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

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<td>CTPA</td>
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<td>THA</td>
<td>Total hip arthroplasty</td>
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<td>TKA</td>
<td>Total knee arthroplasty</td>
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<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor -α</td>
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<td>TPO</td>
<td>Thrombopoietin</td>
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<td>USG</td>
<td>Ultrasonography</td>
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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 berpotensi 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
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<$0.001, 95% CI = -139.71, -83.79). Hubungan positif yang ketara dilihat di antara fibrinogen dan CRP ($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 thromboembolic 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 hyperfibrinogenemia 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).

![Protein C system](image)

**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
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

<table>
<thead>
<tr>
<th>Risk factors associated with VTE in trauma patients</th>
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</thead>
<tbody>
<tr>
<td>Age ≥40 years</td>
</tr>
<tr>
<td>Pelvic fracture</td>
</tr>
<tr>
<td>Lower extremity fracture</td>
</tr>
<tr>
<td>Spinal cord injury with paralysis</td>
</tr>
<tr>
<td>Head injury (abbreviated injury score ≥3)</td>
</tr>
<tr>
<td>Ventilator days &gt;3</td>
</tr>
<tr>
<td>Venous injury</td>
</tr>
<tr>
<td>Shock on admission (BP &lt; 90 mmHg)</td>
</tr>
<tr>
<td>Major surgical procedure</td>
</tr>
</tbody>
</table>

(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 predisposition, 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
3.0 OBJECTIVES

3.1 Objectives of the study

3.1.1 General Objective

To study the association of haemostatic, inflammatory and haematological markers contributing to hypercoagulable state in prolonged immobilized trauma patients.

3.1.2 Specific Objective

- To compare selected haemostatic, inflammatory and haematological parameters among prolonged immobilization (lower limb trauma) patients on Day 1 and Day 8 of hospitalization (PT, APTT, D-dimer, Fibrinogen, AT, Protein C, Protein S, ESR, CRP and Platelet count).

- To correlate the haemostatic parameters and inflammatory parameters (ESR, CRP) in prolonged immobilization (lower limb trauma) patients

- To determine the association of clinical risk factors (age, sex, BMI, smoking and type of injury) and abnormal laboratory parameters among prolonged immobilization patients and the relationship with VTE development.
3.2 Research hypothesis

There are associations between laboratory parameters (haemostatic, haematological and inflammatory markers) and prolonged immobilization and clinical risk factors for VTE development.
3.3 Justification/rationale of the study

The true incidence of VTE post trauma immobilization and the need for prophylaxis are not yet clear in orthopaedic practice involving lower limb injuries. Many centres have started using various methods of thromboprophylaxis for trauma patients routinely. However, considering the large volume of patients seeking treatment and at risk of developing VTE, routine use of thromboprophylaxis would incur financial burden to the health system.

The aims of the present study were to evaluate the hypercoagulable markers (including haemostatic, inflammatory and haematological parameters) in prolonged immobilized trauma patients and their association with VTE development.

Local data on haemostatic, inflammatory and haematology biomarkers in prolonged immobilized trauma patients are grossly limited. Using clinical and laboratory parameters as predictors also have not been established in trauma patients.

The results of this study can be included in the local / national protocol for VTE prophylaxis for prolonged immobilization orthopaedic patients.
3.4 Benefits of the Study

1. Data on prevalence of VTE in prolonged immobilized patients could be estimated.

2. Clinical risk factors and laboratory profile associated with hypercoagulable state could be determined.

3. Correlation between hypercoagulable state with haemostasis and inflammatory markers could be determined.

4. Results of this study could aid in early planning for better management. Only selected prolonged immobilized patients should receive prophylaxis anticoagulant treatment and avoiding the low risk patients for prophylaxis treatment. Thus, preventing unnecessary cost and potential complications.

5. Findings can be incorporated into the national/ local guidelines on the anticoagulant prophylaxis for prolonged immobilized patients with high risk of VTE.
3.5 Conceptual Framework

Figure 3.1: Conceptual framework of the study
CHAPTER 4
RESEARCH
METHODOLOGY
4.0 METHODOLOGY

4.1 Study Design

A prospective cohort study from September 2016 until July 2017 on prolonged immobilization patients was conducted.

4.2 Reference Population and Study Locations

All prolonged immobilization patients in orthopaedic wards in Hospital USM from September 2016 until July 2017 that fulfilled study criteria were included. Patients age and clinical risk factors were recorded which included; gender, BMI, smoking and type of injury.

Type of injury in this study was divided into 2; single lower limb fracture (defined as fracture involving one lower limb only and no other fractures involved at other limbs) or multiple injuries (defined as patient had at least one lower limb fracture and at least one other fracture involving the other lower limb or upper limb).

4.3 Ethical clearance

This study was approved by Human Research Ethics Committee, Universiti Sains Malaysia on 5th September 2016; USM/ JEPeM/ 16030137.

Written informed consent was taken for all the subjects involved in this study.
4.4 Subjects

4.4.1 Inclusion Criteria

1. All prolonged immobilization patients due to fracture involving lower limbs (post trauma) in orthopaedic ward (4S,4U and 2Z) in Hospital USM during the study period
2. Age 12-60 years old.
3. Both gender
4. Confined to bed between 7 to 14 days post trauma with traction and not on anticoagulant prophylaxis (current clinical practice in the study institution, prophylaxis is not given to all young and healthy patients, but is given to high risk (e.g., obese, elderly etc) cases only)

4.4.2 Exclusion Criteria

1. Known case of thrombophilia and anti-phospholipid syndrome (APS)
2. Patient on prophylaxis anticoagulant and antiplatelet therapy
3. Patient with history of VTE or symptomatic patient requiring anticoagulant during the study or at presentation
4. Past medical history (PMH) of Ischaemic Heart Disease (IHD) or Diabetes Mellitus (DM).
5. Female on OCP (oral contraceptive) or pregnant
6. DIC (Disseminated intravascular coagulopathy), liver disease and sepsis
7. Complicated fractures involving spine, pelvic, brain and intraabdominal injuries.
4.5 Eligible Population

Patient who fulfilled the above criteria

4.6 Sampling Method

1. For eligible patient only; convenient sampling
2. All patients fulfilling inclusion criteria and exclusion criteria

4.7 Sample Size

4.7.1 Objective 1

To compare the selected haematological, inflammatory, haemostatic parameters among prolonged immobilization lower limb trauma patient on Day 1 and Day 8 hospitalization (PT, aPTT, D-dimer, Fibrinogen, AT, Protein C, protein S, platelet count, ESR, CRP)

It was concluded that the DVT and inflammation are closely related, increased level of plasma CRP may be a predictor of DVT. Interaction between inflammation and coagulation promotes the incidence of DVT, which may be one of the DVT pathogenesis. The results showed that the mean levels of plasma CRP were significantly higher in deep vein thrombosis group than that in controls CRP (2.67 +/- 0.91) vs (0.14 +/- 0.08) mg/dl)(Wang et al., 2010).
Using PS software
\[ \delta = 2.67 - 0.14 = 2.53 \]

A difference in population means
\[ \sigma = 0.91 \]

For independent tests \( \sigma \) is the within group standard deviation. For paired designs, it is the standard deviation of difference in the response of matched pairs.

\[ \alpha = 0.05 \text{ (level of significance)} \]

\[ P = 0.8 \]

Figure 4.1: Sample size calculation for objective 1

Drop rate: 10%

Based on sample size calculated using PS software

- \[ 3 + (0.1 \times 3) = 3 \]
- Total cases to be sampled = \( 3 \times 2 = 6 \)
4.7.2 Objective 2

To correlate the haemostatic parameters and inflammatory parameters (ESR, CRP) in prolonged immobilization patients.

Using StatsToDo software

The level of plasma CRP was strongly correlated with Fg, FVIII:C and FIX:C ($r(s) = 0.432, 0.571$ and $0.544$, $p < 0.01$). It is concluded that the DVT and inflammation are closely related, increased level of plasma CRP may be a predictor of DVT. Increased plasma levels of Fg, FVIII:C and FIX:C all are important risk factors to DVT. Interaction between inflammation and coagulation promote the incidence of DVT, which may be one of DVT pathogenesis. (Wang et al., 2010)

$r$=correlation coefficient

Figure 4.2: Sample size calculation for objective 2
4.7.3 Objective 3

To determine the association of clinical risk factors (age, sex, BMI, smoking and type of injury) and abnormal laboratory parameters in prolonged immobilization patients and the relationship with VTE development.

Calculated by PS software using two proportion formula

P0 Owings et al evaluated 157 critically injured trauma patients and demonstrated below normal AT-III activity in 61% of patients = 0.61

P1 Estimated proportion of VTE among low AT III =0.34

Power of study = 0.8

Level of significant α= 0.05

Figure 4.3: Sample size calculation for objective 3
• Drop rate: 10%
• Based on sample size calculated using PS software
• $53 + (0.1 \times 53) = 58$
• Total cases to be sampled = $58 \times 2 = 116$

4.7.4 Sample size chosen

Ideally, the highest number of sample size calculation should be selected for the study. Therefore, sample size of 116 patients were chosen from objective 3.

4.8 Sample collection and storage

Patients who were eligible were recruited and consents were obtained at day 1 of admission. Blood was collected within 24 hours following admission for day 1 sample. The samples were collected into 1 EDTA (ethylene diamine tetra acetic acid) bottle (3mls), 1 plain bottle (3mls) and 3 trisodium citrate bottles (2.7mls each bottle). The second sample for each patient was collected on day 8 of immobilization.

The samples from citrated bottles were centrifuged for 15 minutes at 2500g at room temperature to obtained PPP (platelet poor plasma). The PPP was separated from the whole blood. Two out of three of the citrated plasma was divided into aliquots in multiple tubes and immediately stored at -80°C.

A bottle of citrated plasma was immediately tested for PT, aPTT, fibrinogen levels and D-dimer. These samples were run within 2 to 4 hours from the time of venepuncture.
Other remaining PPP stored was used for further analysis of protein C, free protein S and antithrombin which were run in batches. Prior to the analysis, frozen PPP was thawed in 37°C water bath for 10 minutes and capped, to obtain a complete thawing. The samples were mixed thoroughly for at least 5 times inversion to ensure no cryoprecipitate residual that could lead to results error. This thawing procedure followed the Clinical and Laboratory Standard Institute (CSLI) guideline for coagulation test.

The samples from EDTA tube were analysed immediately for full blood count to get platelet count and ESR. FBC was done by automation and ESR by semi-automation or can be manual.

Samples from plain tube which was tested for CRP, fresh serum or samples that were stored at +2°C to +8°C for no longer than 48 hours was used. If the assay is required to be done after this period, the samples were frozen until analysed. Then, the frozen samples should be totally thawed and brought to room temperature before testing. Any haemolyzed or contaminated sera need to be discarded.

All the blood specimen collection, storage and transport followed the Clinical Laboratory Standard Institute – CLSI guidelines, Practical Haematology by Dacie and Lewis and manufacturer insert kits guide.
4.9 Laboratory Methods

Laboratory tests were performed on all samples which included:

- Full blood count (FBC)
- Erythrocyte sedimentation rate (ESR)
- C-reactive protein (CRP)
- Prothrombin time (PT)
- Activated partial thromboplastin time (aPTT)
- Fibrinogen
- D-dimer
- Protein C
- Free Protein S
- Antithrombin

Full blood count was performed on automation; XN series and ESR was performed on semi-automated instrument.

C-reactive protein was performed manually using commercial latex test kit CRP direct Latex Veda Lab France. If CRP was positive, the test was performed on semi-automated analyzer for quantitative results.

All the haemostatic tests were performed on automation STA-R Evolution by Stago (Figure 4.4)
Pre-analytical requirements are very important in laboratory assessment and test for haemostatic and coagulation system. Clinical and Laboratory Standards Institute (CSLI) has already recommended that specimen for PT should be analysed within 24 hrs and within 4 hours for aPTT and other coagulation assays if stored at room temperature (25°C).

All methods discussed followed the laboratory Standard Operation Procedure (SOP) and manufacturer insert kit instructions and guides.
4.9.1 Full Blood Count (FBC)

Principle

The principle of the test is based on Hydro-Dynamic Focusing Method (Direct Current: Sheath Flow DC detection Method) – RBC/PLT Channel. The RBC detector count the RBC and platelet and at same time haematocrit is calculated via RBC pulse height detection method (Figure 4.5).

Inside the detector, the sample nozzle was positioned in front of the aperture and in line with the center. After dilution sample was forced from the nozzle into the conical chamber, surrounded by front sheath reagent and passed through the aperture centre. Then, the sample was sent to catcher tube. This prevented the blood cells in this area from drifting back and prevents generation of false platelet and abnormal blood cells pulses.

Figure 4.5: Schematic representation of Hydrodynamic Focusing System for analysis of RBCs and platelet. Adapted from Sysmex (Ghys et al., 2009).
**Reagents and equipments**

1. Equipments-
   a. Analyzer XN-1000 (Figure 4.6)

   ![Automated analyzer: XN-1000](image)

   **Figure 4.6:** Automated analyzer: XN-1000

   b. Pneumatic Unit
   c. Information Processing Unit
   d. Auto sampler with built in barcode reader

2. Reagents

3. Specimen- Human venous blood sample in K₂ EDTA 2ml/3ml

**Methods**

The XN analyser is a 6-part differential analyser which able to measures up to 43 parameters for whole blood and 7 parameters for body fluid. Automated FBC in this study was performed based on Sheath Flow/ Hydrodynamic Focusing Direct Current
(DC) detection (RBC/PLT Channel). XN series automatically classify cells from the whole blood and carry out processes automatically from aspiration of the sample to outputting the results.

4.9.2 Erythrocyte Sedimentation Rate (ESR)

Principle

The Erythrocyte Sedimentation Rate (ESR) measures the settling of erythrocytes in human plasma over specified time. The reported value was derived from measuring, in milimeters the distance from the bottom of the surface meniscus to the top of erythrocyte sediment in column of anticoagulated blood that has remained in Westergren tube for 1 hour.

Reagents and equipments

1. Disposable plastic transfer pipet
2. Mixrate-X20 ESR analyser (Figure 4.7)

Figure 4.7: Semi-automated analyzer: Mixrate-X20 ESR
3. 3.8% sodium citrate ESR tube

4. Precision-rate @ ESR control

5. Blood sample in K₂ EDTA 2ml/3ml container.

**Methods**

Samples were collected in EDTA tube and diluted accurately in the proportion of 1 volume citrate to 4 volumes of blood or may collect directly into citrate solution. The test should be carried out on the diluted sample within 4 hours of collection or can be up to 6 hours if kept at 4°C.

Samples in the EDTA tube can be used within 24 hours if the specimen is kept at 4°C, provided that 1 volume of trisodium citrate is added to 4 volumes of blood immediately before the test is performed.

The samples must be mixed thoroughly and then draw it into Westergren tube to the 200 milimeter (mm) mark by means of a teat or mechanical device. The tube was place vertically and leave undisturbed for exactly 60 minutes, free from vibrations and draughts and not exposed to direct sunlight. The test was read to the nearest 1 mm the height of the clear plasma above the upper limit of the column of the sedimenting cells. The results is expressed as ESR = X mm in 1 hour.(Bain et al., 2012).
4.9.3 C-Reactive Protein (CRP)

**Principle**

C-Reactive protein is a globulin, also known as acute phase protein which increases in inflammatory processes and it is used as an inflammatory marker. It can be raised up to 300mg in 12-24 hours post inflammatory processes.

**Reagents and equipments**

1. Latex reagent vial (Latex particles coated with goat IgG anti-human CRP, containing 0.095% sodium azide
2. Positive control vial
3. Negative control vial
4. Glass slide
5. Disposable stirrers
6. Instruction leaflet
7. QuikRead go analyzer (Figure 4.8)

![Figure 4.8: Semi-automated analyzer: QuikRead go](image)

45
Methods

Reagents and serum samples need to be put under room temperature. One drop of undiluted sample was placed onto a slide black area. One drop of positive control and one drop of negative control was added in separate circles (Figure 4.8).

The CRP-latex reagent need to be swirled gently before use and one drop of the reagent need to be added next to negative and one drop next to the positive controls. The drops were mixed with stirrer and spread them over the entire surface of the circle. Each sample was using different stirrer. Presence or absence of agglutination were observed within a period not longer than 3 minutes.

![Figure 4.9: Positive and negative control for CRP](image)

If there was agglutination, the sample was then proceed to QuikRead go machine (semi-automated analyzer) for quantification. Twelve μl serum were added into a cuvette together with CRP reagent and subsequently, the cuvette was inserted in the Measurement Well and the result will be displayed on the screen.
4.9.4 Prothrombin time (PT)

**Principle**

Prothrombin time (PT) is a screening test to explore the extrinsic and common coagulation pathway (coagulation factors II, V, VII, X and fibrinogen). The principle consists of the use of calcium thromboplastin to measure the clotting time of patient’s plasma and to compare it with that of a normal standard. Tissue factor reacts with FVIIa to activate the extrinsic pathway and thus will form a clot. Increasing factor VII activity due to cold activation during storage will cause a shortening of PT or aPTT. Samples for PT test are stable for 24 hours or longer.

**Reagents and equipment**

1. STA compact or STA-R Evolution/ STA-R Max
2. Centrifuge
3. Micropipettes
4. Pipette tips
5. Patient and control plasma samples: All most all routine coagulation investigation or test are performed on platelet-poor plasma (PPP), which is prepared by centrifugation at 2000g to 2500g for 15 minutes.
6. Thromboplastin: Nowadays recombinant thromboplastin has been used. They are manufactured using recombinant human tissue factor produced in Escherichia coli and synthetic phospholipids, which do not contain any other clotting factors such as prothrombin, factor VII and factor X.
7. CaCl₂: 0.025ml/l

**Methods**

0.1 ml of control plasma was placed into the test tube and warmed in the waterbath (37°C) for 2 minutes. Then 0.2 ml of thromboplastin reagent pre-warmed to 37°C were added and waited for 1 to 3 minutes to allow the mixture to warm. The thromboplastin used was containing calcium chloride-25mM. The contents of the tube were mixed and the endpoint was recorded. The procedure was repeated for the test sample.

Quality control: Controls was run to ensure accuracy and reproducibility of the results. Two different levels of control were used.

**Reference range**

Normal range PT: 12.6 – 15.7 secs (Normal reference range of Haematology Laboratory Hospital USM during the study period).
4.9.5 Activated partial thromboplastin time (aPTT)

**Principle**

Activated Partial Thromboplastin Time (aPTT) is a screening test to explore the intrinsic and common coagulation pathways (coagulation factor XII, XI, IX, VIII, X, V, II and fibrinogen).

The principle involves the recalcification of plasma in the presence of a standardized amount of cephalin (platelet substitute) and a particular activator (silica). This procedure minimizes test variables by standardizing the contact activation and optimizes the concentration of platelet-like phospholipids.

**Reagents and equipment**

1. STA compact or STA-R Evolution/ STA-R Max
2. Centrifuge
3. Micropipettes
4. Pipette tips
5. Patient and control plasma samples: Using platelet-poor plasma (PPP); this is prepared by centrifugation at 2000g to 2500g for 15 minutes.
6. Koalin: 5 g/l (laboratory grade) in barbitone buffered saline, pH 7.4. A few glass beads were added to aid resuspension. The suspension was stable at the room temperature. Other insoluble surface active substances such silica, celite or ellagic acid can also be used.
7. Phospholipid: Many reagents were available; these contained different contents. When choosing a reagent for aPTT, it is important to establish that reagent was sensitive to deficiencies of factors VIII, IX and XI at concentrations of 0.35-0.4 IU/ml. Reagents which fail to detect at this level were insensitive for routine use.

8. CaCl₂: 0.025mol/l

Methods

The phospholipid reagent and the kaolin suspension were mixed with equal volumes and then it was left in glass tube in the waterbath at 37°C. Subsequently, 0.1 ml of plasma was placed into a second glass tube with 0.2 ml of kaolin-phospholipid solution was added to the plasma and then mixed. The stopwatch was started simultaneously with the mixture was left at 37°C for 5-10 minutes with occasional shaking. At 5-10 minutes, 0.1 ml prewarmed CaCl₂ was added and a second stopwatch was started. The times taken for the mixtures to clot were recorded.

Quality Control: Controls were run to ensure accuracy and reproducibility of the results. Two different levels of control were used.

Reference range

Normal range aPTT: 30.0 – 45.8 secs (Normal reference range of Haematology Laboratory Hospital USM during the study period).
4.9.6 Fibrinogen

Principle

Fibrinogen assay is a quantitative determination of fibrinogen level in plasma by the clotting method of Clauss. In Clauss technique, diluted plasma was clotted with a strong thrombin solution; the plasma must be diluted to give a low level of any inhibitors (e.g. FDPs and heparin). A strong thrombin solution must be used so that the clotting time over a wide range is independent of thrombin concentration.

Reagents and equipments

1. STA – liquid Fib
2. STA-R evolution
3. Centrifuge
4. Micropippetes
5. Pipette tips
6. STA- Owren Koller buffer
7. Thrombin solution: Freshly reconstituted to 100 NIH u per ml in 9 g/l NaCl
8. Patient and control plasma samples: Using platelet-poor plasma (PPP); this was prepared by centrifugation at 2000g to 2500g for 15 minutes.
Methods

The clotting time in seconds against the fibrinogen concentration in g/l was plotted on log/log graph paper. In this study, quantitative measurement of fibrinogen was done by automation; Stago STA-R Evolution.

Patients’ plasmas were tested undiluted. The instrument automatically prepares the dilutions in Owren- Koller buffer.

The fibrinogen assay of the plasma to be tested was automatically carried out by the analyser as soon as the samples have been loaded. If any of the patient’s results falls outside the working range of the assay, the instrument automatically retests the sample in question at the appropriate dilution.

Quality Control: Two different levels of control were used to ensure the accuracy and reproducibility. The controls were used undiluted.

Reference range

Normal range: 2.32-4.44 g/l (Normal reference range of Haematology Laboratory Hospital USM during the study period).
4.9.7 D-dimer

**Principle**

The assay is quantitative determination by the immune-turbidimetric method. It is based on the change in turbidity of the microparticles suspension that is measured by photometry at 540nm.

A suspension of latex microparticles, coated by covalent bonding with monoclonal antibodies specific for D-dimer, is mixed with the test plasma whose D-dimer level is to be assayed. An antigen-antibody reaction takes place, leading to an agglutination of the latex microparticles that induces an increase in turbidity of the reaction medium. The increased in turbidity is reflected by an increase in absorbance which latter being measured photometrically. The increase in absorbance is a function of the D-dimer level present in the sample test.

**Reagents and equipment**

1. Tris buffer
2. Suspension of microlatex particles coated with 2 different mouse monoclonal anti-human D-dimer antibodies then stabilized with bovine albumin. These reagents containing sodium azide (<1 g/l) as a preservative.
Methods

The D-dimer assay of the plasma to be tested was automatically carried out by analyser. The detection of latex immunoassays is based on the absorbance (optical density: O.D.) of monochromatic light at 540nm. An antigen-antibody reaction takes place respectively when monochromatic light passing through the cuvette. If any of the patient results falls outside the working range of the assay, the instruments automatically retests the sample in question at an appropriate dilution.

Quality control: Two different levels of control are used in order to ensure accuracy and reproducibility of the result. The controls are used undiluted.

Normal range

A level of < 0.5 μg/ml as normal range (Normal reference range of Haematology Laboratory Hospital USM during the study period).

4.9.8 Protein C Assay

Principle

Protein C is a vitamin K dependent protein and present in the plasma as a zymogen. Protein C is activated in vivo by thrombin in the presence of thrombomodulin. Protein C
can be activated in vitro by a protein fraction derived from the venom of the copperhead snake.

The Protein C kit is a Protein C assay based on a synthetic chromogenic substrate. The quantification of protein C in patients citrated plasma were done by automated chromogenic assay.

**Reagents**

1. Diluent: concentrated solution containing 0.9% sodium chloride with preservative.
2. Protein C activator: lyophilized fraction from the venom of *Agkistrodon c. contortrix*, buffer, bovine serum albumin and preservative.
3. Chromogenic substrate: lyophilized chromogenic substrate, synthetic thrombin inhibitor and bulking agent

**Methods**

The protein C level in patient plasma was measured automatically in two stages:

1. Incubation of the plasma with Protein C activator.
2. Quantification of activated Protein C with a synthetic chromogenic substrate.

   The paranitroaniline released was monitored kinetically at 405nm and was directly propotional to the Protein C level in the test sample.
Quality control: Two different levels of control are used in order to ensure accuracy and reproducibility of the result.

**Normal range**

A level of 70-140% for male and 67-155% for female (Normal reference range of Haematology Laboratory Hospital USM during the study period).

**4.9.9 Free Protein S Assay**

**Principle**

Protein S is a vitamin K-dependent cofactor for the anticoagulant and the profibrinolytic activity effects of activated Protein C. Two forms of Protein S are present in the plasma: free Protein S (40%) and Protein S linked to the complement C4b-binding protein (C4BP)(60%). Only free Protein S has functional cofactor activity.

Protein S deficiency may be hereditary or acquired. Deficiency of Protein S has been associated with high risk of developing VTE in young people.

The presence of free protein S is determined by measuring the increase of turbidity produced by agglutination of two latex reagents. The quantification of free protein S in patients citrated plasma were done by automated latex ligand immunoassay.
Reagents

1. C4BP buffer containing bovine serum albumin, stabilizers and preservative.
2. C4BP latex; lyophilized suspension of polystyrene latex particles coated with purified human C4BP containing bovine serum albumin, stabilizers and preservative.
3. Anti PS Mab latex; a suspension of polystyrene latex particles coated with a monoclonal antibody directed against human Protein S containing bovine serum albumin, stabilizers and preservative.

Methods

The presence of free Protein S was determined by measuring the increase of turbidity produced by the agglutination of two latex reagents.

Purified C4BP adsorbed onto the first latex reagents react with high affinity for free Protein S of patient plasma in the presence of Ca²⁺ ions. The free protein S adsorbed on the C4BP latex triggers agglutination reaction with the second latex reagent which is sensitized with a monoclonal antibody directed against human Protein S. The degree of agglutination will be directly proportional to the free Protein S concentration in the test sample.

Quality control: Two different levels of control are used in order to ensure accuracy and reproducibility of the result.
Reference range

A level of 72.2-123.3% for male and 57.6-112.5% for female (Normal reference range of Haematology Laboratory Hospital USM during the study period).

4.9.10 Antithrombin Assay

Principle

Antithrombin (AT) or Heparin Cofactor I is the major inhibitor of blood coagulation and is essential for effective heparin therapy. By inhibiting the coagulation proteases, especially thrombin, FXa, FIIa, AT prevents uncontrolled coagulation and thrombosis.

The Liquid Antithrombin kit is an assay based on a synthetic chromogenic substrate and Cofactor Xa inactivation. Therefore, the method is specific and not influenced by Heparin Cofactor II. The quantification of antithrombin in patients citrated plasma were done by automated chromogenic assay.

Reagents

1. Chromogenic substrate: Chromogenic substrate, surfactant and buffer
2. Factor Xa reagent: Solution containing bovine Factor Xa, heparin, buffer, bovine serum and preservatives
Methods

Antithrombin levels in patient plasma are measured automatically in two stages:

1. Incubation of plasma with the Factor Xa reagent in the presence of an excess of heparin.
2. Quantification of the residual Factor Xa activity with a synthethic chromogenic substrate. The paranitroaniline released was monitored kinetically at 405nm and inversely proportional to the antithrombin level in the test sample.

Quality control: Two different levels of control are used in order to ensure accuracy and reproducibility of the result.

Reference range

A level of 83-126% (Normal reference range of Haematology Laboratory Hospital USM during the study period).
4.10 Statistical analysis

Data were recorded and analysed by using the statistical package programme SPSS version 22.

Statistical comparison was done by paired t-test, to determine the mean difference of haematological, inflammatory and haemostatic biomarkers changes between day 1 and day 8.

Correlation between haemostatic and inflammatory parameters was done by using Pearson correlation analysis.

Simple linear regression was conducted to determine the association of clinical risk factors and abnormal parameters among prolonged immobilized patients and multiple linear regression were applied for selected clinical risk factors in order to determine the clinical risk factors that best predict the abnormal markers and possible its relationship to VTE. The interaction, multicollinearity and assumption were analysed stepwise.
4.11 Flowchart of the study

Immobilized orthopaedic patients who fulfilled the inclusion criteria

Blood taking at Day 1 for haematology*, haemostatic** & inflammatory*** markers

- Negative D-dimer
  - No DVT/PE until Day 8

- Positive D-dimer but no symptom or sign
  - Confirmed DVT/PE before Day 8

- Positive D-dimer with symptom or sign
  - Doppler ultrasound /CTPA for confirmation
    - no DVT/PE
    - Confirmed DVT/PE

Blood Taking at Day 8 for haematology*, haemostatic** & inflammatory*** markers

- Negative D-dimer
  - Data analysis

- Positive D-dimer & no symptom or sign

- Positive D-dimer with symptom or sign
  - Doppler ultrasound /CTPA for confirmation
    - +/- DVT/PE

Data analysis

*haematology markers: FBC
**haemostatic markers: PT, APTT, D-dimer, fibrinogen, Protein C, Protein S, AT
***inflammatory markers: ESR, CRP

Figure 4.10: Flowchart of the study
CHAPTER 5
RESULTS
5.0 RESULTS

This is a prospective cohort study conducted at Hospital University Sains Malaysia (USM) from September 2016 to July 2017. During the study period, all lower limb/s trauma patients were screened for eligibility and excluded based on the criteria stated before. A total of 52 patients with lower limb/s fracture who required immobilization more than 7 days and received no anticoagulant prophylaxis were involved.

Several laboratory parameters (haematological, inflammatory, and haemostatic) were measured serially on Day 1 and Day 8 post lower limb/s trauma.

5.1 Characteristics of Patients

The study consisted of 52 trauma patients who met the inclusion criteria. Majority of the patients were males which contributed around 75.0% (n=39) of patients and only 25.0% (n=13) were females (Figure 5.1). The predominant major injury was single lower limb fracture which contributed 61.5% (n=32) as compared to patients with multiple injuries 38.5% (n=20) (Figure 5.2). Multiple injuries were defined as at least one lower limb fracture and at least one other fracture involving the other lower limb or upper limb. The age distribution ranged from 12 to 59 years old. Majority of the patients who were involved in this study aged between 20-40 years old which contributed around 51.9%. Out of all patients involved in this study, 9.6% (n=5) patients were obese and 42.3% patients were smoker. Further details of patients’ characteristics were summarized in Table 5.1.
Interestingly, none of the patients involved developed VTE during study period. However, 2 patients were suspected to have Fat Embolism Syndrome during second and third day of immobilization. Thus, they were excluded from the study.

![Gender distributions](image1)

**Figure 5.1:** Gender distributions

![Type of injury involved](image2)

**Figure 5.2:** Type of injury involved
Table 5.1 Characteristics of patients (n=52)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 20</td>
<td>19</td>
<td>36.5</td>
</tr>
<tr>
<td>20 – 40</td>
<td>27</td>
<td>51.9</td>
</tr>
<tr>
<td>More than 40</td>
<td>6</td>
<td>11.5</td>
</tr>
<tr>
<td>BMI (kg/m)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 25</td>
<td>36</td>
<td>69.0</td>
</tr>
<tr>
<td>25 – 30</td>
<td>11</td>
<td>21.2</td>
</tr>
<tr>
<td>More than 30</td>
<td>5</td>
<td>9.6</td>
</tr>
<tr>
<td>Smoke</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoking</td>
<td>30</td>
<td>57.7</td>
</tr>
<tr>
<td>Smoking</td>
<td>22</td>
<td>42.3</td>
</tr>
</tbody>
</table>

5.2 Haemostatic, inflammatory and haematological changes between Day 1 and Day 8 of immobilization

The targeted haemostatic, inflammatory and haematological parameters were measured serially on day 1 and day 8. The involved factors were then analysed using paired t test, with p value < 0.05 was considered significant.

Among the haemostatic, inflammatory and haematological parameters studied, only fibrinogen, ESR and platelet gave significant mean difference between day 1 and day 8 of immobilization (Table 5.2). The mean fibrinogen was higher in day 8 compared to day 1 with the mean fibrinogen was increased by 1.33 in day 8 of immobilization (p<0.001, 95% CI of mean difference: -1.91, -0.76). The mean ESR was increased by 28.50 in day 8 of immobilization (p<0.001, 95% CI of mean difference: -36.94, -20.06),
while the mean platelet was increased by 111.75 in day 8 of immobilization ($p<0.001$, 95% CI of mean difference: -139.71, -83.79).

Even though D-dimer level was noted to be increased at day 1 post trauma in most of patients and remained increased at day 8, however it was not statistically significant. Two male patients were noted to have significantly high D-dimer at day 1. The first patient had measurement of more than 20 μg/ml while the other patient had 11.04 μg/ml at Day 1 post trauma. The levels were persistently elevated even at Day 8 post trauma and immobilization. Both patients had no signs or symptoms of VTE. Both of them had one lower limb fracture and other fracture elsewhere. One of them was less than 20 years old and the other one was 28 years old.

Other parameters also did not show any significant changes in patients with prolonged immobilization.

### Table 5.2

<table>
<thead>
<tr>
<th>Variable</th>
<th>D1 Mean (SD)</th>
<th>D8 Mean (SD)</th>
<th>Mean difference (95% CI)</th>
<th>t statistic (df)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>14.37</td>
<td>14.29</td>
<td>0.07 (-0.21, 0.36)</td>
<td>0.51 (51)</td>
<td>0.614</td>
</tr>
<tr>
<td>APTt</td>
<td>38.61</td>
<td>39.78</td>
<td>-1.17 (-2.95, 0.61)</td>
<td>-1.32 (51)</td>
<td>0.194</td>
</tr>
<tr>
<td>D-dimer</td>
<td>2.96</td>
<td>3.24</td>
<td>-0.27 (-0.95, 0.41)</td>
<td>-0.80 (51)</td>
<td>0.431</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>4.38</td>
<td>5.71</td>
<td>-1.33 (-1.91, -0.76)</td>
<td>-4.65 (51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>108.40</td>
<td>113.00</td>
<td>-4.59 (-10.78, 1.60)</td>
<td>-1.49 (51)</td>
<td>0.142</td>
</tr>
<tr>
<td>Protein C</td>
<td>105.78</td>
<td>112.16</td>
<td>-6.37 (-16.40, 3.66)</td>
<td>-1.28 (51)</td>
<td>0.208</td>
</tr>
<tr>
<td>Protein S</td>
<td>73.87</td>
<td>74.06</td>
<td>-0.19 (-7.15, 6.78)</td>
<td>-0.05 (51)</td>
<td>0.958</td>
</tr>
<tr>
<td>CRP</td>
<td>71.00</td>
<td>66.42</td>
<td>4.58 (-15.59, 24.74)</td>
<td>0.46 (51)</td>
<td>0.651</td>
</tr>
<tr>
<td>ESR</td>
<td>26.88</td>
<td>55.38</td>
<td>-28.50 (-36.94, -20.06)</td>
<td>-6.78 (51)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>platelet</td>
<td>259.92</td>
<td>371.67</td>
<td>-111.75 (-139.71, -83.79)</td>
<td>-8.02 (51)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Paired t-test was applied
5.3 Correlation between haemostatic parameters and inflammatory parameters (ESR, CRP) in prolonged immobilization patients

There was significant correlation between fibrinogen and both inflammatory parameters. Meanwhile, other haemostatic parameters showed poor correlation with either CRP or ESR (Table 5.3).

There was a fair, positive correlation between fibrinogen and CRP (Pearson correlation=0.35, p-value = 0.012), which suggests increased in fibrinogen correlated with the increased in CRP. At the same time, there was also a positive relationship and moderate correlation between fibrinogen and ESR (Pearson correlation=0.54, p-value <0.001), which suggests increased in fibrinogen also correlated to the increase in ESR levels.

As a conclusion, there were statistically significant positive correlation between fibrinogen and CRP and between fibrinogen and ESR. We observed that when fibrinogen was increased the CRP and ESR were increased as well.

Table 5.3 Descriptive statistics and correlations between haemostatic and inflammatory parameters in prolonged immobilization patients (n=52)

<table>
<thead>
<tr>
<th>Haemostatic</th>
<th>Mean</th>
<th>SD</th>
<th>Inflammatory</th>
<th>CRP</th>
<th>ESR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pearson</td>
<td>p-value</td>
<td>Pearson</td>
</tr>
<tr>
<td>PT</td>
<td>-0.07</td>
<td>1.04</td>
<td>0.254</td>
<td>0.069</td>
<td>-0.118</td>
</tr>
<tr>
<td>APTt</td>
<td>1.17</td>
<td>6.40</td>
<td>0.020</td>
<td>0.890</td>
<td>0.223</td>
</tr>
<tr>
<td>D-dimer</td>
<td>0.27</td>
<td>2.45</td>
<td>0.073</td>
<td>0.606</td>
<td>0.015</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>1.33</td>
<td>2.07</td>
<td>0.347</td>
<td>0.012</td>
<td>0.536</td>
</tr>
<tr>
<td>Antithrombin</td>
<td>4.59</td>
<td>22.23</td>
<td>0.128</td>
<td>0.365</td>
<td>-0.042</td>
</tr>
<tr>
<td>Protein C</td>
<td>6.37</td>
<td>36.03</td>
<td>-0.050</td>
<td>0.723</td>
<td>0.032</td>
</tr>
<tr>
<td>Protein S</td>
<td>0.19</td>
<td>25.03</td>
<td>0.098</td>
<td>0.490</td>
<td>0.053</td>
</tr>
</tbody>
</table>
5.4 Relationship between clinical risk factors (age, sex, BMI, smoking, type of injury) and abnormal laboratory parameters in prolonged immobilization patients and its relation to VTE

Simple linear regression and multiple linear regression were done to determine the relationship between the clinical risk factors (age, gender, BMI, smoking and type of injury) and abnormal laboratory parameters (fibrinogen, ESR and platelet). For fibrinogen and ESR, there were no significant linear relationships between both parameters and all the associated risk factors (Table 5.4 and Table 5.5). This showed that there was no association between clinical risk factors and these parameters (fibrinogen and ESR).

However, for platelet level, in simple linear regression, there was a significant linear relationship of platelet with age, BMI and type of injury (p-value<0.05). However, using multiple linear regression, only two clinical risk factors (BMI and types of injury) were found to have significant linear relationship with platelet count (Table 5.6).

As a conclusion, BMI more than 31 kg/m² (obese patients) with multiple injuries (fractures) showed increased platelet count.
Table 5.4  Associated clinical risk factor and fibrinogen in prolonged immobilization patients by Simple linear regression (n=52)

<table>
<thead>
<tr>
<th>Variable</th>
<th>( b^a ) (95% CI)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 20</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>20 – 40</td>
<td>0.45 (-0.81, 1.71)</td>
<td>0.479</td>
</tr>
<tr>
<td>More than 40</td>
<td>-0.11 (-2.08, 1.86)</td>
<td>0.910</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>-1.27 (-2.56, 0.02)</td>
<td>0.054</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 25</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>25 – 30</td>
<td>-0.39 (-1.84, 1.06)</td>
<td>0.592</td>
</tr>
<tr>
<td>More than 30</td>
<td>-0.21 (-2.23, 1.80)</td>
<td>0.834</td>
</tr>
<tr>
<td>Smoke</td>
<td></td>
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<tr>
<td>Non-smoking</td>
<td>0</td>
<td></td>
</tr>
<tr>
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<tr>
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Note: \( a = \) crude regression coefficient
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<tr>
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<th>p-value</th>
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Note: a = crude regression coefficient
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Note: a = crude regression coefficient; b = adjusted regression coefficient
Forward Linear Regression model was applied (R^2=0.20). The model reasonably fits well and model assumptions are met. Multicollinearity and interaction term are checked and not found.
CHAPTER 6
DISCUSSION
6.0 DISCUSSION

This prospective study sequentially compared haemostatic, inflammatory and haematological parameters in post lower limb trauma patients and evaluated their relationship after prolonged immobilization and possible risk of VTE. These parameters were measured at day 1 and day 8 immobilization period.

None of the patient in this study developed sign and symptom of VTE. Among the sampled patients, two of them were suspected to develop Fat Embolism Syndrome. They developed symptom of drowsiness and reduced in oxygen saturation at day 2 and day 3 post trauma respectively. Both patients were excluded from the study.

This study assessed several laboratory parameters which were potential for biomarkers that could contribute to post trauma hypercoagulable state and VTE development. By definition, a biomarker is a measurable substance that could detect the presence of certain disease state or determine the activity of a particular disease. In general, a biomarker is an indicator of the presence of a disease although it may be detected in the physiological state but is altered as a result of the disease process. Hence its use is to detect the disease by using a specific biomarker (e.g., tumour marker) or disease activity by measuring the levels (Abdullah, 2015). The latter could be a non-disease specific marker and in this study the laboratory changes of the laboratory parameters could be used as a biomarker for assessing the risk of VTE development. The fact that all the participating patients were not on anticoagulant provided a good opportunity to study the involved biomarkers. The measured haematological parameter was platelet count.
For inflammatory parameters, CRP and ESR were measured. While for haemostatic parameters, PT, aPTT, fibrinogen, D-dimer, antithrombin, protein C and protein S were evaluated.

6.1 Characteristic and demographic pattern of patients.

In this study, all the patients were previously healthy with no previous medical illness, not on anticoagulant and for female, they were not on oral contraceptive or hormonal therapy. These exclusion criteria were made to avoid the risk factors which may contribute to abnormal markers that were assessed in the study.

6.1.1 Age factor

Increasing age was one of the important factor for VTE even though majority of patients in this study consisted of predominantly young patients (88.5%). Only six patients which contributed around 11.5% were more than 40 years old.

According to a previous study, patients aged more than 40 years old were at risk of VTE (Stein et al., 2005), while few other studies reported that the incidence rate for VTE were markedly increased with age in both men and women (NÆSs et al., 2007; Silverstein et al., 1998; Spencer et al., 2006). Incidences were also increased in men whose age were more than 45 years old (Heit, 2015).
Possible mechanisms for higher incidence rate of VTE with increased age may be due to cumulative effects of VTE risk factors as one grew older. For example, decreased regular exercise, increasing immobility and increasing systemic activation of blood coagulation that occurred in older people may predispose to hypercoagulable state (Lowe et al., 1997). In fact, one study found that certain coagulation factors such as factor V, VII, VIII, IX and fibrinogen increase progressively with age (Franchini, 2006).

The fact that none of our studied patients were found to have VTE was could be due to 88.5% of them aged less than 40 years old.

This study may indicate that decision for prophylaxis in young patients with low risk of VTE is not warranted. However more data to support is required and current practice is considered acceptable from this study.

6.1.2 Gender

Gender is an associated risk factor for VTE. In our study, majority of the involved patients were male which contributed around 75% (n=39) and only 25% (n=13) were female.

Study by Heit in 2015 mentioned that the incidences of VTE were higher in women of childbearing age (16-44 years) compared with men at similar age. This is most likely because of hormones during pregnancy or due to use of oral contraceptive pill in that
age. Pregnancy and oral contraceptive pill usage are associated with haemostatic changes that include increased concentrations of most procoagulant factors (fibrinogen, V, VII, VIII, IX, X and XII), decreased concentrations of the natural anticoagulants (protein S and antithrombin) and reduced fibrinolytic activity (Previtali et al., 2011).

A recent meta-analysis by Lu et al in 2016 to evaluate gender differences for risk of VTE after total hip arthroplasty (THA) and total knee arthroplasty (TKA) showed female patients have slightly higher risk of VTE than male patients after THA and TKA. Female patients were at risk due to they always have thick layer of fat and at the same time female patients were prone to immobilize after surgery (Lu et al., 2016). Previously, a study by Miyagi et al found that male had higher risk of VTE after TKA (Miyagi et al., 2007), while Mraovic et al found that sex was not a significant risk factor for VTE in post THA and TKA (Mraovic et al., 2010).

However, from these 52 patients involved, none of them developed sign or symptoms of VTE.

### 6.1.3 BMI

In this study, we categorized the patients into 3 categories; normal BMI, overweight and obese patients. For the normal BMI category, there were 36 patients (69%), while 11 patients (21.2%) were overweight and only 5 patients were obese (9.6%).
A study by Stein et al found that obesity was an associated risk factor for VTE in patients who were less than 40 years old (Stein et al., 2005). Lindström et al, reported that obese patients had a 2.5 fold (95% confidence interval (CI) = 2.49-2.51) risk to develop a DVT and 2.2 fold (95% CI = 2.20-2.23) risk to developed PE (Lindström et al., 2017). A very recent study by Musil et al also agreed that obesity was a significant risk factor for VTE (Musil et al., 2017).

The risk of VTE in obesity is due to 2 mechanisms. First, a high body weight can cause mechanical impairment of the valve system in the deep veins of the lower limbs, thus favouring venous stasis (Previtali et al., 2011). Second mechanism is due to the obesity induced proinflammatory state. This condition is caused by cytokines (interleukin-6 and tumour necrosis factor-α) which was released by adipose tissues (Faber et al., 2009; Fontana et al., 2007). Hypercoagulable state may develop due to this chronic inflammatory conditions (Kornblith et al., 2015).

### 6.1.4 Smoking

Smoking is known to be associated with the development of arteriosclerosis and venous thrombosis. The mechanism involved is because of smoking that can cause vascular inflammation. An inflamed vascular wall may increase the production of cytokines such as Interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α). These cytokines play a major regulatory role in the synthesis of acute phase proteins, including fibrinogen (Mendall et al., 1997). Furthermore, IL-6 appears to be the principal procoagulant
cytokine in humans (Stouthard et al., 1996). Smoking has also been reported to increase the level of CRP (Das, 1985). CRP was produced in response to increase in IL-6. Subsequently CRP plays a role to increase the expression of tissue factor on monocytes (Cermak et al., 1993). This tissue factor is the key to initiation of coagulation in vivo and subsequently favouring thrombosis.

However, a recent study by Musil et al found that a family history of VTE, smoking and estrogens alone or in combinations were not a significant risk factors for VTE (Musil et al., 2017). Contrarily, another study in Danish population regarding smoking and VTE found that smoking was an independent risk factor for VTE among middle-aged men and women with a hazard ratio of 1.32 (95% CI, 1.00-1.74) for smoking men and 1.52 (95% CI, 1.15-2.00) for smoking women. They also mentioned that former smokers and person who never smoke had the same risk for VTE. This indicates the possible benefit of cessation of smoking (Severinsen et al., 2009). In this study, 42.3% patients were smoker, however none of them developed VTE.

6.1.5 Types of injury

Multiple injuries in this study were defined as at least one lower limb fracture and at least one other fracture involving the other lower limb or upper limb. Single lower limb trauma was defined as fracture involving one lower limb only and no other fracture involved at other limbs.
The predominant injury in this study was single lower limb fracture which contributed 61.5% (n=32) as compared to patients with multiple injuries 38.5% (n=20)

Niikura et al conducted a study to determine the rate of VTE among Japanese population with fractures of the pelvis and/or lower extremities using physical prophylaxis (graduated compression stockings and intermittent pneumatic compression). They found that patients with multiple fractures have a higher incidence of VTE (Niikura et al., 2012).

6.2 Haemostatic, inflammatory and haematological changes associated with prolonged immobilization patients.

We sequentially assessed several laboratory parameters (haemostatic, inflammatory and haematological) that can contribute to hypercoagulable state post trauma and immobilization. These laboratory parameters were compared between Day 1 and Day 8 of immobilization.

Among all the laboratory parameters assessed, it was found that fibrinogen (haemostatic), ESR (inflammatory) and platelet (haematological) gave a significant mean difference between day 1 and day 8 post trauma and immobilization. The level was increased at day 8 compared to day 1 of trauma and immobilization. These findings were similar when compared to previous studies. However, despite the significant
increase of these biomarkers, none of the patients developed VTE during the study period. This indicates other concomitant risk factors are required to exert the significant effect of hypercoagulable state for these markers.

Fibrinogen is known as an acute phase protein. Its level will increase following trauma or injury. Increased level of fibrinogen signifies a pro-inflammatory state and it also contributes to thrombus formation. Harr et al. in a randomized control trial study found that the level would be increased after day 5 trauma/injury and contributed to hypercoagulability states (Harr et al., 2014). There have been a few studies that found an association between hyperfibrinogenemia with myocardial infarctions, strokes, arterial thrombosis and VTE (Collaboration, 2012; Koenig, 2003; Koster et al., 1994; Maresca et al., 1999).

Regarding ESR, it is an indirect measure of the acute phase protein. In case of post trauma/injury, acute phase protein such as fibrinogen that we discussed earlier will be increased. This fibrinogen is a positively charged protein, and it will interact with erythrocytes which normally have net negative charges, thus favouring rouleaux formation which will then increase ESR. This is how ESR level proves its value in the context of assessing inflammation (Husain and Kim, 2002).

Platelet counts were noted to increase significantly when comparing the level between Day 1 and Day 8. This finding is supported by Valade et al. who also found around 20.4% of patients post trauma/injury developed thrombocytosis. However, they also
mentioned that this reactive thrombocytosis was not associated with VTE unless another risk factor was involved (Valade et al., 2005).

Meanwhile, Harr et al found that platelet was a dominant contributor to hypercoagulable state post injury. Based on in-vitro study, they demonstrated that increased platelet counts caused an increased fibrin production, which in turn caused an increased thrombus formation. From these findings they suggested the important role of considering antiplatelet therapy in VTE prophylaxis following trauma, particularly after 48 hours post trauma (Harr et al., 2013). Looking at the role of antiplatelet therapy, one study was conducted in Orthopaedic Unit at Wellington Hospital involving patients who had Achilles tendon injury and requiring lower limb immobilization for one week or more. These patients were prescribed aspirin as prophylaxis against VTE. It was reported that 6.4% of these patients still developed symptoms of VTE which was later confirmed radiologically. These symptoms occurred within 70 days of immobilization and surprisingly, it was similar to another previous study without aspirin prophylaxis where 6.3% patients developed VTE (Braithwaite et al., 2016). These findings may suggest that platelet alone is not responsible for the pathogenesis of VTE, but with another biomarker the risk of VTE is increased. This was observed in this study whereby fibrinogen, ESR and platelet were found to be significantly increased but not to the extent of VTE development. This is probably due to no added patient factors to further increased the risk of this complication.

In this study, a quantitative assay was used for the measurement of D-dimer. D-dimer (normal range <0.5 μg/ml) was markedly elevated for almost all patients and remained
elevated throughout study period although none of them had signs or symptoms of VTE. However, the increase in the level of this biomarker was not statistically significant.

D-dimer is a degradation product of fibrinogen and cross-linked fibrin. It is comprehensible that an elevation of D-dimer level in trauma patients is caused by tissue damage and is an indicator of hyperfibrinolysis during the early phase of trauma.

However, an important fact to note was that despite not having VTE, the level of D-dimer may also be increased in non-trauma patients with other conditions, either physiological or pathological causes. Physiological causes that increase the D-dimer level include pregnancy and puerperium, increasing age (>65 years), cigarette smoking and postoperative period. Meanwhile, the other pathological causes are liver and renal diseases, infections, chronic inflammatory disease and malignancy. In fact, any bleeding and condition that causes thrombosis may increase the D-dimer level (Pulivarthi and Gurram, 2014). Therefore, a positive D-dimer test alone cannot be used to diagnose VTE, but instead further imaging testing is required to either confirm or exclude VTE.

Findings in this study proposed that D-dimer had no role in predicting VTE in trauma patient. Similarly, Crowther et al in their prospective cohort study involving 197 patients, reported that neither test of hypercoagulability nor D-dimer levels predict patients at risk of DVT and suggested that they should not be used to guide diagnostic testing for DVT (Crowther et al., 2005). This was contrary to another earlier study,
where they used D-dimer level as a predictive value for VTE in trauma patients (Schmidt et al., 1992).

Currently, D-dimer is routinely used in diagnostic algorithm for VTE. D-dimer has a high sensitivity but low specificity (Pulivarthi and Gurram, 2014). A positive D-dimer in patients who are suspected to have VTE may have no value, but a negative D-dimer hold a high negative predictive value that when it is coupled with clinical diagnostic proves to be beneficial to rule out VTE and avoiding unnecessary cost and radiation exposure to patient from radiological testing. However, the role is recommended in non-hospitalized patients without history of trauma.

In this study, other haemostatic markers, protein C, protein S and antithrombin which were anticoagulant proteins had no significant changes between day 1 and day 8. Despite our findings, a study by Engelman et al mentioned that antithrombin level were reduced following trauma, although these changes did not correlate with injury severity. Protein C level was also reduced following a trauma, and it correlated well with severity of injury. Meanwhile, protein S level remained unchanged after multiple trauma (Engelman et al., 1996). Nevertheless, we conclude that these anticoagulant proteins were not suitable for predictor of VTE but can be risk for VTE in the presence of multiple risk factors including hereditary low levels of these natural proteins.
6.3 Correlation between haemostatic parameters and inflammatory parameters (ESR, CRP) in prolonged immobilization patients.

This study showed that there was a statistically significant positive correlation between fibrinogen and CRP and between fibrinogen and ESR. It was observed that when fibrinogen was increased, the CRP and ESR will be increased as well. Meanwhile, other haemostatic parameters (PT, aPTT, D-dimer, protein C, protein S and antithrombin) did not show any correlation either with CRP or ESR.

Deep vein thrombosis and inflammation are closely related. An interaction between inflammation and coagulation promote the incidence of DVT and may be one of DVT pathogenesis. The increased level of CRP may be a predictor of VTE. Study by Wang et al, showed that the mean levels of plasma CRP were significantly higher in DVT group than that in control (Wang et al., 2010).

From the same study, they also found that the inflammatory biomarkers which was CRP, was strongly correlated with coagulation markers (fibrinogen, factor VIII, and factor IX). Concurrently, these coagulation markers were also increased in patients who had DVT (Wang et al., 2010).

Based on the evidence from previous study and observation from this study, we can conclude that patients in this study were at increased risk of VTE. Fortunately, none of
our cohort showed complication post immobilization. This may be due to cumulative effect of haemostatic imbalance towards prothrombotic side is not affected significantly. However, the risk for VTE is evidenced by this study when patient has high platelet, obese and had multiple injuries.

6.4 Association between clinical risk factors (age, sex, BMI, smoking, type of injury) and abnormal laboratory markers in prolonged immobilization patients

In this study, we attempted to determine the association between clinical risk factors which were age, sex, BMI, smoking and type of injury with abnormal markers (fibrinogen, ESR and platelet).

Surprisingly, there were no significant association found between fibrinogen and ESR with the all clinical risk factors studied. Only platelet gave a statistically significant association with a few of the clinical risk factors studied. Using simple linear regression, there was a significant linear relationship between the platelet level with age, BMI and type of injury. However, by multiple linear regression, only BMI and type of injury showed significant relationship towards platelet. From this study data, platelet level would tend to be increased in patients with increased BMI or having multiple injuries involving more than 1 fracture. These factors may compromise the haemostatic system towards prothrombotic effect and lead to VTE development. Thus, the estimated
risk of VTE can be considered high when platelet was increased in multiple injuries and obese patients, although none developed this complication.

One study by Kornblith et al found that, obese patients were found to have higher platelet count, lower D-dimer, and high factor IX compared to patients with normal weight on day of admission. Functional fibrinogen level and clot strength (MA) when measured by thromboelastography (TEG) were also higher on admission for obese patient. The relationship of BMI with clot strength, functional fibrinogen and factor IX persisted for 24 hours post injury, while the relationship of BMI and platelet persisted for 120 hours and more (Kornblith et al., 2015).

Excess of adipose tissue will increase the risk of VTE and arterial thromboembolism by up to 2.5 folds because these adipose tissue acts as proinflammatory and procoagulant (Eichinger et al., 2008; Samad and Ruf, 2013). Cytokines which are released by adipose tissues, including interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) are found to contribute to the obesity induced proinflammatory state (Faber et al., 2009; Fontana et al., 2007). Hypercoagulability condition may be partially due to this chronic low-grade inflammation, but it is likely also due to direct effects of adipose tissue on mediators of coagulation (Kornblith et al., 2015).

In agreement with previous study, platelet count were increased for patients with multiple injuries. Valade et al mentioned in their study, reactive thrombocytosis was a common finding after severe trauma. 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. However, they also mentioned that reactive thrombocytosis was not associated with increased risk of thromboembolic events, unless additional risk factors were present (Valade et al., 2005).
CHAPTER 7
LIMITATIONS
7.0 LIMITATIONS

The main limitation of this study was the small sample size. Therefore, the findings could not represent the whole trauma patients. The targeted sample size could not be reached due to several factors encountered;

1. Only few patients with lower limbs trauma had been admitted. Significant number of them were treated as outpatient hence the sample size was affected in this study.

2. The availability of a new emergency operation room in Trauma Centre Hospital Universiti Sains Malaysia had reduced the hospital stay by early operation. Most of the patients were encouraged for early mobilisation post operation to reduce the risk of VTE, which is the standard practice in surgical patients.

3. The patients were followed for a very short period of time (up to Day 8 only) to determine the VTE development.

4. The inclusion and exclusion criteria that were established had further reduced the sample size.

The small sample had lowered the statistical power of the study, and as a consequence, significant results might escape the analysis. Larger multicentre study is clearly needed, and should be conducted in many trauma centres in Malaysia and hence could represent the true risk of VTE and the usefulness of these biomarkers in clinical practice.

In the study of haemostatic parameters, preanalytical problems are very critical. The venepuncture technique, transportation of blood, plasma separation as well as
processing are very important factors that need to be highlighted. These could affect the results of the tested parameters. To reduce preanalytical error, the samples were collected and processed according to the CLSI (Clinical Laboratory Standards Institute) guidelines.
CHAPTER 8
CONCLUSIONS
AND
RECOMMENDATIONS
VTE post trauma is an acquired form of thrombophilia and potentially a life-threatening condition. Further researches in this area would help to improve patient care in trauma settings. This study attempted to investigate the VTE risk among orthopaedic patients who were immobilized secondary to lower limb trauma.

In conclusion, we found three biomarkers; fibrinogen, ESR and platelet count that showed significant changes after day 8 of immobilization. All of these are prothrombotic parameters which showed body response towards tissue injury following trauma.

There were significant correlations between fibrinogen and both inflammatory biomarkers (CRP and ESR) studied. This showed that CRP and ESR were increased proportionately with fibrinogen level, giving rise to the cumulative risk of hypercoagulable state.

This study showed the prothrombotic role of fibrinogen, ESR and platelet levels in post trauma patients. Thus, fibrinogen, ESR and platelet level are probably useful biomarkers to objectively assess the risk of VTE and follow-up after therapeutic intervention in post trauma patients.
Even though, this study showed significant results for these three prothrombotic biomarkers, but none of the patients developed VTE during the study period. This may be due to small study population and the selection of the subjects involved in this study. Younger and healthy patients are expected to have low risk for VTE. Additional hereditary risk factors were not seen in this study cohort (protein C, protein S and antithrombin deficiency). Therefore, at this stage these biomarkers alone could not be used in deciding the need for prophylaxis to prevent VTE. Future study with good sample size and subjects’ selection are required for confirmation of the role of these biomarkers in clinical practice.

Among abnormal biomarkers 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. There was no significant association between fibrinogen and ESR with all the clinical risk factors. This study hence confirms the previous findings that patients with higher BMI and more severe injuries are at higher risk for VTE. Additionally, we found high platelet count could mark-up the risk of VTE and this is also a potential direct or indirect risk marker for VTE.

Due to study limitations, there is still inadequate evidence to conclude that these three biomarkers could predict VTE related to hypercoagulability state post trauma. Further research is needed to continue similar study on bigger sample size and longer study period. Future study should also focus on scoring system which include clinical risks and biomarkers for a more concise and comprehensive assessment of VTE risk. However, these three biomarkers may be used as additional parameters to support
prophylaxis indication against VTE in high risk patients with multiple injuries involving limb (e.g., older patients, underlying medical conditions etc).
CHAPTER 9
REFERENCES
References


APPENDICES
Appendix A:

STUDY PROTOCOL APPROVAL BY JAWATANKUASA ETIKA PENYELIDIKAN MANUSIA UNIVERSITI SAINS MALAYSIA (JEPeM-USM)

5th September 2016

Dr. Salfarina Iberahim
Department of Hematology
School of Medical Sciences
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.

JEPeM Code : USM/JEPeM/16030137
Protocol Title : Haematological, Haemostatic and Inflammatory Biomarkers among Young Orthopaedic Patients with Prolonged Immobilization and Risk of Venous Thromboembolism.

Dear Dr. ,

We wish to inform you that your study protocol has been reviewed and is hereby granted approval for implementation by the Jawatankuasa Etika Penyelidikan Manusia Universiti Sains Malaysia (JEPeM-USM). Your study has been assigned study protocol code USM/JEPeM/16030137, which should be used for all communication to the JEPeM-USM related to this study. This ethical clearance is valid from 5th September 2016 until 4th September 2017.

Study Site: Hospital Universiti Sains Malaysia.

The following researchers also involve in this study:
1. Assoc. Prof. Dr. Noor Haslina Mohd Noor
2. Prof. Wan Zaidah Abdul lah
3. Dr. Wan Haslindawani Wan Mahmood
4. Assoc. Prof. Dr. Tengku Muzaffar Tengku Muhd Shihbudin

The following documents have been approved for use in the study.
1. Research Proposal

In addition to the abovementioned documents, the following technical document was included in the review on which this approval was based:
1. Patient Information Sheet and Consent Form (English version)
2. Patient Information Sheet and Consent Form (Malay version)
3. Data Collection Form - Proforma

Attached document is the list of members of JEPeM-USM present during the full board meeting reviewing your protocol.

While the study is in progress, we request you to submit to us the following documents:
1. Application for renewal of ethical approval 60 days before the expiration date of this approval through submission of JEPeM-USM FORM 3(B) 2015: Continuing Review Application Form. Subsequently this need to be done yearly as long as the research goes on.
2. Any changes in the protocol, especially those that may adversely affect the safety of the participants during the conduct of the trial including changes in personnel, must be submitted or reported using JEPeM-USM FORM 3(A) 2015: Study Protocol Amendment Submission Form.
3. Revisions in the informed consent form using the JEPeM-USM FORM 3(A) 2015: Study Protocol Amendment Submission Form.
4. Reports of adverse events including from other study sites (national, international) using the JEPeM-USM FORM 3(G) 2014: Adverse Events Report.
5. Notice of early termination of the study and reasons for such using JEPeM-USM FORM 3(E) 2015.
6. Any event which may have ethical significance.
7. Any information which is needed by the JEPeM-USM to do ongoing review.
8. Notice of time of completion of the study using JEPeM-USM FORM 3(C) 2014: Final Report Form.

Please note that forms may be downloaded from the JEPeM-USM website: www.jepem.kk.usm.my


Thank you.

"ENSURING A SUSTAINABLE TOMORROW"

Very truly yours,

PROF. DR. MOHD SHUKRI ÖRMAN
Deputy Chairperson
Jawatankuasa Etika Penyelidikan (Manusia) JEPeM
Universiti Sains Malaysia
Date of meeting: 24th May 2016
Venue: Meeting Room, Division of Research & Innovation, USM Kampus Kesihatan.
Time: 9.00 a.m. – 2.00 p.m
Meeting No: 336

Members of Committee of the Jawatankuasa Etika Penyelidikan (Manusia), JEPeM Universiti Sains Malaysia who reviewed the protocol/documents are as follows:

<table>
<thead>
<tr>
<th>Member (Title and Name)</th>
<th>Occupation (Designation)</th>
<th>Male/Female (M/F)</th>
<th>Tick (✓) if present when above items were reviewed</th>
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<tbody>
<tr>
<td>Deputy Chairperson:</td>
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<tr>
<td>Professor Dr. Mohd Shukri Othman</td>
<td>Deputy Chairperson of Jawatankuasa Etika Penyelidikan (Manusia), JEPeM USM</td>
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<td>✓ (Deputy Chairperson)</td>
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<td>Secretary:</td>
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<tr>
<td>Mr. Mohd Bazlan Haliz Mukrim</td>
<td>Research Officer</td>
<td>M</td>
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</tbody>
</table>

Members:
1. Dr. Azlan Husin Lecturer, School of Medical Sciences M ✓
2. Dr. Haslina Tai Lecture, School of Dental Sciences F ✓
3. Mr. Hj. Ismail Hassan Community Representative M ✓
4. Professor Dr. Narazah Mohd Yusoff Lecturer, Advanced Medical and Dental Institute (AMDI) F ✓
5. Professor Dr. Nik Hazlina Nik Hussain Lecturer, School of Medical Sciences F ✓
6. Associate Professor Siti Hawa Ali Lecturer, School of Health Sciences F ✓
7. Professor Dr. Suzina Sheikh Ab Hamid Lecturer, School of Medical Sciences F ✓
8. Professor Wan Abdul Manan Wan Muda Lecturer, School of Health Sciences M ✓
9. Mrs. Zawiah Abu Bakar Community Representative F ✓


PROFESSOR DR. MOHD SHUKRI OTHMAN
Deputy Chairperson
Jawatankuasa Etika Penyelidikan (Manusia), JEPeM
Universiti Sains Malaysia
Appendix B:

PRESENTATION FROM THIS RESEARCH

POSTER PRESENTATION

1. Title: Haematological and Inflammatory Biomarkers Among Prolonged Immobilised Orthopaedic Patients

Venue: 14th Annual Scientific Meeting
       Malaysian Society of Haematology

Date:  20th - 22nd April 2017
MALAYSIAN SOCIETY OF HAEMATOLOGY
14th Annual Scientific Meeting

20th-22nd April 2017
Holiday Villa, Johor Bahru

"Towards A New Era of Precision Medicine"
HAEMATOLOGICAL AND INFLAMMATORY BIOMARKERS AMONG PROLONGED IMMOBILISED ORTHOPAEDIC PATIENTS

Salfarina I, Noor Haslina MN, Wan Zaidah A, Tengku Munafir TMS, Wan Haslindawani WM
Department of Hematology, School of Medical Sciences, Health Campus, University Sains Malaysia, Kubang Kerian, Kelantan
Email: salfarina78@gmail.com

Background
- Trauma induces hypercoagulable state leading to thrombosis. Based on previous studies there is a close relationship between haematological and inflammatory biomarkers in post trauma patients. About 20% of post trauma patients developed thrombocytosis after a few days/weeks.
- It was hypothesized that progressive postinjury thrombocytosis contributes to a hypercoagulable state.
- C-reactive protein (CRP) and erythrocytes sedimentation rate (ESR) which are an inflammatory biomarker and acute phase protein will increase post trauma.
- However, sequential changes that occur in the haematological and inflammatory markers in the prolonged immobilized patients were not really studied in our setting.

Material/Method
- This is a prospective cohort study conducted at Hospital University Sains Malaysia from June 2016 to January 2017. We measured several biomarkers (haematological and inflammatory) serially in Day 1 and Day 8 post lower limb’s trauma in patients who received no anticoagulant prophylaxis. The involved factors were analysed by using paired t test, with p value < 0.05 as significant results.

Results
- About 23 patients with lower limb’s fracture who required immobilization more than 7 days were included in this study. 17 patients were males. Majority (13) of the patients had only single lower limb fracture. Among haematological and inflammatory markers studied were platelet count and ESR which gave a significant different mean between day 1 and day 8 with mean (SD) for platelet D1 vs D8 was -116(104.9), p <0.001 and mean (SD) for ESR D1 vs D8 was -25.53(24.4), p<0.002. C-reactive protein (CRP) did not show any significant change in patients with prolonged immobilization.

Conclusion
- In conclusion, both platelet count and ESR showed significant changes in relation to prolonged immobilization. Both parameters were responding towards tissue injury following trauma.
- Reactive thrombocytosis is a common finding after severe trauma. Unless additional risk factors are present, reactive thrombocytosis is not associated with an increased risk of thromboembolic events.

Keywords: trauma, immobilised, haematological, inflammatory
INTRODUCTION

• Trauma induces hypercoagulable state leading to thrombosis. Based on previous studies there is a close relationship between haematological and inflammatory biomarkers in post trauma patients. About 20% of post trauma patients developed thrombocytosis after a few days/weeks.

• It was hypothesized that progressive post injury thrombocytosis contributes to a hypercoagulable state.

• C-reactive protein (CRP) and erythrocytes sedimentation rate (ESR) which are inflammatory biomarkers and acute phase protein will increase post trauma.

• However, sequential changes that occur in the haematological and inflammatory markers in the prolonged immobilized patients were not really studied in our setting.

MATERIAL & METHODS

• This is a prospective cohort study conducted at Hospital University Sains Malaysia from June 2016 to January 2017. We measured several biomarkers (haematological and inflammatory) weekly in Day 1 and Day 8 post lower limb’s trauma in patients who received no anticoagulant prophylaxis. The involved factors were analysed by using paired t test, with p value < 0.05 as significant results.

RESULTS

• About 23 patients with lower limb’s fracture who required immobilization more than 7 days were included in this study. 17 patients were males. Majority (15) of the patients had only single lower limb fracture.

• Among haematological and inflammatory markers studied, platelet count and ESR which gave a significant different mean between day 1 and day 8 with mean (SD) for platelet D1 vs D8 was -116 (104.9), p < 0.001 and mean (SD) for ESR D1 vs D8 was -161 (104.9), p < 0.002. C-reactive protein (CRP) did not show any significant change in patients with prolonged immobilization.

DISCUSSIONS

• Trauma and immobilization induced hypercoagulability in which it disturbs the haemostatic balance inducing state, endothelial wall dysfunction and alterations in the clotting system.

• Acute phase proteins will also be increased following trauma. Examples are CRP, ESR and coagulation protein such as fibrinogen and prothrombin. These acute phase proteins can be used as the biomarkers.

• These biomarkers are expected to change due to trauma as well as the prolonged immobilization. Based on the study that was done, platelet and ESR gave a significant changes in relation to post trauma and prolonged immobilization.

• Other parameters such as PT, aPTT and D-dimer, did not give any significant value between Day 1 and Day 8 study.

• Even though platelet and ESR gave significant changes in relation to trauma and immobilization, these two biomarkers were not associated with hypercoagulable state in the patient.

CONCLUSION

• In conclusion, both platelet count and ESR showed significant changes in relation to prolonged immobilization. Both parameters were responding towards tissue injury following trauma.

• Reactive thrombocytosis is a common finding after severe trauma. Unless additional risk factors are present, reactive thrombocytosis is not associated with an increased risk of thromboembolic events.

REFERENCES


• Tariq MI and David HK. C-reactive protein and Erythrocyte Sedimentation Rate in Orthopaedics. Orthopaedic Journal 2001;13-16.

Appendix C:

PROFORMA

TAJUK KAJIAN

Haemostatic and inflammatory biomarkers among young orthopaedic patients with prolonged immobilization and risk of venous thromboembolism.

1. PROFORMA RESPONDEN

RN/ ID : ____________________________________________

No. K/P (Baru) : _____________________

No K/P (Lama) : _____________________

Tarikh Lahir : _____________________

Umur : _____________________

Jantina : ___________________

Alamat Rumah : __________________________________________________________

No. Tel (Rumah): _____________________

No. Tel (Bimbit) : _____________________

Berat badan : ____________ (kg)

Tinggi : ____________ cm

2. SILA JAWAB YA ATAU TIDAK PADA SOALAN-SOALAN DIBAWAH.

1. Adakah anda pernah mendapatkan rawatan di klinik atau di hospital? 
   YA/ TIDAK

2. Jika YA, sila nyatakan jenis penyakit dan rawatan yang diterima.
   _____________________________________________________________________

3. Adakah anda;
   i. Mengidap kencing manis/ Diabetes 
   YA/ TIDAK
   ii. Mengidap darah tinggi/ Hypertension 
   YA/ TIDAK
   iii. Mengidap penyakit jantung/ HeartDisease
   YA/ TIDAK
   iv. Penyakit thrombophilia dan 'lupus anticoagulant'
   YA/ TIDAK
   v. Mengambil ubat anti-pembekuan dan anti-platelet
   YA/ TIDAK
vi. Mengalami masalah salur darah tersumbat atau pesakit yang memerlukan ubat antipembekuan semasa kajian ini dijalankan Y/A/TIDAK
vii. Pesakit perempuan yang mengambil ubat pencegah kehamilan atau yang sedang hamil Y/A/TIDAK
viii. Pesakit yang mengalami ‘disseminated intravascular coagulopathy’, penyakit hati dan sepsi Y/A/TIDAK
ix. Lain-lain (sila nyatakan)

4. Adakah anda pernah mengidap apa-apa penyakit lain? Y/A/ TIDAK
5. Jika YA, sila nyatakan.

6. Adakah anda merokok? Y/A/ TIDAK
7. Jika YA,
   i. Berapa lama anda merokok? _____________________
   ii. Berapa batang rokok sehari (anggaran) _____________
Appendix D:
CONSENT FORM (IN MALAY)

MAKLUMAT KAJIAN

Tajuk Kajian: Biomaker hemostatik dan inflamasi dikalangan pesakit ortopedik muda dan kaitan dengan limitasi pergerakan serta risiko untuk mendapat masalah penyumbatan salur darah.

Nama Penyelidik: DR SALFARINA BINTI IBERAHIM (No. MMC : 42494)

SUPERVISOR: PM DR NOOR HASLINA MOHD NOOR (MMC : 34466)

CO-SUPERVISOR: PROF DR WAN ZAIDAH ABDULLAH (MMC : 30040)
DR WAN HASLINDAWANI WAN MAHMOOD (MMC: 37706)
PM DR TENGKU MUZAFFAR BIN TENGKU MOHAMAD SHIHABUDIN (MMC: 34372)

PENGENALAN

Anda dipelawa untuk menyertai satu kajian penyelidikan secara sukarela yang melibatkan kajian terhadap pesakit ortopedik yang melibatkan limitasi pergerakan yang panjang. Kajian ini bertujuan untuk mengetahui kaitannya dengan biomaker hemostatik dan inflamasi serta risiko untuk mendapat masalah penyumbatan pada salur darah.

Buat masa sekarang, kajian terhadap pesakit ortopedik muda yang melibatkan limitasi pergerakan yang panjang dan kaitannya dengan biomaker hemostatik dan inflamasi serta risiko untuk mendapat masalah penyumbatan salur darah belum lagi dilakukan. Ubat anti pencegahan dari berlakunya penyumbatan salur darah tidak diberi secara rutin kepada pesakit yang sihat dan muda dan hanya pesakit yang mengalami simptom atau yang bergantung kepada justifikasi klinikal yang akan dirawat mengikut kaedah perubatan semasa.

Sebelum anda bersetuju untuk menyertai kajian penyelidikan ini, adalah penting anda membaca dan memahami borang ini. Sekiranya anda menyertai kajian ini, anda akan menerima satu salinan borang ini untuk disimpan sebagai rekod anda.

Penyertaan anda di dalam kajian ini dijangka mengambil masa sehingga beberapa minggu. Seramai 116 pesakit akan menyertai kajian ini.

TUJUAN KAJIAN

Kajian ini dijalankan bertujuan untuk mengetahui kaitan biomarker hemostatik dan inflamasi dikalangan pesakit ortopedik muda yang terlibat dalam limitasi pergerakan yang lama dan kaitannya dengan risiko penyumbatan salur darah. Kami akan menjalankan beberapa jenis
ujian darah serta ujian radiologi seperti ultrasound Doppler (jika perlu) bagi tujuan pengesanan awal masalah penyumbatan salur darah. Sebanyak 15ml darah akan diambil pada hari pertama dan 15ml pada hari ke-8 selepas trauma. Kami berharap dengan adanya kajian ini, kami dapat membuktikan pesakit yang kurang bergerak yang memerlukan ubat antipembekuan, akan diberikan ubat antipembekuan tersebut dan mereka yang tidak mempunyai risiko atau risiko yang rendah dielakkan daripada ubat antipembekuan ini.

Hasil dari kajian ini juga diharap akan membantu untuk dijadikan panduan pada peringkat hospital seterusnya kebangsaan dalam pemberian ubat antipembekuan secara profilaksis kepada pesakit yang berisiko.

Terdapat kemungkinan maklumat yang dikumpulkan semasa kajian ini akan dianalisa oleh pihak penyelidik pada masa depan untuk kegunaan lain yang mungkin untuk tujuan perubatan atau saintifik lain yang selain dari yang kini dicadangkan.

KELAYAKAN PENYERTAAN

Pegawai perubatan yang bertanggungjawab dalam kajian ini atau salah seorang kakitangan kajian telah membincangkan kelayakan untuk menyertai kajian ini dengan anda. Adalah penting anda berterus terang dengan pegawai perubatan dan kakitangan tersebut tentang sejarah diri dan kesihatan anda. Anda tidak seharusnya menyertai kajian ini sekiranya anda tidak memenuhi semua syarat kelayakan.

Syarat-syarat penyertaan;
• Semua pesakit yang mempunyai limitasi pergerakan disebabkan oleh patah tulang pelvis atau kaki (lower limbs) disebabkan trauma. Pesakit ini adalah pesakit diwad otopedik HUSM (4s/4U/2Z) sepanjang jangkamasa kajian.
• Pesakit yang berumur 18-50 tahun..
• Lelaki dan perempuan
• Terpaksa berada dikatil sepanjang masa dengan limitasi pergerakan untuk tempoh 7 hingga 14 hari tanpa ubat pencegahan pembekuan darah.

Syarat-syarat untuk pengecualian penyertaan;
• Pesakit thrombophilia dan ‘lupus anticoagulant’
• Pesakit yang mengambil ubat anti-pembekuan dan anti-platelet
• Pesakit yang mengalami masalah salur darah tersumbat atau pesakit yang memerlukan ubat antipembekuan semasa kajian ini dijalankan
• Mempunyai sejarah penyakit seperti masalah jantung dan diabetes
• Pesakit perempuan yang mengelami kelahiran atau yang sedang hamil
• Pesakit yang mengalami ‘disseminated intravascular coagulopathy’, penyakit hati dan sepsis

PROSEDUR-PROSEDUR KAJIAN

Borang keizinan akan diberikan kepada anda dan anda boleh memilih sama ada bersetuju atau pun tidak untuk melibatkan diri dalam penyelidikan ini.

**RISIKO**

Pada kebanyakan pesakit, proses pengambilan darah tidak akan menyebabkan sebarang komplikasi yang serius. Walau bagaimanapun, pengambilan darah mungkin akan menyebabkan berlakunya pendarahan, kesan lebam, rasa tidak selesa/sakit atau jangkitan pada kawasan terbabit.

Tiada risiko tambahan lain yang akan terlibat selain daripada kesan pengambilan darah.

Jika apa-apa maklumat penting yang baru dijumpai semasa kajian ini yang mungkin mengubah persetujuan anda untuk terus menyertai kajian ini, anda akan diberitahu secepat mungkin.

**MELAPORKAN PENGALAMAN KESIHATAN**


**PENYERTAAN DALAM KAJIAN**

Penyertaan anda dalam kajian ini adalah secara sukarela. Anda berhak menolak untuk menyertai kajian ini atau anda boleh menamatkan penyertaan anda pada bila-bila masa, tanpa sebarang hukuman atau kehilangan manfaat yang sepatutnya anda perolehi.

Penyertaan anda juga mungkin boleh diberhentikan oleh pegawai perubatan yang terlibat dalam kajian ini tanpa persetujuan anda. Sekiranya anda berhenti menyertai kajian ini, pegawai perubatan yang terlibat di dalam kajian ini atau salah seorang kakitangan akan berbincang dengan anda mengenai apa-apa isu perubatan berkenaan dengan pemberhentian penyertaan anda..

**MANFAAT YANG MUNGKIN (Manfaat terhadap Individu, Masyarakat, Universiti)**

Prosedur kajian ini akan diberikan kepada anda tanpa kos. Anda mungkin menerima maklumat tentang kesihatan diri anda hasil daripada pemeriksaan dan ujian makmal yang akan dilakukan dalam kajian ini. Sekiranya perlu, anda akan dirujuk kepada pakar dalam unit tertentu bagi tujuan pemeriksaan lanjut (ultrasound Doppler) dan rawatan lanjut.
Maklumat atau hasil yang bakal diperolehi daripada kajian ini diharap dapat membantu kami memahami keadaan yang dikaji, seterusnya membantu dalam pengesanan awal segala masalah kesihatan yang berkaitan.

Dengan ini diharapkan, ianya sedikit sebanyak dapat memberi manfaat kepada semua pesakit yang mempunyai masalah yang sama pada masa hadapan.

PERSOALAN

Sekiranya anda mempunyai sebarang soalan mengenai prosedur kajian ini atau hak-hak anda, sila hubungi:

DR SALFARINA BINTI IBERAHIM (No. MMC: 42494)
JABATAN HEMATOLOGI/ UPT
HOSPITAL UNIVERSITI SAINS MALAYSIA,
KAMPUS KESIHATAN,
16150 KOTA BHARU KELANTAN
NO. TEL: 09-7673000 ext 6206

Sekiranya anda mempunyai sebarang soalan berkaitan kelulusan Etika atau sebarang pertanyaan dan masalah berkaitan kajian ini, sila hubungi:

En. Mohd Bazlan Hafidz Mukrim
Setiausaha Jawatankuasa Etika Penyelidikan (Manusia) USM
Pusat Inisiatif Penyelidikan -Sains Klinikal & Kesihatan
USM Kampus Kesihatan.
No. Tel: 09-767 2354 / 09-767 2362
Email : bazlan@usm.my/jepem@usm.my

KERAIHSIAAN

Maklumat perubatan anda akan dirahsiaakan oleh pegawai perubatan dan kakitangan kajian. Ianya tidak akan dedahkan secara umum melainkan jika ia dikehendaki oleh undang-undang. Namun begitu, sekitanya anda dirujuk kepada unit perubatan tertentu bagi tujuan rawatan, pakar atau pegawai perubatan yang terlibat dalam rawatan anda dibenarkan untuk meneliti rekod perubatan anda jika perlu.

Data yang diperolehi dari kajian yang tidak mengenalpasti anda secara perseorangan mungkin akan diterbitkan untuk tujuan memberi pengetahuan baru.

Rekod perubatan anda yang asal mungkin akan dilihat oleh pihak penyelidik, Lembaga Etika kajian ini dan pihak berkuasa regulatori untuk tujuan mengesahkan prosedur dan/atau data kajian klinikal. Maklumat perubatan anda mungkin akan disimpan dalam komputer dan diproses dengannya.
Dengan menandatangani borang persetujuan ini, anda membenarkan penelitian rekod, penyimpanan maklumat dan pemindahan data seperti yang dihuraikan di atas.

**TANDATANGAN**

Untuk dimasukkan ke dalam kajian ini, anda atau wakil sah anda mesti menandatangani serta mencatatkan tarikh halaman tandatangan (Lihat contoh Borang Keizin Pesakit di **LAMPIRAN S** atau **LAMPIRAN P**).
Borang Keizinan Pesakit/ Subjek
(Halaman Tandatangan)

Tajuk Kajian: Biomaker hemostatik dan inflamasi dikalangan pesakit ortopedik muda dan kaitan dengan limitasi pergerakan serta risiko untuk mendapat masalah penyumbatan salur darah.

Nama Penyelidik: DR SALFARINA BINTI IBERAHIM (No.MMC : 42494)

SUPERVISOR:
PM DR NOOR HASLINA MOHD NOOR (MMC : 34466)

CO-SUPERVISOR:
PROF DR WAN ZAIDAH ABDULLAH (MMC : 30040)
DR WAN HASLINDAWANI WAN MAHMOOD (MMC: 37706)
PM DR TENGKU MUZAFFAR BIN TENGKU MOHAMAD SHIHABUDIN (MMC: 34372)

Untuk menyetarai kajian ini, anda atau wakil sah anda mesti menandatangani mukasurat ini.

Dengan menandatangani mukasurat ini, saya mengesahkan yang berikut:

- Saya telah membaca semua maklumat dalam Borang Maklumat dan Keizinan Pesakit ini termasuk apa-apa maklumat berkaitan risiko yang ada dalam kajian dan saya telah pun diberi masa yang mencukupi untuk mempertimbangkan maklumat tersebut.
- Semua soalan-soalan saya telah dijawab dengan memuaskan.
- Saya, secara sukarela, bersetuju menyetarai kajian penyelidikan ini, mematuhi segala prosedur kajian dan memberi maklumat yang diperlukan kepada doktor, para jururawat dan juga kakitangan lain yang berkaitan apabila diminta.
- Saya boleh menamatkan penyertaan saya dalam kajian ini pada bila-bila masa.
- Saya telah pun menerima satu salinan Borang Maklumat dan Keizinan Pesakit untuk simpanan peribadi saya.

Nama Pesakit (Dicetak atau Ditaip)          Nama Singkatan & No. Pesakit

No. Kad Pengenalan Pesakit (Baru)          No. K/P (Lama)
| Tandatangan Pesakit atau Wakil Sah | Tarikh (dd/MM/yy)  
(Masa jika perlu) |
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<td>Tarikh (dd/MM/yy)</td>
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<td>Nama Saksi dan Tandatangan</td>
<td>Tarikh (dd/MM/yy)</td>
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</table>

**Nota:**  
i) Semua subjek/pesakit yang mengambil bahagian dalam projek penyelidikan ini **tidak** dilindungi insuran.
LAMPIRAN P

Borang Keizinan bagi Penerbitan Bahan yang berkaitan dengan Pesakit/Subjek
(Halaman Tandatangan)

Tajuk Kajian: Biomaker hemostatik dan inflamasi dikalangan pesakit ortopedik muda
dan kaitan dengan limitasi pergerakan serta risiko untuk mendapat
masalah penyumbatan salur darah.

Nama Penyelidik: DR SALFARINA BINTI IBERAHIM (No. MMC :42494)

SUPERVISOR:
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DR WAN HASLINDAWANI WAN MAHMOOD (MMC: 37706)
PM DR TENGKU MUZAFFAR BIN TENGKU MOHAMAD
SHIHABUDIN (MMC: 34372)

Untuk menyertai kajian ini, anda atau wakil sah anda mesti menandatangani mukasurat ini.
Dengan menandatangani mukasurat ini, saya memahami yang berikut:

- Bahan yang akan diterbitkan tanpa dilampirkan dengan nama saya
dan setiap percubaan yang akan dibuat untuk memastikan
ketanpanamaan saya. Saya memahami, walaubagaimanapun,
ketanpanamaan yang sempurna tidak dapat dijamin. Kemungkinan
sesiapa yang menjaga saya di hospital atau saudara dapat
mengenal saya.
- Bahan yang akan diterbitkan dalam penerbitan mingguan/bulanan/dwibulanan/suku tahunan/dwi tahunan
merupakan satu penyebaran yang luas dan tersebar ke seluruh
dunia. Kebanyakan penerbit ini akan tersebar kepada doktor-
doctor dan juga bukan doktor termasuk ahli sains dan ahli jurnal.
- Bahan tersebut juga akan dilampirkan pada laman web jurnal di
seluruh dunia. Sesetengah laman web ini bebas dikunjungi oleh
semua orang.
- Bahan tersebut juga akan digunakan sebagai penerbitan tempatan
dan disampaikan oleh ramai doktor dan ahli sains di seluruh
dunia.
- Bahan tersebut juga akan digunakan sebagai penerbitan buku
oleh penerbit jurnal.
- Bahan tersebut tidak akan digunakan untuk pengiklanan ataupun
bahan untuk membungkus.

Saya juga memberi keizinan bahawa bahan tersebut boleh digunakan
sebagai penerbitan lain yang diminta oleh penerbit dengan kriteria berikut:

- Bahan tersebut tidak akan digunakan untuk pengiklanan atau
bahan untuk membungkus.
- Bahan tersebut tidak akan digunakan di luar konteks – contohnya: Gambar tidak akan digunakan untuk menggambarkan sesuatu artikel yang tidak berkaitan dengan subjek dalam foto tersebut.

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**Nota:**

i) Semua subjek/pesakit yang mengambil bahagian dalam projek penyelidikan ini **tidak dilindungi insuran**.
Appendix D:

CONSENT FORM (IN ENGLISH)

ATTACHMENT B

RESEARCH INFORMATION

Research Title: Haemostatic and inflammatory biomarkers among young orthopaedic patients with prolonged immobilization and risk of venous thromboembolism

Researcher's Name: DR SALFARINA BINTI IBERAHIM (No. MMC : 42494)

SUPERVISOR:
PM DR NOOR HASLINA MOHD NOOR (MMC : 34466)

CO-SUPERVISOR:
PROF DR WAN ZAIĐAH ABDULLAH (MMC : 30040)
DR WAN HASLINDAWANI WAN MAHMOOD (MMC : 37706)
PM DR TENGKU MUZAFFAR BIN TENGKU MOHAMAD SHIHABUDIN (MMC: 34372)

INTRODUCTION

You are invited to take part voluntarily in a research study regarding haemostatic and inflammatory biomarkers among young orthopaedic patients with prolonged immobilization and risk of venous thromboembolism.

At present, local data on haemostatic and inflammatory biomarkers in prolonged immobilized young trauma patients and risk of venous thromboembolism is not yet available. Thromboprophylaxis is not routinely practice in healthy immobilized patients in our setting and only symptomatic patients will be treated according to the current practice and clinician justification.

Before agreeing to participate in this research study, it is important that you read and understand this form. If you participate, you will receive a copy of this form to keep for your records.

Your participation in this study is expected to last up for a few weeks. Up to 116 patients will be participating in this study.

PURPOSE OF THE STUDY

The aim of the present study is to evaluate the haematological, haemostatic and inflammatory biomarkers in prolonged immobilized young trauma patients and its association with venous
thromboembolism development. We will do some blood tests as well as radiological investigations such as ultrasound Doppler (if needed) for screening/ diagnostic purpose in such patients.

We are hoping the results of this study can be included in the local / national protocol for venous thromboembolism prophylaxis for prolonged immobilization orthopedic patients and allow patients in need to receive prophylaxis anti coagulant treatment, while the low risk patients may avoid the prophylaxis treatment.

It is possible that information collected during this study will be analyzed by the sponsor in the future for other possible uses or other medical or scientific purposes other than those currently proposed.

QUALIFICATION TO PARTICIPATE

The doctor in charge of this study or a member of the study staff has discussed with you the requirements for participation in this study. It is important that you are completely truthful with the doctor and staff about you health history. You should not participate in this study if you do not meet all qualifications.

Inclusion criteria;

- All 18-40 years old prolonged immobilization patients (7-14 days) in orthopaedic ward during the study period
- Both sex

Exclusion criteria;

- Known case of thrombophilia and lupus anticoagulant
- Patient on prophylaxis anticoagulant and anti platelet
- Patient with deep vein thrombosis or symptomatic patient requiring anticoagulant during the study.
- Past medical history of heart disease and diabetes
- Female on oral contraceptive pill or pregnant
- Disseminated intravascular coagulopathy, liver disease and sepsis

STUDY PROCEDURES

A consent form would be given to you and you can choose whether agree or disagree to participate in this research.

If you are agree to participate in this study, you will be asked to provide information about your background, past medical history and social history. Peripheral venous blood will be taken from you for few blood tests, and that blood results will be analysed for research purpose.

RISKS

For most people, needle puncture for blood draws do not cause any serious problems. However, they may cause bleeding, bruising, discomfort, infections and/or pain at the needle site.
Other than that, there are no additional risks involved.

If any important new information is found during this study that may affect you wanting to continue to be part of this study, you will be told about it right away.

REPORTING HEALTH EXPERIENCES.

If you have any injury, bad effect, or any other unusual health experience during this study, make sure that you immediately tell the nurse or Dr. Salfarina Binti Iberahim (MMC Registration No. 42494) at 09-7673000 ext 6206.

You can call at anytime, day or night, to report such health experiences.

PARTICIPATION IN THE STUDY

Your taking part in this study is entirely voluntary. You may refuse to take part in the study or you may stop participation in the study at anytime, without a penalty or loss of benefits to which you are otherwise entitled.

Your participation also may be stopped by the study doctor or sponsor without your consent.

POSSIBLE BENEFITS (Benefit to Individual, Community, University)

Study procedures will be provided at no cost to you. You may receive information about your health from any physical examination and laboratory tests to be done in this study.

If needed, you will be referred to other medical unit for management and treatment purposes.

Thus, we hope that the research performed will help us in terms of understanding further the condition concerned and may help in early detection of any related medical problems.

We also hope that the outcome and information regarding this research will beneficial to future patients.

QUESTIONS

If you have any question about this study or your rights, please contact;

Dr. Salfarina Binti Iberahim (No. MMC: 42494)
Jabatan Hematologi/ UPT
Hospital Universiti Sains Malaysia,
Kampus Kesihatan,
16150 Kota Bharu Kelantan
No. Tel: 09-7673000 ext 6206
If you have any questions regarding the Ethical Approval or any issue / problem related to this study, please contact;

Mr. Mohd Bazlan Hafidz Mukrim  
Secretary of Human Research Ethics Committee USM  
Centre for Research Initiatives, Clinical & Health Sciences  
USM Health Campus  
Tel. No.: 09-767 2354 / 09-767 2362  
Email: bazlan@usm.my/jepem@usm.my

CONFIDENTIALITY

Your medical information will be kept confidential by the study doctor and staff, and will not be made publicly available unless disclosure is required by law. However, if you are referred to other medical unit for treatment purpose, the specialist or medical personnel taking care of you will be allowed to review your records whenever necessary.

Data obtained from this study that does not identify you individually will be published for knowledge purposes.

Your original medical records may be reviewed by the researcher, the Ethical Review Board for this study, and regulatory authorities for the purpose of verifying clinical trial procedures and/or data. Your medical information may be held and processed on a computer.

By signing this consent form, you authorize the record review, information storage and data transfer described above.

SIGNATURES

To be entered into the study, you or a legal representative must sign and data the signature page [ATTACHMENT S or ATTACHMENT P]
Research Title: Haemostatic and inflammatory biomarkers among young orthopaedic patients with prolonged immobilization and risk of venous thromboembolism.

Researcher’s Name: DR Salfarina Binti Iberahim (No. MMC: 42494)

SUPERVISOR:
PM DR Noor Haslina Mohd Noor (MMC: 34466)

CO-SUPERVISOR:
PROF DR Wan Zaidah Abdullah (MMC: 30040)
DR Wan Haslindawani Wan Mahmod (MMC: 37706)
PM DR Tengku Muzaaffar bin Tengku Mohamad Shihabudin (MMC: 34372)

To become a part of this study, you or your legal representative must sign this page. By signing this page, I am confirming the following:

- I have read all of the information in this Patient Information and Consent Form including any information regarding the risk in this study and I have had time to think about it.
- All of my questions have been answered to my satisfaction.
- I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the doctor, nurses, or other staff members, as requested.
- I may freely choose to stop being a part of this study at anytime.
- I have received a copy of this Patient Information and Consent Form to keep for myself.

Patient Name (Print or type) ________________________________

Patient Initials ________________________________

Patient I.C No. (New) ________________________________

Patient I.C No. (Old) ________________________________
Signature of Patient or Legal Representative ____________________________
Date (dd/MM/yy) ____________________________
(Add time if applicable)

Name of Individual
Conducting Consent Discussion (Print or Type)

Signature of Individual
Conducting Consent Discussion ____________________________
Date (dd/MM/yy) ____________________________

Name & Signature of Witness ____________________________
Date (dd/MM/yy) ____________________________

Note: i) All subject/patients who are involved in this study will not be covered by insurance.
Patient’s Material Publication Consent Form
Signature Page

Research Title: Haemostatic and inflammatory biomarkers among young orthopaedic patients with prolonged immobilization and risk of venous thromboembolism

Researcher’s Name: DR Salfarina Binti Iberahim (No. MMC : 42494)

SUPERVISOR:
PM DR Noor Haslina Mohd Noor (MMC : 34466)

CO-SUPERVISOR:
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DR Wan Haslindawani Wan Mahmoood (MMC : 37706)
PM DR Tengku Muzaaffar Bin Tengku Mohamad Shihabudin (MMC : 34372)

To become a part this study, you or your legal representative must sign this page.

By signing this page, I am confirming the following:

- I understood that my name will not appear on the materials published and there has been efforts to make sure that the privacy of my name is kept confidential although the confidentiality is not completely guaranteed due to unexpected circumstances.

- I have read the materials or general description of what the material contains and reviewed all photographs and figures in which I am included that could be published.

- I have been offered the opportunity to read the manuscript and to see all materials in which I am included, but have waived my right to do so.

- All the published materials will be shared among the medical practitioners, scientists and journalist worldwide.

- The materials will also be used in local publications, book publications and accessed by many local and international doctors worldwide.

- I hereby agree and allow the materials to be used in other publications required by other publishers with these conditions:

  - The materials will not be used as advertisement purposes nor as packaging materials.
The materials will not be used out of context – i.e.: Sample pictures will not be used in an article which is unrelated subject to the picture.

<table>
<thead>
<tr>
<th>Patient Name (Print or type)</th>
<th>Patient Initials or Number</th>
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<td>Patient's Signature</td>
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<tr>
<td>Patient's Signature</td>
<td>Date (dd/MM/yy)</td>
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</table>

Name and Signature of Individual
Conducting Consent Discussion

Note:  
1) All subject/patients who are involved in this study will not be covered by insurance.