HAEMOSTATIC, INFLAMMATORY AND HAEMATOLOGICAL BIOMARKERS AMONG ORTHOPAEDIC PATIENTS WITH PROLONGED IMMOBILIZATION AND RISK OF VENOUS THROMBOEMBOLISM

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LIST OF ABBREVIATIONS

APC	Activated protein C
APS	Anti-phospholipid syndrome
aPTT	Activated partial thromboplastin time
ARDS	Acute respiratory distress syndrome
AT	Antithrombin
BMI	Body mass index
C4BP	C4 Binding Protein
CLSI	Clinical and Laboratory Standard Institute
CRP	C-reactive protein
CTPA	Computerised Tomography Pulmonary Angiography
DIC	Disseminated intravascular coagulopathy
DM	Diabetes mellitus
DVT	Deep vein thrombosis
EDTA	Ethylene diamine tetra acetic acid
ESR	Erythrocyte sedimentation rate
FBC	Full blood count
IHD	Ischaemic heart disease

IL-6	Interleukin 6
NSAIDs	Non-steroidal anti-inflammatory drugs
OCP	Oral contraceptive pill
PC	Protein C
PE	Pulmonary embolism
PLT	Platelet
PMH	Past medical history
PPP	Platelet poor plasma
PS	Protein S
PT	Prothrombin time
RBC	Red blood cell
SIRS	Systemic inflammatory response syndrome
TEG	Thromboelastography
TF	Tissue factor
THA	Total hip arthroplasty
TKA	Total knee arthroplasty
TNF-α	Tumour necrosis factor -α
TPO	Thrombopoietin
USG	Ultrasonography

USM	Universiti Sains Malaysia
VTE	Venous thromboembolism

BIOMARKER HEMOSTASIS, INFLAMASI DAN HEMATOLOGI DALAM KALANGAN PESAKIT ORTOPEDIK YANG MEMERLUKAN IMMOBILISASI YANG PANJANG DAN RISIKO UNTUK MENDAPAT PEMBEKUAN SALUR DARAH VENA

ABSTRAK

Trauma dan immobilisasi untuk tempoh yang lama akan menyebabkan kemudah-bekuan darah yang berpontensi untuk menyebabkan thrombosis. Beberapa kajian telah menunjukkan kaitan di antara biomarker hemostasis, inflamasi dan hematologi dengan pesakit trauma.

Tujuan kajian ini dijalankan adalah untuk menyiasat perubahan marker kemudah-bekuan darah (parameter hemostasis, inflamasi dan hematologi) di kalangan pesakit trauma yang memerlukan immobilisasi yang lama dan juga untuk menentukan hubungkait di antara parameter hemostasis dan inflamasi. Hubungan diantara faktor risiko klinikal (umur, jantina, BMI, merokok dan jenis kecederaan) dengan parameter makmal yang abnormal dan perkaitannya dengan pembekuan salur darah vena juga dikaji.

Kajian kohort prospektif telah dijalankan di Hospital Universiti Sains Malaysia dari September 2016 hingga Julai 2017. Seramai 52 pesakit yang berumur antara 12 hingga 59 tahun, mengalami patah kaki dan memerlukan immobilisasi lebih dari 7 hari dan tidak menerima ubat anti pembekuan darah telah dimasukkan dalam kajian ini. Parameter yang telah ditetapkan diukur pada hari pertama dan kelapan tempoh

immobilisasi. Ujian-ujian makmal yang dibuat adalah PT, aPTT, D-dimer, Fibrinogen, Protein C, Protein S, ESR, CRP dan platelet. Ciri-ciri pesakit and faktor risiko klinikal (umur, jantina, BMI, merokok dan jenis kecederaan) telah direkodkan.

Fibrinogen, ESR dan kiraan platelet telah menunjukkan min perbezaan yang ketara di antara hari pertama dan kelapan immobilisasi. Min fibrinogen telah meningkat sebanyak 1.33 pada hari kelapan immobilisasi (p<0.001, 95% CI = -1.91, -0.76), min ESR juga meningkat sebanyak 28.50 (p<0.001, 95% CI = -36.94, -20.06) manakala min kiraan platelet meningkat sebanyak 111.75 pada hari kelapan immobilisasi (p<0.001, 95% CI = -139.71, -83.79). Hubungan positif yang ketara dilihat diantara fibrinogen dan CRP (R = 0.35, p = 0.012) dan begitu juga diantara fibrinogen dengan ESR (R = 0.54, p < 0.001). Sementara itu, parameter yang lain tidak menunjukkan hubungkait yang ketara. Di antara parameter yang abnormal (fibrinogen, ESR, platelet) yang boleh dilihat dalam kajian ini, hanya platelet yang menunjukkan perkaitan ketara dengan faktor risiko klinikal iaitu BMI dan jenis kecederaan.

Walaupun tiada pesakit yang mendapat masalah pembekuan salur darah dalam kajian ini, namun kajian terdahulu mendapati fibrinogen, ESR dan paras platelet sememangnya adalah biomarker prothrombotik. Dengan penemuan daripada kajian ini, boleh disimpulkan bahawa biomarker ini boleh digunakan untuk membantu indikasi pemberian ubat anti-pembekuan dalam menangani masalah pembekuan salur darah di kalangan pesakit yang berisiko tinggi.

Kajian susulan perlu diteruskan dengan melibatkan saiz sampel yang lebih besar dan juga mewujudkan sistem skor (termasuk faktor risiko klinikal dan biomarker) yang lebih menyeluruh untuk menilai risiko masalah pembekuan salur darah di kalangan

pesakit trauma yang mendapat kecederaan pada kaki dan terlibat dengan immobilisasi yang panjang.

HAEMOSTATIC, INFLAMMATORY AND HAEMATOLOGICAL BIOMARKERS AMONG ORTHOPAEDIC PATIENTS WITH PROLONGED IMMOBILIZATION AND RISK OF VENOUS THROMBOEMBOLISM.

ABSTRACT

Trauma and prolonged immobilization induce hypercoagulable state with thrombotic potential. Multiple studies have shown close relationship between haematological, haemostatic & inflammatory markers and post traumatic patients.

The aims of this study were to investigate the changes of hypercoagulable markers (haemostatic, inflammatory and haematological parameters) in prolonged immobilized trauma patients and to determine the correlation between haemostatic parameters and inflammatory parameters (ESR, CRP) among the subjects. The association between clinical risk factors (age, sex, BMI, smoking and type of injury) and the abnormal laboratory parameters were also studied including the relationship with VTE.

A prospective cohort study was conducted at Hospital University Sains Malaysia from September 2016 to July 2017. A total of 52 patients with lower limb/s fracture with age ranged from 12 to 59 years old, who required immobilization more than 7 days and received no anticoagulant prophylaxis were involved in this study. The predetermined parameters were serially measured on day 1 and day 8 of immobilization. The laboratory tests included PT, aPTT, D-dimer, Fibrinogen, AT, Protein C, Protein S, ESR, CRP and platelet count. Subjects' characteristic and clinical risk factors (age, sex, BMI, smoking and type of injury) were recorded.

Fibrinogen, ESR and platelet count gave significant difference in mean between day 1 and day 8 immobilization. The mean for fibrinogen was increased by 1.33 on day 8 of immobilization (p<0.001, 95% CI of mean difference: -1.91, -0.76), the mean ESR was increased by 28.50 (p<0.001, 95% CI of mean difference: -36.94, -20.06) and the mean of platelet count was increased by 111.75 on day 8 immobilization (p<0.001, 95% CI of mean difference: -139.71, -83.79). There were significant positive correlations between fibrinogen and CRP (R = 0.35, p = 0.012) as well as fibrinogen and ESR (R = 0.54, p < 0.001). Other parameters showed no significant correlations to each other. Among the abnormal parameters (fibrinogen, ESR, platelet) observed in this study, only platelet gave a significant association with clinical risk factors. Body mass index and type of injury showed significant relationship towards platelet.

Although no VTE event documented in this study, previous studies have shown that fibrinogen, ESR and platelet levels are prothrombotic biomarkers. With the findings from this study, it can be concluded that these biomarkers could support prophylaxis indication against VTE risk in high risk patients.

Further research to continue similar study with bigger sample size focusing on scoring system (which include clinical risks and biomarkers) is needed for comprehensive assessment of VTE risk among patients with lower limb/s trauma and prolonged immobilization.

CHAPTER 1
INTRODUCTION

1.0 INTRODUCTION

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are collectively known as venous thromboembolism (VTE). These events are common and potentially a life-threatening complication following trauma. The incidences were reported between 5 to 63% by Toker *et al* (Toker *et al.*, 2011).

VTE has grown to become an important public health problem due to the increasing incidence and its many risk factors. The reported incidence of DVT and PE varies based on many studies. This thromboembolic event was contributed by many factors, such as patient factors, nature and site of injury/injuries, severity of the injury/injuries and method of detection of the VTE.(Tai *et al.*, 2013)

Trauma per se may induce hypercoagulable state. This was confirmed in a study by Selby *et al* in 2009 involving multi-system trauma patients. The study reported overall VTE rate was 59%. Both trauma and immobilization post trauma were found to further complicate the condition. However, the sequential changes in coagulation markers and their relationships to clinical thrombosis have been poorly characterized (Selby *et al.*, 2009).

A study by Aduful and Darko found that venous thrombosis usually affects patients who were above 40 years old, obese, bed ridden, had undergone major operations or were having hypercoagulable states (Aduful and Darko, 2007).

Another study by Rasi *et al* found that patients with stable foot/ankle fractures or who had ligaments injuries which were treated with a splint or a short leg cast, were predisposed to DVT in the affected leg due to immobilization and inactivity of the ankle pump mechanism. They also found that the risk of DVT is highest between day 7 to day 14 of immobilization (Rasi *et al.*, 2013).

Niikura *et al* conducted a study involving Japanese patients with fractures of the pelvis and/or lower extremities who were using physical prophylaxis alone (either graduated compression stocking or intermittent pneumatic compression). They found that 24 out of 126 patients had developed VTE (Niikura *et al.*, 2012).

Apart from the above factors, there were also genetic clotting defects that may promote VTE. For example, activated protein C resistance (factor V Leiden mutation), prothrombin G20210A gene mutation, deficiencies of antithrombin, protein C, protein S and hyperhomocysteinemia (Aduful and Darko, 2007).

Although guidelines were made for VTE prophylaxis in the orthopaedic trauma patients, they had insufficient evidence in the literature to make strong recommendations regarding the type and duration of prophylaxis to be given. The risk of associated morbidity of chemical anticoagulants used in the orthopaedic trauma must also be taken into consideration, especially the risk of bleeding (Scolaro *et al.*, 2015).

In our hospital setting, thromboprophylaxis is not routinely practiced for healthy immobilized patients, and only symptomatic patients will be treated according to the current practice and clinician justification. This study basically included orthopaedic

patients who had lower limb trauma. The findings of this study could represent the preliminary multidisciplinary effort to provide recommendation of VTE prophylaxis in the fields of orthopaedic surgery and orthopaedic trauma.

This study includes orthopaedic patients who had lower limb trauma. It can be either single or multiple injuries which required immobilization. This study is attempted to look into the changes of the haematological, haemostatic and inflammatory markers in relation to prolonged immobilization between day 1 and day 8 post trauma, and determine their association with VTE development.

CHAPTER 2
LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 VENOUS THROMBOEMBOLISM (VTE)

Venous thromboembolism encompasses two interrelated conditions which are deep venous thrombosis (DVT) and pulmonary embolism (PE). It is a multifactorial disease, involving interactions between clinical risk factors and predisposition to thrombosis which is either acquired or inherited.

Prolonged immobilization, trauma involving long bones and pelvic fractures, spinal cord and traumatic brain injuries are another risk factors for developing thrombo-embolic complication. These thrombo-embolic complications are significant contributors for morbidity and mortality, and managing these complications will put an unbearable burden on the health systems.

Despite the use of prophylactic protocols, the incidence of VTE is still high during the clinical course post-injury**.** Within the trauma and orthopaedics discipline, VTEs are the most common preventable cause of in-hospital deaths (Lichte *et al.*, 2015a).

2.2 TRAUMA

The word trauma comes from a Greek word meaning wound. Although originally it refers to a physical wound, nowadays the word trauma also refers to an emotional wound. Trauma in this study referred to physical injury/injuries that occurred to patient which required medical attention and hospital admission.

There were many risk factors for developing VTE that have been identified: age, long bone and pelvic fractures, spinal cord and traumatic brain injuries, prolonged immobilization and delay of prophylactic management (Paffrath *et al.*, 2010).

In a study by Lichte *et al*, they observed a significant higher rate of venous thromboembolism (VTE) in patients with injuries to the extremities, especially pelvic body region (Lichte *et al.*, 2015b).

Another study reported that the hypercoagulable state due to immobilization that was induced by trauma had higher risk of VTE compared to those who were immobilized without tissue injury or trauma (Adrichem *et al.*, 2014).

2.3 IMMOBILIZATION

Immobilization is defined as a reduction or elimination of motion of the body part by mechanical means or by strict bed rest to allow healing. Well's criteria define immobilization as restricted movement for three or more days. Immobilization of more than 72 hours is one of the risk factors for venous thromboembolism (VTE).

Well's criteria had been developed as a pre-test probability to guide further diagnostic test. However, it was developed for non-traumatic cases and not for trauma cases. The Wells score was not significantly predictive of PE in patients admitted to the orthopaedic trauma service (Young *et al.*, 2013).

Another study by Rasi *et al* found that the risk of DVT is highest between day 7 to day 14 of immobilization (Rasi *et al.*, 2013). This was supported by study by Brakenridge *et al*, where they report the median times to develop VTE is approximately around 10 days post injury (Brakenridge *et al.*, 2013).

Few study suggested that, cast immobilization, especially immobilization of lower extremity were also known risk factor for VTE (Ettema *et al.*, 2008; Testroote *et al.*, 2008).

In a different study, trauma related indications for below knee cast in non-surgically treated patients were strongly associated with VTE than non-traumatic indications. They also found a clear relationship between duration of immobilization and the development of VTE. They found that twice as many patients were diagnosed with VTE in the

second week of immobilization as in the first week. The finding corresponded with the natural course of the disease, as a venous clot generally takes some time to develop (Adrichem *et al.*, 2014).

2.4 BIOMARKERS

Biomarkers are also known as biological markers. It is a measurable indicator that correlates well with the risk or progression of a disease. It can be blood/ tissue/ gene/ enzyme.

Complex organ functions or general characteristic changes in biological structures can also serve as biomarkers. For example, high blood pressure to determine risk of stroke, C-reactive protein (CRP) to determine risk of infection/ inflammation, or body temperature to determine fever.

2.4.1 Biomarkers and risk of venous thromboembolism

Many studies have shown that haemostatic, inflammatory and haematological markers had significant risk related to development of VTE. One of the examples of haemostatic biomarkers that had been successfully implemented in clinical practice is D-dimer (Abdullah, 2015).

Based on the study in an elderly cohort with 1700 incident cardiovascular events over 9 years of follow-up, interleukin 6 (IL-6), C-reactive protein (CRP), D-dimer,

homocysteine and white blood cell count were independently associated with future cardiovascular diseases (Kritchevsky *et al.*, 2005; Zakai *et al.*, 2007).

2.5 EFFECT OF TRAUMA AND IMMOBILIZATION ON HAEMOSTATIC PARAMETERS

Haemostasis is a complicated and well balanced physiologic process to maintain the blood in a fluid form. Following a ruptured vessel due to trauma, haemostatic proteins will serve to prevent excessive bleeding via clot formation. At the same time, another haemostatic proteins will function as anti-coagulant for lysing and limiting the extension of clot. Thus, any imbalance of this process will result in a complication.

The list of haemostatic biomarkers that have been studied in the case of trauma were Prothrombin Time (PT), activated Partial thromboplastin Time (aPTT), D-dimer, fibrinogen, Protein C (PC), Protein S (PS) and antithrombin (AT) assays.

2.5.1 Prothrombin Time (PT) and activated Partial Thromboplastin Time (aPTT)

The prothrombin time is sensitive for deficiency in factor II, V, VII, X and fibrinogen, while aPTT is sensitive for deficiency in factor XII, XI, X, IX, VIII, V, II and as well as fibrinogen.

Both are standard coagulation test and measures the extrinsic and intrinsic clotting pathway respectively. However, the tests were done on platelet poor plasma and therefore cannot assess the true rate of clot formation, overall clot strength, or degree of clot dissolution (Essell *et al.*, 1993).

A study by Park *et al* showed that hypercoagulopathic state is often not well represented by commonly utilized laboratory assays such as aPTT and PT. However, it can be detected via thromboelastography (TEG). Among all of the patients who had prolonged PT and aPTT but low AT and protein C, only 6% of them developed pulmonary embolism (Park *et al.*, 2009).

2.5.2 D-dimer

D-dimer is one of the screening test that was done in the evaluation of hypercoagulability state and to assess risk of VTE. Many studies had used this screening test as their tools before proceeding with diagnostic test such as Doppler ultra sound for DVT and CT pulmonary angiography (CTPA) for PE.

This test is used as an initial screening test in the emergency department to assist diagnosis in patients who have signs or symptoms suggestive of VTE. It is a marker of endogenous fibrinolysis (Wells *et al.*, 2003).

Studies by Vanfleteren and Wesseling found that in the primary care setting, D-dimer is a useful test for suspected VTE patients. Conducted at their centre in 2007 and 2008, the

diagnostic yield of VTE in patients with positive D-dimer test results was 24% and 21% respectively (Vanfleteren and Wesseling, 2011).

D-dimer test represents an excellent non-invasive triage test with high predictive value in patients with suspected VTE. The combination of both a low pretest clinical probability of disease and a negative D-dimer result can safely exclude VTE and limit the number of patients requiring further evaluation with imaging techniques (Fancher *et al.*, 2004; Tamariz *et al.*, 2004).

Based on one study, D-dimer was found to be a useful investigation to rule out DVT in post surgical patients. A normal level is helpful to eliminate DVT, but elevated level is not confirmatory for DVT. The study also found that D-dimer level will return to normal level if there is no DVT (Zamir *et al.*, 2015).

2.5.3 Fibrinogen

Fibrinogen is one of the acute phase protein. It is synthesized in the liver. In case of trauma this protein will commonly increase up to four-folds from the baseline plasma concentration. A few studies documented this increment as an associated risk of hypercoagulable state and subsequently contributing to VTE. It was reported in a study that hyperfibrinogenaemia will cause thrombosis and resist thrombolysis (Machlus *et al.*, 2011).

Study by Harr *et al* found significant increase in fibrinogen level over 5 day study period among 50 subjects (Harr *et al.*, 2014). Park *et al* also found similar finding of increased fibrinogen level post injury (Park *et al.*, 2009).

The role of fibrinogen in trauma or inflammation were mainly described as proinflammatory. In addition, it has been shown that mice lacking in fibrinogen had a delayed inflammatory response to intravenous endotoxin which suggest that physiologic concentrations of fibrinogen are involved in the initiation of inflammation (Cruz-Topete *et al.*, 2006).

2.5.4 Protein C, Protein S and Antithrombin

Based on several studies, it was established that following trauma tissue factor (TF) and markers of thrombin generations were increased while natural anticoagulants such protein C (PC), protein S (PS) and antithrombin (AT) were reduced (Selby *et al.*, 2009).

Trauma to the blood vessel will release procoagulant substances that may trigger platelet-leukocytes adherence and aggregation. This tissue factor-bearing microparticles released by trauma may trigger endothelial dysfunction to promote thrombin generation and other procoagulant processes that subsequently favour pathological thrombosis (Dahl *et al.*, 2015).

Protein C as well as protein S is a member of the family of vitamin K dependent glycoprotein. Protein C is activated by thrombin to form activated protein C (APC), and once the APC is generated, it will bind to protein S which is non-enzymatic cofactor on

the surface of activated cells. This complex will then inactivate factor Va and factor VIIIa by limited proteolysis. By degrading the activated clotting factors Va and VIIIa, APC will down regulate thrombin generation (A. Victor Hoffbrand *et al.*, 2011).

Protein S circulates as free Protein S in 40% and the other 60% is bound to C4b-binding protein (Dahlback, 2007). Only free Protein S has functional cofactor activity, and study by 2 researcher concluded that their study has an agreement for this hypothesis (Ten Kate and Van Der Meer, 2008).

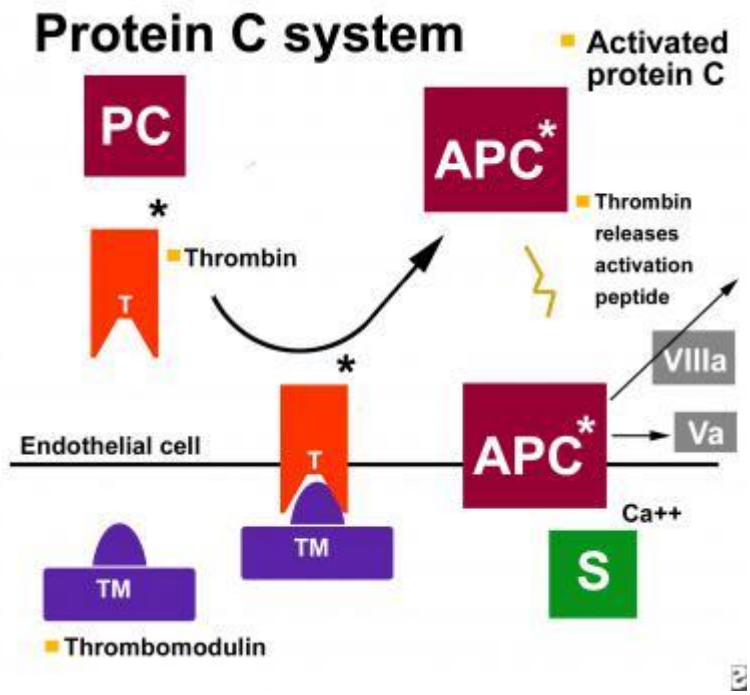

Figure 2.1: Protein C pathway (Adapted from emedicine.medscape.com)

In trauma cases, protein C was noted to be low in early trauma, whether in traumatic brain injury or non-traumatic brain injury (Genét *et al.*, 2013). Similar findings were also noted for protein S and antithrombin. This effect will induce the hypercoagulable state in trauma patients.

Inherited protein C, protein S and antithrombin deficiency are known to be associated with increased number of recurrent thrombosis. A study by Park *et al* found that antithrombin and protein C level was lowered throughout the first 7 days after injury. This may be due to increased tissue injury which contributes to hyperinflammation, thus increased consumption of these anticoagulant proteins (Park *et al.*, 2009).

Owing *et al* evaluated 157 patients who were critically injured post trauma and based on that study, 61% patients were found to have low levels of anti-thrombin. This findings were also supported by Engelman *et al* (Engelman *et al.*, 1996; Owings *et al.*, 1996).

2.6 EFFECT OF TRAUMA AND IMMOBILIZATION ON PLATELET

Platelet is one of the blood component which play a major role together with coagulation factors in haemostasis during injuries and trauma. They are fragments of megakaryocytes cytoplasm that are released from the bone marrow and thus have no nucleus.

Following a trauma or injury, this blood component will be activated for primary haemostasis and followed by secondary haemostasis. Normal platelet count is between 150-400 x 10^9 / L. When the level is beyond the range it is called thrombocytosis.

Thrombocytosis can be divided into two main causes which are primary and secondary thrombocytosis. The primary thrombocytosis is usually due to clonal problem, for example myeloproliferative neoplasm while the secondary causes are due to reactive

events for example acute bleeding, post trauma, infection and inflammations. The level of the platelet count usually will return to normal after the resolution of acute phase.

As mentioned before, thrombocytosis is one of the complications that occur following trauma. Previous study showed around 20- 27 % patient developing thrombocytosis following trauma and this thrombocytosis was one of the causes for thromboembolic event when associated with other clinical risk factors (Valade *et al.*, 2005).

The pathogenesis of this complication is due to the body response to trauma or injury. Following a trauma, the body will respond by increasing the cytokines, such as interleukin 6 (IL-6). IL-6 can promote thrombocytosis through its action on thrombopoietin (TPO) (Kaser *et al.*, 2001).

This TPO is the ligand of c-*mpl* pro-oncogene. It is also the primary regulator and promoter for proliferation and differentiation of megakaryocytes progenitor (Kaushansky, 1995). Interestingly, a study by Olson *et al.* found that platelet count was not associated with risk for VTE (Olson *et al.*, 2014).

2.7 EFFECT OF TRAUMA AND IMMOBILIZATION ON INFLAMMATORY PARAMETERS

C- reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are known as acute phase proteins which reflects the acute phase response following injury or trauma. Tissue injury, bleeding and inflammation will cause local and systemic reactions.

Local response usually involves vasodilatation, platelet aggregation and release of lysosomal enzymes. Systemic responses include fever, leukocytosis and increased in acute phase protein (Husain and Kim, 2002).

2.7.1 C-Reactive Protein

C-reactive protein was discovered in 1930 by Tillet and Francis. It was discovered in the serum of patients with pneumonia. CRP is synthesized by the hepatocytes (Husain and Kim, 2002).

The plasma levels of CRP in healthy subjects is usually 1 mg/L with normal levels being defined as < 10mg/L. CRP will increase within 4-6 hours after initial tissue injury and continue to increase several folds within 24-48 hours. It returns to normal with restoration of tissue structure.

Based on previous study, CRP has been associated with increased risk for cardiovascular disease and CRP levels also can predict future risk for development of symptomatic peripheral artery disease (Krieger *et al.*, 2004).

Apart from the impairment of endothelial function which causes the pro-inflammatory state, CRP also can directly or indirectly contribute to the pro-inflammatory state (Fichtlscherer *et al.*, 2000).

There was a strong evidence in the studies involving mouse model for a causal relationship between an inflammatory process and the development of DVT (Myers *et*

al., 2003). Olson et al reported that higher CRP level was associated with higher risk of VTE, while inflammation may be the potential mechanism underlying VTE (Olson *et al.*, 2014).

High circulating levels of pro-inflammatory adhesion molecule P-selectin were associated with increased thrombosis. P-selectin expression on endothelial cells and platelets may be increased when stimulated by cytokines, which in turn are released from monocytes after CRP stimulation (Myers *et al.*, 2003).

The link between inflammation and thrombosis in the pathogenesis of venous thrombosis was supported by study that demonstrated increased CRP levels in patient with acute DVT (Reiter *et al.*, 2003).

Two group of researchers who were Kritchevsky *et al* and Pearson *et al* also supported that inflammatory and haemostatic biomarkers were associated with cardiovascular risk factor (Kritchevsky *et al.*, 2005; Pearson *et al.*, 2003).

Study by Wang et al on DVT patients and 26 normal control showed that the mean level of plasma CRP, fibrinogen, FVIII:C, and FIX:C were significantly higher in DVT than in control groups. The level of plasma CRP was strongly correlated with fibrinogen, FVIII:C and FIX:C (Wang et al., 2010).

2.7.2 Erythrocyte Sedimentation Rate

ESR was first introduced by Westergren in 1921. The method that was introduced by Westergren, measures the rate of the gravitational settling in 1 hour of anticoagulated red blood cells (RBCs) from a fixed point in a calibrated tube of a defined length and diameter held in an upright position (Ng, 1997).

Erythrocytes usually have net negative charges and therefore repel each other. However, during trauma high molecular weight proteins that are positively charge, such as fibrinogen will have increased therefore favouring rouleaux formation and subsequently increasing the ESR. Based on that value, ESR was used as an indirect measure of the acute phase reaction (Husain and Kim, 2002).

The value of the ESR may be affected by the size/shape of the red blood cells, plasma composition, and fluid status. ESR was also affected by temperature, smoking and drugs such as non-steroidal anti-inflammatory drugs (NSAIDs).

2.8 RISK OF HYPERCOAGULABLE STATE IN PROLONGED IMMOBILIZATION POST TRAUMA

Trauma is one of the major cause to induce hypercoagulable state which can complicate and produce poor outcome for patients. Syndrome of micro-thrombosis, such as disseminated intravascular coagulopathy (DIC), acute respiratory distress syndrome (ARDS) and systemic inflammatory response syndrome (SIRS) are among the

complications that can be seen due to effect of hypercoagulable state (Selby *et al.*, 2009).

Hypercoagulable state associated with prolonged immobilization will further increase the risk of deep vein thrombosis (DVT) and pulmonary embolism (PE). These complications have long been discussed as a complicating factor in the care of trauma patients during and after hospitalization. In view of significant morbidity and high risk for mortality associated with DVT and PE, the role of prevention, early detection and treatment of these complications are very critical in the care for trauma patients (Kelsey *et al.*, 2000).

The incidence of VTE following trauma patients was reported between 5 to 69 % and traditionally, pelvic fracture, lower limb fracture, head injury and prolonged immobilization have been considered high risk factor (Toker *et al.*, 2011).

Given the potential for poor outcome of patients with VTE, and furthermore with the risk of bleeding associated with anticoagulant, correct diagnosis and management should be given when VTE is present and safely excluding it out when absence (Wells and Anderson, 2013).

Table 2.1: Risk factors associated with VTE in trauma patients

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

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

The mechanism by which trauma disturbs the haemostatic balance which can favour hypercoagulability state are stasis, vessel wall dysfunction due to injury, and alterations in the clotting mechanism. These came to be known as Virchow's triad (Figure 2.2). Even though the pathogenesis of DVT in major trauma is a highly complex and multifactorial process which involved both acquired risk factors and genetic pre-disposition, the principle of Virchow's triad remains a valid concept (Tai *et al.*, 2013).

Age more than 40 years old with obesity also contribute to VTE. Many studies were supporting these risk factors (Stein *et al.*, 2005).

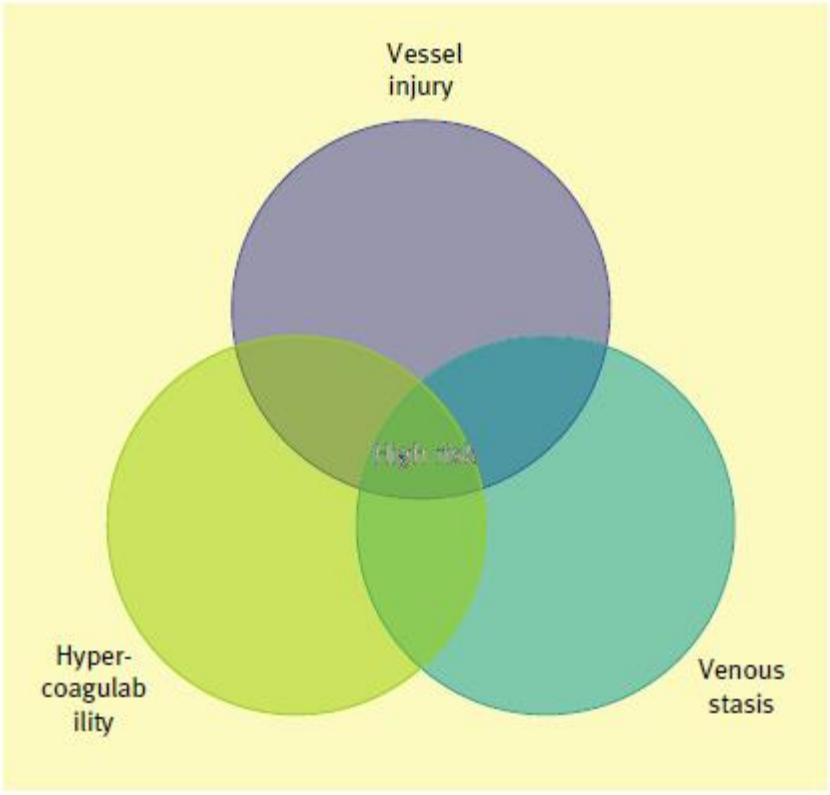

Figure 2.2: Virchow's Triad.

Trauma patients with head injury, spinal cord injury, pelvic and long bone fracture are often immobilized and this immobility renders the patients in static position, causing a reduction in venous blood return.

Stasis alone is already damaging the blood vessels, whereas the endothelial damage caused by direct trauma to the vessels will expose blood to tissue factor, collagen and von Willebrand's factor. All of these factors attract platelets and will stimulate the intrinsic and extrinsic pathways of the coagulation cascade, inducing hypercoagulable state and thrombosis (Kelsey *et al.*, 2000).

According to Meissner *et al*, VTE is associated with obesity and immobilization of more than three days. Because of this they conclude that VTE after injury is a systemic

hypercoagulable disorder with local manifestations of thrombosis related to lower extremity stasis (Meissner *et al.*, 2003).

2.9 OTHER RISK FACTORS CONTRIBUTING TO VTE

Beside older age group and obesity, smoking is another potential risk factor that contribute to VTE. Study by Cheng *et al*, showed cigarette smoking had statistically significant association with risk of VTE among the general population and they also reveal a dose relationship between smoking and VTE risk. Hence, they suggested that smoking behaviour should be considered when screening individuals for VTE and in the prevention of first and subsequent VTE events (Cheng *et al.*, 2013). Zhang et al, in their study as well also supported that smoking has contributed to VTE risk with dose-response relationship (Zhang *et al.*, 2014).

Regarding gender differences, one meta-analysis study evaluating gender differences of VTE after total hip and total knee arthroplasty involving twenty studies with 7,892,585 patients, showed female patients have slightly higher risk for VTE than male patients (Lu *et al.*, 2016). Previous study by Zhang *et al* also supported that female patients have higher risk of VTE than male patients (Zhang *et al.*, 2015).

Other risk factors that might contribute to VTE are inherited blood clotting disorders, women on oral contraceptive use, pregnancy and patients with past medical history of hypertension, Diabetes Mellitus (DM), heart problem, and history of Anti-Phospholipid Syndrome (APS).

CHAPTER 3
OBJECTIVES