risk of Inhibitor Development in Children with Haemophilia A. *European Oncology & Haematology*, 8. https://doi.org/10.17925/eoh.2007.0.0.8

Berntorp, E., Donfield, S., Waters, J., Mattson, E., DiMichele, D., Gringeri, A., & Astermark, J. (2005). The FEIBA® Novoseven® comparative study (FENOC) - a randomized evaluation of by-passing agents in hemophilia complicated by inhibitors [abstract]. *Blood*, *106*(11), Abstract no: 324. https://doi.org/10.1182/blood-2006-04-017988.An

Sébastien Lacroix-Desmazes., Jagadeesh Bayry., Namita Misra., Michael P. Horn., Sylvie Villard., Anastas Pashov., Natalie Stieltjes., Roseline d'Oiron., Jean-Marie Saint-Remy., Johan Hoebeke., Michel D. Kazatchkine., Joseph Reinbolt, Ph.D. (2002). The Prevalence of Proteolytic Antibodies against factor VIII in haemophilia A. *N Eng J Med* 2002;26:662-667. https://doi: 10.1056/NEJMoa011979

Blanchette, V. S., Key, N. S., Ljung, L. R., Manco-Johnson, M. J., van den Berg, H. M., & Srivastava, A. (2014). Definitions in hemophilia: Communication from the SSC of the ISTH. *Journal of Thrombosis and Haemostasis*, *12*(11), 1935–1939. https://doi.org/10.1111/jth.12672

Borhany, M., Kumari, M., Shamsi, T., Naz, A., & Farzana, T. (2012). Frequency of Factor VIII (FVIII) Inhibitor in Haemophilia A. *Journal of the College of Physicians and Surgeons Pakistan*, *22*(5), 289–293. https://doi.org/05.2012/JCPSP.289293

Burckhardt, J. (1994). Amplification of DNA from whole blood. *Genome Research*, *3*(4), 239–243. https://doi.org/10.1101/gr.3.4.239

Carcao, M. D. (2006). Intensive exposure to factor VIII may be a risk factor for inhibitor development in mild hemophilia A. *Seminars in Hematology*, *43*(SUPPL. 1), 1228–1236. https://doi.org/10.1053/j.seminhematol.2005.12.001

Carcao, M., & Goudemand, J. Inhibitors In Hemophilia : A Primer Fifth Edition. *World Federation of Hemophilia*: Canada. *2018.*

Chambost, H. (2010). Assessing risk factors: Prevention of inhibitors in haemophilia. *Haemophilia*, *16*(SUPPL. 2), 10–15. https://doi.org/10.1111/j.1365-2516.2009.02197.x

Chaves, D. G., Velloso-Rodrigues, C., Oliveira, C. A., Teixeira-Carvalho, A., Santoro, M. M., & Martins-Filho, O. A. (2010). A shift towards a T cell cytokine deficiency along with an anti-inflammatory/regulatory microenvironment may enable the synthesis of anti-FVIII inhibitors in haemophilia a patients. *Clinical and Experimental Immunology*, *162*(3), 425–437. https://doi.org/10.1111/j.1365-2249.2010.04258.x

Chong, K. K. L., Chiang, S. W. Y., Wong, G. W. K., Tam, P. O. S., Ng, T. K., Hu, Y. J., Yam, G. H. F., Lam, D. S. C., & Pang, C. P. (2008). Association of CTLA-4 and IL-13 gene polymorphisms with Graves' disease and ophthalmopathy in Chinese children. *Investigative Ophthalmology and Visual Science*, *49*(6), 2409–2415. https://doi.org/10.1167/iovs.07-1433

Chuansumrit, A., Sasanakul, W., Sirachainan, N., Kadegasem, P., Wongwerawattanakoon, P., Mahaklan, L., & Nathalang, O. (2017). Association of factor VIII and factor IX mutations, HLA Class II, tumour necrosis factor-α and interleukin-10 on inhibitor development among Thai haemophilia A and B patients. *Haemophilia*, *23*(6), e518–e523. https://doi.org/10.1111/hae.13344

Colowick, A. B., Bohn, R. L., Avorn, J., & Ewenstein, B. M. (2000). Immune tolerance induction in hemophilia patients with inhibitors: Costly can be cheaper. *Blood*, *96*(5), 1698–1702. https://doi.org/10.1182/blood.v96.5.1698.h8001698_1698_1702

Coppola, a, Santoro, C., Tagliaferri, a, Franchini, M., & DI Minno, G. (2010). Understanding inhibitor development in haemophilia A: towards clinical prediction and prevention strategies. *Haemophilia : The Official Journal of the World Federation of Hemophilia*, *16 Suppl 1*, 13–19. https://doi.org/10.1111/j.1365-2516.2009.02175.x

David, S., Nair, S. C., Singh, G. S., Alex, A. A., Ganesan, S., Palani, H. K., Balasundaram, N., Lakshmi, K. M., Joshi, A., Kannan, S., Korula, A., Nambiatheyil Aboobacker, F., Abraham, A., George, B., Apte, S. J., Srivastava, A., & Mathews, V. (2019). Prevalence of FVIII inhibitors in severe haemophilia A patients: Effect of treatment and genetic factors in an Indian population. *Haemophilia*, *25*(1), 67–74. https://doi.org/10.1111/hae.13633

de Alencar, J. B., Macedo, L. C., de Barros, M. F., Rodrigues, C., Cadide, R. C., Sell, A. M., & Visentainer, J. E. L. (2013). Importance of immune response genes in hemophilia A. *Revista Brasileira de Hematologia e Hemoterapia*, *35*(4), 280–286. https://doi.org/10.5581/1516-8484.20130095

DiMichele. (2000). Inhibitors in haemophilia: A primer. *Haemophilia*, *6*(SUPPL. 1), 38–40. https://doi.org/10.1046/j.1365-2516.2000.00045.x

Eckhardt, C. L., Astermark, J., Nagelkerke, S. Q., Geissler, J., Tanck, M. W. T., Peters, M., Fijnvandraat, K., & Kuijpers, T. W. (2014). The Fc gamma receptor IIa R131H polymorphism is associated with inhibitor development in severe hemophilia A. *Journal of Thrombosis and Haemostasis*, *12*(8), 1294–1301. https://doi.org/10.1111/jth.12631

ElBagoury, M., Omar, N. M., & Kotb, M. (2018). Hemophilia A and incidence of inhibitors. *Journal of Pharmaceutical Sciences and Research*, *10*(3), 506–508.

Ettinger, R. A., James, E. A., Kwok, W. W., Thompson, A. R., & Pratt, K. P. (2010). HLA-DR-restricted T-cell responses to factor VIII epitopes in a mild haemophilia A family with missense substitution A2201P. *Haemophilia*, *16*(102), 44–55. https://doi.org/10.1111/j.1365-2516.2008.01905.x

Goudemand, J., Rothschild, C., Laurian, Y., & Calvez, T. (2006). Influence of the type of factor VIII concentrates on the incidence of factor VIII inhibitors in previously untreated patients with severe hemophilia A: Further clarifications on the cohorts' follow-up [4]. *Blood*, *107*(9), 3810. https://doi.org/10.1182/blood-2005-04-1371.J.G.

Gouw, S. C., Van Den Berg, H. M., Fischer, K., Auerswald, G., Carcao, M., Chalmers, E., Chambost, H., Kurnik, K., Liesner, R., Petrini, P., Platokouki, H., Altisent, C., Oldenburg, J., Nolan, B., Garrido, R. P., Mancuso, M. E., Rafowicz, A., Williams, M., Clausen, N., Van Der Bom, J. G. (2013). Intensity of factor VIII treatment and inhibitor development in children with severe hemophilia A: The RODIN study. *Blood*, *121*(20), 4046–4055. https://doi.org/10.1182/blood-2012-09-457036

Gouw, S. C., Van Den Berg, H. M., Oldenburg, J., Astermark, J., De Groot, P. G., Margaglione, M., Thompson, A. R., Van Heerde, W., Boekhorst, J., Miller, C. H., Le Cessie, S., & Van Der Bom, J. G. (2012). F8 gene mutation type and inhibitor development in patients with severe hemophilia A: Systematic review and meta-analysis. *Blood*, *119*(12), 2922–2934. https://doi.org/10.1182/blood-2011-09-379453

Gouw, S. C., Van Der Bom, J. G., Ljung, R., Escuriola, C., Cid, A. R., Claeyssens-Donadel, S., Van Geet, C., Kenet, G., Mäkipernaa, A., Molinari, A. C., Muntean, W., Kobelt, R., Rivard, G., Santagostino, E., Thomas, A., & Van Den Berg, H. M. (2013). Factor VIII products and inhibitor development in severe hemophilia A. *New England Journal of Medicine*, *368*(3), 231–239. https://doi.org/10.1056/NEJMoa1208024

Gouw, S. C., Van Der Bom, J. G., & Van Den Berg, H. M. (2007). Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: The CANAL cohort study. *Blood*, *109*(11), 4648–4654. https://doi.org/10.1182/blood-2006-11-056291

Gringeri, A., Mantovani, L. G., Scalone, L., & Mannucci, P. M. (2003). Cost of care and quality of life for patients with hemophilia complicated by inhibitors: The COCIS study group. *Blood*, *102*(7), 2358–2363. https://doi.org/10.1182/blood-2003-03-0941

Hay, C. R., Ollier, W., Pepper, L., Cumming, A., Keeney, S., Goodeve, A. C., Colvin, B. T., Hill, F. G., Preston, F. E., & Peake, I. R. (1997). HLA class II profile: a weak determinant of factor VIII inhibitor development in severe haemophilia A. UKHCDO Inhibitor Working Party. Thrombosis and haemostasis, 77(2), 234–237.

Hedner, U., & Kisiel, W. (1983). Use of human Factor VIIa in the treatment of two hemophilia A patients with high-titer inhibitors. *Journal of Clinical Investigation*, *71*(6), 1836–1841. https://doi.org/10.1172/JCI110939

Iorio, A., Halimeh, S., Holzhauer, S., Goldenberg, N., Marchesini, E., Marcucci, M., Young, G., Bidlingmaier, C., Brandao, L. R., Ettingshausen, C. E., Gringeri, A., Kenet, G., Knöfler, R., Kreuz, W., Kurnik, K., Manner, D., Santagostino, E., Mannucci, P. M., & Nowak-Göttl, U. (2010). Rate of inhibitor development in previously untreated hemophilia A patients treated with plasma-derived or recombinant factor VIII concentrates: A systematic review. *Journal of Thrombosis and Haemostasis*, *8*(6), 1256–1265. https://doi.org/10.1111/j.1538-7836.2010.03823.x

Jacquemin, M. G., Desqueper, B. G., Benhida, A., Elst, L. Vander, Hoylaerts, M. F., Bakkus, M., Thielemans, K., Arnout, J., Peerlinck, K., Gilles, J. G. G., Vermylen, J., & Saint-Remy, J. M. R. (1998). Mechanism and kinetics of factor VIII inactivation: Study with an IgG4 monoclonal antibody derived from a hemophilia A patient with inhibitor. *Blood*, *92*(2), 496–506. https://doi.org/10.1182/blood.v92.2.496.414k16_496_506

Jr, H. H. K., Negrier, C., Vinciguerra, C., Gitschier, J., Goossens, M., Girodon, E., Ghanem, N., Plassa, F., Lavergne, J. M., Vidaud, M., Costa, J. M., Laurian, Y., Lin, S., Shen, M., Lillicrap, D., Taylor, S. a M., Windsor, S., Valleix, S. V, Nafa, K., Hospi-, C. (1995). *Factor VI11 Gene Inversions in Severe Hemophilia*. *86*(6), 2206–2212.

Karabon, L., Kosmaczewska, A., Bilinska, M., Pawlak, E., Ciszak, L., Jedynak, A., Jonkisz, A., Noga, L., Pokryszko-Dragan, A., Koszewicz, M., & Frydecka, I. (2009). The CTLA-4 gene polymorphisms are associated with CTLA-4 protein expression levels in multiple sclerosis patients and with susceptibility to disease. *Immunology*, *128*(1 PART 2), 787–796. https://doi.org/10.1111/j.1365-2567.2009.03083.x

Kempton, C. L., & White, G. C. (2009). How we treat a hemophilia A patient with a factor VIII inhibitor. *Blood*, *113*(1), 11–17. https://doi.org/10.1182/blood-2008-06-160432

Laws, S. M., Perneczky, R., Wagenpfeil, S., Müller, U., Förstl, H., Martins, R. N., Kurz, A., & Riemenschneider, M. (2005). TNF polymorphisms in Alzheimer disease and functional implications on CSF beta-amyloid levels. *Human Mutation*, *26*(1), 29–35. https://doi.org/10.1002/humu.20180

Leissinger, C., Gringeri, A., Antmen, B., Berntorp, E., Biasoli, C., Carpenter, S., Cortesi, P., Jo, H., Kavakli, K., Lassila, R., Morfini, M., Neǵrier, C., Rocino, A., Schramm, W., Serban, M., Uscatescu, M. V., Windyga, J., Zulfikar, B., & Mantovani, L. (2011). Anti-inhibitor coagulant complex prophylaxis in hemophilia with inhibitors. *New England Journal of Medicine*, *365*(18), 1684–1692. https://doi.org/10.1056/NEJMoa1104435

Lu, W., Pan, K., Zhang, L., Lin, D., Miao, X., & You, W. (2005). Genetic polymorphisms of interleukin (IL)-1B, 1L-1RN, IL-8, IL-10 and tumor necrosis factor α and risk of gastric cancer in a Chinese population. *Carcinogenesis*, *26*(3), 631–636. https://doi.org/10.1093/carcin/bgh349

Lu, Y., Ding, Q., Dai, J., Wang, H., & Wang, X. (2012). Impact of polymorphisms in genes involved in autoimmune disease on inhibitor development in Chinese patients with haemophilia A. *Thromb Haemost*, *107*(1), 30–36. https://doi.org/10.1160/TH11-06-0425\r11-06-0425 [pii]

Mahlangu, J., Powell, J. S., Ragni, M. V., Chowdary, P., Josephson, N. C., Pabinger, I., Hanabusa, H., Gupta, N., Kulkarni, R., Fogarty, P., Perry, D., Shapiro, A., Pasi, K. J., Apte, S., Nestorov, I., Jiang, H., Li, S., Neelakantan, S., Cristiano, L. M., … Pierce, G. F. (2014). Phase 3 study of recombinant factor VIII Fc fusion protein in severe hemophilia A. *Blood*, *123*(3), 317–325. https://doi.org/10.1182/blood-2013-10-529974

McDevitt, H., Munson, S., Ettinger, R., & Wu, A. (2002). Multiple roles for tumor necrosis factor-alpha and lymphotoxin alpha/beta in immunity and autoimmunity. *Arthritis research*, *4 Suppl 3*(Suppl 3), S141–S152. https://doi.org/10.1186/ar570

Mikaeili, A. S., Bolhassani, A., & Nasouhi, N. (2018). Investigating the IGSF2 and TNFα genes polymorphism and the risk of inhibitor development in patients with Hemophilia A. *Journal of Mazandaran University of Medical Sciences*, *28*(162), 59–68. https://doi.org/10.1182/blood-2006-05-024711

Miller, C. H., Rice, A. S., Boylan, B., Payne, A. B., Kelly, F. M., Escobar, M. A., Gill, J., Leissinger, C., Soucie, J. M., Abshire, T. C., Dunn, A. L., Kempton, C. L., Bockenstedt, P. L., Brettler, D. B., Di Paola, J. A., Radhi, M., Lentz, S. R., Massey, G., Barrett, J. C., … Yaish, H. (2015). Characteristics of hemophilia patients with factor VIII inhibitors detected by prospective screening. *American Journal of Hematology*, *90*(10), 871–876. https://doi.org/10.1002/ajh.24104

Mudla, M. I., Rahman, M. A., & Thye, S. L. (2012). *Management of Haemophilia* (Vol. 12).

Oldenburg, J., & Pavlova, A. (2006). Genetic risk factors for inhibitors to factors VIII and IX. Haemophilia: the official journal of the World Federation of Hemophilia, 12 Suppl 6, 15–22. https://doi.org/10.1111/j.1365-2516.2006.01361.x

Oliveira, C. A., Velloso-Rodrigues, C., Machado, F. C. J., Carvalho, B. N., Gentz, S. H. L., Martins-Filho, O. A., & Chaves, D. G. (2013). Cytokine profile and FVIII inhibitors development in haemophilia A. *Haemophilia*, *19*(3), 139–142. https://doi.org/10.1111/hae.12096

Osooli, M., & Berntorp, E. (2015). Inhibitors in haemophilia: What have we learned from registries? A systematic review. *Journal of Internal Medicine*, *277*(1), 1–15. https://doi.org/10.1111/joim.12301

Owaidah, T., Al Momen, A., Alzahrani, H., Almusa, A., Alkasim, F., Tarawah, A., Al Nouno, R., Al Batniji, F., Alothman, F., Alomari, A., Abu-Herbish, S., Abu-Riash, M., Siddiqui, K., Ahmed, M., Mohamed, S. Y., & Saleh, M. (2017). The prevalence of factor VIII and IX inhibitors among Saudi patients with hemophilia: Results from the Saudi national hemophilia screening program. *Medicine (United States)*, *96*(2), 1–7. https://doi.org/10.1097/MD.0000000000005456

Paskulin, D. D. Á., Fallavena, P. R. V, Paludo, F. J. O., Borges, T. J., Picanço, J. B., Dias, F. S., & Alho, C. S. (2011). TNF -308G > A promoter polymorphism (rs1800629) and outcome from critical illness. *Brazilian Journal of Infectious Diseases*, *15*(3), 231–238. https://doi.org/10.1016/S1413-8670(11)70181-7

Pavlova, A., Delev, D., Lacroix-Desmazes, S., Schwaab, R., Mende, M., Fimmers, R., Astermark, J., & Oldenburg, J. (2009). Impact of polymorphisms of the major histocompatibility complex class II, interleukin-10, tumor necrosis factor-α and cytotoxic T-lymphocyte antigen-4 genes on inhibitor development in severe hemophilia A. *Journal of Thrombosis and Haemostasis*, *7*(12), 2006–2015. https://doi.org/10.1111/j.1538-7836.2009.03636.x

Peyvandi, F., Mannucci, P. M., Garagiola, I., El-Beshlawy, A., Elalfy, M., Ramanan, V., Eshghi, P., Hanagavadi, S., Varadarajan, R., Karimi, M., Manglani, M. V., Ross, C., Young, G., Seth, T., Apte, S., Nayak, D. M., Santagostino, E., Mancuso, M. E., Sandoval Gonzalez, A. C., Rosendaal, F. R. (2016). A randomized trial of factor VIII and neutralizing antibodies in hemophilia A. *New England Journal of Medicine*, *374*(21), 2054–2064. https://doi.org/10.1056/NEJMoa1516437

Pinto, P., Ghosh, K., & Shetty, S. (2012). Immune regulatory gene polymorphisms as predisposing risk factors for the development of factor VIII inhibitors in Indian severe haemophilia A patients. *Haemophilia*, *18*(5), 794–797. https://doi.org/10.1111/j.1365-2516.2012.02845.x

Pinto, P., Shelar, T., Nawadkar, V., Mirgal, D., Mukaddam, A., Nair, P., Kasatkar, P., Gaikwad, T., Ali, S., Jadli, A., Patil, R., Parihar, A., Shanbhag, S., Kulkarni, B., Ghosh, K., & Shetty, S. (2014). The Epidemiology of FVIII Inhibitors in Indian Haemophilia A Patients. *Indian Journal of Hematology and Blood Transfusion*, *30*(4), 356–363. https://doi.org/10.1007/s12288-014-0342-z

Qian, J., Collins, M., Sharpe, A. H., & Hoyer, L. W. (2000). Prevention and treatment of factor VIII inhibitors in murine hemophilia A. *Blood*, *95*(4), 1324–1329. https://doi.org/10.1182/blood.v95.4.1324.004k25_1324_1329

Repessé, Y., Slaoui, M., Ferrandiz, D., Gautier, P., Costa, C., Costa, J. M., Lavergne, J. M., & Borel-Derlon, A. (2007). Factor VIII (FVIII) gene mutations in 120 patients with hemophilia A: Detection of 26 novel mutations and correlation with FVIII inhibitor development. *Journal of Thrombosis and Haemostasis*, *5*(7), 1469–1476. https://doi.org/10.1111/j.1538-7836.2007.02591.x

Rosendaal, F. R., Nieuwenhuis, H. K., Van den Berg, H. M., Heijboer, H., Mauser- Bunschoten, E. P., Van der Meer, J., Smit, C., Strengers, P. F. W., & Briet, E. (1993). A sudden increase in factor VIII inhibitor development in multitransfused hemophilia A patients in The Netherlands. *Blood*, *81*(8), 2180–2186. https://doi.org/10.1182/blood.v81.8.2180.bloodjournal8182180

Sh, M., J, J., Sh, H., Sh, S., M, I. U., & S, A. (2012). Detection of Factor VIII Inhibitors in Hemophilia A Patients. *Iranian Journal of Blood and Cancer*, *4*(4), 163–168. http://ijbc.ir/article-1-385-en.html

Shi, Q., Wilcox, D. A., Fahs, S. A., Weiler, H., Wells, C. W., Cooley, B. C., Desai, D., Morateck, P. A., Gorski, J., & Montgomery, R. R. (2006). Factor VIII ectopically targeted to platelets is therapeutic in hemophilia A with high-titer inhibitory antibodies. *Journal of Clinical Investigation*, *116*(7), 1974–1982. https://doi.org/10.1172/JCI28416

Shin, S. P. (2015). Association between hepatocellular carcinoma and tumor necrosis factor alpha polymorphisms in South Korea. *World Journal of Gastroenterology*, *21*(46), 13064. https://doi.org/10.3748/wjg.v21.i46.13064

Silveira, A. C. O., Santana, M. A. P., Ribeiro, I. G., Chaves, D. G., & Martins-Filho, O. A. (2015). The IL-10 polarized cytokine pattern in innate and adaptive immunity cells contribute to the development of FVIII inhibitors. *BMC Hematology*, *15*(1), 1–8. https://doi.org/10.1186/s12878-014-0019-8

Valentino, L. A., Mamonov, V., Hellmann, A., Quon, D. V., Chybicka, A., Schroth, P., Patrone, L., & Wong, W. Y. (2012). A randomized comparison of two prophylaxis regimens and a paired comparison of on-demand and prophylaxis treatments in hemophilia A management. *Journal of Thrombosis and Haemostasis*, *10*(3), 359–367. https://doi.org/10.1111/j.1538-7836.2011.04611.x

van den Berg, H. M. (2016). Different impact of factor VIII products on inhibitor development? *Thrombosis Journal*, *14*(Suppl 1). https://doi.org/10.1186/s12959-016-0102-4

Van Helden, P. M. W., Van Den Berg, H. M., Gouw, S. C., Kaijen, P. H. P., Zuurveld, M. G., Mauser-Bunschoten, E. P., Aalberse, R. C., Vidarsson, G., & Voorberg, J. (2008). IgG subclasses of anti-FVIII antibodies during immune tolerance induction in patients with hemophilia A. *British Journal of Haematology*, *142*(4), 644–652. https://doi.org/10.1111/j.1365-2141.2008.07232.x

White, G. C., Courter, S., Bray, G. L., Lee, M., & Gomperts, E. D. (1997). A multicenter study of recombinant factor VIII (Recombinate®) in previously treated patients with hemophilia A. *Thrombosis and Haemostasis*, *77*(4), 660–667. https://doi.org/10.1055/s-0038-1656030

Wojdasiewicz, P., Poniatowski, Ł. A., Nauman, P., Mandat, T., Paradowska-Gorycka, A., Romanowska-Próchnicka, K., Szukiewicz, D., Kotela, A., Kubaszewski, Ł., Kotela, I., Kurkowska-Jastrzębska, I., & Gasik, R. (2018). Cytokines in the pathogenesis of hemophilic arthropathy. *Cytokine and Growth Factor Reviews*, *39*, 71–91. https://doi.org/10.1016/j.cytogfr.2017.11.003

ZemaniFodil, F. (2014). Factor 8 Gene Mutations and Risk of Inhibitor Development in Hemophilia A Algerian Patients. *Journal of Pharmacogenomics & Pharmacoproteomics*, *05*(01), 1–4. https://doi.org/10.4172/2153-0645.1000124