THE USE OF CLOT WAVEFORM ANALYSIS AS AN ADDITIONAL PARAMETER FOR VENOUS THROMBOEMBOLISM RISK ASSESSMENT IN PATIENT WITH PROLONGED IMMOBILIZATION

BY

DR ROSMANIZA BINTI MUHAMAT YUSOFF

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER IN PATHOLOGY (HEMATOLOGY)

SCHOOL OF MEDICAL SCIENCES

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SUPERVISORS:

DR SALFARINA IBERAHIM

ASSOC PROF DR NOOR HASLINA MOHD NOOR

PROF DR ROSLINE HASSAN

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LIST OF ABBREVIATIONS

aPTT	Activated Partial Thromboplastin Time
CWA	Clot Waveform Analysis
DVT	Deep Vein Thrombosis
DIC	Disseminated Intravascular Coagulation
JEPem	Jawatankuasa Etika Penyelidikan Manusia
PE	Pulmonary Embolism
PT	Prothrombin Time
SPSS	Statistical Package for the Social Sciences
VTE	Venous Thromboembolism

ABSTRAK

Objektif: Imobilisasi yang berpanjangan adalah faktor yang diketahui boleh meningkatkan risiko terjadinya darah beku di dalam salur darah. Kajian ini bertujuan untuk membandingkan parameter pada graf gelombang pembekuan darah antara pesakit dengan imobilisasi dan kontrol normal. Kami juga berhasrat untuk melihat perbezaan parameter pada graf pembekuan darah bagi pesakit pada hari pertama imobilisasi dan pada hari ketiga dan ke atas.

Metodologi: 30 orang pesakit dengan imobilisasi yang berpanjangan dari Hospital USM terlibat dalam kajian ini. Plasma daripada pesakit diambil pada hari pertama imobilisasi dan pada hari ketiga ke atas. Ujian pembekuan darah (PT dan aPTT) dilakukan dengan menggunakan mesin ACL TOP 300 CTS (Bedford,USA) yang menggunakan kaedah optikal bagi mengesan pembekuan darah. Sebanyak 20 sampel plasma daripada kontrol normal diambil daripada penderma darah yang sihat. Analisis graf gelombang pembekuan darah bagi PT dan aPTT yang terhasil semasa proses pembentukan darah beku dilakukan. Bagi perbandingan parameter pada graf pembekuan darah antara kontrol normal dan pesakit, 'Mann-Whitney test' digunakan kerana data yang diperolehi tidak mempunyai taburan yang normal. 'Paired t-test' digunakan untuk membuat perbandingan parameter pada graf gelombang pembekuan darah antara hari pertama imobilisasi dan hari ketiga ke atas. Kajian ini telah mendapat kelulusan etika daripada Universiti Sains Malaysia (Kod JEPem:USM/JEPem/20010032).

Keputusan: Purata PT dan aPTT bagi kontrol normal adalah 11.66 saat dan 33.98 saat. Tiada perbezaan ketara pada purata PT dan aPTT antara pesakit dan kontrol normal. Purata parameter pada graf pembekuan darah PT iaitu perubahan delta, masa untuk halaju puncak dan jumlah penyerapan cahaya sewaktu halaju puncak adalah lebih tinggi bagi pesakit dengan imobilisasi berbanding kontrol normal. Untuk parameter pada graf pembekuan darah aPTT, perubahan delta dan jumlah penyerapan cahaya sewaktu halaju puncak bagi pesakit adalah lebih tinggi berbanding dengan kontrol normal. Parameter pada graf gelombang pembekuan darah PT bagi pesakit menunjukkan tiada pebezaan ketara antara hari pertama imobilisasi dan pada hari ketiga ke atas. Bagi parameter pada graf gelombang pembekuan darah aPTT, kesemua parameter adalah lebih tinggi pada hari ketiga ke atas kecuali pada masa titik akhir pembekuan darah.

Kesimpulan: Pesakit dengan imobilisasi yang berpanjangan mempunyai parameter pada graf gelombang permbekuan darah PT dan aPTT yang lebih tinggi berbanding kontrol normal. Parameter pada graf gelombang pembekuan darah mempunyai potensi untuk membantu dalam mengenal pasti pesakit yang berisiko untuk mendapat darah beku di dalam saluran darah melalui analisis pada graf gelombang pembekuan darah ini. Walaubagaimanapun, lebih banyak kajian lanjut diperlukan supaya data tambahan yang ada di dalam ujian rutin ini dapat digunakan sepenuhnya.

ABSTRACT

Objectives: Prolonged immobilization is a well-known risk factor for thromboembolism. This study aims to compare the clot waveform analysis (CWA) parameters between patients with prolonged immobilization and normal controls. We also intended to compare patients' clot waveform parameters at day one and after day three of immobilization.

Methods: A total of 30 patients with prolonged immobilization from Hospital USM were recruited for this study. The patients' plasma were taken on day one of admission and were repeated after a period of immobilization (on day three and above). The coagulation profile (prothrombin time, PT and activated partial thromboplastin time, aPTT) was performed using ACL TOP 300 CTS (Bedford,USA) coagulation analyser that utilize optical method for clot detection. Blood sample for 20 normal controls were recruited from healthy blood donors. The clot waveform for PT and aPTT generated during clot formation was then analysed. For comparison of clot waveform analysis between healthy controls and patients with prolonged immobilization, Mann-Whitney test was used. Paired t-test was used for the comparison of clot wave parameters between day one of immobilization and day three and above. This study was approved by Universiti Sains Malaysia Research Ethics Committee (JEPeM code: USM/JEPeM/20010032).

Result: Mean PT and aPTT for healthy controls were 11.66 seconds and 33.98 seconds, respectively. There was no significant difference in mean PT and aPTT between patients and controls. However, the mean PT CWA parameters were significantly higher among

patients with prolonged immobilization than controls, which were the delta change, peak time velocity and height velocity. While for aPTT CWA, the delta change and height velocity were significantly higher than controls. PT CWA parameters among patient with prolonged immobilization showed no significant difference between day one and day three of immobilization, while for aPTT CWA, all parameters were higher after day three of immobilization except for time of endpoint.

Conclusion: Patients with prolonged immobilization had elevated PT and aPTT CWA parameters compared to normal controls. CWA parameters may help to recognize patient at risk of developing thrombosis through the changes at the clot waveform. However, we need more study on the clot waveform so that additional information from routine coagulation testing can be fully utilize.

CHAPTER 1
INTRODUCTION

1.1 Clot waveform analysis in patient with prolonged immobilization

Venous thromboembolism (VTE) that comprises both deep vein thrombosis (DVT) and pulmonary embolism (PE) is a major health problem. The estimated incidence rate of venous thromboembolism in general population is 1-2 per 1000-person-years.[1,2] About two-thirds of VTE episodes manifest as DVT and one-thirds as PE with or without DVT. [3] Several studies found that the incidence of VTE increases with age. The rates increase sharply after the age of around 45 years and are slightly higher in men than in women. [3]

VTE results from blood clots formation in the vein and mainly composed of fibrin and platelet plug. Their occurrence is due to imbalance between thrombogenic stimuli and protective factors such as coagulation factor inhibitors. [4] This condition is worsened by additional risk factors such as age, surgery, immobility, pregnancy, trauma, malignancy and *et cetera*.[3,4] Immobility is a well- recognized risk factor for VTE. Patients that previously confined to bed rest, whether in a hospital or nursing home, have an 8-fold increase in the odds of developing VTE complications.[5] Based on the Wells' criteria, immobilization is defined as reduced mobility for three days and above.

Current practice to assess the coagulation system is by performing screening test for coagulation. Prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen levels are routinely performed to determine the coagulation activation, whereas D-Dimer is performed to determine fibrinolysis activation. Hypercoagulable state is indicated when coagulation system is active, which will result in shortened PT and APTT, increased fibrinogen, D-Dimers and platelet count. [6]

PT assay is an in vitro measurement of the extrinsic and common coagulation pathways. Tissue factors, calcium and phospholipids are added to the test plasma and the time for the formation of fibrin clot is recorded. In aPTT assay, contact factor activator (eg silica, kaolin) combined with calcium and phospholipids are added to the sample to initiate the clotting process. Three methods are available for measuring the PT and aPTT coagulation assay which are the manual tilt tube method, semi - automated coagulation analyser and automated coagulation analyser. Currently most laboratories are using the automated coagulation analyser that use two distinct methods to detect clot formation which are either optical or mechanical method.

In this study, we are using ACL TOP 300 automated coagulation analyser that employs optical method for clot detection. The ACL series analyser detect light absorbance during clot formation. As shown in Figure 1, delay phase indicate mixing of the sample and reagent and the data acquired during this phase is not used for the analysis. The baseline phase begins after the sample and reagents are mixed. Fibrin formation represented by the acceleration phase and the optical change during this phase is rapid resulting in the steep slope rise. As fibrin formation progress, the sample becomes more turbid, hence reducing the amount of light falling on a photosensitive detector. Deceleration phase indicate reduction in the rate of clot formation and during this time, the fibrinogen has been converted to fibrin causing the optical change and the slope plateau off. End point indicate the clot formation is complete and data acquisition stopped at this point. The delta change represents the changes in light absorbance between the baseline and end point. [7]

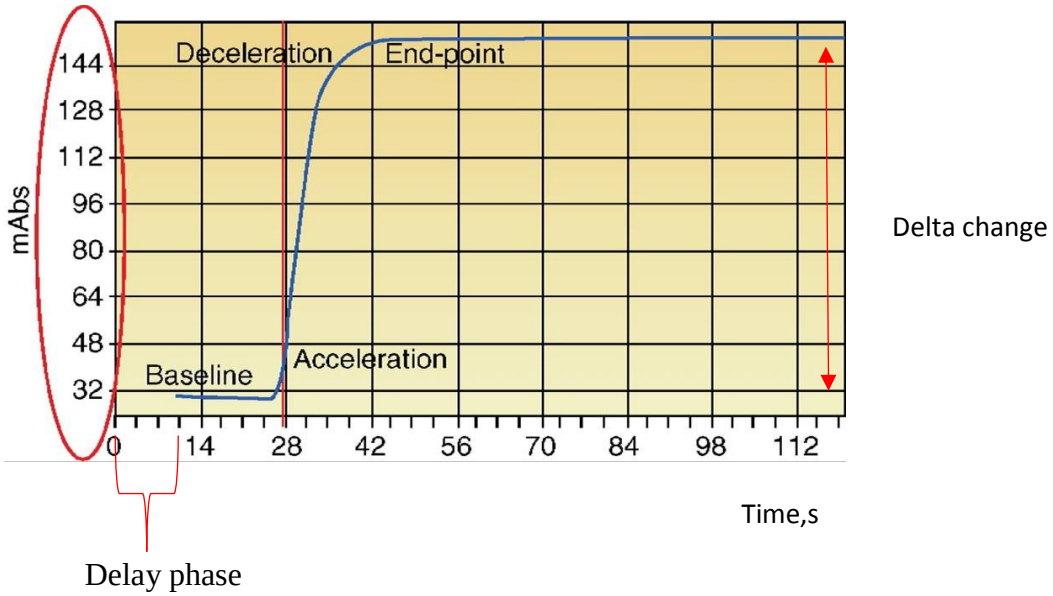

Figure 1: Normal clot signature curve for PT and aPTT (Image courtesy of Beckman-Coulter, CA).

From the PT clot signature curve shown in Figure 1, the first derivative curve (shown in pink line in Figure 2) can be derived from the clot signature curve. The first derivative curve represents the velocity of clotting process. Peak time velocity (shown by red arrow) is the time at which maximum velocity of the clotting process is achieved, and height velocity (shown by yellow arrow) represents the amount of light absorbance during the clot formation.

Figure 2: Example of normal clot waveform for PT and its derivative

While for aPTT clot curve as shown in Figure 3, additional data obtained were the first derivate curve (shown in pink line) that represent velocity of the clotting process and second derivative (shown in turquoise line) that represent acceleration of the clotting process. For first derivative curve, the yellow arrow is the height velocity that represent the maximum amount of light absorbed during clot formation and the red arrow is the peak time velocity which is the time at which maximum velocity of the clotting process is achieved. While for second derivative curve, the green arrow indicates the maximum acceleration of the clotting process.

Figure 3: Example of normal clot waveform for aPTT and its derivatives

This additional information can be obtained from the routine test of PT and aPTT without additional cost. However, the PT and aPTT clot waveform is not well utilized and more study is needed to explore other potential use of CWA. Therefore, in this study we will evaluate the CWA parameters of PT and aPTT in patient with prolonged immobilization by comparing the CWA parameters between patients and normal controls, and identify

the difference in clot waveform parameters at day one and after day three and above of immobilization.

1.2 Justification of study

Considering the large number of patients who are at risk of developing VTE, especially among hospitalized patients who are confined to bed, routine use of the anticoagulant prophylaxis would put an unbearable financial burden on the health care systems.

The use of CWA can give information about the coagulation state of the patient. Hence, it may be included as one of the parameters for VTE screening. Furthermore, this CWA data is readily available from routine coagulation testing and confer no additional cost.

To date, majority of published works on its utility have focused on congenital bleeding disorder, disseminated intravascular coagulation (DIC) and sepsis. Existing literature on thrombotic disorder is still lacking. One study by Ruberto et al in 2018 reported a higher clot wave analysis (CWA) parameters were shown to be associated with high Padua Prediction Score, a VTE risk assessment tool for hospitalized medical patient, suggesting its utility as a surrogate for hypercoagulability. This study is going to assess the clot wave parameters in patient with prolonged immobilization and the potential use for VTE screening. This may help in early diagnosis for a better management and allow the patients in need to receive prophylaxis anti-coagulant treatment, while the low-risk patients may avoid the unnecessary prophylaxis treatment.

1.1 Dissertation Organization

This dissertation was arranged based on Format B (Manuscript ready format) according to the guideline by the Postgraduate Office, School of Medical Sciences (2016). The second chapter is the study objectives. Chapter three is the manuscript that is ready for submission to Oman Medical Journal. The manuscript title is "Clot waveform analysis parameters in patient with prolonged immobilization". Study protocol submitted and the obtained ethical approval from JEPeM, USM (JEPeM code: USM/JEPeM/20010032) is in the chapter four. Chapter five contains elaboration on methodology and additional references. The raw data was included in the attached CD.

CHAPTER 2

STUDY OBJECTIVES

Objectives

2.1 General Objective

To study the Clot Wave Analysis (CWA) parameters among patient with prolonged immobilization.

2.2 Specific Objective

a) To verify reference range of CWA parameters among healthy individuals.

b) To compare the clot wave analysis parameters between patient with prolonged immobilization and healthy individual.

c) To compare the mean of the CWA parameters between day one of admission and after day three of immobilization.

CHAPTER 3

MANUSCRIPT

3.1 Title page

Coagulation status using Clot Wave Analysis (CWA) in patient with prolonged immobilization.

Authors:

Rosmaniza Muhamat Yusoff [1,2], Salfarina Iberahim [1,2], Noor Haslina Mohd Noor [1,2] Rosline Hassan [1,2]

[1] Department Haematology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

[2] Hospital Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

Word count excluding abstract, acknowledgements and references: 3500 words.

Name and address for corresponding author:

Name: Dr Salfarina Iberahim

Address: Department of Hematology, School of Medical Science, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

Phone number: +6097676187. Fax: +6097673333

Email address – salfarina@usm.my

3.2 Abstract

Objectives:

Prolonged immobilization is a well-known risk factor for thromboembolism. This study aims to determine the changes of clot waveform analysis (CWA) parameters in a patient with prolonged immobilization following lower limb trauma. We also intended to determine the CWA parameters between day one and after day three of immobilization.

Methods:

A total of 30 patients with prolonged immobilization from Hospital USM were recruited for this study. The patients' plasma were taken on day one of admission and repeated after a period of immobilization (on day three and above). PT-based CWA and aPTT-based CWA was performed using ACL TOP 300 CTS (Bedford,USA) coagulation analyser that utilize optical method for clot detection. Plasma samples for 20 normal controls were recruited from a healthy blood donor. The CWA parameters generated during clot formation were analysed. For comparison of CWA parameters between healthy controls and patients with prolonged immobilization, Mann-Whitney test was used. Paired t-test was used for the comparison of clot wave parameters between day one and after day three of immobilization. This study was approved by Universiti Sains Malaysia Research Ethics Committee (JEPeM code: USM/JEPeM/20010032).

Result:

Mean PT and aPTT for healthy controls were 11.66 seconds and 33.98 seconds, respectively. There was no significant difference in mean PT and aPTT between patients and controls. However, the mean PT CWA parameters were significantly higher among patients with prolonged immobilization than controls, which were the delta change, peak time velocity and height velocity. While for aPTT CWA parameters, the delta change and height velocity were significantly higher than controls. PT CWA parameters among patient with prolonged immobilization showed no significant difference between day one and after day three of immobilization, while for aPTT CWA, all parameters were higher in day three except for time of endpoint.

Conclusion:

Patients with prolonged immobilization had elevated PT and aPTT CWA parameters compared to normal controls. CWA parameters may help to recognize patient at risk of developing thrombosis through the changes at the clot waveform. However, further study are needed so that additional information from routine coagulation testing can be fully utilized.

Keywords: Prothrombin Time (PT), activated Partial Thromboplastin Time (aPTT), CWA, prolonged immobilization

3.3 Introduction

Venous thromboembolism (VTE) is a major health problem, and it comprises both deep vein thrombosis (DVT) and pulmonary embolism (PE). It contributes as one of significant cause of mortality and morbidity in hospitalized patients and is costly to the healthcare system in term of management including treatment following VTE. After myocardial infarction and stroke, it is the third most common cardiovascular disease in the Western parts of the world.[1]

The overall VTE rates are 100 per 100,000 populations per year, and 70% are hospital acquired. [2] About two-thirds of VTE episodes manifest as DVT and one-thirds as PE with or without DVT. The rates of VTE increase sharply after the age of around 45 years and are slightly higher in men than in women. [3] Immobility is a recognized risk factors for VTE not only in surgical patients but also a potential risk factors in medical patients. Risk of VTE increases significantly with two times higher in immobilized patients than in those with normal mobility. [4] According to Malaysian clinical practice guideline on prevention and treatment of venous thromboembolism issued in 2013, immobilization is when patient is bedridden for more than three days. [5,6]

Diagnosis of VTE is confirmed from the radiological examination. DVT diagnosis is established from the compressive ultrasonography, while PE is confirmed from the computerized tomography pulmonary angiogram (CTPA). Clinical prediction models are available to help identify patients at risk for VTE and the most widely used is the Wells' score. DVT is likely if the Wells' score is ≥ 2, and PE is likely when the score is >4.2.

VTE results from the blood clots formation in the vein, mainly composed of fibrin and platelet plug. Current practice to assess the coagulation system is by performing the screening test for coagulation which are the Prothrombin Time (PT) and activated partial thromboplastin time (aPTT). Hypercoagulable state is indicated when coagulation system is active, which will result in shortened PT and aPTT, increased fibrinogen level, D-dimers and platelet count. [7]

CWA provide additional information from the routine PT and aPTT testing beside clotting time, and it can be applied in various clinical setting. Many studies focused on application of CWA on disseminated intravascular coagulation (DIC), congenital bleeding disorders and sepsis. [8,9] Biphasic pattern of aPTT waveform was observed in lupus anticoagulant (LA) positive patient, haemophilia case with and without inhibitor and patients with DIC. [8] Suzuki et al reported the height of the first derivative peak and second derivative peak is useful for the diagnosis of DIC and prediction of the bleeding risk or outcome in patients with DIC. [10] In thrombotic disorder, patients with acute VTE had elevated aPTT based CWA parameters compared to control. [11] \Another study found CWA values were higher in patients with high Padua Prediction Score (PPS). [12]

Clot waveforms can be derived from automated coagulation analyser that utilize the optical testing principle. It has an extra advantage over mechanical method by providing the clot waveform during the dynamic of the clotting process. Two types of automated coagulation analyser are available which analyses the changes in light transmittance or light absorbance that occur during the process of clot formation. [8]

The ACL series coagulation analyser detect light absorbance during clot formation. The CWA parameters that were produced by ACL series consist of delay phase, baseline phase, acceleration, deceleration, end point and delta change as shown in Figure 1. Delay phase indicate the mixing process between the sample and the reagent, and the data acquired during this phase is not used for the analysis. The baseline begins after the sample and reagents are mixed. Fibrin formation represented by the acceleration phase and the optical change during this phase is rapid resulting in the steep slope rise. As fibrin formation progress, the sample becomes more turbid, hence reducing the amount of light falling on the photosensitive detector. Deceleration phase indicate reduction in the rate of clot formation and during this time, the fibrinogen has been converted to fibrin causing the optical change and the slope plateau off. Data acquisition stop at the endpoint. Delta change represents the change in light absorbance between the baseline and end point. [13]

Normal PT clot signature curve has first derivative curve (pink line in Figure 2) that represent the velocity of clotting process, which automatically derived from the software. Peak time velocity (red arrow in Figure 2) is the time at which maximum velocity of the clotting process is achieved, and height velocity (yellow arrow) represents the amount of light absorbance during the clot formation. APTT clot signature curve (Figure 3) provides additional data apart from clotting time which include the first derivate (yellow arrow) and second derivative (green arrow) that represent the maximum velocity and maximum acceleration of the clotting process, respectively. Other coagulation analyser such as MDA and Sysmex CS series detect light transmittance during clot formation. So far, there is no study of transmittance curve in the PT that was produced by MDA and Sysmex CS series. Most study focused on the aPTT transmittance curve.

In aPTT clot transmittance curve, the upper waveform shows the recording of changes in transmitted light intensity (T) over time (t) (Figure 4). Point 'A' indicate the beginning of the signal while point 'B' is the onset of coagulation. Point 'C' and 'D' represent the midpoint and end of the coagulation, respectively. Point E indicate the end of the signal. The data is separated into the pre-coagulation phase (A, B), the coagulation phase (B–D), and the post-coagulation phase (D, E). The red and blue curves was derived from the clot transmittance curve. The middle waveform shows the first derivative of transmittance reflecting maximum coagulation velocity (min1) that indicates the conversion of fibrinogen to a fibrin clot. While lower waveform is the second derivative of transmittance data that reflect the coagulation acceleration (min2) and maximum coagulation deceleration (max2). [14]

This additional information can be obtained from the routine test of PT and aPTT without additional cost. However, the PT and aPTT clot waveform parameters is not well utilized to explore other potential use of CWA parameters. Therefore, in this study we will evaluate the CWA parameters of PT and aPTT in patient with prolonged immobilization by comparing the CWA parameters between patients and normal controls and identify the difference in clot waveform parameters at day one and after day three of immobilization.

3.4 Method

This is a prospective cohort study involving patients with prolonged immobilization due to lower limb injuries in orthopaedic ward Hospital USM, from November 2020 until October 2021. This study was approved by Universiti Sains Malaysia Research Ethics Committee (JEPeM code: USM/JEPeM/20010032). In this study, 30 patients from age 18

to 48 years old were included. The clinical and laboratory data consist of age, gender, BMI and smoking status were traced from patients' folder.

Patient with baseline coagulation profile taken on admission was followed-up until repeat sample were taken after day three of immobilization. The samples for PT and aPTT were collected in a trisodium citrate bottle (2.7 ml of blood) and was sent immediately to Haematology Laboratory Hospital USM. To obtain the platelet poor plasma (PPP), samples were centrifuged for 15 minutes at 2500 g at room temperature. The PPP was then separated from the whole blood.

In this study, conventional coagulometer that utilize optical method was used. Clot waveform derived simultaneously from ACL TOP 300 CTS (Bedford,USA) coagulation analyser during routine PT and aPTT testing. It is based on the continuous monitoring of light absorbance during clot formation. The CWA was automatically constructed by plotting changes in light absorbance against time. The parameters that had been studied for the PT is baseline length, time of endpoint, delta change and first derivative peak (represented by peak time velocity and height velocity) of the clot formation. For aPTT, the baseline length, time of endpoint, delta change, first derivate peak (represented by peak time velocity and height velocity) and second derivative (represented by peak time acceleration were studied. All the parameters were manually recorded and analysed.

Data was recorded and analysed by using the Statistical Package for the Social Sciences (SPSS) Statistics for Windows (Version 25.0. Armonk, NY: IBM Corp.). The statistical analysis was performed using Mann-Whitney test for comparing parameters between patients with prolonged immobilization and normal controls. While for comparison of

clot wave pattern between day one and after day three of immobilization, paired t-test was used.

3.5 Results

A total of 30 patients with prolonged immobilization aged between 18-48 years old were included in this study. Socio-demographic characteristics of patients was summarized in Table 1. Majority of the patients aged between 18-29 years old which is 20 (66.7%) patients, followed by 7 (23.3%) patients for aged 30-39 years, and between 40-49 years there is 3 (10%) patients. The mean age of study population was 26.7-year-old. Among them, 28 (93.3%) were male and 2 (6.7%) were female. 60% of the patients were smoker. Since this data was taken during the movement control order (MCO) period, there was limited number of patients for this study and majority of them were male. All of the patients were from Malay ethnicity and had normal BMI.

Clot Waveform analysis for Prothrombin Time (PT) and aPTT in healthy controls

The mean PT clotting time for healthy control was 11.66 seconds (Table 2). The mean for baseline length was 10.01 seconds, which indicate the time taken to activate the coagulation cascade from the addition of all reagents up to thrombin production. The mean endpoint for healthy control was 36.39 seconds. During this time, the data acquisition stopped, and all fibrinogens were converted to fibrin. The mean peak time velocity was 11.68 seconds, which indicate the time taken for conversion of fibrinogen to fibrin.

The mean aPTT clotting time for healthy controls was 33.98 seconds. The mean baseline length was 31.71 seconds. The coagulation stopped at 73.19 seconds. The mean peak time velocity and the mean peak time acceleration were 36.23 seconds and 33.60 seconds, respectively.

Clot waveform analysis in patient with prolonged immobilization in comparison with normal controls.

Mann-Whitney Test was used in this analysis as the data was not normally distributed. Mean PT clotting time for patients was 12.35 seconds. The delta change, peak time velocity and height velocity were significantly higher in CWA for PT in patients with prolonged immobilization compared to healthy individuals ($p<0.05$). Meanwhile, baseline length and endpoint showed no significant difference between patients and healthy controls ($p>0.05$).

Mean aPTT for patients was 34.90 seconds. The delta change and height velocity were significantly higher compared to controls ($p<0.05$). Meanwhile, no significant difference were found in the baseline length, end point, peak time velocity and peak time acceleration between healthy individuals and patients with prolonged immobilization ($p>0.05$).

Clot waveform analysis at day one and after day three of immobilization.

Mean PT and aPTT after day three of immobilization were 12.46 seconds and 34.03 seconds, respectively. For PT CWA parameters, only end point showed significant higher level in day one compared to prolonged immobilization ($p=0.004$) (Table 4). Meanwhile, other CWA parameters for PT showed no statistical differences between day one and after day three of immobilization.

For aPTT CWA parameters, the aPTT clotting time, baseline length, delta change, peak time velocity, height velocity as well as peak time acceleration of the patients were significantly different between day one and day three and above ($p<0.05$). Meanwhile, end points showed no significant different between day one and day three and above ($p=0.286$).

3.6 Discussion

This study looks into the potential utility of CWA parameters to detect hypercoagulable state in patients who are at risk of developing acute VTE. Patients with prolonged immobilization due to lower limb trauma following motor vehicle accident (MVA) were involved in this study. Clot waveform parameters of both PT and aPTT were analysed in these patients serially at day one and after day three of immobilization. Four PT parameters from the curve (baseline length, endpoint, delta change and maximum velocity) and aPTT CWA parameters (baseline length, endpoint, delta change, maximum velocity, and peak time acceleration) were studied and analysed.

There was no significant difference in the standard mean aPTT between patient with prolonged immobilization and controls, but the PT of the patient is slightly prolonged

than the controls. Trauma can induce hypercoagulable state that frequently manifested by pathological thrombosis. [15] Previous studies in selected patient populations suggested that shorter aPTT were associated with an increased risk of VTE. [16,17,18] This association of shortened aPTT with thrombosis could be explained by increased activity of coagulation factors in the intrinsic or common pathways, or due to resistance to activated protein C. [15,17] Injury due to trauma exposes tissue factor to the circulation and initiates clot formation. Platelets activation amplifies thrombin generation and the clotting process, causing coagulation factors consumption. Fibrinolysis is also activated from the release of tissue plasminogen activator. [19] Excess consumption of platelets and coagulation factors will result in decompensation where the coagulation factors from extrinsic and intrinsic pathways decreased causing the PT and APTT to become normal or prolonged. [17,18] as seen in this study. This study also noticed a significant increase in PT CWA parameters among patient with prolonged immobilization in terms of delta change, peak time velocity and height velocity of clot formation. Increased velocity of clot formation means that the clot increased in strength and ultimately had greater density compared to clots in healthy controls. [14] Only few literatures found on the CWA of PT. A study by Matsumoto et al. suggested that the PT CWA parameters might be useful in predicting and monitoring haemorrhagic symptoms. [20] However, the study on PT CWA in thrombotic disorder is still lacking.

Most of the time, aPTT waveform was used in the study of the CWA. The aPTT clot waveform had been studied in haemophilia cases, disseminated intravascular coagulation (DIC) and sepsis. Study done by Ruberto et al. found that clot waveform analysis could give useful information to recognize a hypercoagulable state in patients with high Padua Prediction Score (PPS). Higher aPTT CWA parameters were shown to be associated with high PPS, suggesting its utility as a surrogate for hypercoagulability. They suggested that

the first and second derivative aPTT, D-dimer and fibrinogen levels could be added to PPS for better assessment of the global thromboembolic risk of the patient. [10] Another study of aPTT CWA utilizing light transmittance method among covid 19 patients found that CWA profiles showed a significantly higher mean aPTT, min1, min2, max2, and median delta change than in healthy controls. These CWA variables showed hypercoagulopathy and this is more pronounced in critically ill patients. [12] For aPTT CWA, we noted the delta change and height velocity is higher compared to normal controls. The delta change correlates to fibrin concentration and represent to some extent fibrin thickness and clot density. The height of the velocity (first derivative curve) is considered to reflect the 'thrombin burst'. Low height of the velocity curve suggests the bleeding risk and the increased heights of the first and seconds derivatives curve may be a marker of hypercoagulability. [9]

In this study, we found the PT endpoint was higher on day one compared to after day three of immobilization. This may indicate as the immobilization is more prolonged, the time taken for clot completion became shorter and indicate risk of thrombosis. [5] Other PT CWA parameters showed no difference between the day of immobilization. For aPTT waveform, all the CWA parameters studied were higher in prolonged immobilization patients except for endpoint time.

Recent study among covid-19 patients found that all the CWA parameters (maximum velocity, maximum acceleration) became markedly elevated from second day of admission onwards. These markedly raised CWA parameters are possibly consistent with hypercoagulability. The same study also showed that CWA parameters markedly raised when the ICU stay prolonged, which suggested that there is positive association between raising CWA parameters and the worsening covid-19 infection.[21]

CWA parameters has been studied in different types of infection. It has been shown to predict poor outcomes in severe infections with disseminated intravascular coagulopathy. The biphasic waveform detected in CWA has been studied extensively in the setting of DIC arising from different causes including infection. This waveform is caused by the formation of calcium-dependent precipitates of very low-density lipoprotein and C-reactive protein (CRP) upon recalcification of plasma in vitro and it has been demonstrated to be an early marker of DIC and was correlated with poor clinical outcomes.[22]

This study has some limitations. Firstly, analysis of the PT and aPTT CWA parameters was based on the optical method, hence the lipemic or haemolyze samples could impact on the CWA results and interpretation. Secondly, no other parameters were used to investigate a hypercoagulable state such as thrombin time and fibrinogen level. Thirdly, small population of patients were studied. There was only a small number of prolonged immobilization patient who were not on thrombosis prophylaxis. Most of the patients who were at risk for thrombosis were started on thrombosis prophylaxis early. There was also limitation in evaluating clot waveform parameters of the PT and APTT because it was manually recorded in this study. Development of software in future for recording the parameters will give more accurate view on data acquisition