HAEMOSTATIC, INFLAMMATORY AND HAEMATOLOGICAL BIOMARKERS AMONG ORTHOPAEDIC PATIENTS WITH PROLONGED IMMOBILISATION AND HYPERCOAGULABLE RISK

NOOR NABILA BINTI RAMLI

UNIVERSITI SAINS MALAYSIA

2024

HAEMOSTATIC, INFLAMMATORY AND HAEMATOLOGICAL BIOMARKERS AMONG ORTHOPAEDIC PATIENTS WITH PROLONGED IMMOBILISATION AND HYPERCOAGULABLE RISK

by

NOOR NABILA BINTI RAMLI

Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

February 2024

ACKNOWLEDGEMENT

In the name of Allah, the Most Merciful and the Most Beneficent

Alhamdulillah, all praises to Allah for the strengths and His blessing in completing this thesis. I would like to take this opportunity to express my deepest gratitude to all those who have supported and contributed to the completion of this thesis. First and foremost, I am immensely grateful to my supervisor, Dr. Salfarina Iberahim, for her guidance, expertise, and continuous support throughout the research process. Her insightful feedback and valuable suggestions have greatly shaped this thesis and enriched my understanding of the subject matter. Not forgotten, my appreciation to my co-supervisors, Assc. Prof. Dr. Noor Haslina Mohd Noor, Prof. Dr. Tengku Muzaffar Tengku Mohamad Shihabudin and Dr. Ahmad Hadif Zaidin Samsudin, for their support and knowledge regarding this topic. I extend my sincere appreciation to all the lecturers and staff from both the haematology department and orthopaedic department of Hospital Universiti Sains Malaysia for their kindness and support. I would like to express my heartfelt gratitude to my family for their unwavering support and encouragement throughout this journey. Their love, patience, and understanding have been my constant motivation, and I am deeply grateful for their presence in my life. To everyone who has contributed in one way or another, no matter how big or small, I offer my sincerest thanks. Your support and encouragement have made this thesis possible, and I am truly grateful for your involvement.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

<	Less than
>	More than
≥	More than or equal to
±	Plus minus
α	Alpha
β	Beta
δ	Delta
°C	Celcius
µ	Micro
%	Percentage
g/L	Gram per liter
kDa	Kilodaltons
kg/m^2	Kilogram per square meter
mg/ml	Milligrams per milliliter
mg/L	Milligrams per liter
mm/hr	Millimeters per hour
mmHg	Millimeters of mercury
nm	Nanometers
ug/mL	Microgram per milliliter
s	Seconds

LIST OF ABBREVIATIONS

AIS	Abbreviated injury scale
APC	Activated protein C
aPTT	Activated partial thromboplastin time
AUC	Area under curve
BMI	Body mass index
BP	Blood pressure
C4BP	C4b Binding Protein
CI	Confidence interval
CLSI	Clinical and Laboratory Standard Institute
CRP	C-reactive protein
CTPA	Computed Tomography Pulmonary Angiography
DIC	Disseminated intravascular coagulation
DM	Diabetes mellitus
DVT	Deep vein thrombosis
EDTA	Ethylene diamine tetra acetic acid
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
hs-CRP	High-sensitivity C-reactive protein
HR	Hazard ratio
IHD	Ischemic heart disease
IL-1	Interleukin-1
IL-6	Interleukin-6
ISS	Injury Severity Score
OCP	Oral contraceptive pill
OR	Odds ratio
PE	Pulmonary embolism
PLT	Platelet
PMH	Past medical history
PPP	Platelet poor plasma
PT	Prothrombin time
RBC	Red blood cell

RR	Risk ratio
SD	Standard deviation
SOP	Standard operating procedure
SPSS	Statistical Package for the Social Sciences
TEG	Thromboelastography
TGF-β	Transforming growth factor-β
THA	Total hip arthroplasty
TKA	Total knee arthroplasty
TNF-α	Tumor necrosis factor-α
V/Q	Ventilation-perfusion scan
VTE	Venous thromboembolism
WHR	Waist-to-hip ratio
USM	Universiti Sains Malaysia

LIST OF APPENDICES

BIOPENANDA HEMOSTATIS, KERADANGAN DAN HEMATOLOGI DALAM KALANGAN PESAKIT ORTOPEDIK DENGAN IMOBILISASI BERPANJANGAN DAN RISIKO HIPERKUAGULABEL

ABSTRAK

Pembekuan salur darah vena merupakan kebimbangan kesihatan awam yang utama kerana insiden morbiditi dan kematian yang tinggi. Pesakit yang mengalami trauma anggota bawah dengan imobilisasi yang berpanjangan akan menyebabkan keadaan hiperkuagulabel yang akhirnya menyumbang kepada perkembangan pembekuan salur darah vena. Oleh itu, kajian ini bertujuan untuk menilai perubahan dalam penanda hiperkuagulabel (hemostatis, keradangan dan hematologi) dan faktor risiko klinikal (jantina, umur, jenis kecederaan, BMI dan status merokok) dalam pesakit trauma ortopedik tidak bergerak yang berpanjangan dan risiko hiperkuagulabel. Ini adalah kajian kohort prospektif pesakit trauma ortopedik imobilisasi berpanjangan yang dimasukkan ke wad ortopedik di Hospital Universiti Sains Malaysia dari Ogos 2020 hingga Mac 2022. Sejumlah 54 pesakit yang mengalami patah tulang anggota bawah, berumur antara 11 hingga 50 tahun, yang memerlukan imobilisasi selama lebih daripada 5 hari tanpa menerima ubat anti pembekuan darah terlibat dalam kajian ini. Ujian makmal termasuk D-dimer, fibrinogen, PT, aPTT, protein S, protein C, antitrombin, CRP, ESR dan kiraan platelet diukur secara bersiri pada hari 1 dan hari ke-5 imobilisasi. Sementara itu, faktor risiko klinikal yang merangkumi jantina, umur, jenis kecederaan, BMI dan status merokok telah direkodkan semasa kemasukan. Analisis statistik menunjukkan bahawa beberapa biopenanda memberikan perbezaan min yang ketara antara hari 1 dan hari ke-5 imobilisasi, yang termasuk fibrinogen, protein C, antitrombin, CRP,

ESR dan kiraan platelet. Peningkatan perbezaan min bagi setiap biopenanda dengan fibrinogen meningkat sebanyak 0.66g/L (p<0.001), protein C meningkat sebanyak 14.07% (p<0.001), antitrombin meningkat sebanyak 11.67% (p<0.001), ESR meningkat sebanyak 17.98mm/hr (p<0.001), dan kiraan platelet telah meningkat sebanyak 128.59×10^{9}/L (p<0.001) pada hari ke-5 imobilisasi. Sebaliknya, perbezaan min untuk CRP telah menurun sebanyak 19.53mg/L (p=0.022) pada hari ke-5 imobilisasi. Terdapat korelasi positif sederhana yang signifikan di antara parameter CRP dengan D-dimer (korelasi pearson, r =0.45, nilai p=0.002), fibrinogen (r =0.53, nilai p<0.001) dan PT (r =0.42, nilai p=0.005). Sebaliknya, parameter ESR menunjukkan korelasi positif sederhana dengan D-dimer (r =0.40, nilai p=0.003), korelasi positif yang kuat dengan fibrinogen (r =0.75, nilai p<0.001) dan korelasi positif yang sederhana dengan aPTT (r =0.38, nilai p=0.005). Antara parameter tidak normal (fibrinogen, protein C, antitrombin, CRP, ESR dan kiraan platelet) yang diperhatikan dalam kajian ini, hanya protein C yang memberikan perkaitan yang signifikan dengan umur (31-40) dan status merokok. Kesimpulannya, kajian ini menemui beberapa biopenanda (fibrinogen, ESR, dan kiraan platelet) yang menunjukkan perubahan ketara selepas hari ke-5 imobilisasi. Walaupun tiada kejadian pembekuan salur darah vena yang didokumenkan dalam kajian ini, kajian terdahulu telah menunjukkan bahawa biopenanda ini adalah parameter protrombik yang menunjukkan tindak balas badan terhadap kecederaan tisu berikutan trauma. Oleh itu, biopenanda ini mungkin berguna dalam menilai risiko pembekuan salur darah vena berkaitan dengan keadaan hiperkuagulabel dan boleh menyokong petunjuk profilaksis terhadap pembekuan salur darah vena dalam pesakit berisiko tinggi sekiranya berlaku imobilisasi trauma.

HAEMOSTATIC, INFLAMMATORY AND HAEMATOLOGICAL BIOMARKERS AMONG ORTHOPAEDIC PATIENTS WITH PROLONGED IMMOBILISATION AND HYPERCOAGULABLE RISK

ABSTRACT

Venous thromboembolism is a major public health concern due to its high incidence of morbidity and mortality. Patients who experienced lower limb/s trauma with prolonged immobilisation will induce the hypercoagulable state that finally contributes to the VTE development. Therefore, this study aims to evaluate the changes in hypercoagulable markers (haemostatic, inflammatory and haematological) and clinical risk factors (gender, age, type of injury, BMI and smoking status) in prolonged immobilised orthopaedic trauma patients and hypercoagulable risk. This is a prospective cohort study of prolonged immobilised orthopaedic trauma patients admitted to the orthopaedic ward at Hospital Universiti Sains Malaysia from August 2020 to March 2022. A total of 54 patients with lower limb/s fractures, ages ranging from 11 to 50 years old, who required immobilisation for more than 5 days without receiving anticoagulant prophylaxis were involved in this study. The laboratory tests included D-dimer, fibrinogen, PT, aPTT, free protein S, protein C, antithrombin, CRP, ESR and platelet count were serially measured on day 1 and day 5 of immobilisation. Meanwhile, clinical risk factor that include gender, age, type of injury, BMI and smoking status were recorded during the admission. The paired t-test analysis demonstrated that several biomarkers gave a significant mean difference between day 1 and day 5 of immobilisation, which included fibrinogen, protein C, antithrombin, CRP, ESR and platelet count. The mean differences increased for each biomarker with fibrinogen was increased by

0.66g/L (p<0.001), protein C was increased by 14.07% (p<0.001), antithrombin was increased by 11.67% (p<0.001), ESR was increased by 17.98mm/hr (p<0.001), and platelet count was increased by 128.59×10^9/L (p<0.001) on day 5 of immobilisation. On the contrary, the mean difference for CRP decreased by 19.53mg/L (p=0.022) on day 5 of immobilisation. There was a significant moderate positive correlation between the CRP parameter with D-dimer (Pearson correlation, r =0.45, p=0.002), fibrinogen (r =0.53, p<0.001) and PT (r =0.42, p=0.005). On the other hand, ESR parameters showed a moderate positive correlation with D-dimer (r =0.40, p=0.003), strong positive correlation with fibrinogen (r =0.75, p<0.001) and moderate positive correlation with aPTT (r =0.38, p=0.005). Among the abnormal parameters (fibrinogen, protein C, antithrombin, CRP, ESR and platelet count) observed in this study, only protein C showed a significant association with age (31-40) and smoking status. In conclusion, this study found several biomarkers (fibrinogen, ESR, and platelet count) that showed significant changes after day 5 of immobilisation. Even though there was no VTE incident documented in this study, previous studies have shown that these biomarkers are prothrombic parameters that show the body's response towards tissue injury following trauma. Thus, these biomarkers are probably useful in assessing the risk of VTE related to hypercoagulable state and could support prophylaxis indications against VTE in a high-risk patient in the case of trauma immobilisation.

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Venous thromboembolism (VTE) is a medical condition in which a blood clot develops in a vein. It is the third-most prevalent vascular diagnosis after stroke and heart attack, affecting 300,000 to 600,000 Americans each year (Beckman et al., 2010). This disease comprises two interrelated conditions that form a continuous spectrum, which are deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT is worsened in 20% to 60% of patients by post-thrombotic syndrome, which is characterised by chronic swelling, discomfort, and oedema in the afflicted limb (Ashrani & Heit, 2009). Meanwhile, individuals diagnosed with PE are subjected to an elevated risk of long-term complications, such as right ventricular dysfunction and severe pulmonary hypertension (Grewal et al., 2022). DVT and PE both have a negative impact on quality of life (Hogg et al., 2013).

VTE incidents are common and can lead to life-threatening complications in trauma patients. Toker et al. reported an incidence rate of VTE ranging from 5% to 63% in trauma patients without receiving prophylaxis (Toker et al., 2011). The likelihood of developing VTE is in direct proportion to the number of predisposing factors present in the patient. In the context of orthopaedic trauma, several risk factors have been identified for VTE, including advanced age, previous VTE history, heart failure, comorbidities, obesity, polytrauma, longer operation times, and prolonged immobilisation (Tan et al., 2016). As a result, the reported occurrence rates of DVT and PE varies across different studies.

Apart from trauma, temporary immobilisation of the lower limb also contributes to the risk of VTE. According to the Cochrane review published by Zee et al., the incidence rate of VTE ranged from 4% to 40% in patients with lower limb immobilisation (Zee et al., 2017). The use of temporary lower limb immobilisation, such as fiberglass, plaster, or air casts, for treating patients with ankle fractures is reported to predispose the afflicted leg to DVT due to inactivity of the ankle pump mechanism and immobilisation (Rasi et al., 2013). Moreover, in a case-control study involving patients with lower limb immobilisation, researchers found a strong connection between the duration of immobilisation and VTE development. The number of patients with VTE was nearly twice as high during the second week of immobilisation compared with the first week. They came to the conclusion that it was connected to the disease's natural course since blood clots take time to form (Van Adrichem et al., 2014).

The combination of orthopaedic trauma and prolonged immobilisation increases the risk of VTE. A study found that individuals who were immobilised as a result of trauma exhibited a greater prevalence of VTE compared with those who were immobilised without trauma (Van Adrichem et al., 2014). The use of pharmacological thromboprophylaxis could minimise the future incidence of VTE in these patients. However, given the lack of knowledge regarding actual VTE risk and scarce evidence on risk stratification, there is a lot of diversity in the global practices of thromboprophylaxis (Grewal et al., 2022).

DVT is often diagnosed during emergency room visits, and various studies have been undertaken to analyse its risk factors. Due to the significant morbidity and mortality risks associated with VTE, prevention, diagnosis, and management of this disease in orthopaedic trauma patients are crucial. Despite the fact that there is a

huge amount of information on VTE in the overall trauma population, there is limited reliable information regarding VTE prophylaxis and treatment in the orthopaedic trauma group. Furthermore, the research lacks sufficient data to support firm recommendations regarding the type and duration of prophylaxis that should be given to these patients (Scolaro et al., 2015). In addition, the relationship between the clinical risk factor for thrombosis and sequential changes in coagulation markers for VTE development following trauma have also been poorly established (Selby et al., 2009).

In Malaysia, providing routine thromboprophylaxis to all patients is not feasible as it will impose an unbearable financial burden on the health system. Thus, thromboprophylaxis is not routinely administered to healthy and low-risk immobilised patients in the hospital setting, and only patients with symptoms and underlying comorbidities will be treated according to current practices and physician's justification. Therefore, the findings from this study could offer recommendations on VTE prophylaxis in orthopaedic trauma and surgery.

This study involves orthopaedic patients who were admitted to the hospital due to lower limb injuries. Patients can either have single or multiple injuries that require immobilisation for more than five days. This study looked into changes in haemostatic, inflammatory, and haematological markers, as well as related clinical risk factors, in response to prolonged immobilisation between day one and day five post-trauma, and risk of hypercoagulability.

1.2 Justification of the Study

VTE is one of the most severe and underdiagnosed diseases, despite its preventable nature. Occult asymptomatic VTE can go unnoticed and heighten the risk of VTE complications. Pulmonary embolism is usually associated with occult VTE of the lower limb, and this particular complication poses substantial morbidity and mortality risks.

There is a lack of a clear study on the true incidence of post-trauma immobilisation VTE and the need for prophylaxis in healthy immobilised trauma patients. Many hospitals have been regularly administering different forms of thromboprophylaxis to these patients. However, considering the massive number of patients seeking medical treatment for these conditions, routine use of VTE prophylaxis might impose an unsustainable economic burden on the healthcare system.

This study evaluated the changes in the hypercoagulable markers (haemostatic, inflammatory and haematological parameters) in orthopaedic trauma patients with prolonged immobilisation and risk of hypercoagulability related to VTE. At the same time, this study also emphasised that VTE can develop not only in symptomatic and older people, but also in healthy and low-risk immobilised patients.

Additionally, there are limited studies in Malaysia that provide local data on haemostatic, inflammatory and haematological biomarkers in prolonged immobilised orthopaedic trauma patients. No established VTE studies employing clinical and laboratory parameters as diagnostic markers exist for these patients. Therefore, the findings of this study can be recommended in the local or national

protocol/guidelines for VTE prophylaxis in high-risk orthopaedic trauma patients with prolonged immobilisation.

1.3 Research Hypothesis

There are associations between laboratory parameters (haemostatic, inflammatory and haematological markers) and prolonged immobilisation, as well as clinical risk factors for hypercoagulable states related to VTE development.

1.4 Research Questions

1. Is there any significant haemostatic (D-dimer, fibrinogen, PT, aPTT, free protein S, protein C, antithrombin), inflammatory (CRP, ESR) or haematological marker (platelet count) change that could be associated with prolonged immobilised orthopaedic trauma patients?
2. Is there any correlation between haemostatic parameters (D-dimer, fibrinogen, PT, aPTT, free protein S, protein C, antithrombin) and inflammatory parameters (CRP, ESR) in these prolonged immobilised orthopaedic trauma patients?
3. Is there any significant relationship between clinical risk factors and laboratory parameters that could be associated with prolonged immobilised orthopaedic trauma patients?

1.5 Objectives of the Study

General Objective:

To study the association between haemostatic, inflammatory, and haematological markers contributing to a hypercoagulable state in prolonged immobilised orthopaedic trauma patients.

Specific Objectives:

1. To compare selected haemostatic, inflammatory and haematological markers in prolonged immobilised orthopaedic trauma patients on day 1 and day 5 of hospitalisation (D-dimer, fibrinogen, PT, aPTT, free protein S, protein C, antithrombin, CRP, ESR and platelet count).
2. To correlate haemostatic parameters (D-dimer, fibrinogen, PT, aPTT, free protein S, protein C, antithrombin) and inflammatory parameters (CRP, ESR) in prolonged immobilised orthopaedic trauma patients.
3. To determine the association between clinical risk factors (gender, age, type of injury, BMI and smoking status) and abnormal laboratory parameters among prolonged immobilised orthopaedic trauma patients and risk of hypercoagulable states.

1.6 Significance of the Study

The findings of this study could be beneficial for society, given that VTE has become a significant public health concern due to its growing prevalence. This study aims to provide crucial information about the relationship between VTE and a particular clinical risk factor as well as blood biomarker in prolonged immobilised orthopaedic trauma patients.

Through this research, the clinical risk factor and laboratory profile associated with hypercoagulable states and VTE development could be determined. The results of this research could also evaluate the correlation between haemostatic parameters and inflammatory parameters.

Additionally, the results could aid in early planning for better management of VTE patients, as it will help physicians decide on the prophylaxis treatment for VTE patients. Only selected prolonged immobilised trauma patients should receive prophylaxis anti-coagulant treatment, while low-risk patients should be excluded from such treatment. This will prevent unnecessary costs and further potential complications.

Finally, the data presented in these research findings can be incorporated into the national or local guidelines on anti-coagulant prophylaxis for prolonged immobilised orthopaedic trauma patients who are at a high risk of developing VTE.

1.7 Conceptual Framework

A conceptual framework of prolonged immobilised orthopaedic trauma patients with clinical risk factors and blood biomarkers as a predictor for VTE development is shown in Figure 1.1. The framework demonstrates the clinical risk factors, which include gender, age, type of injury, BMI and smoking status, and blood biomarkers (haemostatic, inflammatory and haematological) that propose a potential risk factor in developing VTE.

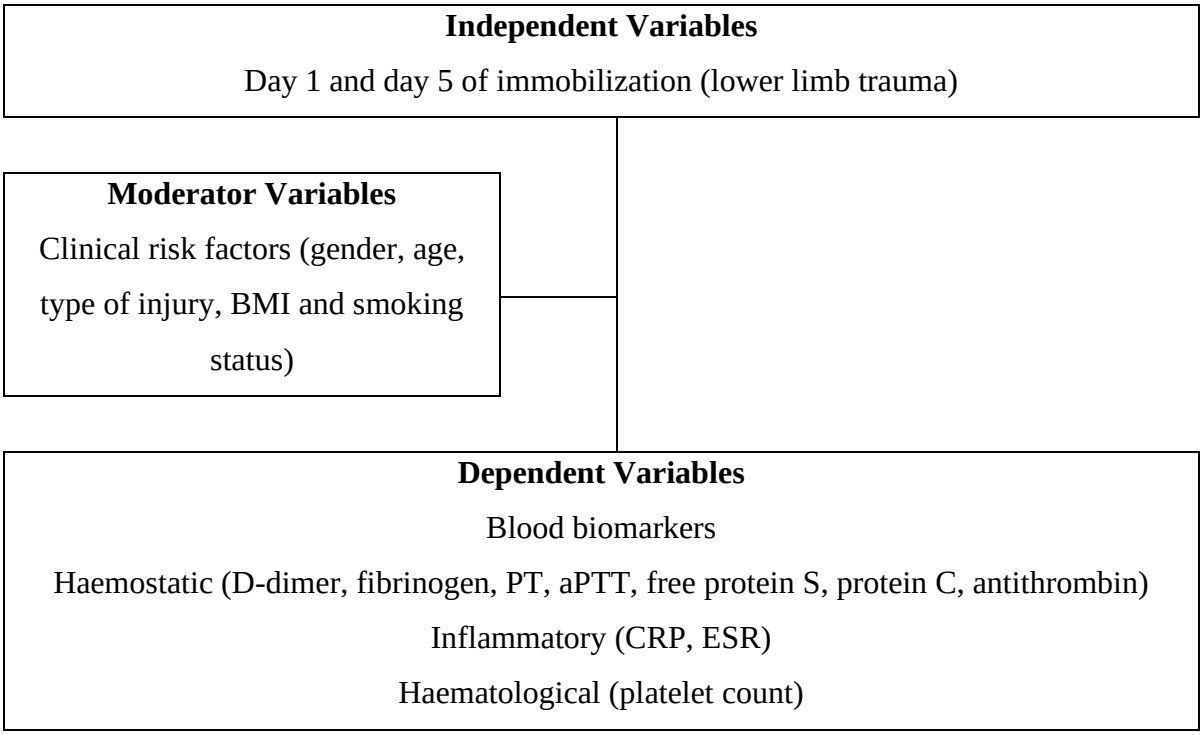

Figure 1.1 Conceptual framework of the study

1.8 Outlines/Overview of the Thesis

The thesis consists of six chapters:

Chapter 1 provides information on the study's background, research objective, significance of the study and conceptual framework of the study.

Chapter 2 discusses in more detail about the venous thromboembolism disease in orthopaedic trauma and prolonged immobilised patients, clinical risk factors and blood biomarkers in predicting the risk of VTE.

Chapter 3 outlines the experimental work carried out in this research study to assess the blood biomarker and clinical risk factor in orthopaedic trauma patients with prolonged immobilisation.

Chapter 4 presents the results and data analyses of the study.

Chapter 5 discusses the results obtained from the study.

Chapter 6 includes conclusions, limitations and recommendations for future research.

Appendix A contains the consent form.

Appendix B contains the ethical approval.

Appendix C contains the proforma of the study.

Appendix D contains list of the reagent and methods.

CHAPTER 2

LITERATURE REVIEW

2.1 Venous Thromboembolism

Venous thromboembolism (VTE) comprises two clinical manifestations, which are deep vein thrombosis (DVT) and pulmonary embolism (PE). VTE affects millions of people globally and is one of the primary causes of morbidity and mortality among inpatients (Akamine et al., 2022). According to a study by Bahl et al., 10% to 40% of patients undergoing medical treatment or general surgery who did not receive proper thromboprophylaxis treatment developed VTE (Bahl et al., 2010). Annually, VTE is accountable for inducing an approximated fatality rate of 100,000 to 300,000 individuals within the territory of the United States (Wakefield et al., 2009).

2.1.1 Deep vein thrombosis

DVT is defined as the development of a blood clot within a deep vein. It most commonly develops in the deep veins of the lower limbs; however, it can also form in the deep veins of the upper limbs, visceral veins, and even the vena cava (Olaf & Cooney, 2017). This disease commonly manifests with symptoms such as pain, erythema, tenderness, and swelling in the afflicted limb. As a result, in cases of lower limb DVT, the afflicted leg is often swollen, causing the calf circumference to be larger than the unaffected side. DVT in the lower leg commonly begins in the calf veins. Approximately 10% to 20% of thrombosis extend proximally, with an additional 1% to 5% causing fatal pulmonary embolism (Turpie et al., 2002).

2.1.2 Pulmonary embolism

PE occurs when a blood clot from DVT dislodges, becoming an embolus and travelling through the blood stream. The embolus continues to travel through the circulation until it reaches a smaller-diameter artery, at which point it becomes trapped and block distal blood flow (Tarbox & Swaroop, 2013). Small PE lyses naturally and may not have any clinical impact, whereas a large embolus might induce pulmonary vascular occlusion, increase pressure on the right heart, and potentially lead to hypotension and sudden death (Giordano et al., 2017). Symptoms of PE include dyspnea, haemoptysis, cyanosis, and pleuritic chest pain (Turpie et al., 2002).

2.2 Incidence of VTE

In the United States, the annual occurrence rate of new VTE cases is estimated to be between 73 and 133 per 100,000 individuals (Huang et al., 2014). Based on multiple studies, the overall VTE occurrence might be higher among African-American populations (Zakai et al., 2014) and lower in Asian (Cheuk et al., 2004), Asian-American (White et al., 2005), and Hispanic populations (Hooper et al., 2002). In 2015, Heit conducted a narrative review, combining a few papers, and found that the incidence rates for DVT and PE varied from 45 to 117 and 29 to 78 per 100,000 person-years, respectively (Heit, 2015). The reported incidence rate of VTE differs between research due to demographic variables and the existence of genetic and acquired risk factors.

2.3 Pathogenesis of VTE

Virchow's triad, initially identified in 1856, involves three main factors in the development of thrombosis: irregular blood flow (venous stasis), vascular endothelial damage (vessel wall injury), and an increase in the blood's tendency to clot (hypercoagulability) (Bagot & Arya, 2008). Figure 2.1 shows the Virchow's triad concept for VTE pathogenesis (Ramli et al., 2023).

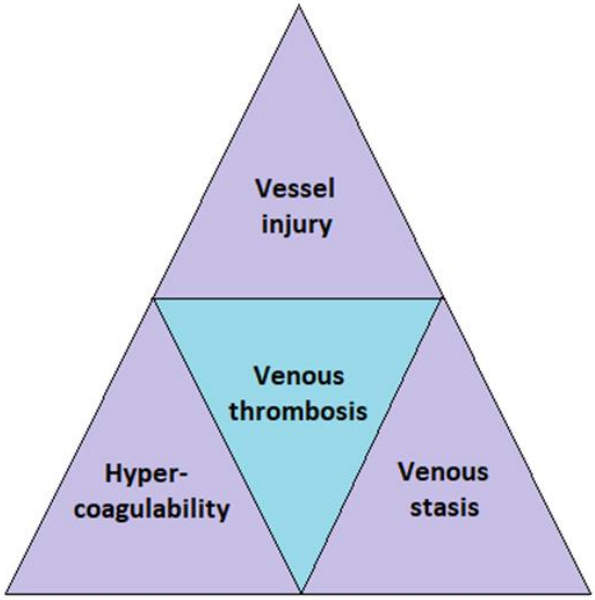

Figure 2.1 Virchow's triad (Ramli et al., 2023)

The pathogenesis of VTE is significantly influenced by venous stasis and hypercoagulability, whereas in arterial thromboembolism, vessel wall injury plays a crucial role. Venous stasis is induced by local factors (mechanical constriction of the venous system and prolonged immobilisation) and systemic factors (increased blood viscosity). The second component of Virchow's triad, vessel wall injury, can occur in the form of endothelial injury in the context of surgery, trauma or the presence of indwelling venous catheters. Endothelial injury exposes tissue factor to blood

circulation, causing blood coagulation to be stimulated and enhanced by the fast production of thrombin (Becattini & Agnelli, 2002).

Hypercoagulability, the third component of Virchow's triad, occurs when there is an imbalance in haemostatic system, favouring procoagulant factors and promoting blood coagulation. This imbalance can either be inherited or acquired. In cases where the imbalance is due to an inherited condition, such as activated protein C resistance, prothrombin mutation, or antithrombin deficiency, the resulting hypercoagulable state remains a lifelong risk factor for thrombosis (Khan & Dickerman, 2006; Kroegel & Reissig, 2003). On the other hand, hypercoagulability resulting from an acquired condition, such as autoimmune diseases, cancer, pregnancy, and certain medications (such as estrogen treatment), should be treated only for as long as the risk factor is present (Lippi & Franchini, 2008).

2.4 Risk Factor of VTE

VTE is a multi-causal disease that involves an interplay of acquired and genetic risk factors. Genetic risk factors are typically associated with hypercoagulability, while acquired risk factors generally involve both blood stasis and hypercoagulability. The known genetic factors for VTE are prothrombin 20210A mutation, factor V Leiden mutation, and a deficiency of natural anticoagulants, such as antithrombin, protein C and protein S. Conversely, acquired risk factors include immobilisation, trauma, major surgery, hormonal replacement therapy, oral contraceptives, pregnancy, antiphospholipid syndrome, advanced age, obesity (Rosendaal & Reitsma, 2009), gender (Lu et al., 2016) and smoking status (Severinsen et al., 2009).

2.4.1 Trauma

Trauma in this study can be defined as a physical injury that can result in wounds or fractured bones. According to Heit et al., trauma patients requiring medical attention and hospital admissions are associated with a 12-fold increase in the risk of VTE (Heit et al., 2000). Factors indicating an increased risk of VTE in trauma patients include the number of operations, pelvic injuries, concurrent medical problems, and the severity of the injury (Paffrath et al., 2010).

The classification of injuries based on type and severity is essential for studying their magnitude, distribution, and contributing factors, particularly in diagnosing VTE in trauma patients (Stevenson et al., 2001). Injury scoring systems, like the Injury Severity Score (ISS) and the Abbreviated Injury Score (AIS), are frequently used to determine the severity of a patient's trauma. The ISS is an established anatomical scoring system that provides a comprehensive score to patients afflicted with multiple injuries. Each injury is rated using an AIS and categorised into one of six body regions. In each body area, only the highest AIS is used. The ISS is calculated by squaring the total scores of the three most severely injured body areas (Javali et al., 2019). Studies have shown that an elevated ISS has been positively correlated with an increased likelihood of VTE. In a study by Azu et al., involving a sample size of 10,150 trauma patients, an elevated ISS was significantly linked to a higher frequency of VTE incidences (Azu et al., 2007).

Several studies have also discovered that the occurrence of DVT ranges from 5% to 80% in trauma patients (Scolaro et al., 2015; Toker et al., 2011). Moreover, lower limb or pelvic fractures, head injuries or prolonged immobilisation have been widely recognised as high-risk factors for VTE in trauma patients (Table 2.1) (Tai et al., 2013; Toker et al., 2011).

Table 2.1 VTE risk factors in trauma patients

VTE risk factors in trauma patients
Lower limb fracture
Pelvic fracture
Venous injury
Major surgical procedure
Ventilator days >3
Spinal cord injury with paralysis
Shock on admission (BP <90 mmHg)
Head injury (abbreviated injury score ≥3)
Age ≥40 years

(Adapted from (Tai et al., 2013))

Major trauma frequently contributes to some or all of the risk factors in Virchow's triad, which ultimately results in an elevated susceptibility to thromboembolic events (Hak, 2001; Ruiz et al., 1991). The mechanism by which trauma induces thrombus formation may be accelerated by blood vessel damage, blood composition changes, and changes in venous blood flow or stasis (Tai et al., 2013).

Trauma patients suffering from a lower limb fracture, pelvic fracture, head injury, or spinal cord injury, are often immobilised. Immobility causes the patient to be in a static position, which reduces the venous blood return and results in venous stasis. Moreover, direct trauma to the blood vessel causes endothelial damage by exposing blood to collagen, basement membrane, tissue factor, and von Willebrand's factor. All of these factors attract platelets and stimulate the extrinsic and intrinsic coagulation cascades, thereby inducing hypercoagulable states and subsequently leading to thrombosis (Kelsey et al., 2000). Trauma-induced hypercoagulability can worsen a patient's condition and result in a negative prognosis (Selby et al., 2009)

Yumoto et al. discovered in their cohort study that approximately one out of every three severe trauma patients had VTE 10 days after being admitted to the intensive care unit. Additionally, it was observed that the presence of a lower limb fracture and a higher ISS were both significant risk factors for VTE (Yumoto et al., 2017).

2.4.2 Immobilisation

Immobilisation is the process of securing a joint or bone in a fixed position through the use of a splint, cast, or brace. This approach aims to limit or reduce movement while an injured part recovers. A few studies define immobilisation as "bedrest" or "confinement to bed" for more than three days. Pottier et al. discovered that immobilisation significantly increases the risk of VTE, with immobilised patients being approximately twice as likely to develop VTE compared with ambulatory individuals (Pottier et al., 2009). According to Well's criteria, cast immobilisation of the lower limb and immobilisation for more than three days are risk factors for VTE. In a recent study by Modi et al., they discovered that Well's criteria can serve as a pre-test tool for risk stratification, which can effectively rule out the potential likelihood of DVT in patients suffering from traumatic injuries (Modi et al., 2016).

Immobilisation of the extremities, such as during bed rest or plaster casts, reduces movement in cases of prolonged travel and, due to paralysis, is linked to an elevated risk of VTE. The common denominator is that immobility interferes with the calf musculature's function of pumping blood upstream via the veins. This promotes venous stasis that leads to DVT development (Rosendaal, 2005). Venous stasis can therefore cause hypercoagulability by activating the extrinsic and intrinsic coagulation pathways (Kelsey et al., 2000).

In a meta-analysis consisting of six randomised controlled studies, it was determined that DVT incidences varied significantly, ranging from 4.3% to 40%, among patients who had undergone immobilisation through the use of a cast for a minimum duration of one week without receiving prophylactic measures (Testroote et al., 2008). Another studies also found a considerably greater prevalence of VTE in patients with a lower extremity injury, particularly in the pelvic body area (Lichte et al., 2015), and those with cast immobilisation (Ettema et al., 2008; Van Adrichem et al., 2014).

2.4.3 Gender

Gender may have an influence on the development of first and recurrent VTE. Studies on the effect of gender as a VTE risk factor has produced contradictory findings. Epidemiological studies have shown that women of childbearing age had a higher incidence of first VTE due to pregnancy and the influence of oestrogen-containing contraceptives (Tormene et al., 2011). Oral contraceptive use and pregnancy were reportedly associated with haemostatic changes that included a reduction in fibrinolytic activity, an increase in coagulation factors, and a decrease in natural anticoagulants, all of which resulted in VTE development (Previtali et al., 2011).

In a meta-analysis study comprising 7,892,585 individuals undergoing total knee arthroplasty (TKA) or total hip arthroplasty (THA), it was shown that female patients had a slightly greater incidence of VTE compared with male patients. Female patients were more likely to develop VTE due to their proclivity to immobilise after surgery (Lu et al., 2016). Prior research has also demonstrated that female patients exhibited a greater susceptibility to the development of VTE compared with their male counterparts (Zhang et al., 2015).

Contrastingly, in cohort studies by Ageno et al. and Lind et al., they reported that unprovoked events of VTE are more common in males rather than females (Ageno et al., 2013; Lind et al., 2022). Romero et al., meanwhile, concluded that gender cannot be considered as an independent risk factor for VTE. Instead, the absolute risk is typically associated with particular conditions that are unique to women (Romero et al., 2005).

2.4.4 Age

Age is a notable risk factor for the development of VTE. As indicated earlier, the prevalence of VTE rise substantially with age. However, the specific age at which the risk starts to increase is unknown (Toker et al., 2011) and slightly differs according to different study. Naess et al., reported that around 60% of all VTE incidents occur in those aged 70 and above (Naess et al., 2007). In a recent study, they found that an age of more than 50 years was independently associated with VTE following Achilles tendon rupture (Oliver et al., 2022).

Selby et al. in their prospective cohort study discovered that increasing age was the most significant clinical predictor of VTE following trauma (Selby et al., 2009). The reasons why the risk of developing thrombosis tends to increase as a person gets older are not fully understood. However, it might be due to the existence of other diseases that predispose individuals to thrombosis, an increase in coagulation factor levels, or a combination of these two factors (Cushman, 2007). Furthermore, venous valves and leg muscle tone might decline with age, contributing to impaired venous return and increasing the risk of VTE (Rosendaal, 2005).

2.4.5 Body mass index

Body size and shape are primarily determined by weight and height, and are sometimes measured using the waist-to-hip ratio (WHR) or body mass index (BMI). BMI has been studied as a possible risk factor for DVT or as a predictor of coagulation factor concentrations. Two independent case-control studies have demonstrated that individuals who are clinically obese with a BMI exceeding 30 kg/m^2 exhibit a twofold increase in the likelihood of VTE (Abdollahi et al., 2003; Samama, 2000). Moreover, a recent study reported that obese patients are at a 6.2-fold greater risk of developing VTE, and it confirmed that obesity is an independent and moderately significant risk factor for VTE (Hotoleanu, 2020).

Obesity is associated with increased intra-abdominal pressure, impaired fibrinolysis, a chronic low-grade inflammatory state, and high levels of fibrinogen, factor VIII, and von Willebrand factor. These factors collectively contribute to a prothrombotic state and an increased risk of VTE (Lentz, 2016). According to a study by Stein et al., obese patients were found to have a relative risk of 2.50 for acquiring DVT and a relative risk of 2.21 for PE (Stein et al., 2005). In three separate Mendelian randomisation studies investigating the relationship between BMI and VTE, strong evidence of a causal connection between higher BMI and the risk of VTE was identified. Additionally, these studies suggested that reducing obesity rates could potentially help lower the incidence of VTE (Klovaite et al., 2015; Lindström et al., 2017; Tan et al., 2021).

2.4.6 Smoking status

Smoking is a known risk factor for atherosclerosis, but its relevance as an independent risk factor for VTE is debateable. In a Danish follow-up study, researchers discovered a substantial correlation between current smoking and VTE, with hazard ratios of 1.52 and 1.32 for smoking women and men, respectively (Severinsen et al., 2009). Furthermore, in a Tromsø study, it was reported that heavy smokers consuming more than 20 packs per year had a hazard ratio of 1.46 for total VTE and a hazard ratio of 1.75 for provoked VTE (Enga et al., 2012).

Cheng et al. revealed that cigarette smoking is correlated with a marginally higher risk of VTE, and they observed a dose-response relationship between smoking and VTE risk. Therefore, they suggested that the consideration of smoking habits should be incorporated into the screening process for individuals who may be at risk for VTE (Cheng et al., 2013). Zhang et al. reported a similar finding, in which smoking can contribute to the risk of VTE with a dose-response relationship (Zhang et al., 2014). The connection between smoking and VTE risk may be explained by a procoagulant condition, decreased fibrinolysis, inflammation, and increased blood viscosity (Miller et al., 1998).

In a recent investigation by Paulsen et al., it was discovered that smoking was associated with a 50% increased risk of VTE in cancer patients. However, no correlation was established in cancer-free patients (Paulsen et al., 2021).

2.5 Biomarker and VTE Development

2.5.1 Biomarker

Biomarkers, also known as biological markers, are a characteristic that is objectively tested and analysed as an indication of normal biological processes, pathogenic processes, or pharmacologic reactions to a therapeutic intervention. Biomarkers are effectively classified based on their intended use as: a) a diagnostic tool, which includes disease detection, b) a tool for disease staging or disease extent classification, c) a prognostic indicator, which predicts the course of a disease (i.e. recurrence, progression, and survival), and d) a predictive tool, which allows for therapy response prediction (Group, 2001).

The perfect biomarker should possess specific, sensitive, and predictive qualities. It should also be rapid, cost-effective, stable both in vitro and in vivo environments, non-invasive, and easily measurable. Additionally, the biomarker should hold sufficient preclinical and clinical value to influence decisions related to the disease process to which it is applied (Torres Courchoud & Pérez Calvo, 2016).

2.5.2 Haemostatic, inflammatory and haematological parameters as potential biomarkers for diagnosing VTE

Multiple studies have reported that haemostatic, inflammatory and haematological biomarkers were associated with the VTE development. A haemostatic biomarker, such as D-dimer, has been successfully utilised in clinical practice to diagnose VTE (Abdullah, 2015). Ma et al. conducted a retrospective study exploring the association between haematological biomarkers and DVT development. Their found that D-dimer levels higher than 0.5 mg/L and platelet distribution width lower than 12% were related to DVT development (Ma et al., 2020).

According to a Chinese study, trauma patients commonly exhibited elevated plasma D-dimer levels, and this increase was closely associated with the number of fractures. The study indicated that D-dimer serves not only as a marker for identifying DVT and PE, but also as an indicator for assessing the severity of trauma in patients with acute injuries. In other words, D-dimer alone cannot be utilised as a predictive marker for VTE in trauma patients (Zhang et al., 2012). Given its limitations, more research into potential additional biomarkers for identifying VTE is required.

2.6 Haemostatic Biomarkers

Haemostasis is a physiologic process that maintains blood fluidity and facilitates the rapid generation of a haemostatic plug, a platelet-rich mass encased in fibrin, outside an injured blood vessels to arrest blood flow (coagulation). Haemostasis is carefully regulated in healthy individuals through several anticoagulant mechanisms that balance procoagulant forces and thereby avoid improper vascular blood clotting (Segers et al., 2007). In the event of a physiologic necessity for blood loss to be stopped, the dynamic balance between procoagulant and anticoagulant factors can be quickly altered in favour of coagulation (Vine, 2009).

Haemostatic biomarkers, including D-dimer, fibrinogen, prothrombin time (PT), activated partial thromboplastin time (aPTT), free protein S, protein C and antithrombin, could possibly be used as alternative biomarkers to improve the accuracy and effectiveness of VTE diagnosis.

2.6.1 D-dimer

D-dimer is a soluble fibrin degradation product produced by the fibrinolytic system as a result of the orderly breakdown of thrombi. Numerous studies have demonstrated that D-dimer functions as an established and valuable marker for assessing coagulation and fibrinolysis activity. This biomarker has been extensively utilised as a screening test to evaluate hypercoagulability and the risk of VTE (Favresse et al., 2018). D-dimer testing measures the current state of fibrinolysis and possesses a high negative predictive value, allowing the exclusion of an ongoing clot forming process, hence it may be utilised to rule out DVT in patients who have a low pre-test probability (Anghel et al., 2020).

The D-dimer test is useful in the primary care setting for patients with suspected VTE. According to studies in 2007 and 2008, the diagnostic yield of VTE in patients with a positive D-dimer test was 24% and 21%, respectively (Vanfleteren & Wesseling, 2011). Several studies found that screening criteria based on D-dimer levels can be used as a reference to rule out VTE in severe trauma patients (Iyama et al., 2018) and postoperative lower limb fracture patients (Yang et al., 2017). Additionally, Japanese researchers observed that D-dimer levels on day 10 can be useful in predicting VTE in major trauma patients (Yumoto et al., 2017). Thus, researchers suggested that high levels of plasma D-dimer are linked with a higher occurrence of VTE (Hansen et al., 2021).

The D-dimer test is an effective non-invasive triage test with a high degree of predictability for those who may be experiencing a thromboembolic episode. VTE can be ruled out in a patient who has both a negative D-dimer value and a low clinical pre-test probability of disease. Therefore, it could help in reducing the number of

individuals who require additional ultrasound testing (Fancher et al., 2004; Tamariz et al., 2004; Wells et al., 2003).

Based on the prospective cross-sectional study involving 114 total knee arthroplasty (TKA) patients, D-dimer testing was shown to be an effective method for ruling out DVT in post-surgical patients. A normal D-dimer level was found to be beneficial in excluding DVT, but an elevated level alone was not definitive evidence of DVT. Furthermore, the D-dimer level will return to normal in cases with no underlying DVT. They also found that relying solely on the D-dimer test is insufficient for the diagnosis of early DVT in post-TKA patients (Zamir et al., 2015).

2.6.2 Fibrinogen

Fibrinogen is a glycopeptide that aids in blood clot formation. It has a molecular weight of 340 kDa and is synthesised in hepatocytes (Hayakawa, 2017). Plasma fibrinogen is an important predictor of blood viscosity and blood flow, and a key component of the coagulation cascade (Kamath & Lip, 2003). In healthy people, the concentrations of plasma fibrinogen may vary from 2 to 5 mg/mL. However, it should be noted that fibrinogen is classified as an acute phase protein. In the occurrence of traumatic incidents, there is potential for these protein concentrations to escalate up to four times beyond their normal levels (Kattula et al., 2017).

According to Machlus et al., elevated fibrinogen levels promote the development of thrombus that is resistant to proteolytic degradation (Machlus et al., 2011). Another study also reported that a significant increase in plasma fibrinogen was linked to a higher risk of PE when combined with DVT (Klovaite et al., 2013). Furthermore, numerous research has been carried out to investigate the complex relationship between fibrinogen concentration and VTE risk. They suggest that elevated levels of plasma fibrinogen can cause thrombosis through multiple