UNIVERSITI PUTRA MALAYSIA

FLOWCYTOMETRIC ASSESSMENT OF PLATELET MICROPARTICLES CD41 AND CD62P IN E/BETA-THALASSEMIA PATIENTS IN PUBLIC HOSPITALS IN SELANGOR, MALAYSIA

BAHAA HADI JABER ALMHANAWI

FPSK(m) 2016 52

FLOWCYTOMETRIC ASSESSMENT OF PLATELET MICROPARTICLES CD41 AND CD62P IN E/BETA-THALASSEMIA PATIENTS IN PUBLIC HOSPITALS IN SELANGOR, MALAYSIA

By

BAHAA HADI JABER ALMHANAWI

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

November 2016

COPYRIGHT

DEDICATION

To my Family

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

FLOWCYTOMETRIC ASSESSMENT OF PLATELET MICROPARTICLES CD41 AND CD62P IN E/BETA-THALASSEMIA PATIENTS IN PUBLIC HOSPITALS IN SELANGOR, MALAYSIA

By

BAHAA HADI JABER ALMHANAWI

November 2016

Chairman : Bahariah Binti Khalid, PhD
Faculty : Medicine and Health Science

The hypercoagulability complications and an increase in thrombosis risk have been reported in B-thalassemia patients. Despite the fact that the life expectancy of B-thalassemia has been improved, thalassemic patients still suffer from many complications including thrombotic risk. High level of platelet microparticles (PMPs) in the circulation of B-thalassemia patients is believed to be responsible for the presence of hypercoagulability state in B-thalassemia patients. The main objective of this research was to assess the level of platelet microparticles in Hb E/B-thalassemia patients and normal individuals in the Malaysian population. The specific objectives were to determine the level of platelet microparticles CD41 and CD62P in Hb E/B-thalassemia and normal individuals, determine the Annexin-5 level in both, Hb E/B-thalassemia and normal individuals, and to correlate the levels of platelet microparticles with the blood parameters. A case-control study was carried out to assess the level of platelet microparticles in Hb E/beta-thalassemia patients (cases) and normal individuals (control) in the Malaysian population. A convenience sample of 37 patients with Hb E/beta-thalassemia (12 paediatrics and 25 adults) were investigated and compared with 28 normal individuals (3 paediatrics and 25 adults) who were studied in the same period. The samples were analyzed using immunophenotyping application in flow cytometer platform. PMPs were processed and analyzed directly after labeling by BD FACS-CantoII^{TM} flow cytometer (Becton Dickinson, USA), using FlowJo software (version 10.1r1). Platelet microparticles defined as MPs that were smaller than 1.0 µm, had a positive staining for A-5, and exposed platelet-specific markers namely, CD41 and/or CD62P. However, in this research, the mean event of CD62P Vs. A-5 were significantly higher in Hb E/B-thalassemia compared to the normal individuals in the adults group (p= 0.006) respectively. There was a strong association between the phospholipid (PS) and platelet activation markers CD41 and CD62P on the activated platelet cells. In conclusion, platelet microparticles are significantly increased in Hb E/B-thalassemia patients compared to the normal individuals, and there is a strong association between platelet microparticle and some blood parameters.

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

PENILAIAN ALIRAN SITOMETRIK MIKROPARTIKAL PLATLET CD41 DAN CD62P DALAM KALANGAN PESAKIT HB E/B-THALASSEMIA DI HOSPITAL AWAM SELANGOR, MALAYSIA

Oleh

BAHAA HADI JABER ALMHANAWI

November 2016

Pengerusi : Bahariah Binti Khalid, PhD
Fakulti : Perubatan dan Sains Kesihatan

Komplikasi hypergumpalan dan peningkatan risiko thrombosis telah dilaporkan berlaku kepada pesakit thalassemia. Meskipun terbukti bahawa jangka hayat pesakit B-thalassemia telah bertambah baik, namun pesakit thalassemia masih menhadapi banyak komplikasi lain seperti risiko thrombotic. Peningkatan jumlah mikropartikal platlet (PMPs) yang tinggi dikalangan pesakit B-thalassemia adalah dipercayai berpunca daripada kewujudan hypergumpalan pada pesakit B-thalassemia. Objektif utama kajian ini adalah untuk mengetahui tahap mikroplatlet pesakit Hb E/B-thalassemia dan individu normal dalam populasi di Malaysia. Kajian ini lebih mengfokuskan bagi mengenalpasti tahap mikropartikal platlet mikropartikal CD41 dan CD62P dalam Hb E/B-thalassemia dan individu normal, mengenalpasti tahap Annexin-5 pada Hb E/B-thalassemia dan individu normal, dan untuk mengetahui hubungan tahap mikropartikal dengan parameter klinikal dan faktor lain yang berkait. Satu kajian kes-kontrol telah dijalankan untuk mengukur tahap mikropartikal platlet pada pesakit Hb E/beta-thalassemia (kes) dan individu normal(kontrol) dalam kalangan populasi masyarakat Malaysia. Sampel yang mudah didapati diambil daripada 37 orang pesakit yang menghidap Hb E/beta-thalassemia (12 pediatrik dan 25 dewasa) dan telah dijalankan ujikaji keatas sampel tersebut , dengan membandingkannya dengan 28 individu sihat (3 pediatrik dan 25 orang dewasa) yang dikaji serentak. Analasis sampel dilakukan dengan mengunakan aplikasi immunophenotyping dalam dataran aliran sitometer. PMPs terus dianalisis selepas di tanda oleh BD FACS-CantoII^{TM} aliran sitometer (Becton Dickinson, Amerika syarikat), mengunakan perisisan FlowJo (versi 10.1r1). Mikropartikal platlet didefinisikan sebagai MP adalah lebih kecil daripada 1.0 µm, mempunyai lekatan tanda positif untuk A-5, dan menanda platlet-spesifik terdedah dinamakan CD41 dan/atau CD62P. Meskipun begitu, min acara bagi kedua-dua CD62P dan A-5 adalah ketara lebih tinggi pada Hb E/B-thalassemia berbanding individu normal dalam kalangan golongan kumpulan orang dewasa (p= 0.006) secara respektif. Terdapat jalinan yang kuat antara phospholipid (PS) dan mengaktifan petanda platlet CD41 dan CD62P pada sel platlet yang telah diaktifkan. Sebagai konklusi, mikropartikal platlet

meningkat secara ketara pada pesakit Hb E/B-thalassemia berbanding individu normal, dan terdapat jalinan yang kuat antara mikropartikal platlet and beberapa parameter darah.

AKNOWLEDGEMENTS

I would like to thank my family and my supervisory committee for their tremendous support.

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

Bahariah Binti Khalid, PhD
Medical Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

Elizabeth George, PhD
Professor (Medicine)
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Eusni Rahayu Mohd Tohit, PhD
Medical Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Zainina Seman, PhD
Medical Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Salmiah Md. Said, PhD
Medical Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Dr. Bahariah Binti Khalid

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Elizabeth George

Signature: _____
Name of Member of Supervisory Committee: Dr. Eusni Rahayu Mohd Tohit

Signature: _____
Name of Member of Supervisory Committee: Dr. Zainina Seman

Signature: _____
Name of Member of Supervisory Committee: Dr. Salmiah Md. Said

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	Alpha
A-5	Annxin-5
ATP	Adenosine Triphosphate
APC	Activated Protein C
APC*	Allophycocyanin
β	Beta
CBC	Complete Blood Count
Ca^{+}	Calcium
CD	Clusters Of Differentiation
CRF	Clinical Report Form
DVT	Deep Venous Thrombosis
DLS	Dynamic Light Scatter
ER	Endoplasmic Reticulum
ELISA	Enzyme-Linked Immunosorbent Assay
EM	Electron Microscopy
FITC	Fluorescein Isothiocyanate
FSC	Forward Scatter
FMO	Fluorescent Minus One
GP	Glycoprotein
GCP	Good Clinical Practice
Hb	Hemoglobin
HIT	Heparin-Induced Thrombocytopenia
HCT	Hematocrit
ITP	Primary Immune Thrombocytopenia

MPs	Microparticles
mAbs	Monoclonal Antibodies
MFI	Mean Fluorescence Intensity
MCV	Mean Cell Volume
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
PMPs	Platelets Microparticles
PE	Pulmonary Embolism
PVT	Portal Vein Thrombosis
PRP	Platelet Rich Plasma
PPP	Platelet Poor Plasma
PFP	Platelet-Free Plasma
PS	Phosphatidylserine
PE	Phosphatidylethanolamine
PC	Phosphatidylcholine
PKC	Protein Kinase C
PNH	Paroxysmal Nocturnal Hemoglobinuria
PE	Phycoerythrins
Plt	Platelets
RBCs	Red Blood Cells
RT	Room Temperature
RDW	Red Cell Distribution Width
SM	Sphingomyelin
SSC	Side Scatter
SPSS	Statistical Package For Social Sciences

TM	Thalassemia Major
TI	Thalassemia Intermedia
TEE	Thromboembolic Events
TEM	Transmission Electron Microscope
TBV	Total Blood Volume
TMPs	Total Microparticles
VTE	Venous Thromboembolism
WAS	Wiskott-Aldrich Syndrome
WBCs	White Blood Cells

CHAPTER 1

INTRODUCTION

1.1 Introduction to the Chapter

This research is about the platelets microparticles (PMPs) and their role in hypercoagulability status in Hb E/β-thalassemia in comparison with normal individuals. The current chapter presents the general study background, describes problem statement, research significance, hypothesis, objectives, and research questions. Moreover, the chapter summary represented in the conceptual framework of the study which is provided at the end of this chapter.

1.2 Thalassemias

Thalassemias are groups of heterogenic inherited disorders which occur as a result of, reduced or absence of globin chain synthesis, which is a protein molecule that is responsible for carrying the oxygen in the red blood cells (RBCs). There are two types of thalassemia disorder around the world: alpha (α) thalassemia and beta (β) thalassemia. Epidemiological studies show that α-thalassemia is more dominant in the Far East region while β-thalassaemia is more common in the Mediterranean region (Hoffbrand, V., & Moss, P. A. 2011).

1.3 β-thalassaemia

β-thalassemia has two main classes: β+ thalassemia, in which there is a variable reduction in the synthesis of β globin chain and β0 thalassemia, in which there is an absence of β globin chain production. β-thalassemia causes a variable anemia range from minor symptoms to life-threatening anemia. It can be classified based on the clinical symptoms into two phenotypes: thalassemia major (TM) or Cooley's anemia in which the patient suffer from severe and life-threatening anemia that occurs within months after birth, and thalassemia intermedia (TI) which manifested less clinical severity than TM (Provan, D., & Gribben, J. Eds. 2010).

1.4 Hb E/β-thalassemia

The homozygote of Hb E/β-thalassemia has the phenotype of β thalassemia trait due to abnormal hemoglobin production, and thalassemia, because of the generation of the alternative splicing site by the mutation. The populations in the joint borders of Thailand, Cambodia, and Laos have the highest incidence of Hb E/β-thalassemia. Patients who inherit Hb E and β-thalassemia trait manifest TM or TI (Provan, D., & Gribben, J. Eds. 2010).

1.5 Hb E/β-thalassemia in Malaysian Population

Hb E represents the most common Hb variant in the Southeast Asia with the frequency of 50% in many different areas (Fucharoen, S., & Winichagoon, P. 1997). In Malaysia, the Hb E is quite common in Malays with 5% carrier rate and the Orang Asli of Peninsular Malaysia manifest higher rate of Hb E disorder (Traeger, J., Wood, W. G., J. B., D. J., & Wasi, P. 1980). Hb E/β-thalassemia is an extreme clinical condition that resultss from the interaction of Hb E with β-thalassemia. It is considered a public health problem in the Malaysian population and the most frequent type of thalassemia in Malays (George, E. 2013).

1.6 Hypercoagulable State in Thalassemia Patients

The chronic hypercoagulability state in Hb E/ Beta-thalassemia has been observed in these patients. The disturbance in the circulatory of the thalassemia patients can be manifested by peripheral arterial and venous thrombosis, transient ischemic attacks, and microcirculatory obstruction (Grisaru, D., & Rachmilewitz, E. A. 1992). The presence of PMPs in the circulation has been showed to support the procoagulant activity (Mallat, Z., Benamer, H., Hugel, B., Benessiano, J., Steg, P. G., Freyssinet, J. M., & Tedgui, A. 2000). Importantly, the procoagulant activity was corroborated by clinical research manifesting increased level of MPs in patients with risk of thromboembolic events (TEE) (VanWijk, M. J., VanBavel, E., Sturk, A., & Nieuwland, R. 2003).

1.7 Platelet Microparticles and Hypercoagulability

The exposure of PS on the PMPs surface led to binding of the coagulation factors via Ca^{2+} ions; that enable the formation of prothrombinase and tenase complex. PMPs are enriched in binding sites for activated factor Va, factor VIIIa, and factor IXa and provide the surface for thrombin formation (Sims, P. J., Faioni, E. M., Wiedmer, T., & Shattil, S. J. 1988).

1.8 Problem Statement

Increased in the level of CD41 and CD62P in thalassemic patients has been proven by recent studies in regard to thrombus formation. Studies have shown that CD41 and CD62P are increased in thrombotic patients at a significant rate. CD62P is exclusively expressed by platelet in contrast to other microparticles. CD41 has been studied on its clinical relevance in certain thrombogenic conditions. However, much mysterious about the role of these microparticles still unknown about clot formation. To our best knowledge, CD62P, and CD41 which is derived from platelet have not been studied in E/beta-thalassemic patients in Malaysia. To date, there have been efforts of risk stratification of hypercoagulable state in cancer by using CD62P that is a cell adhesion molecule found in platelet and appears to be playing a key role in response to tissue injury and inflammation and subsequently thrombus formation. However, the platelet microparticles formation will be affected by two main factors, namely serum calcium as the enzymes that responsible for the release of platelet microparticles are calcium-

dependent enzymes and the platelet number that is essential for platelet microparticles formation.

1.9 Research Significance

There were hardly any studies conducted to assess the platelet microparticles CD41 and CD62P in Hb E/β thalassemic patients and normal individuals in Southeast Asia countries, particularly in Malaysia. Very few studies have been conducted in the Middle East countries on thalassemic patients that have shown an increase in their level of platelet microparticles. It is known that thalassemia has a high prevalence in Malaysian population namely E/beta thalassemia 4.5% (George, 2001) and they are at risk to develop hypercoagulability state because they manifest a high incidence of blood clotting. Thus, this research is necessary to prove whether the platelet microparticles CD41 and CD62P are significantly increased in selected groups (cases and controls) of Malaysian patients. Moreover, to establish a new data for platelet microparticles level in thalassemic patients in Malaysia population.

1.10 Research Hypothesis

1.10.1 Alternative Hypothesis

The level of platelet microparticles, namely CD41 and CD62P are significantly increased in Hb E/β-thalassemia patients.

1.10.2 Null Hypothesis

The level of platelet microparticles, namely CD41 and CD62P are not significantly increased in patients with Hb E/β-thalassemia.

1.11 Research Objectives

1.11.1 General Objective

To evaluate the level of platelet microparticles (CD41 and CD62P) in Hb E/β-thalassemia patients and normal individuals.

1.11.2 Specific Objectives

i. To determine the level of platelet microparticles CD41 and CD62P in Hb E/β-thalassemia and normal individuals in the Malaysian population.
ii. To determine the phospholipid level in both, normal and Hb E/β-thalassemic subjects.
iii. To correlate the levels of platelet microparticles CD41 and CD62P, and Annexin-5 with complete blood count parameters.

1.12 Research Question

Is there any association between CD41, CD62P, and A-5 with Hb E/β-thalassemia?

1.13 Conceptual Framework

Figure 1:1. Provides a detailed description of the conceptual framework of the research. Two groups were chosen to be investigated regard to platelet microparticles(CD41 and CD62P) level in this research. The first group is Hb E/β thalassemia patients and the second is normal individuals. Platelet-derived microparticles (CD41 and CD62P) were set as dependent variables. And the other factors that may affect the level of platelet-derived microparticle in these two groups which are demographic factors(age, gender and ethnicity), patients characteristics(splenectomy status, and Iron chelation status), clinical severity (Blood transfusion status) and other factors(Complete blood count (CBC)) were set as independent variables . Importantly, CD41 and CD62P are platelet-derived microparticles that are blebing from activated platelets. Sources provide that patients with thalassemia and thrombosis have thrombotic complication combined with a notable augmentation in the level of platelet microparticles.

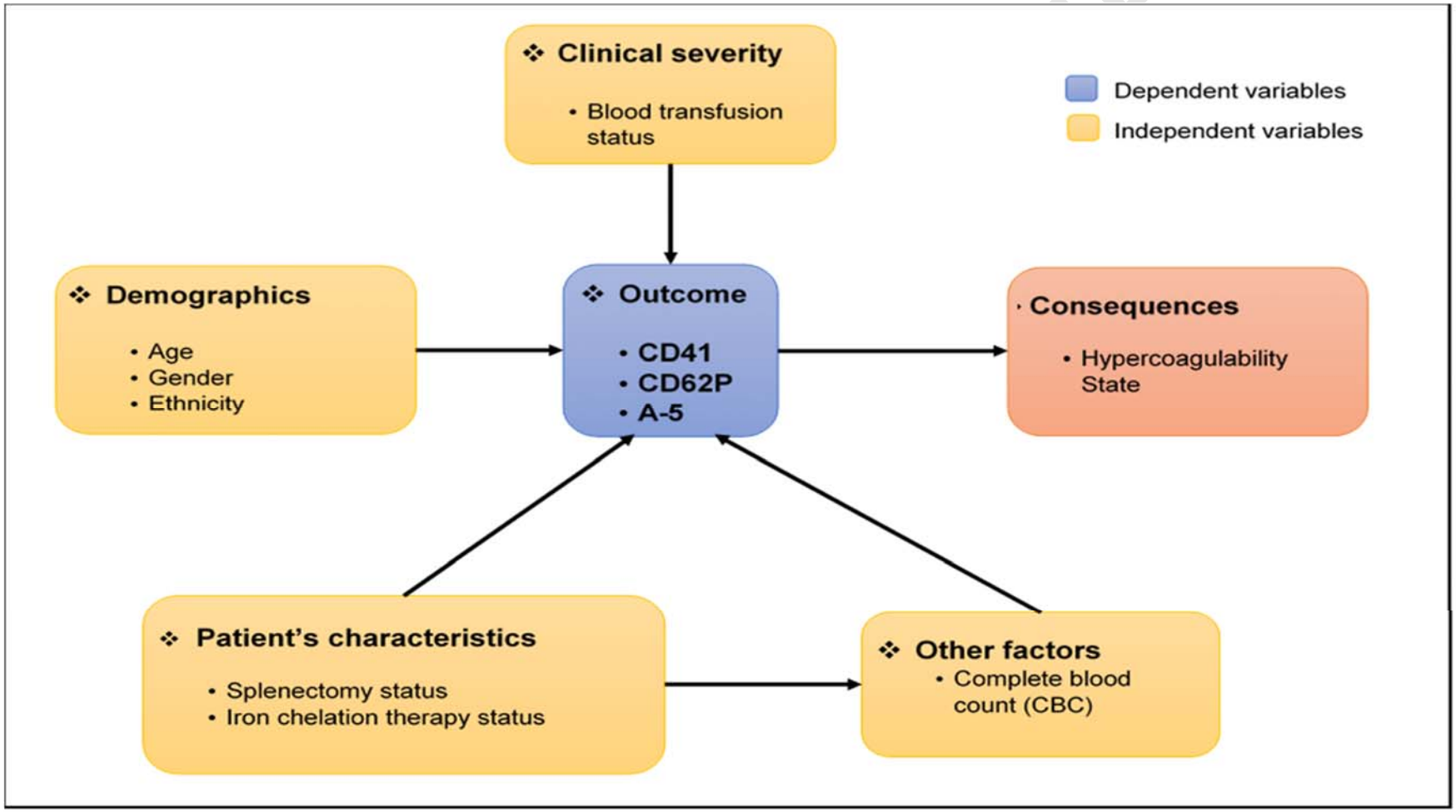

Figure 1.1 : Flow chartt of Conceptual Framework.

REFERENCES

Aday, L. A., & Cornelius, L. J. (2011). *Designing and conducting health surveys: A comprehensive guide* John Wiley & Sons.

A., & Mallat, Z. (2001). Circulating microparticles from patients with myocardial infarction cause endothelial dysfunction. *Circulation, 104*(22), 2649-2652.

Almhanawi, B. H., Khalid, B., Ibrahim, T. A., & Tohit, E. R. M. (2016). A transmission electron microscopy study of anticoagulant-induced platelet vesiculation. Porto Biomedical Journal.

Atichartakarn, V., & Angchaisuksiri, P. (2002). Relationship between hypercoagulable state and erythrocyte phosphatidylserine exposure in splenectomized haemoglobin E/β-thalassaemic patients. *British Journal*.

Augoustaki, O., Bilek, M. (1972). Thalassemia major (homozygous betathalassemia). A survey of 138 cases with emphasis on neurologic and muscular aspects. *Neurology, 22*(3), 294-304.

Ahnadi, C. E., Chapman, E. S., Lpine, M., Okrongly, D., Pujol-Moix, N., Hernndez, A., Grant, A. M. (2003). Assessment of platelet activation in several different anticoagulants by the Advia 120 hematology system, fluorescence flow cytometry, and electron microscopy. *Thrombosis and Haemostasis*, *90*(5), 940–948.

Barry, O. P., Kazanietz, M. G., Pratico, D., & FitzGerald, G. A. (1999). Arachidonic acid in platelet microparticles up-regulates cyclooxygenase-2-dependent prostaglandin formation via a protein kinase C/mitogen-activated protein kinase-dependent pathway. *The Journal of Biological Chemistry, 274*(11), 7545-7556.

Barry, O. P., Pratico, D., Lawson, J. A., & FitzGerald, G. A. (1997). Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. *The Journal of Clinical Investigation, 99*(9), 2118-2127. doi:10.1172/JCI119385 [doi]

Berckmans, R. J., Nieuwland, R., Boing, A., Romijn, F., Hack, C. E., & Sturk, A. (2001). Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. *Thrombosis and Haemostasis-Stuttgart-, 85*(4), 639-646.

Borgna-Pignatti, C., Rugolotto, S., & Stefano, P. De. (2004). Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine.

Berckmans, R. J., Nieuwland, R., Boing, A., Romijn, F., Hack, C. E., & Sturk, A. (2001). Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation. *Thrombosis and Haemostasis-Stuttgart-, 85*(4), 639-646.

Bettache, N., Gaffet, P., Allegre, N., Maurin, L., Toti, F., Freyssinet, J. M., & Bienvenüe, A. (1998). Impaired redistribution of aminophospholipids with distinctive cell shape change during Ca2 -induced activation of platelets from a patient with scott syndrome. *British Journal of Haematology, 101*(1), 50-58.95

Bevers, E. M., Comfurius, P., Dekkers, D. W., & Zwaal, R. F. (1999). Lipid translocation across the plasma membrane of mammalian cells. *Biochimica Et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1439*(3), 317-330.

Bevers, E. M., Comfurius, P., van Rijn, J. L., & Hemker, H. C. (1982). Generation of Prothrombin-Converting activity and the exposure of phosphatidylserine at the outer surface of platelets. *European Journal of Biochemistry, 122*(2), 429- 436.

Bevers, E. M., Comfurius, P., & Zwaal, R. F. (1983). Changes in membrane phospholipid distribution during platelet activation. *Biochimica Et Biophysica Acta (BBA)-Biomembranes, 736*(1), 57-66.

Biro, E., Nieuwland, R., Tak, P. P., Pronk, L. M., Schaap, M. C., Sturk, A., & Hack, C. E. (2007). Activated complement components and complement activator molecules on the surface of cell-derived microparticles in patients with rheumatoid arthritis and healthy individuals. *Annals of the Rheumatic Diseases, 66*(8), 1085-1092.

Bode, A., & Knupp, C. L. (1994). Effect of cold storage on platelet glycoprotein ib and vesiculation. *Transfusion, 34*(8), 690-696.

Bode, A. P., Orton, S. M., Frye, M. J., & Udis, B. J. (1991). Vesiculation of platelets during in vitro aging. *Blood, 77*(4), 887-895.

Borgna-Pignatti, C., Rugolotto, S., & Stefano, P. De. (2004). Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine.

Boilard, E., Nigrovic, P. A., Larabee, K., Watts, G. F., Coblyn, J. S., Weinblatt, M. E., Lee, D. M. (2010). Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science (New York, N.Y.), 327*(5965), 580-583. doi:10.1126/science.1181928 [doi]

Borgna Pignatti, C., Carnelli, V., Caruso, V., Dore, F., De Mattia, D., Di Palma, A., Musumeci, S. (1998). Thromboembolic events in beta thalassemia major: An italian multicenter study. *Acta Haematologica, 99*(2), 76-79. doi:40814 [pii] 96

Boulanger, C. M., Scoazec, A., Ebrahimian, T., Henry, P., Mathieu, E., Tedgui, Brown, M., & Wittwer, C. (2000). Flow cytometry: Principles and clinical applications in hematology. *Clinical Chemistry, 46*(8 Pt 2), 1221-1229.

Cappellini, M. D., Motta, I., Musallam, K. M., & Taher, A. T. (2010). Redefining thalassemia as a hypercoagulable state. *Annals of the New York Academy of Sciences, 1202*(1), 231-236.

Cappellini, M. D., Musallam, K. M., Poggiali, E., & Taher, A. T. (2012). Hypercoagulability in non-transfusion-dependent thalassemia. *Blood Reviews, 26*, S20-S23.

Cappellini, M., Motta, I., & Musallam, K. (2010). Redefining thalassemia as a hypercoagulable state. *New York Academy*

Cappellini, M. D., Musallam, K. M., & Taher, A. T. (2011). Thalassemia as a hypercoagulable state.

Cappellini, M., Robbiolo, L., Bottasso, B., Coppola, R., & Fiorelli, G. (2000). Venous thromboembolism and hypercoagulability in splenectomized patients with thalassaemia intermedia. *British Journal of Haematology, 111*(2), 467-473.

Castaman, G., Yu-Feng, L., Battistin, E., & Rodeghiero, F. (1997). Characterization of a novel bleeding disorder with isolated prolonged bleeding time and deficiency of platelet microvesicle generation. *British Journal of Haematology, 96*(3), 458-463.

CHAN, J. K. C.; NG, C. S.; HUI, P. K. (1988). "A simple guide to the terminology and application of leucocyte monoclonal antibodies".*Histopathology*. 12 (5): 461–480.

Castaman, G., Yu-Feng, L., Battistin, E., & Rodeghiero, F. (1997). Characterization of a novel bleeding disorder with isolated prolonged bleeding time and deficiency of platelet microvesicle generation. *British Journal of Haematology, 96*(3), 458-463. 97

Castaman, G., Yu-Feng, L., & Rodeghiero, F. (1996). A bleeding disorder characterised by isolated deficiency of platelet microvesicle generation. *The Lancet, 347*(9002), 700-701.

Castaman, G., Yu-Feng, L., & Rodeghiero, F. (1996). A bleeding disorder characterised by isolated deficiency of platelet microvesicle generation. *The Lancet, 347*(9002), 700-701.

Celi, A., Pellegrini, G., Lorenzet, R., De Blasi, A., Ready, N., Furie, B. C., & Furie, B. (1994). P-selectin induces the expression of tissue factor on monocytes. *Proceedings of the National Academy of Sciences of the United States of America, 91*(19), 8767-8771.

Chang, C. P., Zhao, J., Wiedmer, T., & Sims, P. J. (1993). Contribution of platelet microparticle formation and granule secretion to the transmembrane migration of phosphatidylserine. *The Journal of Biological Chemistry, 268*(10), 7171-7178.

Chargaff, E., & West, R. (1946). The biological significance of the thromboplastic protein of wood. *Journal of Biological Chemistry, 166*, 189-197.

Chirinos, J. A., Heresi, G. A., Velasquez, H., Jy, W., Jimenez, J. J., Ahn, E., Ahn, Y. S. (2005). Elevation of endothelial microparticles, platelets, and leukocyte activation in patients with venous thromboembolism. *Journal of the American College of Cardiology, 45*(9), 1467-1471.

Connolly, G. C., & Khorana, A. A. (2010). Emerging risk stratification approaches to cancer-associated thrombosis: Risk factors, biomarkers and a risk score. *Thrombosis Research, 125*, S1-S7.

Connor, D. E., Ma, D. D., & Joseph, J. E. (2013). Flow cytometry demonstrates differences in platelet reactivity and microparticle formation in subjects with thrombocytopenia or thrombocytosis due to primary haematological disorders. *Thrombosis Research, 132*(5), 572-577. 98

Connor, J., Pak, C. H., Zwaal, R. F., & Schroit, A. J. (1992). Bidirectional transbilayer movement of phospholipid analogs in human red blood cells. evidence for an ATP-dependent and protein-mediated process. *The Journal of Biological Chemistry, 267*(27), 19412-19417.

Dachary-Prigent, J., Pasquet, J., Fressinaud, E., Toti, F., Freyssinet, J., & Nurden, A. T. (1997). Aminophospholipid exposure, microvesiculation and abnormal protein tyrosine phosphorylation in the platelets of a patient with scott syndrome: A study using physiologic agonists and local anaesthetics. *British Journal of Haematology, 99*(4), 959-967.

Del Conde, I., Shrimpton, C. N., Thiagarajan, P., & Lopez, J. A. (2005). Tissuefactor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. *Blood, 106*(5), 1604-1611. doi:2004-03-1095

Diamant, M., Nieuwland, R., Pablo, R. F., Sturk, A., Smit, J. W., & Radder, J. K. (2002). Elevated numbers of tissue-factor exposing microparticles correlate with components of the metabolic syndrome in uncomplicated type 2 diabetes mellitus. *Circulation, 106*(19), 2442-2447.

Diaz, C., & Schroit, A. (1996). Role of translocases in the generation of phosphatidylserine asymmetry. *Journal of Membrane Biology, 151*(1), 1-9. Eldor, A., & Rachmilewitz, E. A. (2002). The hypercoagulable state in thalassemia. *Blood, 99*(1), 36-43.

Elsayh, K. I., Zahran, A. M., El-Abaseri, T. B., Mohamed, A. O., & El-Metwally, T.H. (2013). Hypoxia biomarkers, oxidative stress, and circulating microparticles in pediatric patients with thalassemia in upper egypt. *Clinical and Applied thrombosis/hemostasis : Official Journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis, 20*(5), 536-545.

Fitzgerald, D. J., Roy, L., Catella, F., & FitzGerald, G. A. (1986). Platelet activation in unstable coronary disease. *New England Journal of Medicine, 315*(16), 983-989.

Flaumenhaft, R., Dilks, J. R., Richardson, J., Alden, E., Patel-Hett, S. R., Battinelli, E., Italiano, J. E.,Jr. (2009). Megakaryocyte-derived microparticles: Direct visualization and distinction from platelet-derived microparticles. *Blood, 113*(5), 1112-1121.

Ferraris, V. A. (2015). Microparticles: the good, the bad, and the ugly. *The Journal of Thoracic and Cardiovascular Surgery*, *149*(1), 312–3.

Forlow, S. B., McEver, R. P., & Nollert, M. U. (2000). Leukocyte-leukocyte interactions mediated by platelet microparticles under flow. *Blood, 95*(4), 1317-1323.

Fox, J. E., Austin, C. D., Boyles, J. K., & Steffen, P. K. (1990). Role of the membrane skeleton in preventing the shedding of procoagulant-rich microvesicles from the platelet plasma membrane. *The Journal of Cell Biology, 111*(2), 483-493.

Fox, J. E., Austin, C. D., Reynolds, C. C., & Steffen, P. K. (1991). Evidence that agonist-induced activation of calpain causes the shedding of procoagulantcontaining microvesicles from the membrane of aggregating platelets. *The Journal of Biological Chemistry, 266*(20), 13289-13295.

Ferraris, V. A. (2015). Microparticles: the good, the bad, and the ugly. *The Journal of Thoracic and Cardiovascular Surgery*, *149*(1), 312–3.

Fucharoen, S., & Winichagoon, P. (1997). Hemoglobinopathies in southeast asia: Molecular biology and clinical medicine. *Hemoglobin, 21*(4), 299-319.

Furie, B., & Furie, B. C. (2004). Role of platelet P-selectin and microparticle PSGL-1 in thrombus formation. *Trends in Molecular Medicine, 10*(4), 171-178.

Gemmell, C. H., Ramirez, S. M., Yeo, E. L., & Sefton, M. V. (1995). Platelet activation in whole blood by artificial surfaces: Identification of plateletderived microparticles and activated platelet binding to leukocytes as 100 material-induced activation events. *The Journal of Laboratory and Clinical Medicine, 125*(2), 276-287.

Gemmell, C. H., Sefton, M. V., & Yeo, E. L. (1993). Platelet-derived microparticle formation involves glycoprotein IIb-IIIa. inhibition by RGDS and a glanzmann's thrombasthenia defect. *The Journal of Biological Chemistry,268*(20), 14586-14589.

George, E. (2013). HbE β-thalassaemia in malaysia: Revisited. *Journal of Hematology & Thromboembolic Diseases, 2013.*

George, E. (2001). Beta-thalassemia major in malaysia, an ongoing public health problem. *The Medical Journal of Malaysia, 56*(4), 397-400.

Heijnen, H. F., Debili, N., Vainchencker, W., Breton-Gorius, J., Geuze, H. J., & Sixma, J. J. (1998). Multivesicular bodies are an intermediate stage in the formation of platelet alpha-granules. *Blood, 91*(7), 2313-2325.

Heijnen, H. F., Schiel, A. E., Fijnheer, R., Geuze, H. J., & Sixma, J. J. (1999). Activated platelets release two types of membrane vesicles: Microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. *Blood, 94*(11), 3791-3799.

Hoffbrand, V., & Moss, P. A. (2011). *Essential haematology* John Wiley & Sons. Holme, P. A., Orvim, U., Hamers, M. J., Solum, N. O., Brosstad, F. R., Barstad,

R. M., & Sakariassen, K. S. (1997). Shear-induced platelet activation and platelet microparticle formation at blood flow conditions as in arteries with a severe stenosis. *Arteriosclerosis, Thrombosis, and Vascular Biology, 17*(4), 646-653.

Holme, P. A., Solum, N. O., Brosstad, F., Egberg, N., & Lindahl, T. L. (1995). Stimulated glanzmann's thrombasthenia platelets produced microvesicles. microvesiculation correlates better to exposure of procoagulant surface than to activation of GPIIb-IIIa. *Thrombosis and Haemostasis, 74*(6), 1533-1540.101

Horstman, L. L., & Ahn, Y. S. (1999). Platelet microparticles: A wide-angle perspective. *Critical Reviews in oncology/hematology, 30*(2), 111-142.

Horstman, L. L., & Ahn, Y. S. (1999). Platelet microparticles: A wide-angle perspective. *Critical Reviews in oncology/hematology, 30*(2), 111-142.

Helley, D., Eldor, A., Girot, R., & Ducrocq, R. (1996). Increased procoagulant activity of red blood cells from patients with homozygous sickle cell disease and beta-thalassemia. *Thrombosis.*

Hugel, B., Socie, G., Vu, T., Toti, F., Gluckman, E., Freyssinet, J. M., & Scrobohaci, M. L. (1999). Elevated levels of circulating procoagulant microparticles in patients with paroxysmal nocturnal hemoglobinuria and aplastic anemia. *Blood, 93*(10), 3451-3456.

Italiano, J. E.,Jr, Mairuhu, A. T., & Flaumenhaft, R. (2010). Clinical relevance of microparticles from platelets and megakaryocytes. *Current Opinion in Hematology, 17*(6), 578-584. doi:10.1097/MOH.0b013e32833e77ee [doi]

Iwamoto, S., Kawasaki, T., Kambayashi, J., Ariyoshi, H., & Monden, M. (1996). Platelet microparticles: A carrier of platelet-activating factor? *Biochemical and Biophysical Research Communications, 218*(3), 940-944.

Joop, K., Berckmans, R., Nieuwland, R., Berkhout, J., Romijn, F., Hack, C. E., & Sturk, A. (2001). Microparticles from patients with multiple organ dysfunction syndrome and sepsis support coagulation through multiple mechanisms.*Thrombosis and Haemostasis-Stuttgart-, 85*(5), 810-820.

Jy, W., Horstman, L. L., Arce, M., & Ahn, Y. S. (1992). Clinical significance of platelet microparticles in autoimmune thrombocytopenias. *J Lab Clin Med,119*(4), 334-345. 102

Kelton, J. G., Moore, J. C., Warkentin, T. E., & Hayward, C. P. (1996). Isolation and characterization of cysteine proteinase in thrombotic thrombocytopenic purpura. *British Journal of Haematology, 93*(2), 421-426.

Kim, H. K., Song, K. S., Chung, J., Lee, K. R., & Lee, S. (2004). Platelet microparticles induce angiogenesis in vitro. *British Journal of Haematology,124*(3), 376-384.

Lee, K., Hong, K., & Papahadjopoulos, D. (1992). Recognition of liposomes by cells: In vitro binding and endocytosis mediated by specific lipid headgroups and surface charge density. *Biochimica Et Biophysica Acta (BBA)-Biomembranes, 1103*(2), 185-197.

Lee, Y. J., Jy, W., Horstman, L. L., Janania, J., Reyes, Y., Kelley, R. E., & Ahn, Y. S. (1993). Elevated platelet microparticles in transient ischemic attacks, lacunar infarcts, and multiinfarct dementias. *Thrombosis Research, 72*(4),295-304.

Lacroix, R., Judicone, C., Poncelet, P., Robert, S., Arnaud, L., Sampol, J., & Dignat-George, F. (2012). Impact of pre-analytical parameters on the measurement of circulating microparticles: Towards standardization of protocol. *Journal of Thrombosis and Haemostasis*, *10*(3), 437–446.

Leventis, P., & Grinstein, S. (2010). The distribution and function of phosphatidylserine in cellular membranes. *Annual Review of Biophysics*.

Lemke, G., & Burstyn-Cohen, T. (2010). TAM receptors and the clearance of apoptotic cells. *Annals of the New York Academy of Sciences, 1209*(1), 23-29.

Logothetis, J., Constantoulakis, M., Economidou, J., Stefanis, C., Hakas, P., Mallat, Z., Benamer, H., Hugel, B., Benessiano, J., Steg, P. G., Freyssinet, J. M., & Tedgui, A. (2000). Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation, 101*(8), 841-843.

Manfre, L., Giarratano, E., Maggio, A., Banco, A., Vaccaro, G., & Lagalla, R. (1999). MR imaging of the brain: Findings in asymptomatic patients with thalassemia intermedia and sickle cell-thalassemia disease. *AJR.American* 103 *Journal of Roentgenology, 173*(6), 1477-1480.

Mause, S. F., Ritzel, E., Liehn, E. A., Hristov, M., Bidzhekov, K., Muller-Newen, G., Weber, C. (2010). Platelet microparticles enhance the vasoregenerative potential of angiogenic early outgrowth cells after vascular injury. *Circulation, 122*(5), 495-506.

Merten, M., Pakala, R., Thiagarajan, P., & Benedict, C. R. (1999). Platelet microparticles promote platelet interaction with subendothelial matrix in a glycoprotein IIb/IIIa-dependent mechanism. *Circulation, 99*(19), 2577-2582.

Michaeli, J., Mittelman, M., Grisaru, D., & Rachmilewitz, E. (1992). Thromboembolic complications in beta thalassemia major. *Acta Haematologica, 87*(1-2), 71-74.

Mannucci, P. (2010). Red cells playing as activated platelets in thalassemia intermedia. *Journal of Thrombosis and Haemostasis*.

Morel, O., Toti, F., Hugel, B., Bakouboula, B., Camoin-Jau, L., Dignat-George, F., & Freyssinet, J. M. (2006). Procoagulant microparticles: Disrupting the vascular homeostasis equation? *Arteriosclerosis, Thrombosis, and Vascular Biology*, *26*(12), 2594–2604.

Miyazaki, Y., Nomura, S., Miyake, T., Kagawa, H., Kitada, C., Taniguchi, H., Fukuhara, S. (1996). High shear stress can initiate both platelet aggregation and shedding of procoagulant containing microparticles. *Blood, 88*(9), 3456-3464.

Mannucci, P. (2010). Red cells playing as activated platelets in thalassemia intermedia. *Journal of Thrombosis and Haemostasis*.

Morel, O., Toti, F., Hugel, B., Bakouboula, B., Camoin-Jau, L., Dignat-George, F., & Freyssinet, J. M. (2006). Procoagulant microparticles: Disrupting the vascular homeostasis equation? *Arteriosclerosis, Thrombosis, and Vascular Biology*, *26*(12), 2594–2604.

Mobarrez, F., Antovic, J., Egberg, N., Hansson, M., Jörneskog, G., Hultenby, K., & Wallén, H. (2010). A multicolor flow cytometric assay for measurement of platelet-derived microparticles. *Thrombosis Research, 125*(3), e110-e116.

Morel, O., Jesel, L., Chauvin, M., Freyssinet, J., & Toti, F. (2003). Eptifibatide-induced thrombocytopenia and circulating procoagulant platelet-derived microparticles in a patient with acute coronary syndrome. *Journal of Thrombosis and Haemostasis, 1*(12), 2685-2687.

Nagalla, S., Shaw, C., Kong, X., Kondkar, A. A., Edelstein, L. C., Ma, L., Bray, P. F. (2011). Platelet microRNA-mRNA coexpression profiles correlate with platelet reactivity. *Blood, 117*(19), 5189-5197. doi:10.1182/blood-2010-09-299719.

Nieuwland, R., & Sturk, A. (2002). Platelet-derived microparticles. *Platelets,* , 255-265.

Nielsen, M. H., Beck-Nielsen, H., Andersen, M. N., & Handberg, A. (2014). A flow cytometric method for characterization of circulating cell-derived microparticles in plasma. *Journal of Extracellular Vesicles*, *3*, 1–12.

Nieuwland, R., Berckmans, R. J., McGregor, S., Boing, A. N., Romijn, F. P., Westendorp, R. G., Sturk, A. (2000). Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood, 95*(3), 930-935.

Nomura, S., Komiyama, Y., Miyake, T., Miyazaki, Y., Kido, H., Suzuki, M., . . . Fukuhara, S. (1994). Amyloid beta-protein precursor-rich platelet microparticles in thrombotic disease. *Thrombosis and Haemostasis, 72*(4), 519-522.

Owens, A. P.,3rd, & Mackman, N. (2011). Microparticles in hemostasis and thrombosis. *Circulation Research, 108*(10), 1284-1297.

Pasquet, J., Toti, F., Nurden, A. T., & Dachary-Prigent, J. (1996). Procoagulant activity and active calpain in platelet-derived microparticles. *Thrombosis Research, 82*(6), 509-522.

Polack, B., Schved, J. F., & Boneu, B. (2001). Preanalytical recommendations of the "Groupe d"Etude sur l'Hémostase et la Thrombose' (GEHT) for venous blood testing in hemostasis laboratories. *Haemostasis*, *31*(1), 61–8.

Pattanapanyasat, K., Gonwong, S., Chaichompoo, P., Noulsri, E., Lerdwana, S., Sukapirom, K.,Fucharoen, S.(2007).Activated platelet-derived microparticles in thalassaemia. *British Journal of Haematology, 136*(3), 462-471.

Polack, B., Schved, J. F., & Boneu, B. (2001). Preanalytical recommendations of the "Groupe d"Etude sur l'Hémostase et la Thrombose' (GEHT) for venous blood testing in hemostasis laboratories. *Haemostasis*, *31*(1), 61–8.

Piccin, A., Murphy, W. G., & Smith, O. P. (2007). Circulating microparticles: Pathophysiology and clinical implications. *Blood Reviews, 21*(3), 157-171.

Provan, D., & Gribben, J. (2010). *Molecular hematology* John Wiley & Sons.

Ramacciotti, E., Hawley, A. E., Farris, D. M., Ballard, N. E., Wrobleski, S. K., Myers, D. D.,Jr, Wakefield, T. W. (2009). Leukocyte- and platelet-derived microparticles correlate with thrombus weight and tissue factor activity in an experimental mouse model of venous thrombosis. *Thrombosis and Haemostasis,*

RODMAN, N. F.,Jr, PAINTER, J. C., & McDEVITT, N. B. (1963). Platelet disintegration during clotting. *The Journal of Cell Biology, 16*, 225-241.

Rosing, J., Speijer, H., & Zwaal, R. F. (1988). Prothrombin activation on phospholipid membranes with positive electrostatic potential. *Biochemistry,27*(1), 8-11.

Ruf, A., Pick, M., Deutsch, V., Patscheke, H., Goldfarb, A., Rachmilewitz, E. A., & Eldor, A. (1997). In-vivo platelet activation correlates with red cell anionic phospholipid exposure in patients with β-thalassaemia major. British journal of haematology, 98(1), 51-56.

Schroit, A. J., & Zwaal, R. F. (1991). Transbilayer movement of phospholipids in red cell and platelet membranes. *Biochimica Et Biophysica Acta (BBA)-Reviews on Biomembranes, 1071*(3), 313-329.

Shcherbina, A., Rosen, F. S., & Remold-O'Donnell, E. (1999). Pathological events in platelets of Wiskott-Aldrich syndrome patients. *British Journal of Haematology, 106*(4), 875-883.

Siljander, P. R. (2011). Platelet-derived microparticles–an updated perspective. *Thrombosis Research, 127*, S30-S33.

Siljander, P., Carpen, O., & Lassila, R. (1996). Platelet-derived microparticles associate with fibrin during thrombosis. *Blood, 87*(11), 4651-4663.

Sims, P. J., Faioni, E. M., Wiedmer, T., & Shattil, S. J. (1988). Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor va and express prothrombinase activity. *The Journal of Biological Chemistry, 263*(34), 18205-18212.

Sims, P. J., Wiedmer, T., Esmon, C. T., Weiss, H. J., & Shattil, S. J. (1989). Assembly of the platelet prothrombinase complex is linked to vesiculation of the platelet plasma membrane. studies in scott syndrome: An isolated defect in platelet procoagulant activity. *The Journal of Biological Chemistry, 264*(29), 17049-17057.

Singer, S. T., Kuypers, F. A., Styles, L., Vichinsky, E. P., Foote, D., & Rosenfeld, H. (2006). Pulmonary hypertension in thalassemia: Association with platelet activation and hypercoagulable state. *American Journal of Hematology, 81*(9), 670-675.

Sitar, G., Balduini, C. L., Manenti, L., Castello, A., Balanzin, D., Ascari, E., & Matteo, I. P. S. (1999). Pulmonary thromboembolism in thalassemia intermedia patients. *Haematologica, 84*, 10.

Sonakul, D., Pacharee, P., Laohapand, T., Fucharoen, S., & Wasi, P. (1980). Pulmonary artery obstruction in thalassaemia. *The Southeast Asian Journal of Tropical Medicine and Public Health, 11*(4), 516-523.

Sumiyoshi, A., Thakerngpol, K., & Sonakul, D. (1992). Pulmonary microthromboemboli in thalassemic cases. *The Southeast Asian Journal of Tropical Medicine and Public Health, 23 Suppl 2*, 29-31.

Taher, A. T., Otrock, Z. K., Uthman, I., & Cappellini, M. D. (2008). Thalassemia and hypercoagulability. *Blood Reviews, 22*(5), 283-292.

Taher, A., Isma'eel, H., Mehio, G., Bignamini, D., Kattamis, A., Rachmilewitz, E. A., & Cappellini, M. D. (2006). Prevalence of thromboembolic events among 8,860 patients with thalassaemia major and intermedia in the Mediterranean area and iran. *Thrombosis and Haemostasis, 96*(4), 488-491.

Tans, G., Rosing, J., Thomassen, M. C., Heeb, M. J., Zwaal, R. F., & Griffin, J. H. (1991). Comparison of anticoagulant and procoagulant activities of stimulated platelets and platelet-derived microparticles. *Blood, 77*(12), 2641-2648.

Tantawy, A. A., Adly, A. A., Ismail, E. A., & Habeeb, N. M. (2013). Flow cytometric assessment of circulating platelet and erythrocytes microparticles in young thalassemia major patients: Relation to pulmonary hypertension and aortic wall stiffness. *European Journal of Haematology, 90*(6), 508-518.

Taube, J., McWilliam, N., Luddington, R., Byrne, C. D., & Baglin, T. (1999). Activated protein C resistance: Effect of platelet activation, platelet-derived microparticles, and atherogenic lipoproteins. *Blood, 93*(11), 3792-3797.

Tehrani, S., Mobarrez, F., Antovic, A., Santesson, P., Lins, P., Adamson, U., Jörneskog, G. (2010). Atorvastatin has antithrombotic effects in patients with type 1 diabetes and dyslipidemia. *Thrombosis Research, 126*(3), e225-e231.

Théorêt, J., Yacoub, D., Hachem, A., Gillis, M., & Merhi, Y. (2011). P-selectin ligation induces platelet activation and enhances microaggregate and thrombus formation. *Thrombosis Research, 128*(3), 243-250.

Toti, F., Satta, N., Fressinaud, E., Meyer, D., & Freyssinet, J. M. (1996). Scott syndrome, characterized by impaired transmembrane migration of procoagulant phosphatidylserine and hemorrhagic complications, is an inherited disorder. *Blood, 87*(4), 1409-1415.

Traeger, J., Wood, W., Clegg, J., Weatherall, D., & Wasi, P. (1980). Defective synthesis of HbE is due to reduced levels of βE mRNA.

van der Zee, P. M., Biro, E., Ko, Y., de Winter, R. J., Hack, C. E., Sturk, A., & Nieuwland, R. (2006). P-selectin- and CD63-exposing platelet microparticles reflect platelet activation in peripheral arterial disease and myocardial infarction. *Clinical Chemistry, 52*(4), 657-664.

van Dieijen, G., Tans, G., Rosing, J., & Hemker, H. C. (1981). The role of phospholipid and factor VIIIa in the activation of bovine factor X. *The Journal of Biological Chemistry, 256*(7), 3433-3442.

van Teunenbroek, A., Wijburg, F. A., ten Cate, J. W., van den Berg, W., & Weening, R. S. (1989). Thromboembolic complications in an asplenic HbEbeta-thalassaemia patient. *The Netherlands Journal of Medicine, 35*(3-4),123-127.

VanWijk, M. J., Svedas, E., Boer, K., Nieuwland, R., VanBavel, E., & Kublickiene, K. R. (2002). Isolated microparticles, but not whole plasma, from women with preeclampsia impair endothelium-dependent relaxation in isolated myometrial arteries from healthy pregnant women. *American Journal of Obstetrics and Gynecology, 187*(6), 1686-1693.

VanWijk, M. J., VanBavel, E., Sturk, A., & Nieuwland, R. (2003). Microparticles in cardiovascular diseases. *Cardiovascular Research, 59*(2), 277-287.

Warkentin, T. E. (1996). Heparin-induced thrombocytopenia: IgG-mediated platelet activation, platelet microparticle generation, and altered procoagulant/anticoagulant balance in the pathogenesis of thrombosis and venous limb gangrene complicating heparin-induced thrombocytopenia.*Transfusion Medicine Reviews, 10*(4), 249-258.

Warkentin, T. E., Hayward, C. P., Boshkov, L. K., Santos, A. V., Sheppard, J. A., Bode, A. P., & Kelton, J. G. (1994). Sera from patients with heparin-induced thrombocytopenia generate platelet-derived microparticles with procoagulant activity: An explanation for the thrombotic complications of heparin-induced thrombocytopenia. *Blood, 84*(11), 3691-3699.

Weber, A., Köppen, H. O., & Schrör, K. (2000). Platelet-derived microparticles stimulate coronary artery smooth muscle cell mitogenesis by a PDGFindependent mechanism. *Thrombosis Research, 98*(5), 461-466.

Weiss, H. J., Vicic, W. J., Lages, B. A., & Rogers, J. (1979). Isolated deficiency of platelet procoagulant activity. *The American Journal of Medicine, 67*(2),206-213.

Wiedmer, T., Shattil, S. J., Cunningham, M., & Sims, P. J. (1990). Role of calcium and calpain in complement-induced vesiculation of the platelet plasma membrane and in the exposure of the platelet factor va receptor. *Biochemistry, 29*(3), 623-632.109.

Winichagoon, P., Fucharoen, S., & Wasi, P. (1981). Increased circulating platelet aggregates in thalassaemia. *The Southeast Asian Journal of Tropical Medicine and Public Health, 12*(4), 556-560.

Wolf, P. (1967). The nature and significance of platelet products in human plasma. *British Journal of Haematology, 13*(3), 269-288.

Woywodt, A., Blann, A. D., Kirsch, T., Erdbruegger, U., Banzet, N., Haubitz, M., & Dignat-George, F. (2006). Isolation and enumeration of circulating endothelial cells by immunomagnetic isolation: Proposal of a definition and a consensus protocol. *Journal of Thrombosis and Haemostasis*, *4*(3), 671–677.

Wolf, B. B., Goldstein, J. C., Stennicke, H. R., Beere, H., Amarante-Mendes, G. P., Salvesen, G. S., & Green, D. R. (1999). Calpain functions in a caspaseindependent manner to promote apoptosis-like events during platelet activation. *Blood, 94*(5), 1683-1692.

Yashar, V., Barenholz, Y., & Hy-Am, E. (1993). Phosphatidylserine in the outer leaflet of red blood cells from β-thalassemia patients may explain the chronic hypercoagulable state and thrombotic episodes. *American Journal*.

Zwaal, R. F., Comfurius, P., & Bevers, E. M. (1992). Platelet procoagulant activity and microvesicle formation. its putative role in hemostasis and thrombosis. *Biochimica Et Biophysica Acta (BBA)-Molecular Basis of Disease, 1180*(1), 1-8.

Zwaal, R. F., Comfurius, P., & Bevers, E. M. (2004). Scott syndrome, a bleeding disorder caused by defective scrambling of membrane phospholipids. *Biochimica Et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1636*(2), 119-128.

Zwaal, R. F., & Bevers, E. M. (1983). Platelet phospholipid asymmetry and its significance in hemostasis. *Sub-Cellular Biochemistry, 9*, 299-334.

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION: _____

TITLE OF THESIS / PROJECT REPORT:

FLOWCYTOMETRIC ASSESSMENT OF PLATELET MICROPARTICLES CD41 AND CD62P IN E/BETA-THALASSEMIA PATIENTS IN PUBLIC HOSPITALS IN SELANGOR, MALAYSIA

NAME OF STUDENT : BAHAA HADI JABER ALMHANAWI

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as :

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
☐	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for :

☐	**PATENT**	Embargo from _____ (date) until _____ (date)

Approved by:

_____
(Signature of Student)
New IC No/ Passport No.:

Date :

_____
(Signature of Chairman of Supervisory Committee)
Name:

Date :

[Note : If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]