Evaluation of Coagulation profile in Primary Brain tumors in the Perioperative period using Thromboelastography

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled "**Evaluation of Coagulation profile in Primary Brain tumors in the Perioperative period using Thromboelastography**" is a bonafide research work carried out by me under the guidance of Dr. Manikandan S, Professor and Head, Division of Neuroanaesthesia and Critical Care, Department of Anaesthesia, SCTIMST.

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CERTIFICATE BY THE GUIDE

This is to certify that the dissertation titled "**Evaluation of Coagulation profile in Primary Brain tumors in the Perioperative period using Thromboelastography**" is a bonafide research work done by Dr. Gautham N S in partial fulfilment of the requirement for the degree of DM Neuroanaesthesia, SCTIMST. This project is externally funded by the Indian Society of Neuroanaesthesiology and Critical Care (ISNACC), and has been done under my guidance.

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CONTENTS

Abbreviations

TF	:	Tissue Factor
HMWK	:	High-Molecular-Weight Kininogen
EDTA	:	Ethylene Diamine Tetra Acetic acid
FDP	:	Fibrin Degradation Products
TT	:	Thrombin Time
POC	:	Point of Care
aPTT	:	Activated Partial Thromboplastin Time
PT	:	Prothrombin Time
INR	:	International Normalized Ratio
IRP	:	International Reference Preparation
LMWH	:	Low Molecular Weight Heparin
TEG	:	Thromboelastography
ROTEM	:	Thromboelastometry
DAPTTIN	:	Double activated APTT reagent
PPP TEG	:	Platelet-Poor Plasma- TEG
PRP TEG	:	Platelet-Rich Plasma- TEG
MA	:	Maximum Amplitude

PMA	:	: Projected MA
TMA	:	Time to MA
SEMS	:	Shear Elastic Modulus Strength
CI	:	Coagulation Index
DIC	:	Disseminated Intravascular Coagulation
TPA	:	Tissue Plasminogen Activator
DVT	:	Deep Vein Thrombosis
PE	:	Pulmonary Embolism
TBI	:	Traumatic Brain Injury
UFH	:	Unfractionated Heparin
ADP	:	Adenosine Diphosphate
PCC	:	Prothrombin Complex Concentrate
SAH	:	Subarachnoid Hemorrhage
ICH	:	Intracranial Hemorrhage
TTG	:	Total Thrombin Generation
AIS	:	Acute Ischemic Stroke
VTE	:	Venous Thromboembolism
SMR	:	Standardized Mortality Rate

BBB	:	Blood Brain Barrier
DNA	:	Deoxyribonucleic Acid
ATP	:	Adenosine Triphosphate
VEGF	:	Vascular Endothelial Growth Factor
GBM	:	Glioblastoma Multiforme
ICU	:	Intensive Care Unit
OT	:	Operation Theatres
ASA	:	American Society of Anesthesiologists
GCS	:	Glasgow Coma Scale
OCP	:	Oral Contraceptive Pills
PEEP	:	Positive End Expiratory Pressure
S.D	:	Standard Deviation
FDP	:	Fibrin Degradation Products

Introduction

Primary brain tumors account for most patients coming for neurosurgery. According to reviews, primary brain tumors account for most of the deaths occurring due to cancer. 11 to 12 per 100,000 adults are being diagnosed every year with primary brain tumors, of which 6 to 7 per 100,000 are malignant. Deaths attributable to primary brain tumors is said to be 13,000 every year. Factors like age, histological type, genetic and environmental factors, geographic and ethnic variation play a role in the risk and prognosis of primary brain tumors[1]. Though brain tumors may account for only 2% of all cancers, they have a disproportionately higher share of mortality and morbidity due to cancers.

Brain tumors are responsible for about 30% of all cancer related deaths in children and about 20% of all cancer related deaths in young adults[2]. One of the main reasons for this is the presence of coagulation related abnormalities seen in patients with primary brain tumors. A study evaluating the frequencies and causes of death following surgery for primary intracranial tumor found that events such as CNS hemorrhage, CNS infarction, acute myocardial infarction, pulmonary embolism and deep vein thrombosis were among the leading causes of death in the perioperative period[3].

Brain tumors are known to be associated with abnormalities of the coagulation system, which may vary from a hypocoagulable state to a hypercoagulable state from patient to patient as well as within the same patient at different times during the course of the disease. Studies have shown that an alteration in the coagulation parameters occurs in the perioperative period in patients with brain tumors, and that these changes are aggravated in the post-operative period[4]. Although there have been several studies and reviews which have attempted to study coagulation abnormalities in this population, these studies do not provide concrete evidence on the coagulation changes, due to variability in the types of tests used, timing of blood sampling etc.

Therefore, it is important to undertake a study to assess the coagulation profile, using an appropriate test, in the perioperative period to study the temporal course of coagulation changes during this time. Thromboelastography is a viscoelastic test of coagulation which measures all the components of the coagulation pathway, and it would be appropriate to study the coagulation changes in the perioperative period using Thromboelastography.

Review of literature

Mechanisms of coagulation: Classical theories and current concepts

Blood has several functions, the most important of which is to carry oxygen. It exists in fluid form in the blood vessels. When an injury to the vessel occurs, blood gets exposed to the extravascular components, leading to coagulation of blood to a gel, which plugs the site of injury, till the injury on the vessel is repaired. The process by which blood in fluid form gets converted to gel is called hemostasis. The process of hemostasis was described for the first time in 1964 by Davie et al[4] and Mc Farlane et al[5]. They described the "Classical Cascade Theory", which has been accepted for many years. According to this theory, sequential activation of proenzymes by proteases in the blood leads to formation of thrombin, which in turn, breaks fibrinogen into fibrin. This theory divides coagulation cascade into two arms. The extrinsic pathway is initiated by components which lie outside the intravascular space. Factor VII is activated in the presence of its cofactor, the Tissue Factor (TF), forming the factor VIIa/TF complex, which leads to the activation of factor X[6]. The intrinsic pathway is initiated by blood components within the intravascular space. When blood encounters a negatively charged surface, factor XII becomes activated, in the presence of plasma components like Prekallikrein and High-Molecular-Weight Kininogen (HMWK). Activated factor XII activates factor XI, which in turn activates factor IX. Activated factor IX in the presence of factor VIII activated by traces of thrombin and in the presence of calcium ions (Tenase Complex) activates coagulation factor X, generating thrombin and subsequently fibrin formation. A pictorial representation of the Classical Cascade is shown in Figure 1.

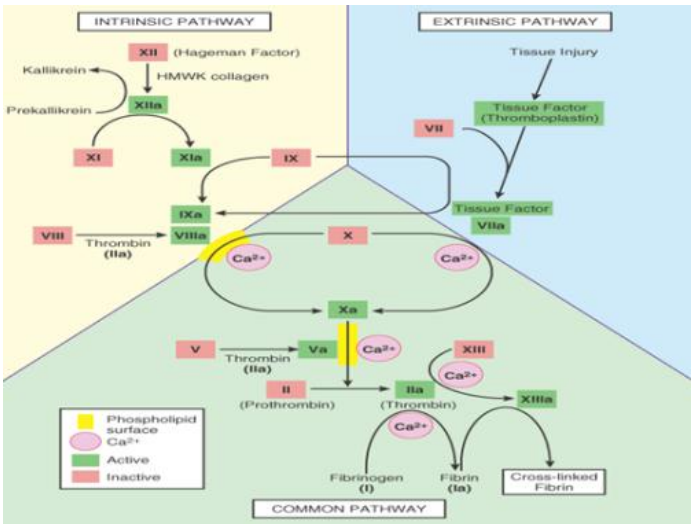

Figure 1 shows a pictorial representation of the Classical Coagulation Cascade.

This model of coagulation has been widely accepted until recently. Some drawbacks have been pointed out in this description of coagulation. Some investigators recently presented evidence that Factor XII deficiency did not cause increased bleeding, nor did its absence protect against pathological thrombosis[7]. Also, it has been shown in studies that exposure of blood to cells which express tissue factor on their surface is necessary as well as sufficient for coagulation to occur[8]. Recently, a cell based pathway for coagulation has been described[6], which proposes that tissue factor is expressed on the surface of cells outside the vascular system, like smooth muscle cells and fibroblasts surrounding blood vessels. When these are exposed to blood following injury to vascular endothelium, the coagulation pathway is initiated. Coagulation then proceeds through phases of initiation, amplification, propagation and termination. In the Initiation phase, Factor VIIa in blood interacts with tissue factor and forms a complex. This complex, further activates small quantities of Factor IX and Factor X and leads to formation of small quantities of thrombin, mainly in the extravascular space. When there is an injury to vascular endothelium, the small quantities of activated factors IX

and X interact with platelets and Factor VIII and proceed to the Amplification phase, where thrombin activates platelets, which in turn leads to activation of Factor V and Factor VIII on its surface. In the Propagation phase, large number of platelets gets recruited to the site of vessel injury. In the Termination phase , the clotting process is limited to the site of injury by anticoagulants such as Antithrombin III, Protein C and Protein S. The Cell based pathway of Coagulation is pictorially represented in Figure 2.

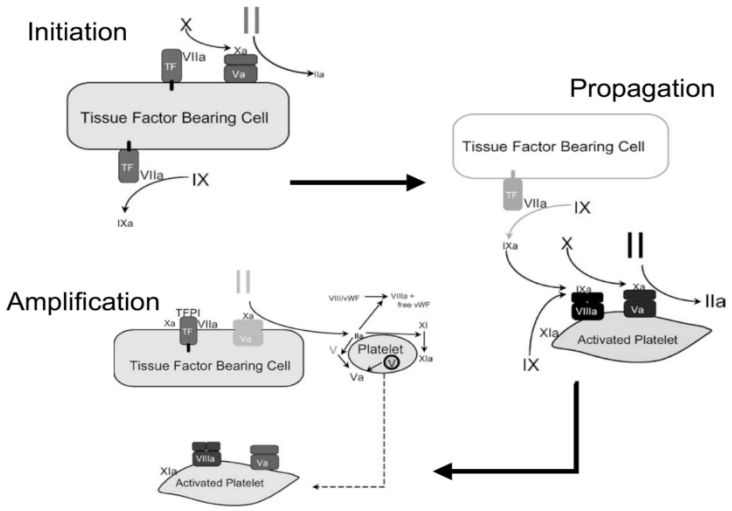

Figure 2 shows a pictorial representation of the Cell based theory of Coagulation. The phases of Initiation, Amplification and Propagation are shown.

Tests for coagulation and their drawbacks in perioperative application

An ideal coagulation test should be easy to perform, should be done rapidly, while giving reliable and robust results. It should also accurately estimate the risk of both bleeding and thrombosis. Further, it should incorporate the influences of physiological

phenomenon such as endothelial interaction, flow conditions, effects of temperature and pH. The classical tests for evaluating coagulation abnormalities were developed at a time when the mechanisms of coagulation were still very unclear and the pathways were still being elucidated. Hence, they are not ideal for screening patients with coagulation abnormalities in the perioperative period. Platelet count is a quantitative measurement of platelets in EDTA solution. As it is only quantitative, it does not provide any information about the function of platelets. Hence, a normal platelet count does not ensure a normal function or lack of bleeding, nor does low platelet count mean that the patient will have bleeding tendencies. It is also difficult to detect hypercoagulable abnormalities. Tests of platelet function maybe better for monitoring coagulation. Fibrinogen level is measured, most commonly, using the Clauss method. In this method, the plasma is diluted tenfold, so that Fibrinogen becomes the rate limiting factor, and thus, the time for coagulation is inversely proportional to the fibrinogen concentration.. Inaccuracies in the test can be produced by Fibrin Degradation Products (FDP), inhibitors of Fibrin formation and polymerization, such as Heparin. Thrombin Time (TT) is the time required for the conversion of Fibrinogen into Fibrin when plasma is exposed to Thrombin. It can be measured by both standard laboratory techniques as well as Point of Care (POC) methods. It is useful in detecting conditions such as Hypofibrinogenemia, Antibodies to Thrombin, use of Heparin or Thrombolytic agents. Activated partial thromboplastin time (aPTT) evaluates integrity of the combined intrinsic and common pathway for coagulation. In this test, blood is taken in a Citrated tube to arrest coagulation. To this, partial Thromboplastin, a surface activator such as Kaolin or Celite, and Calcium are added, and the time taken to clot is noted. Thromboplastin is a tissue extract which contains both Tissue factor and Phospholipid, which is used as a substitute for Platelet membrane. This test uses only the Phospholipid component, and is therefore called partial Thromboplastin. Surface activators of coagulation are used to initiate the coagulation and activate the Intrinsic pathway of Coagulation by directly activating Factor XII. Thus, this test

evaluates the factors involved in the intrinsic and the common pathway. Any deficiency in factors XII, XI, IX, VIII, High Molecular Weight Kininogen(HMWK) or Kallikrein will lead to a prolongation of aPTT. This test is also very sensitive to Heparin, which affects factors IX and X, and therefore, is commonly used to monitor therapy with Heparin. Prothrombin time (PT) uses Thromboplastin, containing both Tissue factor and Phospholipid, to activate the Extrinsic pathway. Calcium is also added, and the time to clot evaluates the function of the Extrinsic and Common pathways of coagulation. PT leads to rapid clotting of plasma, which reduces the sensitivity of the assay. Therefore, the reagent is diluted to prolong the PT to make it more sensitive. Different laboratories dilute the reagent to different degrees, and use different sources of Thromboplastin, leading to different values of PT from different laboratories from the same source of the blood sample. To overcome this, International Normalized Ratio (INR), which is the ratio of patient's PT to the value that would have been obtained, if International Reference Preparation (IRP) had been used in place of the test reagent. Prolongation of PT is seen with deficiencies of factors of Extrinsic and Common pathway, mainly Factor VII. It is also seen in Warfarin therapy, Vitamin K deficiency, Liver disease and DIC.

Prolongation of PT or aPTT indicates a quantitative or qualitative problem with one or multiple Coagulation factors in the pathways, but will not be able to identify the actual cause, for which further assays are required. Also, aPTT or PT cannot be accurately interpreted in isolation, and ideally, must be used together, along with Fibrinogen concentration. This is because both these tests conclude with generation of thrombin bursts, and therefore, very low levels of Fibrinogen leads to a prolongation of aPTT and PT independent of other clotting factors. Since both these tests use reagents whose compositions may vary between laboratories, there may be marked differences in sensitivity to coagulation Factor deficiencies, which, in some cases, may lead to abnormalities in the tests only when the coagulation factor deficiencies reach 30%. In

addition, since these tests may involve multiple Coagulation factors, the tests may detect deficiencies of certain factors leading to prolongation, without any clinical bleeding disorder, leading to unnecessary utilization of resources for further evaluation of the cause for these abnormalities or unnecessary transfusion of blood products. Further, these tests may also not be able to detect some clinically significant bleeding disorders such as Von Willebrand's disease, Hemophilia A, FXIII deficiency and α2-antiplasmin deficiency. These tests are also very sensitive to errors of sampling such as small volume, difficult venipuncture leading to activation of coagulation, contamination with heparin etc.

In clinical practice, laboratory assays for coagulation are used in 3 main situations; for monitoring patients on anticoagulants, investigating a possible bleeding disorder, and for routine perioperative screening for coagulation disorders[9]. In patients on anticoagulants, PT is generally used for monitoring patients on Vitamin K antagonists such as Warfarin, and aPTT is used for monitoring patients on Heparin. In addition to the problem caused by marked variability of sensitivity of these tests to accurately detect coagulation abnormalities due to variations in sensitivity of the reagents used both these tests, INR can only be accurate when the prolongation of PT is due to use of Vitamin K antagonists, and cannot be relied upon when the patient has other conditions which cause prolonged PT such as Liver disease, Congenital Factor deficiencies and DIC. INR is only accurate in the range 0f 1.5-4.5, because the standardization for PT values has been done only for this range. Values outside this range are not reliable. Unlike PT, the aPTT reagent doesn't even have standardization, and is not very sensitive when Low Molecular Weight Heparin (LMWH) is used. The newer anticoagulants have the advantage that their use does not usually warrant monitoring, but, in some situations such as recurrent thrombosis while on treatment with anticoagulants, or when patients on anticoagulants need to undergo a surgery, it may be necessary to monitor the coagulation in such patients. In such situations, PT

and aPTT may not be very useful because of the variability of the reagents to different coagulation factors, as described above. While investigating bleeding disorders, it is important to keep in mind the various components of the coagulation system, and their interactions in the coagulation pathways to arrive at the correct diagnosis. Due to the numerous cellular and plasma factors involved in the coagulation process and their interplay, the assessment of coagulation requires evaluation of the role of the different components like Platelets, the Coagulation Factors, calcium etc. Traditionally, Coagulation system has been evaluated using many tests like Platelet counts, Platelet function tests, Clotting factor levels, PT, aPTT, TT, FDP etc. For a comprehensive assessment of coagulation profile, many of these tests must be performed simultaneously, and therefore, may prolong the time for diagnosis of the bleeding disorder and the variability in the sensitivity of the different tests may contribute to the diagnostic dilemma. In pre-operative assessment of patients undergoing surgery, PT and aPTT is very commonly used to screen patients for bleeding disorders. The problem with the use of these tests for screening is that there can be a large number of false positive tests, due to prolongation of values by factor XII deficiency and Lupus Anticoagulant, which may not cause any clinically significant bleeding, but will lead to unnecessary evaluation or a risk of litigation if not evaluated further. Also, they might miss some bleeding disorders such as Von Willebrand's disease and Hemophilia A. Further, the traditional tests of coagulation are only useful in identifying a hypocoagulable state. Many studies have shown that they cannot very effectively identify a hypercoagulable state in vitro[10]. More recently, aPTT has been evaluated for its role as a screening tool for hypercoagulable states. Some studies have demonstrated shortened aPTT in patients with a history of vascular thrombosis in their event free intervals also[11]. McKenna et al[12] showed that the presence of a shortened aPTT was associated with 10 fold increased risk of thromboembolic events. Shortened aPTT has been shown in arterial as well as venous thrombotic events. aPTT is said to primarily reflect the function of factor VIII. Because of very low sensitivity to

coagulation factors other than factor VIII, and other aspects of coagulation, which are not addressed by aPTT, such as the role of platelets, the endothelium and possible role of inflammation in hypercoagulable states, its role as a screening tool in hypercoagulable states is yet unknown.

Viscoelastic Point of Care (POC) coagulation assays

The main drawback pertaining to the use of traditional tests of coagulation such as PT and aPTT is that they end when there is a generation of thrombin. Therefore, it is merely representative of a part of the whole process of coagulation, which involves formation of fibrin, its interaction with platelets leading to formation of a stable clot, and the effects of the fibrinolytic system. These tests, therefore, fail to address aspects of coagulation such as Kinetics of Clot formation, Strength of the Clot formed, and Viscoelastic properties of Clot, which play a very important role in determining whether the process of clot formation in a given situation is effective in achieving hemostasis or not. They also fail to address the effects of the Fibrinolytic system on Clot formation, and are unable to detect hypercoagulable tendencies because they are not dynamic tests of coagulation. To address these issues, Viscoelastic studies of coagulation were developed. The first instance in literature of using viscoelastic studies of coagulation was in 1889 by Hayem. He suggested that the coagulation function could be monitored by quantifying changes in blood viscosity during the clotting process. Viscoelastic studies of coagulation are based on the premise that a clot has two important properties, Elasticity and Viscosity[13]. Elasticity refers to a reversible deformation of a material when it is subjected to stress. It is indicative of the firmness of the clot, and by quantifying this, it gives us an idea about its stiffness. The Elastic property of a clot is mainly governed by the fibrin in the clot and its interaction with platelets. Viscosity on the other hand, refers to the thickness of a liquid, which provides resistance to flow. As described by Virchow, viscosity of blood is

a very important determinant of the coagulation process. Therefore, by studying these two properties of a clot, we get a very comprehensive idea about the coagulation process. The Viscoelastic tests of coagulation involve subjecting a sample of blood to the process of coagulation, while the properties of the clot such as its kinetics of formation, tensile strength, and nature of dissolution of clot are quantified and studied. Clot kinetics gives us an idea about the adequacy of clotting factors for coagulation. Strength of clot indicates the capacity to achieve hemostasis, and the time to dissolution of clot assesses the role of the Fibrinolytic system. Unlike the traditional tests of coagulation, these tests use whole blood, which provides cell surfaces and platelets, which interact with the clotting factors. Therefore, these tests incorporate all the processes of Coagulation such as Initiation, Amplification, Propagation, and Termination. The advent of viscoelastic studies of coagulation has led to more effective management of hemostasis during surgery, more judicious use of blood products[14], early identification of post-operative bleeding tendencies[15], and its appropriate treatment, better monitoring of anticoagulant therapy[14], and detection of hypercoagulability[10].

Currently, there are 3 instruments available for studying the Viscoelastic properties of coagulation; the Sonoclot coagulation and platelet function analyzer or Sonoclot (Sienco Inc., Arvada, CO, USA), the ROTEM (Pentapharm GmbH, Munich, Germany) and the TEG Thrombelastograph hemostasis analyzer or TEG (Haemonetics Corporation, Braintree, MA, USA). The basic principles of all the 3 instruments remains the same, in that, they subject a sample of blood to the process of coagulation, and study the Viscoelastic properties of whole blood during the process of clotting. All 3 instruments measure the rate of formation of Fibrin, the Strength of clot, and the Lysis of clot. Each of these instruments have their own terminologies and normal ranges for these measured variables. Thromboelastography and Thromboelastometry (ROTEM) study the viscoelastic properties under low shear conditions. The Sonoclot and ROTEM

measure changes in impedance to movement of a vibrating probe immersed in a blood sample, whereas TEG utilizes an oscillating cup with a fixed probe or piston.

Thromboelastography: Principle, technical aspects, and parameters

Thromboelastography was first described by Hartert in 1948. In 1966, the Haemoscope corporation registered trademarks for the terms Thromboelastography and TEG, and therefore, these terms are now used for evaluations done with TEG Haemoscope analyzers. The Haemoscope corporation is now a part of Haemonetics corporation, Braintree, USA. As mentioned earlier, Thromboelastography studies the Viscoelastic properties of whole blood under conditions of low shear, and provides information about the global hemostatic function, starting from Clot formation, proceeding to Clot retraction, and ending with Clot lysis. Blood is taken in a cylindrical cup and heated to 37^{o}C. The cylinder is them made to oscillate at an angle of $4^{o}45'$ at cycles of 10 seconds. A pin is suspended in the cylindrical cup containing blood, and is connected to a torsion wire, which acts as a sensor for any movement of the pin. When the cup oscillates, the pin doesn't move initially, but when the blood in the cylindrical cup starts to clot, strands of fibrin start to form between the pin and the inner wall of the cup, leading to an exertion of torque on the pin when the cup oscillates. The torsion wire detects this torque, which is converted into an electric signal, and represented in a graphical form. As clotting proceeds, the fibrin strands become stronger, leading to increasing movement of the pin. As the clot begins to lyse, the strands begin to dissolve, and the movement of the pin begins to decrease. From the graph obtained, a number of parameters can be studied. Each of these parameters represents a specific step of the clotting process, and allows us to perform a detailed assessment of the coagulation process.

Thromboelastography is generally performed on the native Whole blood. Many additional reagents can be added to speed up the clotting process or to study a specific part of the coagulation process. Also, components of blood, such as Platelet-rich Plasma, or Platelet-poor Plasma can be used to test for specific parameters. In general, the basic TEG® measurements of Kinetics, Strength and Stability of a coagulum can be determined by using a native Whole blood sample. Native Whole blood samples can be modified by addition of reagents to the in vitro sample to determine if a possible therapy might be effective for a coagulopathy, to improve speed of analysis, or to reverse a clinical condition (e.g., heparinization). These techniques involve addition of the following reagents to the native whole blood sample:

- Activators (Kaolin, Celite, Tissue factor, Thrombin, DAPTTIN, etc.)
- Heparin neutralizers (Heparinase, Protamine)
- Platelet blockers (ReoPro®, Integrilin®, Aggrastat®, etc.)
- Antifibrinolytic drugs (Epsilon-amino-caproic acid, Tranexamic acid, Aprotinin)

Celite- or Kaolin-activated TEG methods are used to reduce variabilities and to reduce the running time of a native whole blood TEG sample. Celite and Silica particles (diatomaceous earth) shorten coagulation time because it acts as a contact surface activator (Intrinsic pathway), which activates Factor XII and platelets and stimulates the reserve clotting ability of a blood sample. Similar to Celite is Kaolin (Hydrated Aluminum Silicate), which also activates the intrinsic pathway via Factor XII. Citrated TEG samples are used for conditions where it is difficult to transport the native or modified whole blood to the TEG machine within four to six minutes of phlebotomy. The Citrated TEG sample requires a citrated whole blood specimen, which is recalcified at some later time using Calcium Chloride. Sodium citrated techniques are also useful when using differential centrifugation to isolate Platelet-Poor Plasma (PPP TEG) or

Platelet-Rich Plasma (PRP TEG). Tissue Factor (TF) is an enzyme that, together with factor VII, shortens coagulation time by activating factors IX and X (Extrinsic pathway). Thrombin is an enzyme that shortens coagulation time (Common pathway) by cleaving fibrinogen to form the fibrin clot, and activates platelets. Heparinase I, from Flavobacterium heparinum, is an enzyme that rapidly and specifically neutralizes the anticoagulant properties of heparin. It acts by cleaving the heparin molecule into small inactive fragments without affecting the function of other blood components involved in coagulation. Adding Heparinase to the blood allows visualization of any developing coagulopathies during perioperative cardiopulmonary bypass that are masked by high levels of therapeutic Heparin or are masked by Heparin released from the mast cell of the donor liver during liver transplantation. Heparinase also eliminates any problems or concerns associated with drawing blood from a heparinized line. Since all Platelet-Fibrin interaction is mediated by the Platelet Integrin GPIIb/IIIa receptor, it is possible to negate the Platelet contribution to TEG tracing with anti-platelet drugs such as c7E3 Fab (ReoPro®), an antibody fragment that inhibits clot retraction and abolishes platelet aggregation by binding to fibrinogen receptors GPIIb/IIIa on platelets. Adding a Platelet blocker drug to a whole blood sample can be used to measure the effect of Platelets on the TEG profile. Adding antifibrinolytic drugs, such as Amicar, Tranexamic acid or Aprotinin in vitro to a whole blood sample can be used to identify how a previously identified fibrinolytic TEG profile will respond to this inhibitor. A summary of the different types of blood samples and reagents used for Thromboelastography is given in Table 1.

Table 1 shows a summary of different types of samples and reagents that can be used for TEG. The purpose for which these modifications can be used is also shown.

Sample Type	Blood/Reagent	Purpose
Native	Native whole blood (NWB)	Global evaluation of coagulation
Activated	NWB & Celite, TF, Kaolin, Thrombin, DAPTTIN, etc.	Speed analysis
Antifibrinolytic drugs	WB & εACA, Aprotinin, Tx	Reverse fibrinolysis
Heparinase	WB & Heparinase	Reverse heparin effects
Citrated	Citrated WB (CWB)	Prolonged storage
Activated citrated	CWB & Celite, Kaolin, TF, Thrombin, DAPTTIN, etc.	Speed analysis
PRP	Citrated Platelet Rich Plasma	Enriched platelet function
PPP	Citrated Platelet Poor Plasma	Plasma coagulation
Platelet blockers	WB & ReoPro, Integrilin, Aggrastat, etc.	Reduced or abrogated platelet function

The computerized Thrombelastograph® Hemostasis System (TEG®) automatically records the Kinetic changes in a sample of Whole blood, Plasma, or Platelet-Rich-Plasma as the sample clots, retracts and/or lyses. The overall coagulation profile can be qualitatively or quantitatively interpreted in terms of the hypo, normal, or hypercoagulable state of the sample and the degree of lysis. To evaluate the graphic information displayed by the TEG analyzer, five main parameters of clot formation and lysis are measured. They Include:

- R time
- K time

- α angle
- Maximum amplitude (MA)
- Area parameter LY30

The normal range for all the TEG parameters using different reagents is shown in Table 2.

Table 2 showing the normal range for all the TEG parameters, as defined by the manufacturer, using different reagents.

Sample Type	R min	K min	Angle deg	MA mm	G kd/sc
Celite / Kaolin	4 - 8	0 - 4	47 - 74	54 - 72	6.0 - 13.2
Sodium Citrate Celite / Kaolin	2 – 8	1 – 3	55 – 78	51 – 69	4.6 - 10.9
Native	12 – 26	3 – 13	14 – 46	42 – 63	3.2 - 7.1
Sodium Citrate Native	9 – 27	2 – 9	22 – 58	44 – 64	3.6 - 8.5
Tissue Factor	1 – 3	1 – 3	57 – 78	55 – 75	6.0 - 13.0
Sodium Citrate plus TF	0 – 2	0 – 5	52 – 82	46 – 72	2.7 - 12.5
Tissue Factor Kaolin	17 – 38	30 – 118	66 – 82	54 – 72	5.3 - 12.4
Citrated Tissue Factor Kaolin	22 – 44	34 – 138	64– 80	52 – 71	5.0 - 11.6
Tissue Factor plus Functional Fibrinogen	–	–	–	9 – 29	0 - 2.0
Citrated Tissue Factor plus Functional Fibrinogen	–	–	–	10 – 25	0.5 - 1.7

R or R time is the period of latency from the time that the blood is placed in the TEG analyzer until the initial detectable clot formation occurs, which is when the amplitude of the tracing reaches 2 mm. It is measured in minutes. This is the point at which most traditional coagulation assays reach their end-points. It represents the Enzymatic portion of Coagulation, and represents the role of Coagulation factors and the Coagulation pathways. R-time is prolonged by anticoagulants and factor deficiencies and shortened by hypercoagulable conditions.

K or K time is the time from the measurement of R (Beginning of Clot formation) until a fixed level of Clot firmness is reached (Amplitude = 20 mm). It is measured in minutes. Therefore, K-time is a measure of the speed or Clot kinetics to reach a certain level of Clot strength. It therefore, represents an interaction between Fibrin and Platelets, with a majority of the contribution coming from Fibrin, because it measures how fast Fibrin bonds are being formed. K is shortened by increased Fibrinogen level and, to a lesser extent, by Platelet function, and is prolonged by anticoagulants that affect both. If the amplitude does not reach 20mm, K is undefined.

α angle represents the Kinetics of clot development. It is measured in degrees. The angle is closely related to K-time, since both are a function of the rate of polymerization of Fibrin. The angle is more comprehensive than K-time, as there are hypocoagulable conditions in which the final level of clot firmness does not reach an amplitude of 20 mm (in which case K is undefined). Similar to K, α is increased by increased Fibrinogen levels and, to a lesser extent, by Platelet function, and is decreased by anticoagulants that affect both.

Maximum Amplitude is a measurement of maximum strength or stiffness (maximum shear modulus) of the developed clot. It is measured in mm. Clot strength is the result of two components — the modest contribution of Fibrin to clot strength and the much more significant contribution of the Platelets. In addition to MA, there are various

other parameters which are used to describe clot kinetics and clot strength, and stability. Projected MA, or PMA is an estimator of MA, that is, whether the MA value will achieve at least the lower limit of the normal value for samples treated with Kaolin or Celite or not. PMA facilitates earlier detection of platelet dysfunction. PMA begins to display when amplitude reaches 5 mm, and is finalized when the rate of clot formation slows (i.e., when α is final). PMA is displayed as 0 when it is likely that MA will reach the lower limit of normal and 1 when MA is unlikely to reach the lower limit of normal. Time to MA, or TMA is a global measurement of the dynamics of clot kinetics and can be described as the time needed to form a stable clot. TMA combines the rate of clot development from the start of a sample run until the clot reaches its maximum strength. The A parameter measures the width of the tracing at the latest time point. It is equal to MA until MA is determined. Amplitude (A) is a function of clot strength or elasticity and is measured in mm. The A parameter can be transformed into the actual measure of clot strength, or G parameter, or G (shear elastic modulus strength, SEMS) and is measured as Kilo dynes/cm^2). The absolute SEMS of the sample can be calculated from A, using the formula:

G = (5000A/(100-A))/1000

The calculation of G continues till A becomes equal to MA, after which the software stops calculating G. The value G increases exponentially as the value of A of the sample keeps increasing. Thus, it provides a measurement of Clot firmness in force units. It is also more indicative of small changes in the Clot strength or Clot breakdown than is the amplitude in mm because it is an exponential reflection of A.

The four above mentioned parameters are used to calculate an index to describe the patient's overall coagulation function. The Coagulation Index (CI) is derived from the R, K, MA, and Angle (α) of Native or Kaolin/Celite-activated Whole blood tracings. The formula for calculating the Coagulation Index depends on the type of blood sample

used. The equation for the TEG Coagulation Indices are simple linear combinations of the variables R, K, α and MA values, and is shown in Table 3:

Table 3 shows the linear equations for calculating CI values from R, K, Alpha and MA values with different types of blood samples.

Index	Equation
Native Whole Blood	$CI = -0.2454R + 0.0184K + 0.1655MA - 0.0241\alpha - 5.0220$
Celite-activated WB	$CI = -0.6516R_c - 0.3772K_c + 0.1224MA_c + 0.0759\alpha_c - 7.7922$
Combined	$CI = -0.112R - 0.222K + 0.040MA - 0.042\alpha - 0.578R_c + 0.370K_c + 0.111MA_c + 0.097\alpha_c - 8.397$

Normal values for the Coagulation Index lie between -3.0 and +3.0, which is equivalent to three standard deviations about the mean of zero. Positive values outside this range (CI > +3.0) indicate that the sample is hypercoagulable, whereas negative values outside this range (CI < -3.0) indicate that the sample is hypocoagulable. Hypercoagulable conditions like cancer or deep-vein thrombosis are detected at CI values of +5.0 and above.

There are several Clot Lysis parameters measured by Thromboelastography. Fibrinolysis leads to Clot dissolution, depending on the severity and stage (early or late) of the Fibrinolytic process. Therefore, several sets of parameters are computed to quantify the fibrinolytic state. They are similar in that they rely on the loss of clot strength with time after the maximum clot strength (MA) is reached. The LY30 and LY60 parameters measure Percent Lysis at 30 minutes and 60 minutes after MA respectively, and are based on the reduction of the area under the TEG tracing from the time MA is measured until 30 or 60 minutes after the MA. The A30 and A60 parameters are the Amplitudes of the TEG tracing at 30 minutes and 60 minutes after

MA is measured. A30 and A60 are sometimes presented in an alternate form called the Whole Blood Clot Lysis Index (CL30 or CL60), which presents the values of A30 or A60 relative to MA. The formulas are:

CL30 = 100 x (A30 / MA)

CL60 = 100 x (A60 / MA)

The smaller the value of CL30 or CL60, the greater the severity of the Fibrinolytic process. Therefore, CL30 and CL60 measure Fibrinolysis inversely to the way it is measured by the LY30 and LY60 parameters. Generally, when LY30 and LY60 are high, CL30 and CL60 are low, and vice versa. CL30 and CL60 represent point measurements of the Fibrinolytic status at exactly 30 and 60 minutes after MA is achieved. LY30 and LY60 represent the Fibrinolytic process that took place during those 30 or 60 minutes.

Each TEG parameter, R, K, α, MA and LY30, represents a different aspect of the clot's physical properties. However, due to the interactive nature of hemostasis, these parameters are interrelated. In general, an elongated R means that it takes longer for the first Fibrin strands to be formed and therefore it represents a deficiency in coagulation factors, inhibitors, and/or activators, which results in a slow rate of Thrombin generation. The α parameter measures the rapidity (kinetics) of Fibrin buildup and cross-linking, that is the speed of Clot strengthening. K, or K time, is a measure of the rapidity of reaching a certain level of Clot strength (20 mm amplitude). K and α both measure a similar process, and both are affected by the availability of Fibrinogen, which determines the rate of Clot buildup, and, to a lesser extent, by Platelets. Therefore, an elongated K and a reduced α represents a low level of fibrinogen. MA measures the strength of clot and is affected by Platelet number and function and, to a lesser extent, by Fibrinogen level. Therefore, a small MA and normal R, K, and α represents Thrombocytopenia or Platelet dysfunction. MA, K, and α are interrelated due to the interaction between fibrinogen fiber and platelets, which

together form the Fibrin-Platelet bonding to produce the final Clot. LY30 greater than 7.5% represents hyper fibrinolysis.

Clinical applications of Thromboelastography

Thromboelastography has been used for a very long time as an experimental tool to study coagulation in human as well as veterinary medicine. Of late, many clinical applications for Thromboelastography have been coming up. The most common application of TEG was in Cardiac surgeries, with Cardiopulmonary bypass. In such patients, the management of coagulation is very critical and complex, because, anticoagulation is required for cardiopulmonary bypass, and adequate hemostasis is required when the patient is taken off bypass. In addition, a patient undergoing cardiac surgery, is likely to be on antiplatelet drugs, which can produce abnormalities of Platelet function. TEG has been used to guide the complex process of anticoagulation with Heparin, its reversal with Protamine, and final hemostasis towards the end of surgery. TEG has been found to have a high negative predictive value for significant coagulopathy, and thus, is useful in early detection and treatment of abnormalities in a bleeding patient[15]. It has also been shown that when transfusion algorithms made from parameters of TEG are used, it leads to a significant reduction in transfusion of blood products, allowing a judicious use of blood and blood products in adults and children undergoing cardiac surgery[16,17].

Patients undergoing Orthotopic liver transplantation have a significant derangement in their coagulation function. This is because, an impaired organ function due to liver disease leads to a decreased production and increased clearance of coagulation factors, and Platelet function abnormalities. Systemic complications that maybe seen in such patients, like sepsis, and Disseminated Intravascular Coagulation (DIC) further contribute to this. Also, during the anhepatic phase of transplantation and reperfusion, there is a release of endogenous Heparin and Heparin like substances, and

accumulation of Tissue Plasminogen Activator (TPA), leading to coagulation abnormalities. In addition to the intraoperative hemorrhagic risk due to these coagulation abnormalities[6,18], the post-operative period can be complicated by thrombotic complications[19]. Thromboelastography has been successfully used in such situations to tailor specific management.

Major surgery has been shown to be associated with a hypercoagulable state, which is responsible for the pathogenesis of some of the postoperative thrombotic complications such as Deep Vein Thrombosis (DVT), Pulmonary Embolism (PE), Graft thrombosis, Myocardial ischemia, Ischemic stroke etc. In such situations, where it is difficult to detect hypercoagulability using the traditional tests of coagulation, viscoelastic tests such as TEG are being increasingly used to diagnose hypercoagulable states. Although the experience with the use of TEG in such clinical situations is limited, there are some reviews and studies which have shown that the use of TEG may help improve management in these cases. O'Donnell et al[20] conducted a study with 87 patients with a prior history or family history of thrombotic events. They compared TEG with biochemical screening for Thrombophilia and found that TEG identified a larger number of patients with Thrombophilia. A TEG parameter, MA >68 mm has been shown to significantly increase the incidence of occurrence of thrombotic complications like Myocardial infarction, DVT, PE and Stroke[21]. Papa et al[22] have shown that the different TEG parameters can provide real time information to identify hypercoagulability in digestive tract malignancies.

Thromboelastography been used very commonly in trauma patients. Majority of patients with trauma can present with coagulopathies, especially in the early phase, and may cause substantial mortality in this group of patients. The etiology is very complex and multifactorial, and may be due to processes like Hypothermia, Acidosis, consumption of Platelets and Coagulation factors, Tissue factor release, DIC, Hypoperfusion etc. TEG has been shown to be helpful in such situations, to identify

hyperfibrinolysis[23]. TEG used for identifying specific coagulopathies, followed by appropriate correction can be lifesaving, and is a much more useful tool than the traditional tests of coagulation. A study found that TEG detected a hypercoagulable state in patients admitted to burns and trauma ward, some of whom went on to develop thrombotic complications, whereas the traditional tests like PT and aPTT failed to identify any of these abnormalities[24]. A recent study by Massaro et al evaluated coagulation in the early (<48hrs) and late (>48 hrs) phases of moderate to severe Traumatic Brain Injury (TBI), and found that patients with TBI developed a delayed, and progressive hypercoagulable state, which they attributed to the function of platelets[25]. Theodoro et al[26] conducted a review of literature on the practical aspects of the use of TEG in trauma, and found that TEG is very useful as a point of care test, provided it is performed by experienced personnel, and adequate attention is paid to calibration and standardization. They also stated that though performing the entire test takes as long as the other traditional tests of coagulation, partial results available in minutes can give us an idea about the Clot kinetics and strength, which can be effectively incorporated into transfusion algorithms, and can also point towards platelet dysfunction and a hypercoagulable state[25].

Viscoelastic tests like TEG have also been used to monitor anticoagulation therapy with Unfractionated Heparin (UFH), LMWH, or Heparinoids such as Danaparoid. TEG can be used in patients undergoing Antiplatelet therapy. A sophisticated test called Platelet mapping® has been developed with TEG for monitoring platelet function for patients on antiplatelet therapy[27]. A Kaolin activated whole blood sample is used to measure the maximal hemostatic activity of the blood sample. Further measurements are performed in the presence of Heparin to eliminate thrombin activity: Reptilase and Factor XIII generate a crosslinked Fibrin clot to isolate the Fibrin contribution to the clot strength. The contribution of the Adenosine Diphosphate (ADP) or Thromboxane A2 receptors to the clot formation is provided by the addition of the appropriate

agonists, such as ADP or Arachidonic Acid. The results from these different tests are then compared with each other and the platelet function is calculated.

Modern therapy with blood product transfusion is based on the principle of identification of the specific bleeding abnormality, followed by therapy with a specific product. Basing such therapies on clinical judgement alone, or on traditional tests of coagulation leads to unnecessary transfusion of some pro coagulant agents, thereby increasing the risk of complications. For example, it has been shown that perioperative platelet transfusion after coronary artery bypass graft surgery has been associated with many complications[28,29]. Therefore, Viscoelastic tests such as TEG are very useful in predicting which patient will benefit from which therapy. In fact, The Society of Thoracic Surgeons and Society of Cardiovascular Anesthesiologists recommends that transfusion of coagulation factors should preferably be guided by Point of care Viscoelastic tests, which assess the global coagulation function rapidly and accurately[30]. TEG parameters have been used to guide and monitor transfusion of Fibrinogen, Prothrombin Complex Concentrate (PCC), Recombinant Factor VII and Factor XIII.

Role of Thromboelastography in Neurosurgery

As of now, data pertaining to the application of TEG in neurosurgical practice has been limited. But, when the pathophysiology of many of the neurosurgical conditions is considered, such as Traumatic Brain Injury (TBI), Subarachnoid Hemorrhage (SAH), Intracranial Hemorrhage (ICH), Brain tumors etc., it appears that these conditions can produce coagulopathies as well as hypercoagulability. Moreover, the etiologies for the coagulation abnormalities also may vary, highlighting the complex interactions between different components of the coagulation system. Therefore, it appears that TEG may have a definite role to play in the management of these conditions. TEG has been used to identify patients with ICH who are at a risk for hematoma expansion. In a

study done on 64 patients with ICH, it was found that patients who eventually developed an expansion of ICH had a longer baseline K and delta value compared to those who did not have a hematoma expansion[31]. Ramchand et al[32] compared TEG parameters of 14 patients with moderate to severe SAH with that of healthy individuals on days 1,3,5,7 and 10 after SAH and found that there was a significant increase in the coagulation parameters such as MA and Total Thrombin Generation (TTG) between the 3rd and 10th day of SAH, indicating the existence of a hypercoagulable state. Frontera et al[33] demonstrated a higher MA in poor grade SAH patients within 72 hours of bleed, indicating a role for inflammation, and platelet activation following brain injury in SAH. In TBI, there have been a few studies which have studied coagulation abnormalities using TEG with mortality. Davis et al[34] performed modified TEG wit platelet mapping in 50 patients with TBI and compared them to healthy controls, and found that patients with severe TBI had higher platelet inhibition, and among the TBI patients, it was found that the degree of platelet inhibition correlated with the severity of TBI. Further, it was also found that the percentage of ADP inhibition was higher among non survivors compared to survivors. Windelow et al[35] found that patients with ICH and TBI having TEG abnormalities suggestive of a hypocoagulable state had a higher 30 day mortality than those who did not. A prospective, observational cohort study of 3 groups of patients was done by Manoel et al[36] to study the theory that TBI was associated with a higher risk for coagulopathies due to release of tissue factor from the brain. The three groups were isolated TBI, TBI with multisystem trauma, and multisystem trauma without TBI. They found that the group with isolated TBI had the lowest incidence of coagulopathy among the 3 groups, and concluded that isolated TBI is not an independent risk factor for coagulopathy. As mentioned previously, Massaro et al demonstrated that patients with TBI show abnormalities in TEG parameters, suggestive of hypercoagulability during the late phase of TBI[25]. Thromboelastography may also play an important role in prognostication and management of Acute Ischemic Stroke (AIS). Yao et al[37]

performed Thromboelastography at baseline in 211 patients with AIS and followed them up for neurological outcome. They found that hypercoagulability at baseline (MA>69mm) was associated with more severe stroke at presentation and a worse neurological outcome at 1 year. They also found that hypercoagulability on TEG was an independent predictor of worse outcome after AIS. In addition, TEG with platelet function mapping has also been used to identify patients with AIS who do not respond to Aspirin, and in such patients, TEG is very useful because it enables us to modify our treatment accordingly.

Limitations and criticisms of Thromboelastography

Although Thromboelastography has generated a lot of interest among clinicians leading to increased application of TEG and represents a significant advance in our knowledge and understanding of the process Coagulation, a few criticisms and drawbacks have prevented it from being accepted as a substitute for the traditional tests of coagulation, and therefore it is still more of an experimental tool rather than a clinical one. First, the TEG parameters seem to correlate poorly with the conventional coagulation parameters such as PT and aPTT. Some studies have shown a poor correlation between ROTEM derived parameters and PT and aPTT[38]. Other studies have shown a very weak correlation between R time of TEG and PT and aPTT[39]. This could be due to number of factors such as variations in pre-analytical conditions, differences in the reagents used, differences in the samples used for both the tests etc. There is a major problem is with the standardization of TEG. It has been shown that the values and results of TEG will vary depending on whether an arterial or a venous blood sample is used[39]. Another issue is with the use of citrated blood sample to increase the storage time before TEG can be performed. It has been shown that TEG values with Native whole blood and Citrated whole blood are different, and this difference was maximum in the first 30 mins of storage with sodium citrate[18,40–42]. Also

repeated sampling has been shown to give variable results[18,42]. In addition, differences in values are also seen with age as well as gender[18]. Therefore, tests performed by different persons within the same institute may give different values based on the factors mentioned above. Also, standardizations of testing in different institutes also is very difficult because of the differences the instrument, testing conditions, reagents etc. At present, we do not have a wide standardization for performing the tests. Therefore, comparing and analyzing results from literature, and modifying practice is not possible. At present, attempts are on to achieve standardization so that the test can be more meaningfully applied to clinical practice. In tests which use Kaolin as an activator, analysis with Kaolin alone will not help us distinguish between coagulopathy due to dilution and thrombocytopenia or platelet dysfunction. Thus, transfusion algorithms based on this alone may lead to unwarranted transfusion of platelets. Finally, TEG requires constant calibration of the machine, careful maintenance, supervision and quality control. Cost maybe a prohibiting factor as the reagents are expensive. Despite all the limitations of TEG, it appears to be an attractive tool for studying coagulation abnormalities in a given situation. If the issues of standardization, sampling, use of reagents etc. are adequately addressed, TEG can provide valuable information, which can be incorporated into management protocols.

Brain tumors and coagulation abnormalities

It is very well known that cancer is associated with an increased risk of coagulation abnormalities, especially Thrombotic complications. This association between cancer and thrombotic complications was first described in 1823 by Trousseau[43]. Malignancies have been found to independently increase the risk for venous thromboembolism[44–48], and Venous Thromboembolism (VTE) has been found to be one of the most common complications of malignancy[49]. It appears that the risk of thromboembolic complications varies with the site of cancer. A population based study by Petterson et

al[50] found that malignancies of pancreas, brain, digestive tract, leukemias and lymphomas have the highest risk for thrombotic complications. They found that the Standardized Morbidity Ratio (SMR) for brain tumors was 47.25, the highest for any cancer site in their study, similar to findings in another record linkage study[44], and a systemic meta analysis[51]. The coagulation system and cancer are believed to have a very close link with each other. There is believed to be a very complex interaction between the tumor and the vascular system in the brain which is responsible for evolution of the tumor process, as well as alterations in the vascular environment and coagulation systems within the brain as well as at sites distant from the brain. In brain tumors, there is a very close anatomical relationship between the tumor cells and blood vessels. Characteristics of the tumors such as invasion into blood vessels, compression of blood vessels due to tumor growth and occlusion of microvasculature within the tumor by cell debris and localized activation of coagulation by tumor cells is believed to be responsible for the release of vascular growth factors leading to angiogenesis, leading to formation of new blood vessels and capillary tufts within the tumors and their vicinity[52]. This explains some of the tumor characteristics such as loss of blood brain barrier function in the blood vessels in the tumor, leading to peri tumor edema. In addition, vascular factors such as inflammation and immune response plays a vital role in tumor progression[53]. Conversely, tumor growth may have a role to play with the coagulation system within the vicinity of the tumor as well as at distant sites. The interface between the brain and the blood vessel is generally tightly regulated by an effective Blood Brain Barrier (BBB), which helps exchange of nutrients, transport of substances, clearing of metabolites and protection of neurons from toxins. The invasive nature of brain tumors as well as alterations of the vascular microenvironment by tumors leads to a disruption of this brain vascular interface, giving rise to various abnormalities such as Angiogenesis, loss of BBB, Edema, and activation of the Coagulation systems[54,55]. Therefore, there are many points along the interface where the procoagulant brain tissue encounters coagulation factors present

in the blood. Brain is said to be a procoagulant tissue because it is rich in platelet activators (Von Willebrand's Factor, ADAMTS 13), Tissue Factor(TF) and Phospholipids (Phosphatidylserine, Phosphatidylethanolamine) which contribute to the initiation and propagation of coagulation[56]. Experimental studies have revealed several scenarios in which interactions between a Procoagulant Brain tissue or tumor and the Platelets and Coagulation factors in the blood can give rise to localized and generalized activation of coagulation. First, Coagulation cascade can get activated within the Tumor itself, followed by a spillover of the activated Coagulation factors and microparticles containing tissue factors to the periphery, which in turn initiate and propagate coagulation at distant sites[57,58]. Activation could also occur due to the release of biological material from tumors such as necrotic material, viable cells, DNA, ATP, membrane components, apoptotic material etc., especially following chemotherapy[59]. Finally, it has also been postulated that tumor cells may release mediators such as Vascular Endothelial Growth Factors (VEGF) and cytokines that could stimulate the expression of TF and activation of coagulation pathways at distant endothelial cells[60]. From the above discussion, it appears that the interaction between brain tumor cells and vasculature can lead to activation of coagulation locally as well as at distant sites, leading to a state of hypercoagulability. Chronic insults to the vasculature and coagulation systems by such an interaction may also lead to coagulopathies. Therefore, such patients are likely to experience bleeding tendencies as well as thrombotic events, which may not be restricted just to the perioperative period.

Many studies have evaluated the relationship between Coagulation abnormalities and Primary brain tumors in humans. Many of the early studies on this subject had reported predominantly hemostatic abnormalities in the perioperative period. Singh et al[61] found that compensated DIC and fibrinolysis were the main coagulation abnormalities seen in the preoperative period in patients with primary brain tumors, followed by a hypercoagulable state. They also found that surgical intervention led to

an alteration in the preexisting abnormality or the development of a new abnormality, which they attributed to a transient acute phase reaction. Prasad et al[62] studied coagulation profiles in 45 patients with Primary Brain tumors pre and post operatively, and found that hemostatic abnormalities suggestive of a hypocoagulable state was found in 44 of them, with chronic DIC and fibrinolysis being the most common form of abnormality, although none of them were found to be clinically relevant, and not affected by tumor histology. They also observed that such compensated hemostatic abnormalities may get manifested due to the stress of surgical exposure. Goh et al[63] evaluated the coagulation profile of 50 adults with primary brain tumors using traditional parameters and TEG, and found that there existed a general trend towards hypercoagulability, although, majority of patients who had an intraoperative finding of hypocoagulability subsequently developed hematomas requiring surgical evacuation.

Various studies have described the risk of Pulmonary Embolism (PE) between 0-5%[64]. The mortality is said to range 9-50%. The risk of clinically evident DVT in the general neurosurgical population is 4.3%[65]. But studies on Primary Brain tumors have shown a more substantial risk for DVT and PE. Incidence of DVT on autopsy was found to be 27.5% whereas incidence in patients was found to be 45%. Incidence of PE at autopsy was found to be 8.4%, and in patients with brain tumors was found to be 3-3.8%[64]. Many studies have attempted to evaluate the risk of various factors such as age, tumor type, presence of comorbidity, duration of surgery and ICU stay etc. on DVT and PE. From these studies, some risk factors for development of thromboembolic complications have been identified. Age has been identified as an important risk factor, with some studies identifying age greater than 60 years as a risk factor[66]. Semrad et al identified an incidence of DVT of 30% in patients with age more than 75 years[67]. Female sex was also found to be a risk factor for development of DVT in Brain tumor patients[67]. Tumor histology is another important risk factor. In general, higher grade malignancies have a higher risk of developing DVT. High grade gliomas,

oligodendrogliomas, and metastasis were found to have the highest risk for DVT in a retrospective single center review[67]. They also found that patients who had higher-grade tumors like Glioblastoma Multiforme (GBM) and Anaplastic Astrocytoma had a lower 2-year survival than other tumor types. Other lesions which have been shown to have high risk for DVT are Lymphomas and Hemangiopericytomas[68]. Presence of comorbidities has been found to increase the risk of DVT, with three or more comorbidities having a significantly higher risk than no comorbidity[67]. Comorbidities that are associated with a risk of VTE are heart disease, previous Cancer, prior VTE and seizures[68]. Surgical intervention is another known risk factor, with the highest incidence of VTE within the first two months of a surgical intervention[67]. Another study showed that most DVTs (84%) occurred within the first week, and 92% of DVTs occurred within the first 2 weeks of surgery[69]. Other factors which were found to be associated with an increased risk of VTE are impaired preoperative sensorium, poor functional status, prolonged operating time, post-operative pneumonia, sepsis, UTI, prolonged ventilation[66], immobility, chronic steroid use[70] etc.

Development of thromboembolic complications in brain tumors not only alters the management plan significantly, it also has an adverse impact on survival and outcome. As such, patients developing an acute thromboembolic event without cancer have a 4 to 8 times higher chance of death, but, the survival of patients with cancer developing acute thromboembolic events was found to be lower still[71]. A study found that when VTE occurred in cancer, the risk of death was increased by 20-40%[72]. Further, patients who developed venous thromboembolism were also at a higher risk to develop hemorrhagic complications in the future[73]. Patients who had a thromboembolic complication were also found to have a poorer quality of life[74]. The economic impact of thromboembolic complications is also significant. A study found that the development of venous thromboembolism was associated with a 1.5-fold increase in the length of hospital stay, and a 1.7 fold increase in the hospital costs at 90 days[75].

Due to this significant adverse impact of thromboembolic events in Brain tumors, the focus has been on its prevention in those patients who are believed to be at risk. Of all the preventive measures, Thromboprophylaxis using anticoagulants has received a lot of attention. Despite this, its role in dealing with the problem of venous thromboembolism in brain tumors has been far from clear. There are many questions regarding the use of anticoagulants in these situations which need answering, such as, the usefulness of anticoagulants, the balance between benefit and risk, and timing and nature of such treatment. Although there have been some studies that have found no significant difference in the occurrence of DVT between those who receive thromboprophylaxis, and those who do not[68], a majority of studies in literature report a favorable role for pharmacological prophylaxis in reducing the incidence of DVT. Use of subcutaneous Heparin at 24 or 48 hours has been shown to reduce the lower extremity DVT by 43%[69], although there was no correlation between the use of heparin and the occurrence of Pulmonary Embolism (PE). Hamilton et al, in their meta-analysis found that 5 of the 6 trials included showed a significant reduction in the incidence of symptomatic and asymptomatic VTE with the use of prophylaxis with Heparin compared to placebo, with a pooled RR of 0.58. They also found that Heparin was useful irrespective of whether mechanical thromboprophylaxis was used or not[76]. It was inconclusive as to which among Unfractionated Heparin (UFH) or Low Molecular Weight Heparin (LMWH) was better. Systematic reviews by others also had similar conclusions regarding the effectiveness of Heparin prophylaxis in reducing the incidence of VTE[77,78]. The most feared complication with the use of chemoprophylaxis for prevention of thromboembolism is intracranial hemorrhage, and to a lesser extent, extracranial hemorrhage, which can have devastating consequences. Intracranial hemorrhage can occur spontaneously with high grade tumors like high grade gliomas, and metastatic tumors such as melanoma, choriocarcinoma, thyroid and renal carcinoma in the absence of anticoagulants[79]. Therefore, it is plausible that use of anticoagulants may increase the risk of ICH. Agnelli et al[80] evaluated the efficacy and

safety of Enoxaparin for prevention of clinically evident DVT in a multi-center, randomized, double blinded trial, and found that Enoxaparin was not associated with an increased risk of ICH. A meta-analysis by Collen et al[77] found that the pooled rates for ICH and minor hemorrhage were higher with Heparin therapy, but were not higher with LMWH, compared to treatment not involving these two modalities. In Hamilton et al.'s meta-analysis, ICH was more common in the patient population which received Heparin, although, the increase in this risk was found to be minimal[76]. They made some interesting observations in their discussion. They observed that, based on absolute risk reduction rate for VTE with the use of Heparin of 9.1% and an absolute risk increase of 0.7% and 2.8% for ICH and minor bleeding respectively, the number needed to treat for preventing VTE was 11, and that needed for ICH was 143, suggesting a safety margin in terms of risk to benefit analysis. But, most of the studies included in the analysis did not differentiate between the distal, asymptomatic DVT, from proximal, symptomatic DVT, which were more dangerous and would warrant anticoagulation. If adjustments for proximal, symptomatic DVT were to be made based on available data on the proportion of these in the total DVT population, the number needed to treat to cause ICH would be much lower, and the benefit would not far outweigh the risks of complications. It is probably for this reason that many neurosurgeons are wary of the benefits of using anticoagulants for preventing VTE, and are therefore reluctant to start Thromboprophylaxis in patients with brain tumors for the fear of ICH. Based on current evidence, anticoagulation for Thromboprophylaxis alone or in combination with mechanical thromboprophylaxis has been recommended for prevention of VTE. But the risk of ICH and minor bleeding needs to be kept in mind and it becomes useful to evaluate in each individual situation whether a patient is hypercoagulable and likely to develop VTE or is hypocoagulable and likely to develop bleeding manifestations and does not warrant pharmacological Thromboprophylaxis. Viscoelastic studies of the coagulation system, such as TEG may be of some value here, and may aid in decision making.

From the above discussion, some important inferences can be drawn. First, although brain tumors account for a small percentage of all cancers, in terms of mortality and morbidity produced, they are a significant subgroup. One of the most common and devastating complications of brain tumors is coagulation abnormalities, which may manifest in the form of bleeding tendencies or thromboembolic complications. Second, among all cancers, Brain tumors have one of the most common incidences of thromboembolic complications, and the risk is further increased by factors which are very commonly seen in the perioperative period such as long surgical time, immobility, ventilator dependence, sepsis, chronic steroid use etc. Thirdly, various methods have been shown to reduce the incidence of these thromboembolic complications such as mechanical methods, anticoagulation, IVC filters etc. Anticoagulants have been found to be effective in reducing the risk of VTE, but it comes with a risk of ICH and hemorrhage outside the CNS, the consequences of which can be very devastating; a factor, which is responsible for the reluctance on the part of surgeons to use anticoagulants for thromboprophylaxis routinely. To some extent, this situation could be mitigated if we are able to identify whether a patient is in a hypocoagulable state or a hyper coagulable state at any time in the perioperative period, so that the risk of the use of anticoagulants can be balanced against perceived benefits. Fourth, traditional parameters of coagulation only provide us with incomplete information on coagulation, and are inadequate in identifying whether a patient is at risk of bleeding or thromboembolic complications. Finally, viscoelastic studies of coagulation, such as TEG comprehensively evaluate the different aspects of Coagulation, and are being increasingly applied in studying coagulation in various scenarios. Evidence has shown that TEG is useful in detecting bleeding disorders and identifying risk for hypercoagulability in Neuroanaesthesia and Neurocritical care. Therefore, we attempted to study coagulation parameters in patients with Primary Brain tumors in the perioperative period using TEG, to identify whether there were any trends in coagulation parameter change in the perioperative period, and whether there was any

risk of bleeding tendency or thromboembolic complications in the perioperative period in this population.

Hypothesis

Primary brain tumors have been found to modify the coagulation system. Literature review shows that a variable pattern of coagulation abnormalities exists, making it difficult to have strong conclusions regarding the management of coagulation in these patients. We postulate that the use of more specific tests of Viscoelastic properties of coagulation, like Thromboelastography, over the perioperative period, will be more reliable in identifying these coagulation abnormalities, compared to the traditional tests of coagulation.

Aims and Objectives

The primary objectives of the study were:

1) To study the temporal trends in Coagulation profile over time in the perioperative period in patients with Supratentorial Primary Brain tumors using TEG

The secondary objectives of the study were:

1) To study the pre-operative hemostatic profile of patients with Supratentorial primary brain tumors using traditional coagulation parameters, and compare them with post-operative coagulation profile

2) To identify the presence of any specific patterns of Coagulation abnormality in patients with Supratentorial Brain tumors in the perioperative period.

Materials and methods

Methodology

We designed a Prospective Observational study in patients with Primary Brain tumors, undergoing craniotomy for surgical management of the same. The primary objective of the study was to observe the temporal trends in coagulation profile in the perioperative period in such patients, and to identify specific patterns in coagulation abnormalities in the perioperative period in patients with primary brain tumors. The study was approved by the Institutional Ethics Committee (IEC). Informed written consent was obtained from all the patients enrolled in the study. The study was conducted in the Neurosurgical Intensive Care Unit (ICU) and Neurosurgery Operation Theatres (OT) at Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram.

A total of 25 patients were enrolled in this study from the elective neurosurgical operation list of our hospital with the diagnosis of Primary Brain tumors between October 2016 and September 2017. The inclusion criteria for enrollment into the study were:

- Age 19-60 years.
- Surgery for newly diagnosed Primary Brain tumors in Supratentorial area with tumor size >3 cm.
- American Society of Anesthesiologists (ASA) Physical Status grades 1,2.
- Glasgow Coma Scale (GCS) score 14-15.

Patients excluded from the study included:

- Age below 18 years and greater than 60 years.
- GCS score <13.

- ASA grades 3&4.
- Tumors other than supratentorial primary brain tumors.
- Recurrent tumors.
- Patients who had prolonged immobilization due to paralysis or other debilitating conditions.
- Patients with significant co-morbid conditions such as Diabetes, Hypertension, Liver disease, Ischemic Heart Disease, Stroke, Chronic Renal disease etc. which are known to affect the coagulation system.
- Patients on anti-platelet or anticoagulant therapy or other drugs affecting the coagulation system in the preoperative period.
- Patients on chronic hyperosmolar therapy.
- Patients on Oral Contraceptive Pills (OCP).
- Pregnant patients.
- Patients with hematological disorders.
- Patients who had massive blood loss and transfusion, prolonged preoperative immobility, patients requiring mechanical ventilation and prolonged immobilization post operatively.
- Post-operative re exploration.

Overview of the study protocol

- Blood samples were taken for Thromboelastography on the day before surgery.
- The patient was shifted to the OT and standard monitors were connected, as described in the text below.
- Under Inj. Lignocaine (2%) skin infiltration, intravenous access, and arterial access with 20G arterial cannula was obtained.
- General anesthesia was induced in all patients using a standard protocol, as described clearly in the text below in the methodology section.

- Hemodynamic variables like heart rate, blood oxygen saturation and systolic blood pressure were monitored continuously.
- Anesthesia monitoring included measurements of peak airway pressure, end tidal carbon dioxide and end tidal anesthetic agent (Sevoflurane) concentration.
- All patients were mechanically ventilated using Volume Controlled Ventilation (Aestiva/5, Datex Ohmeda) with a Square wave (Constant inspiratory flow), an inspiratory/ expiratory ratio of 1:2, with respiratory rate of 10 breaths / min, a tidal volume of 8 ml/kg, and Positive End Expiratory Pressure (PEEP) of 0 cm of H_2O. Ventilation was then adjusted to achieve an end tidal carbon dioxide of 35 mm of Hg.
- Sample collection for Thromboelastography was done at four time points; Pre operatively (T0), at 24 hours Post operatively (T1), at 48 hours Post operatively (T2), and at 72 hours Post operatively (T3).

Detailed discussion of the study protocol

All the patients eligible were explained about the study and the investigations involved in the study. In addition, they were also explained about the protocol for anesthetic management, following which a written informed consent was taken from the legal representative of the patients who were willing to participate in the study. The patients were shifted to the OT after confirming their identity and standard anesthetic monitoring was instituted using the following:

- ECG
- Pulse oximetry
- Invasive Blood pressure monitoring
- Temperature monitoring.
- End tidal CO2 and anesthetic gas monitoring.

- Neuromuscular Transmission monitoring.

Under local anesthesia using Inj. Lignocaine (2%), wide bore intravenous access and arterial access with 20 G arterial cannula were obtained and the pressure transducer was positioned at the mid axillary line.

General anesthesia was induced in all the patients using a standard protocol. The patients were first pre-oxygenated with 100% Oxygen at 6 l/min for 5 minutes, following which, they were pre-medicated with Fentanyl 2µg/kg. Anesthesia was induced with Inj. Propofol 2mg/kg, and intubation was facilitated with an intermediate acting muscle relaxant Vecuronium 0.1mg/kg. Maintenance of anesthesia was achieved using a combination of Oxygen (50% FiO2) and Sevoflurane to maintain an end tidal concentration equivalent to .8 MAC to 1 MAC. Analgesia and muscle relaxation was maintained with infusions of Fentanyl at 2µg/kg/min and Atracurium at .3mg/kg/hr. Blankets were used for maintenance of body temperature. Mechanical ventilation was instituted with Volume Controlled mode (Aestiva/ 5, Datex Ohmeda) with a Square wave (constant inspiratory flow) adjusted to obtain an end tidal carbon dioxide of 35 mm of Hg. Additional boluses of Fentanyl 2µg/kg and Propofol 1mg/kg were given at the time of applying head clamps. 300 ml of 3% Saline were used to reduce brain volume and facilitate adequate exposure. Intraoperative blood loss was replaced with a combination of crystalloids, colloids, Packed cells, and other blood products using standard institutional protocol. At the end of the surgery, patients were assessed for extubation. If extubation was planned, muscle relaxation was reversed with Neostigmine .05mg/kg and Glycopyrrolate .01mg/kg, followed by extubation when the patient was fully conscious, moving all limbs and following commands. The patients were then monitored in the ICU in the post-operative period.

Measurement of study parameters

Thromboelastography Parameters

Thromboelastography was performed using the TEG® 5000 Thrombelastograph® Hemostasis System, which is a registered trademark of Haemoscope Corporation, Niles IL 60714 USA. It is distributed in India by Steranco Healthcare Pvt Ltd, Andheri (East), Mumbai. The cups and pins, as well as the Kaolin reagents for performing the tests were procured from the distribution company. The software used for running the Thromboelastography machine was TEG Analytical Software (TAS) Version 4.2.101, also procured from the distribution company. To observe the temporal trends in coagulation profile, Thromboelastography was performed at 4 time periods in all the patients, as follows:

- Preoperatively, on the day before or of surgery (T0).
- Post operatively at 24 hours (T1).
- Post operatively at 48 hours (T2).
- Post operatively at 72 hours (T3).

Blood sample was collected in a Sodium citrate vial. 1 ml of blood was then taken from this citrated sample and transferred into a Kaolin vial. The TEG machine was switched on and the software was run as described in the manufacturer's user manual. After performing the initial daily maintenance tests, cup and pin was mounted on the Thromboelastography machine as described in the manufacturer's user manual. 20 µl of Calcium Chloride was added to the cup placed in its slot in the TEG machine using a micropipette. 340 µl of Kaolin sample was then added to the cup containing Calcium Chloride, and the slot containing the cup was raised to engage the pin, and the test was run. The parameters measured, and the curves were displayed on the monitor. These were then saved into the software's database.

Data Collection

The following data were collected and entered manually by the Principal Investigator for statistical analysis:

A) Demographic parameters:

Demographic parameters such as age, sex and weight were recorded for all the patients at the time of enrollment. Also noted were the type of tumor and the size of the tumor.

B) Coagulation parameters

The following investigations were noted pre-operatively and in the post-operative period:

- Platelet count
- Prothrombin Time (PT)
- International Normalized Ratio (INR)
- Activated partial Thromboplastin time (aPTT).

C) Other perioperative parameters

Amount of intraoperative bleeding was noted, as was the quantity of each blood product transfused. Occurrence of post-operative intracranial bleeding was documented from the patients' post-operative CT scans. Occurrence of Deep Vein Thrombosis (DVT) or Pulmonary Embolism (PE) during the study was also documented.

D) TEG parameters

The TEG parameters that were documented for this study were the following:

- Time to initial thrombin formation (R time)
- Time to clot formation (K time)

- Speed of solid clot formation (α angle)
- Absolute strength of clot (MA)
- Coagulation Index (CI)
- Percent Lysis at 30 minutes (Ly 30)

The TEG parameters have been shown in the Figure 3.

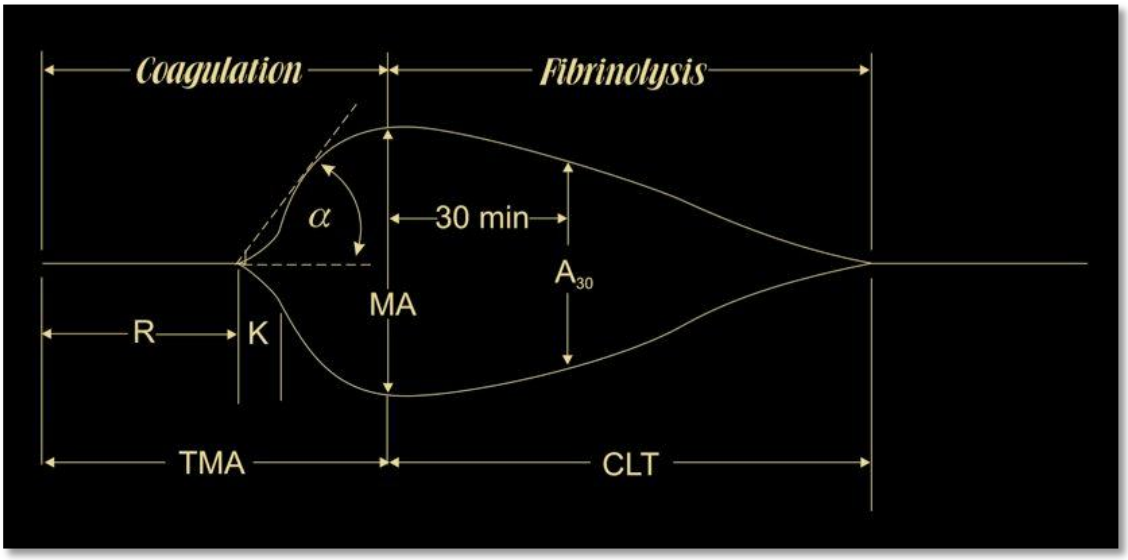

Figure 3 showing a normal TEG trace with the depiction of the TEG parameters.

Time from the start of the study to the formation of a detectable clot (an amplitude of 2mm in the TEG tracing, as defined by the manufacturer) is called R time. Manufacturer defined normal range for R time is 3-6 minutes. R time values outside this range were considered abnormal in this study. Time from the measurement of R to a fixed level of clot firmness (20 mm on the TEG tracing, as defined by the manufacturer) is called K time. Manufacturer defined normal for K time is 1-3 minutes. K time values outside this range were considered abnormal in this study. The normal range for α defined by the manufacturer is 55°-75°, and that for MA is 50-70 mm. Values outside this range in the study were considered abnormal. Manufacturer

defined normal range for Coagulation Index (CI) is -3 to 3. Values less than -3 were considered hypocoagulable, and those above 3 were considered hypercoagulable.

Statistics

Sample size calculation

The purpose of the study was to observe the temporal trends in the hemostatic profile of patients with primary brain tumors in the perioperative period, and to identify patterns of coagulation abnormality. The Thromboelastography parameters described above have a normal distribution in the population. Therefore, the normal values and standard deviations of each of the TEG parameters described in a previous study[63] was used to calculate the power of the study to detect abnormalities in each of the TEG parameters. The normal values and standard deviations of each of the TEG parameters, and the precision at different sample sizes is shown in the Table 4. We selected a sample size of 25 for our study.

Table 4 showing the normal values of R, K, Alpha angle and MA, and the precision at different sample sizes.

Parameter	Normal	S.D	Precision at n=20	Precision at n=25	Precision at n=30
R (min)	11.8	2.4	±1.07	±0.96	±0.88
K (min)	5.3	1.4	±0.63	±0.56	±0.51
A angle(°)	36	7.4	±3.3	±2.96	±2.70
MA (mm)	54	5.6	±2.5	±2.25	±2.04

Numerical data such as age, size of tumors, amount of bleeding, transfusions, coagulation parameters and Thromboelastography parameters were expressed as

mean and standard deviation, if they were normally distributed. Skewed data were expressed as median and interquartile range. Statistical analysis was done using IBM SPSS statistics software; version 20. Tables and figures were constructed using GraphPad Prism software, version 7. Comparison of pre and post-operative values was done with parametric tests (Student's t test) if the values were normally distributed, and with non-parametric tests (Wilcoxon signed-rank test) if the values were not normally distributed. Repeated measures of Thromboelastography parameters was analyzed using repeated measures Analysis of Variance (ANOVA). Logistic regression analysis was used to study the trends for each of the Thromboelastography parameters over time.

Results and Observations

We evaluated the changes in the coagulation function of patients with supratentorial primary brain tumors undergoing neurosurgery using serial Thromboelastography, comparing the preoperative values with postoperative data obtained at time periods of 24 hrs, 48 hrs and 72 hrs. A total of 25 patients were recruited and TEG tests were completed successfully in all patients. The parameters evaluated were R time, K time, α angle, Maximum Amplitude (MA), Coagulation index (CI) and the fibrinolysis parameter Ly30. We analyzed the data to identify patterns of coagulation abnormalities seen in the perioperative period.

Demographic and perioperative data

The demographic and tumor characteristics are summarized in Table 5.

Table 5 showing demographic and tumor characteristics

Admission parameters	Number (n)
Age (mean, S.D)	42.6, 7.7
Tumor Size (mean, S.D)	5.4, 1.3 (cm)
Gender	
Male	16/25
Female	9/25
Diagnosis	
Glioma	15/25
Meningioma	10/25
ASA grade	
I, II	25/25
III,IV	0/25
GCS	
14-15	25/25

We enrolled 25 patients with supratentorial primary brain tumors, of which 15 were glioma and 10 were meningioma. There were 16 male and 9 female patients in the study. All the patients belonged to American Society of Anesthesiologists (ASA) Physical Status grades I and II. All the patients had a Glasgow Coma Scale (GCS) score of 14 or 15. The mean age of patients in the study was 42.6 (±7.7) years. The size of tumor was 5.4 (±1.3) cm. Other perioperative parameters have been summarized in Table 6.

Table 6 showing Perioperative parameters

	Number (n)	Mean (ml)	S.D (ml)
Intraoperative bleeding	25	708.0	309.5
RBC transfusion	10	400.0	210.8
FFP transfusion	3	333.3	115.5
Platelet transfusion	1	100.0	NA

Intraoperative bleeding was 708.0 (±309.5) ml. RBC was transfused in 10 patients, FFP in 3, and Platelets in 1 patient.

Coagulation parameters

Coagulation data have been summarized in Table 7. There was a significant reduction in the Post-Operative Platelet count compared to the Pre-operative values ($p<0.001$). But, both the pre-operative and post-operative platelet counts were within the normal range. There was no significant change in Prothrombin Time (PT), International

Normalized Ratio (INR) and Activated Partial Thromboplastin Time (aPTT) in the post-operative period compared to the pre-operative value.

Table 7 showing a comparison between pre-operative and post-operative coagulation parameters in the perioperative period.

	Pre-Op		Post-Op		
	Mean	S.D	Mean	S.D	p
Platelet (cells/cumm)	**366920**	**36034.6**	**312160**	**38414.1**	**<0.001**
PT (Sec)	15.2	0.7	15.2	0.8	0.843
INR	1.02	0.07	1.04	0.06	0.138
aPTT (Sec)	35.1	4.2	35.6	3.7	0.289

Thromboelastography Parameters

R time, which represents the time to initial thrombin formation, was measured at four time points, T0, T1, T2, and T3. The mean R at T0, T1, T2 and T3 were 5.03 (±1.19) mins, 5.08(±1.17) mins, 4.02(±0.95) mins, and 3.90(±0.85) mins respectively. Table 8 summarizes the R time values.

Table 8 shows R time values at the four time points.

R (min)	n	Mean (± S.D)	Median (Interquartile range)
T0	25	5.03 (±1.19)	4.80 (4.35- 5.80)
T1	25	5.08 (±1.17)	5.30 (4.35- 5.65)
T2	25	4.02 (±0.95)	4.20 (3.20- 4.75)
T3	25	3.90 (±0.85)	3.90 (3.30- 4.75)

Figure 4 shows a box and whisker plot of the R values at the four time points. The median, interquartile range, minimum and maximum values are represented in the box and whisker plot.

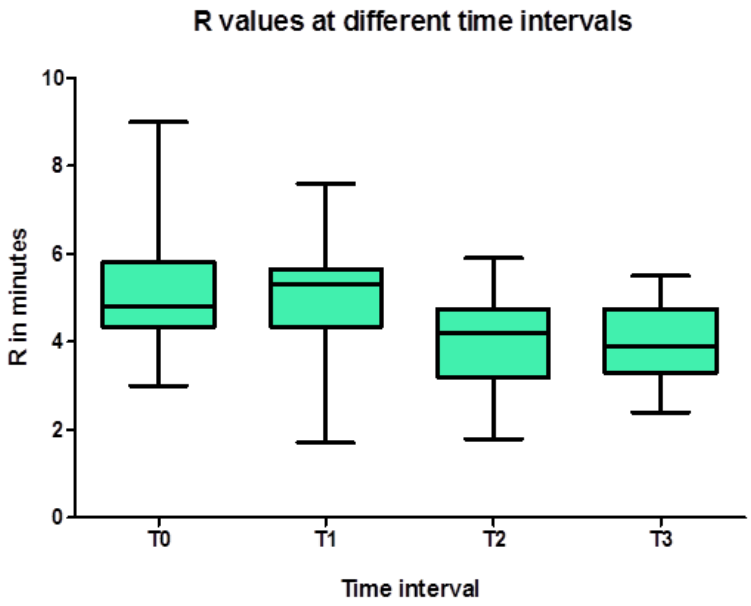

Figure 4 shows a Box and Whisker plot of R time values at the four time points.

Figure 5 shows a scatter plot for R time at the 4 time points. There was a decreasing trend of R values from T0 to T3, with clustering of values close to the lower limit of normal at T2 and T3.

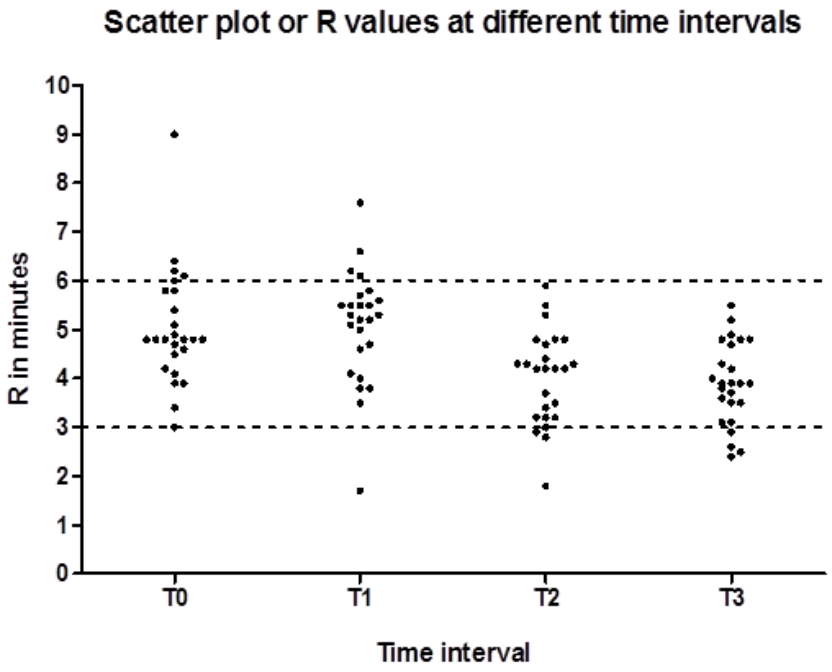

Figure 5 shows a scatter plot of R time values at the four time points. The dotted lines denote the lower and upper limits of normal range.

Paired comparisons of R time values at different time points was done using repeated measures ANOVA, and is summarized in the Table 9. Mean R values at T1 did not change significantly compared to T0, but, there was a significant reduction in R time at 48 hr (T2) and 72 hr (T3), compared to the pre-operative (T0) and 24 hr values (T1). Logistic regression analysis to evaluate the time trends from T0 to T4 showed an average reduction in R time of 0.45 minutes/ day ($p<0.01$).

Table 9 shows Paired comparisons of R time at the four time points.

Paired comparison (R Time)	Mean Difference (minutes)	Std. Error	p
T0 VS T1	-.044	.286	.879
T0 VS T2	1.008*	.299	.003
T0 VS T3	1.132*	.280	<0.001
T1 VS T2	1.052*	.204	<0.001
T1 VS T3	1.176*	.298	.001
T2 VS T3	.124	.241	.611

** denotes a significant difference*

K time, which represents the time to clot formation, was also measured at four time points, T0, T1, T2 and T3. The mean K time at T0, T1, T2 and T3 were 2.54 (±0.74) mins, 2.26 (±0.84) mins, 1.76 (±0.51) mins, 1.53 (±0.35) mins respectively. Table 10 summarizes the K values.

Table 10 shows K time values at the four time points.

K (min)	N	Mean (±S.D)	Median (Interquartile range)
T0	25	2.54 (±0.74)	2.60 (2.15-2.90)
T1	25	2.26 (±0.84)	2.20 (1.70-2.65)
T2	25	1.76 (±0.51)	1.60 (1.30-2.20)
T3	25	1.53 (±0.35)	1.50 (1.25-1.75)

Figure 6 shows a box and whisker plot of the K values at the four time points. The median, interquartile range, minimum and maximum values are represented in the box and whisker plot.

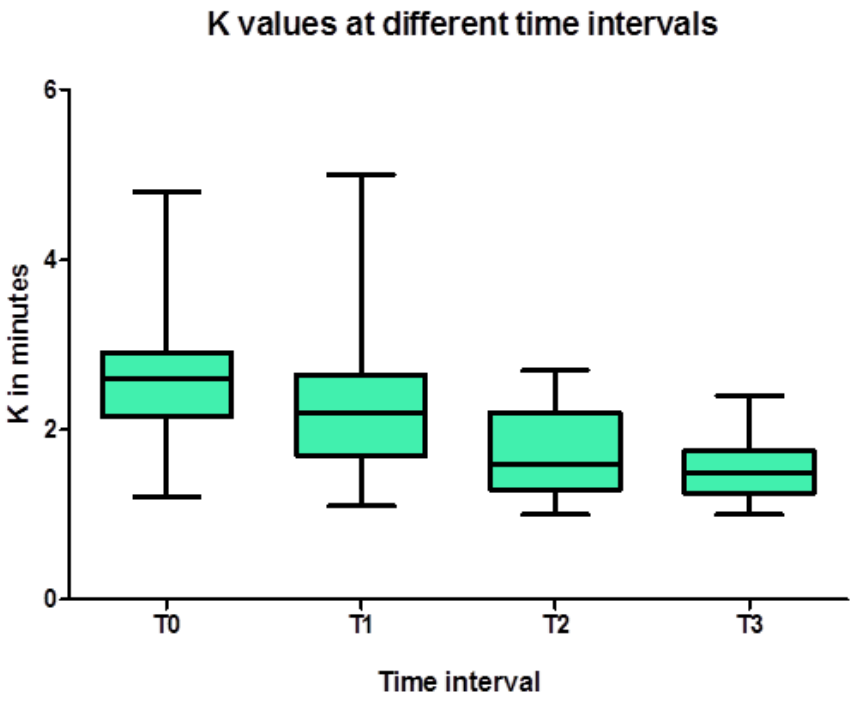

Figure 6 shows a Box and Whisker plot of R time values at the four time points.

Figure 7 shows a scatter plot for K time at the 4 time points. There was a decreasing trend of K values from T0 to T3, with clustering of values close to the lower limit of normal at T3.

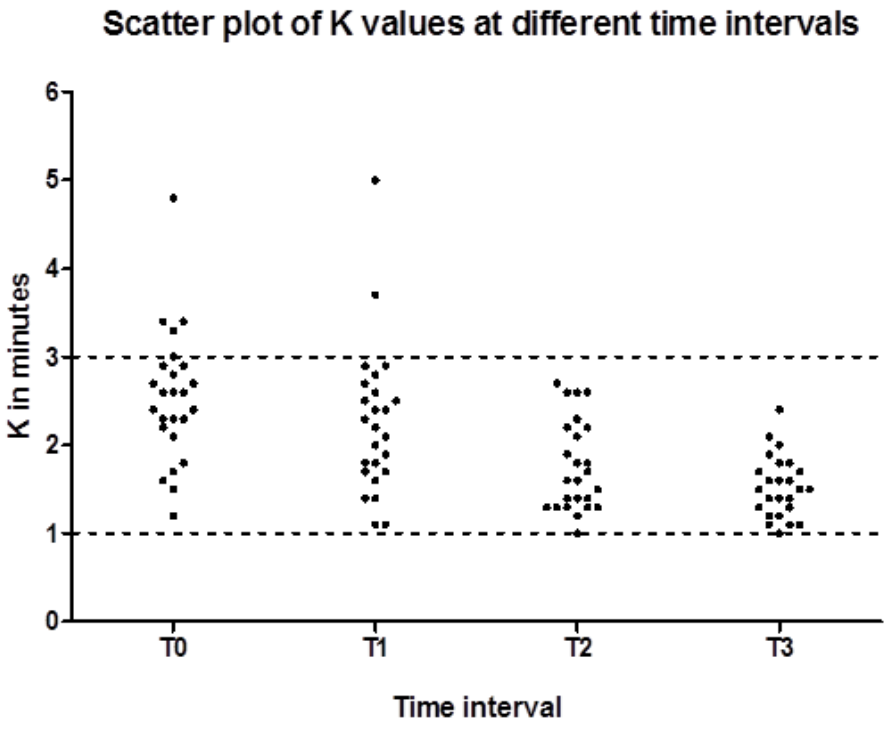

Figure 7 shows a scatter plot of K values at the four time points. The dotted lines denote the lower and upper limits of normal range.

Paired comparisons of K time values at different time points was done using repeated measures ANOVA, and is summarized in the Table 11. Mean K values at T1 did not change significantly compared to T0, but, there was a significant reduction in K time at 48 hr (T2) and 72 hr (T3), compared to the pre-operative (T0) and 24 hr values (T1). Logistic regression analysis to evaluate the time trends from T0 to T4 showed an average reduction in K time of 0.35 minutes/ day ($p<0.01$).

Table 11 shows Paired comparisons of K Time at the four time points.

Paired comparison (K Time)	Mean Difference (minutes)	Std. Error	p
T0 VS T1	.280	.222	.220
T0 VS T2	.776*	.171	<0.001
T0 VS T3	1.012*	.167	<0.001
T1 VS T2	.496*	.134	.001
T1 VS T3	.732*	.162	<0.001
T2 VS T3	.236*	.088	.013

** denotes a significant difference*

α angle, which represents the speed of solid clot formation, was also measured at four time points, T0, T1, T2 and T3. The mean α angle at T0, T1, T2 and T3 were 56.0 (±9.4) degrees, 62.1 (±7.4) degrees, 67.4 (±4.7) degrees, 69.6 (±5.4) degrees respectively. Table 12 summarizes the α angle values.

Table 12 showing Alpha angle values at the four time points.

Alpha angle (Degree)	n	Mean (± S.D)	Median (Interquartile range)
T0	25	56.0 (±9.4)	58.4 (52.6- 62.3)
T1	25	62.1 (±7.4)	63.1 (58.8- 67.45)
T2	25	67.4 (±4.7)	68.3 (66.25- 69.45)
T3	25	69.6 (±5.4)	71.3 (68.4- 72.95)

Figure 8 shows a box and whisker plot of the α angle values at the four time points. The median, interquartile range, minimum and maximum values are represented in the box and whisker plot.

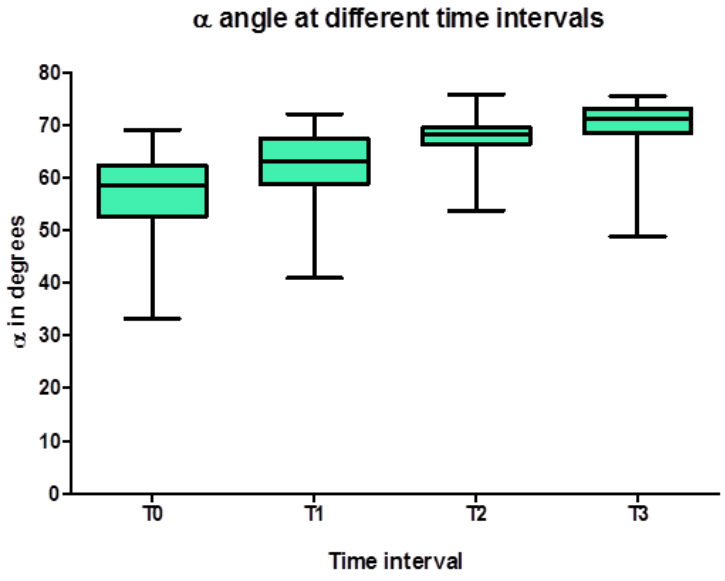

Figure 8 shows a Box and Whisker plot of Alpha angle values at the four time intervals.

Figure 9 shows a scatter plot for α angle at the 4 time points. There was an increasing trend of α angle values from T0 to T3, with clustering of values close to the upper limit of normal at T3.

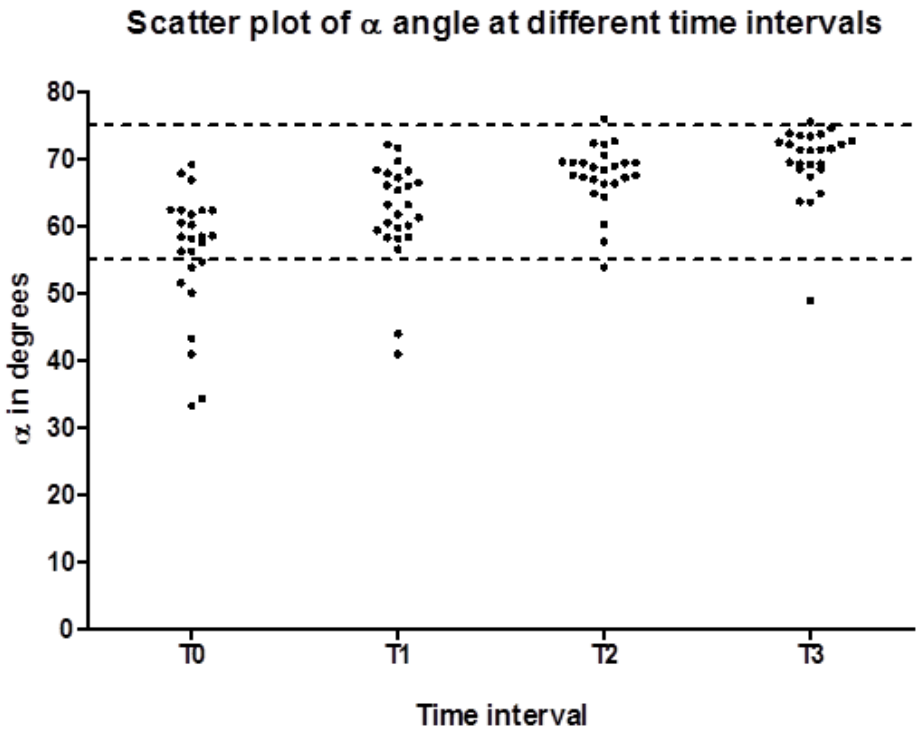

Figure 9 shows a Scatter plot of Alpha angle at the four time points. The dotted lines denote the lower and upper limits of normal range.

Paired comparisons of α angle values at different time points was done using repeated measures ANOVA, and is summarized in the Table 13. Mean α angle values increased significantly compared to the previous time point from T0 to T4. Logistic regression analysis to evaluate the time trends from T0 to T4 showed an average increase in α angle of 4.6 degrees/ day ($p<0.01$).

Table 13 shows Paired comparisons of Alpha angle values at the four time points.

Paired comparison (α angle)	Mean Difference (Degrees)	Std. Error	p
T0 VS T1	-6.112*	2.106	.008
T0 VS T2	-11.428*	2.030	<0.001
T0 VS T3	-13.620*	1.749	<0.001
T1 VS T2	-5.316*	1.058	<0.001
T1 VS T3	-7.508*	1.317	<0.001
T2 VS T3	-2.192	1.243	.091

** denotes a significant difference*

MA, which is a measure of clot strength, was also measured at four time points, T0, T1, T2 and T3. The mean MA values at T0, T1, T2 and T3 were 59.3 (±7.5) mm, 61.4 (±8.6) mm, 67.8 (±6.8) mm, and 72.8 (±4.9) respectively. Table 14 summarizes the MA values.

Table 14 shows MA values at the four time points.

MA (mm)	n	Mean (± S.D)	Median (Interquartile range)
T0	25	59.3 (±7.5)	59.4 (57.05-63.05)
T1	25	61.4 (±8.6)	61.7 (57.65-65.4)
T2	25	67.8 (±6.8)	67.4 (63.4-72.1)
T3	25	72.8 (±4.9)	72.1 (69.25-75.95)

Figure 10 shows a box and whisker plot of the MA values at the four time points. The median, interquartile range, minimum and maximum values are represented in the box and whisker plot.

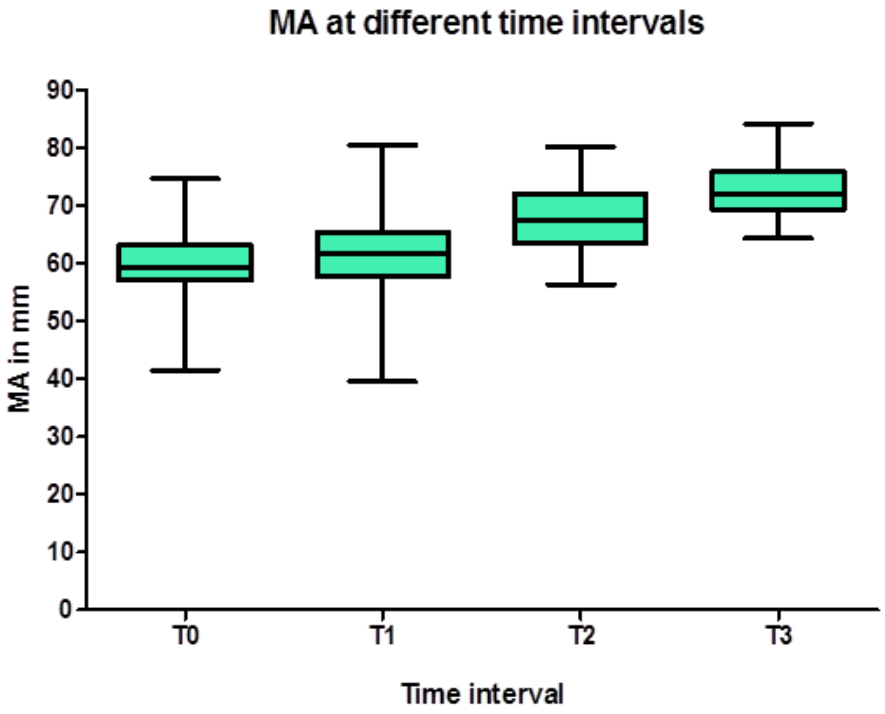

Figure 10 shows a Box and Whisker plot of MA values at the four time points.

Figure 11 shows a scatter plot for MA at the 4 time points. There was an increasing trend of MA from T0 to T3, with clustering of values close to the upper limit of normal at T2 and T3. Many of the MA values at T2 and T3 were above the upper limit of the normal limits.

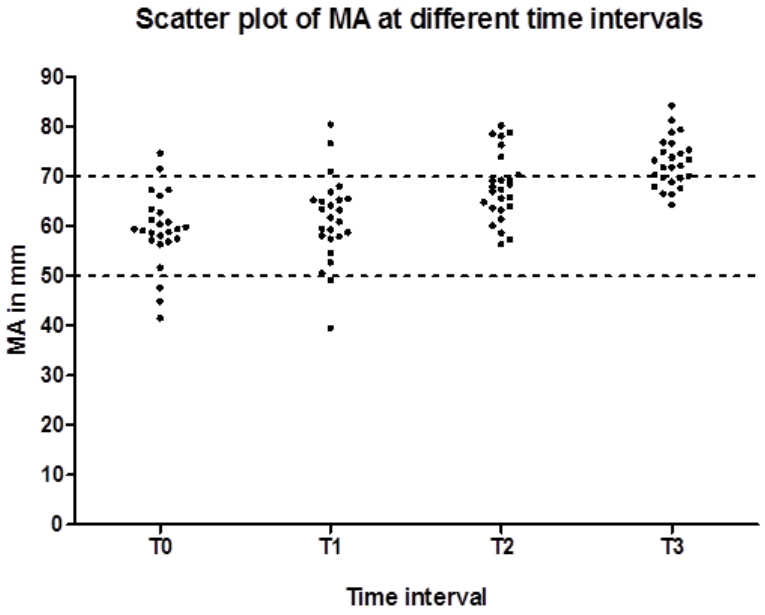

Figure 11 shows a Scatter plot of MA at the four time points. The dotted lines denote the lower and upper limits of normal range.

Paired comparisons of MA values at different time points was done using repeated measures ANOVA, and is summarized in the Table 15. There was no significant change in MA at T0 compared to T1, but, MA increased significantly compared to the previous time point at T3 and T4. Logistic regression analysis to evaluate the time trends from T0 to T4 showed an average increase of MA by 4.7 mm/ day ($p<0.01$).

Table 15 shows Paired comparisons for MA at the four time points.

Paired comparison (MA)	Mean Difference (mm)	Std. Error	p
T0 VS T1	-2.096	2.267	.364
T0 VS T2	-8.512*	1.944	<0.001
T0 VS T3	-13.484*	1.767	<0.001
T1 VS T2	-6.416*	1.535	<0.001
T1 VS T3	-11.388*	1.539	<0.001
T2 VS T3	-4.972*	1.073	<0.001

** denotes a significant difference*

CI, the Coagulation Index, was also measured at four time points, T0, T1, T2 and T3. The mean CI values at T0, T1, T2 and T3 were -0.52 (±1.64), 0.28 (±1.67), 2.34 (±1.36), 3.28 (±0.90) respectively. Table 16 summarizes the CI values.

Table 16 shows CI values at the four time points

CI	N	Mean (± S.D)	Median (Interquartile range)
T0	25	-0.52 (±1.64)	-0.06 (-1.38 - 0.40)
T1	25	0.28 (±1.67)	0.42 (-0.50 - 1.12)
T2	25	2.34 (±1.36)	2.40 (1.71 - 3.33)
T3	25	3.28 (±0.90)	3.23 (2.88 - 3.98)

Figure 12 shows a box and whisker plot of the CI values at the four time points. The median, interquartile range, minimum and maximum values are represented in the box and whisker plot.

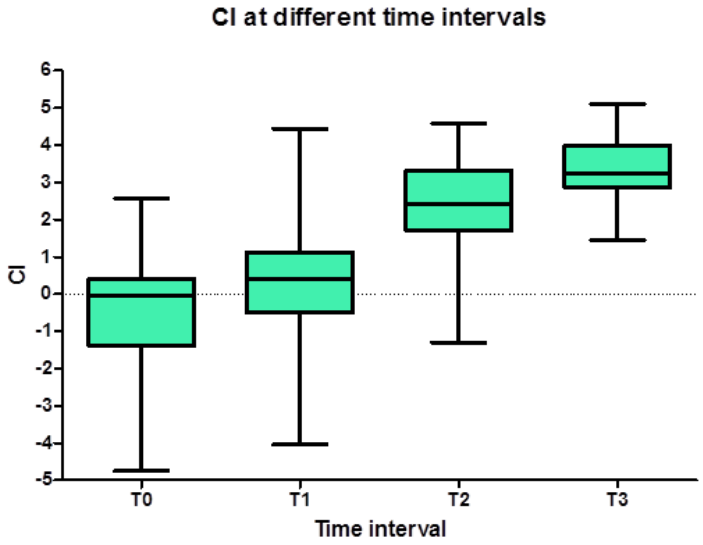

Figure 12 shows a Box and Whisker plot of the CI values at the four time points.

Figure 13 shows a scatter plot for CI at the 4 time points. There was an increasing trend of CI from T0 to T3, with clustering of values close to the upper limit of normal at T2 and T3. Many of the CI values at T2 and T3 were above the upper limit of the normal range.

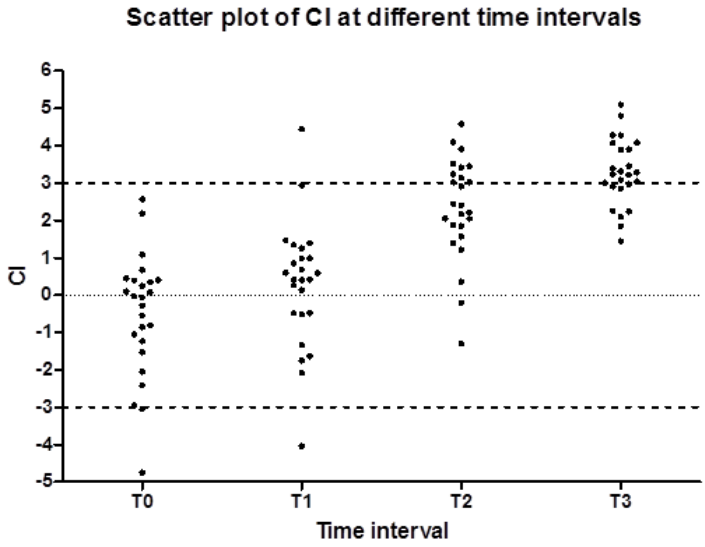

Figure 13 shows a Scatter plot for CI values at the four time points. The dotted lines denote lower and upper limits of normal range.

Paired comparisons of CI values at different time points was done using repeated measures ANOVA, and is summarized in the Table 17. There was no significant change in CI at T1 compared to T0, but, CI increased significantly compared to the previous time point at T3 and T4. Logistic regression analysis to evaluate the time trends from T0 to T4 showed an average increase of CI by 1.34/ day ($p<0.01$).

Table 17 shows the paired comparisons of CI values at the four time points.

Paired comparison (CI)	Mean Difference	Std. Error	p
T0 VS T1	-.797	.483	.112
T0 VS T2	-2.859*	.422	<0.001
T0 VS T3	-3.804*	.393	<0.001
T1 VS T2	-2.061*	.212	<0.001
T1 VS T3	-3.006*	.319	<0.001
T2 VS T3	-.945*	.293	.004

** denotes a significant difference*

The fibrinolysis parameter, Lysis percent at 30 minutes (Ly30) was also measured at four time points, T0, T1, T2 and T3. Table 18 shows the Ly30 values at the four time points.

Table 18 shows Ly30 values at the four time points.

LY30 (%)	N	Mean (± S.D)	Median (Interquartile range)
T0	25	1.27 (±1.55)	0.50 (0.00 - 2.25)
T1	25	0.91 (±1.09)	0.50 (0.00 - 1.80)
T2	25	1.17 (±1.57)	0.60 (0.20 - 1.50)
T3	24	1.21 (±1.51)	0.65 (0.30 - 1.30)

Paired comparisons of Ly30 at different time points using repeated measures ANOVA, as summarized in the Table 19 showed no significant differences in Ly30 at any time points between T0 and T3.

Table 19 shows Paired comparisons of Ly30 at the four time points. There is no significant difference in the Ly30 values at any time point.

Paired comparison	Mean Difference (%)	Std. Error	p
T0 VS T1	.346	.281	.231
T0 VS T2	.171	.430	.695
T0 VS T3	-.042	.433	.924
T1 VS T2	-.175	.281	.539
T1 VS T3	-.388	.367	.302
T2 VS T3	-.213	.360	.561

Complications

None of the patients enrolled in this study had any evidence of intracranial hemorrhage in their post-operative CT scans. None of the patients developed Deep Vein Thrombosis (DVT) during the study, and none of the patients enrolled developed Pulmonary Embolism (PE) during the study.

Discussion

Primary brain tumors are a major subset of patients requiring neurosurgical procedures. Neuro surgery in these patients is associated with morbidity and mortality. Coagulation abnormalities have been described in literature to occur in the perioperative period in these patients. However, the literature is not sufficient to provide a conclusive evidence on the type of coagulation abnormalities. This is due to the tests employed as well as the timing of tests. Literature describes normal coagulation, hypercoagulability as well as hypocoagulability in the perioperative period in this population. Hypocoagulability can lead to complications such as postoperative intracranial hematomas as well as hemorrhage outside the CNS, whereas hypercoagulability, can lead to thromboembolic complications such as DVT and PE. As these disorders form complete opposite ends of the spectrum of coagulation related complications occurring in the perioperative period, effective treatment of these, and successful management of the coagulation disorders requires accurate and prompt identification of these abnormalities across the spectrum of coagulation. As traditional tests of coagulation have not been successful in identification of all types of coagulation abnormalities, Viscoelastic tests of coagulation such as Thromboelastography has generated a lot of interest. Thromboelastography has been used extensively in areas like Cardiac surgery, Liver transplantation and Trauma, but its application in the field of Neurosurgery and Neurocritical care is limited. We studied the temporal trends in coagulation profile of patients with Primary Brain tumors in the perioperative period using Thromboelastography to identify specific patterns of coagulation abnormalities that may occur in the perioperative period.

Summary of the results

In our prospective study of 25 patients with primary brain tumors, we measured the coagulation parameters using TEG at 4 time points in the perioperative period, namely,

Pre operatively, at 24 hours, 48 hours and 72 hours Post operatively. We found that traditional tests of coagulation such as PT, INR and aPTT did not change between the pre-operative and post-operative period. Although the platelet count was found to be reduced post operatively, it was within the normal range.

Thromboelastography parameter R time is the time taken for the formation of initial clot, and represents the enzymatic function of the coagulation factors and their interaction with each other. Our study shows that there was no change in R values in the first 24 hour sample. However, it reduced significantly thereafter till 72 hours post operatively, with an average reduction in R time by 0.45 minutes/ day across the study duration. Although the mean R time values at all 4 time points were within the normal range, there was a clustering of values close to the lower limit of normal range at 48 and 72 hours. The trends in R time observed in our study seem to suggest a tendency towards hypercoagulability in the post-operative period, which implies that tendency to initiation of clot formation is increased in the perioperative period.

We found similar trends with K time, which is a measure of the clot kinetics, and represents predominantly, the function of Fibrin. It did not change significantly at 24 hours, but reduced significantly thereafter till 72 hours post operatively, with an average reduction in K time by 0.35 minutes/day across the duration of the study. There was a clustering of K time values close to the lower limit of normal range at 48 and 72 hours, although the mean K time values at all the studied time points were within the normal range. These trends also seem to suggest a tendency towards hypercoagulability in the post-operative period.

α angle, which also represents the rate of Fibrin formation, had an increasing trend across the time points measured. α angle increased significantly from the pre-operative period, and continued to increase at every time point measured, up to 72 hours, with an average increase of 4.6^{o}/day, indicating a trend towards

hypercoagulability, although the mean α angle values at all the 4 time points measured were within normal limits. But, there was a clustering of α angle values close to the upper limit of normal range at 72 hours.

Analysis of MA, which is a measure of clot strength, representing predominantly, the function of Platelets, and the interaction between Platelets and Clotting factors, showed an increasing trend throughout the study period. There was a significant increase in MA from the pre-operative period, which continued to increase at every time point measured in the study. There was an average increase of MA by 4.7 mm/day. There was a clustering of MA values close to the upper limit of normal range, and many values were also above the upper limit of normal range at 48 hours and 72 hours. The mean MA at 72 hours was 72.8(±4.9) mm, which was above the upper limit of normal, indicating a hypercoagulable state in our patients at this time point.

CI, which is a composite index involving R, K, α and MA also had an increasing trend during the time points measured. Although the change at 24 hours was not significant, the increase in CI thereafter at each time point was significant, with an average increase in CI of 1.34/day. These trends in CI reflect the corresponding changes in the other Thromboelastography parameters at these time points, and indicate a general trend towards hypercoagulability after 24 hours. There was a clustering of CI values close to the upper limit of normal range at 48 and 72 hours, and many values were also found to be above the upper limit of normal range, with the mean CI at 72 hours above the upper limit of normal, indicating the existence of a hypercoagulable state.

The Fibrinolysis parameter Ly30 did not change significantly in the perioperative period, and the mean Ly30 values at all the time points were within the normal range, indicating that there was no perioperative Hyperfibrinolysis in the patients studied.

We found in our study that there was a universal trend towards hypercoagulability in all the TEG parameters measured after 24 hours post operatively. Among all the

Thromboelastography parameters, MA showed the most consistent increase, and the value at 72 hours was in the hypercoagulable range. MA and CI increased throughout the study period, and appeared to be still rising at the last time point measured, and it is possible that they would continue to increase further with time. All these trends suggest that the patients in our study had a change in their coagulation parameters in the perioperative period, with a gradual development of a hypercoagulable picture at 72 hours. Since MA reflects the Platelet function to a great extent, and Fibrin formation and the interaction between Fibrin and Platelets to a small extent, the consistent increase in MA towards hypercoagulability suggests that Platelets and their interaction with Fibrin may play a major role in the development of a hypercoagulable picture. This is also supported by trends towards hypercoagulability seen with K time and α angle, which represent Fibrin formation to a great extent, and Platelet function and its interaction with Fibrin to a small extent. Therefore, Platelets and their interaction with Fibrin appears to play a key role in the development of hypercoagulability in the post-operative period.

Relevance of our findings considering the available evidence

As discussed earlier, there have not been many studies investigating the perioperative coagulation disorders in brain tumors. Singh et al[61] were one of the first to study the coagulation abnormalities in brain tumors. They evaluated the coagulation abnormalities in 25 patients with brain tumors pre operatively, intraoperatively, and post operatively using traditional coagulation parameters such as Platelet count, aPTT, PT, Thrombin Time, Fibrinogen, Fibrin Degradation Products (FDP), and compared them with controls undergoing non neurological surgeries. They found elevated Platelet counts post operatively, in contrast to our study. They classified the abnormalities as acute Disseminated Intravascular Coagulation (DIC), compensated DIC, Fibrinolysis and Hypercoagulability based on the combination of individual

derangements of the parameters measured. They found coagulation abnormalities in 40% of patients pre operatively, the most common ones being compensated DIC, fibrinolysis and a hypercoagulable state. In contrast, we did not find coagulation abnormalities pre operatively in our patients. They also found that the patients became more hypocoagulable during surgery, with compensated DIC becoming acute DIC and the hypercoagulability getting compensated or overcompensated. Our findings are not in concurrence with these findings, as we found the coagulation parameters to proceed towards a hypercoagulable state. Another study by Prasad et al[62] using similar coagulation parameters found coagulation abnormalities pre operatively as well as intra operatively, though most of the abnormalities were hypocoagulable in nature. These differences could be because the traditional tests of coagulation used in these studies may not have been able to identify a hypercoagulable state effectively.

Vukovic et al[81] studied traditional coagulation parameters and specific coagulation activation marker D-Dimer in 19 patients with Meningioma and Glioma, who are considered to be at high risk for thromboembolic complications post operatively, and compared them with patients with metastasis, who are considered to be low risk to develop thromboembolic complications. They found a decrease in post-operative Platelet count, similar to our finding. They also found that D-Dimer levels were elevated at baseline in patients with metastasis, as compared to Meningiomas and Gliomas. There was a significant increase in the D-Dimer levels at 24 hours post operatively in Meningiomas and Gliomas compared to metastasis, indicating an activation of coagulation, following which D-Dimer levels decreased. Patients with Meningiomas and Gliomas had higher D-Dimer values at day 7, indicating that this population had hypercoagulability in the post-operative period, even up to day 7 post operatively. We also found patients to be hypercoagulable at 72 hours post operatively. The findings from this study suggest that coagulation activation begins at

the time of surgery, probably due to release of activation factors from brain tumors, which leads to the existence of a hypercoagulable state post operatively, which may continue up to day 7. D-Dimer is a nonspecific marker of coagulation activation and can be elevated by other causes such as surgery, infections etc. In contrast, TEG parameters used in our study are more specific for coagulation functions. Therefore, our results maybe more accurate.

Goh et al[63] studied the Thromboelastography parameters in 50 patients undergoing surgery for primary brain tumor on the day before surgery, intraoperatively, and 24 hours after surgery. In their study, in 9 patients who developed Intracranial Hematomas post operatively, TEG parameters could identify the coagulation abnormalities in these patients. 7 patients had a reduction in MA, suggestive of poor Clot strength, due to deficiency of Platelets or Clotting factors, whereas 2 had abnormal Fibrinolysis parameters, suggestive of DIC. Hence, TEG proved useful in guiding treatment in complications due to coagulation disorders in this study. The study of trends also showed a tendency towards hypercoagulability from the pre-operative period to the post-operative period, with a reduction in R and K times, and increase in α angle and MA 24 hours post operatively. The changes in R time and MA were significant. The study also showed that the degree of change towards hypercoagulability was less in patients who eventually developed hematomas. Our findings are in concurrence with the findings of this study to the extent that both studies demonstrate the development of a hypercoagulable state in the post-operative period, and that abnormalities in MA are more common and significant, but our findings at 24 hours only showed a significant increase in α angle and MA, but no significant change in R or K. This study also demonstrated the usefulness of TEG parameters not only for identifying coagulation disorders, but also for guiding treatment.

Abrahams et al[82] studied Thromboelastography parameters in 46 neurosurgical patients; including Brain tumors, Aneurysm surgery, and Spine surgery, before, during and after surgery. The traditional parameters were normal, like we found in our study. They found that the Coagulation Index suggested a hypocoagulable picture in the pre-operative period, which increased in the post-operative period, suggesting a hypercoagulable picture. This hypercoagulable picture was not only seen in patients with brain tumors, but also those with SAH and Spine surgery, although the craniotomy patients appeared to be more hypercoagulable, with a higher Coagulation Index. Therefore, it is not clear whether the hypercoagulability seen could only be attributed to brain tumors, or it could be due to other factors common to most neurosurgical procedures, such as, long duration of surgery, immobilization, stress response of surgery etc. We also had an increase of CI values in the post-operative period, with the values at 72 hours being abnormal, and highly suggestive of hypercoagulability. We did not evaluate patients with any other type of neurosurgical procedure. Therefore, it may not be possible to rule out the possibility that the other surgical factors mentioned above could influence the post-operative coagulation profile. An interesting finding in their study was that the Coagulation index increase was maximum from intubation to skin incision and marginal from skin incision to tumor removal or aneurysm clipping. It is interesting because it would be expected that the coagulation abnormalities would be more common at a time when the brain manipulation would be maximum, but their findings were contrary to this hypothesis.

Unlike for brain tumors, there is more data available regarding the use of TEG in other neurosurgical conditions, especially TBI. Analyzing some of the data on coagulation abnormalities in TBI may shed some light on the pathophysiology of coagulation abnormalities in brain tumors. Studies have reported both hypocoagulable and hypercoagulable complications in TBI. Kunio et al[83] prospectively analyzed the coagulation abnormalities in 69 patients with TBI using TEG parameters and found that

patients with R time greater than 9 minutes had a higher mortality, fewer ICU free days , longer hospital stays and greater neurosurgical interventions. MA less than 35 mm was also associated with a higher mortality, indicating that the major coagulation derangement in their population was hypocoagulable nature, and was associated with worse outcomes. They also found that the patients who had R greater than 9 minutes were the ones who had higher head injury scores as well as higher trauma scores. The prospective observational study by Manoel et al[36] found that R time was higher in patients with TBI associated with multisystem injury compared to those with isolated TBI. These two studies, therefore, suggest that coagulopathy is a part of the pathogenesis of TBI, and that the extent of injury to the brain as well as outside the brain influence the degree of coagulopathy. Interestingly, Brohi et al[84] found that development of coagulopathy in patients with TBI in the form prolonged PT and aPTT was associated with systemic hypoperfusion.

Schreiber et al[85] found that the prevalence of hypercoagulability, as identified from TEG parameters, was the highest in the first 24 hours following TBI, with subsequent reduction in the prevalence of hypercoagulability. On the other hand, Massaro et al[25], who evaluated TEG parameters at 24 hour epochs in TBI patients, and compared to controls, found that the TEG parameters in the late phase of TBI (after 48 hours) were more hypercoagulable compared to those in the early phase (up to 48 hours). Parameters like MA and Thrombin Generation were found to be above the upper limit of normal range after 72 hours, and were found to be climbing further, suggesting that a state of hypercoagulability was setting in after 48-72 hours after TBI. Our study also showed a similar pattern.

On examining these studies of coagulation abnormalities in TBI, it appears that both hypocoagulable and hypercoagulable abnormalities can occur with equal frequency, and have equally poor outcomes. Whether they occur as part of a spectrum of coagulation disorders is not known, and the timing of occurrence of either of the two

types of coagulation abnormalities is not clear. Hypocoagulable abnormalities tend to occur early in TBI, and factors such as severe injury, hypotension etc. may contribute to the development of these, whereas hypercoagulable abnormalities tend to occur later, and the risk of hypercoagulable abnormalities, and the risk of subsequent development of thromboembolic complications increases with time. These findings were reflected in our study also. There was a trend towards hypercoagulability in all the TEG parameters measured in our study with time, and parameters like MA were found to be abnormal at 72 hours. Hypocoagulable abnormalities were mostly not seen in our study, which is in contrast with some of the studies on TBI. As we have noted, hypocoagulable abnormalities tend to be associated with greater degree of head injury and hypotension. As these are unlikely to be seen in patients undergoing elective brain tumor surgery, the likelihood of such abnormalities is less, as seen in our study.

Evaluation of temporal trends in coagulation parameters like R, K, α angle, MA, and CI in the perioperative period in Supratentorial Brain tumors in our study showed the progression of all the TEG parameters towards hypercoagulability, with MA and CI values showing the most significant change. It is worthwhile to note that these values were still increasing at 72 hours, and it is plausible that they would continue to rise further. We did not find any instance of thromboembolic complications like DVT or Pulmonary Embolism (PE). Our sample size was not large enough to evaluate the influence of the abnormalities in TEG parameters on thromboembolic complications. Therefore, it is difficult to infer about the relationship between abnormal TEG parameters and the risk of thromboembolic complications. As has been discussed previously, brain tissue is very rich in procoagulant substances such as Tissue factor, Thromboplastins, Fibrinolysins etc. Any form of brain injury or brain manipulation is likely to release these substances into circulation, which may then interact with the coagulation system and lead to different types of coagulation abnormalities. Tumor

surgery, could, therefore, produce derangements in coagulation by this mechanism. In the post-operative period, the interaction between the factors released from the tumor or brain and the coagulation system becomes stronger, and could contribute towards the development of a hypercoagulable state. Platelets seem to play a major role in this process, and as our study shows, Platelet function, and its interaction with the Coagulation factors is an important contributing factor to the development of hypercoagulability. There are many other perioperative factors which contribute to the process of development of post-operative hypercoagulability, such as long surgery times, higher blood loss, with more transfusions, use of Steroids, post-operative mechanical ventilation, higher duration of immobility in the post-operative period, delay in starting chemoprophylaxis for VTE for the fear of Intracranial Hemorrhage etc. Therefore, patients undergoing surgery for Supratentorial Primary Brain tumors are prone to develop hypercoagulability and are at risk for thromboembolic complications. Thromboelastography has been very useful in identifying these abnormalities. Further evaluation of the role of Thromboelastography in identifying those patients who will be at risk to develop thromboembolic complications will improve our perioperative management of patients with brain tumors.

Limitations of our study

There may have been some shortcomings in our study, which will be enumerated below.

- Our study was primarily designed to detect coagulation abnormalities in terms of Thromboelastography parameters. Our sample size was not large enough to evaluate the outcomes of coagulation abnormalities in terms of bleeding or thromboembolic complications such as DVT and Pulmonary Embolism. Outcome of coagulation abnormalities are more important clinically than the mere presence of these abnormalities. Therefore, more studies are required to investigate this relationship. Nevertheless, as there are few studies which evaluate coagulation abnormalities in brain tumors, this study can be used as a blueprint for further studies.

- We did not have controls in our study with whom the coagulation abnormalities of patients could be compared. As discussed previously, there are many factors in the perioperative period which can affect the coagulation independently of the brain tumor pathology, the influence of these factors cannot be ruled out. A detailed study will need to account for these factors.

- We followed up the coagulation profile of patients up to 72 hours post operatively. As some of the studies mentioned earlier have shown that changes of coagulation towards hypercoagulability continue much later into the post-operative period, our follow up may not have been long enough to detect thromboembolic complications.

- The impact of Blood product transfusions on the Thromboelastography parameters, especially at the 24-hour time point is unclear, as each of these blood products can directly affect the Thromboelastography parameters,

especially R and K time, which represent the function of coagulation factors and fibrin generation respectively. Further studies may clarify the effects of blood transfusions on TEG parameters in brain tumor patients.

Conclusions

The following conclusions can be drawn from our study:

- Patients with Supratentorial Primary brain tumors have a trend towards the development of a hypercoagulable state in the post-operative period, especially after 24 hours.
- The change towards hypercoagulability in the post-operative period was most consistent with MA, which suggests that platelets and their interaction with coagulation factors plays a major role in the development of a hypercoagulable state in the post-operative period.
- Based on our results, we believe that the safe period to start anticoagulation medication post operatively for prophylaxis of DVT or Pulmonary Embolism without the fear of development of Intracranial Hematoma in these patients would be after 48 hours.
- Compared to traditional tests of coagulation like PT, aPTT and Platelet count, Thromboelastography is a useful diagnostic tool to identify coagulation abnormalities in the perioperative period, and maybe useful in guiding treatment in the perioperative period.
- The spectrum of coagulation abnormalities occurs well beyond the immediate post-operative period, as seen in our study. Evaluation of coagulation parameters only in the early post-operative period may miss the hypercoagulable picture that occurs after 24 hours. Therefore, it is important to perform TEG at frequent intervals to follow up patients who are at a high risk of developing thromboembolic complications beyond 24 hours to identify a hypercoagulable state so that appropriate therapeutic decisions can be taken.
- From our study, it is not clear when the coagulation status returns to normal in the post-operative period. Further studies are needed to address this issue.

Bibliography

1. Wrensch M, Minn Y, Chew T, Bondy M, Berger MS. Epidemiology of primary brain tumors: current concepts and review of the literature. *Neuro Oncol*. 2002;4(4):278-299.
2. McNeill KA. Epidemiology of Brain Tumors. *Neurol Clin*. 2016;34(4):981-998.
3. Solheim O, Jakola AS, Gulati S, Johannesen TB. Incidence and causes of perioperative mortality after primary surgery for intracranial tumors: a national, population-based study. *J Neurosurg*. 2012;116(4):825-834.
4. Davie EW, Ratnoff OD. Waterfall Sequence for Intrinsic Blood Clotting. *Science*. 1964;145(3638):1310-1312.
5. Palta S, Saroa R, Palta A. Overview of the coagulation system. *Indian J Anaesth*. 2014;58(5):515-523.
6. Ferreira CN, Sousa MDO. A cell-based model of coagulation and its implications. *Rev Bras Hematol e Hemoter*. 2010;32(5):416-421.
7. Maly MA, Tomasov P, Hajek P, et al. The role of tissue factor in thrombosis and hemostasis. *Physiol Res*. 2007;56(6):685-695.
8. Riddel JPJ, Aouizerat BE, Miaskowski C, Lillicrap DP. Theories of blood coagulation. *J Pediatr Oncol Nurs*. 2007;24(3):123-131.
9. Chee YL. Coagulation. *J R Coll Physicians Edinb*. 2014;44(1):42-45.
10. Park MS, Martini WZ, Dubick MA, et al. Thromboelastography as a Better Indicator of Postinjury Hypercoagulable State Than Prothrombin Time or Activated Partial Thromboplastin Time. *J Trauma*. 2009;67(2):266-276.
11. Legnani C, Mattarozzi S, Cini M, Cosmi B, Favaretto E, Palareti G. Abnormally short activated partial thromboplastin time values are associated with increased

risk of recurrence of venous thromboembolism after oral anticoagulation withdrawal. *Br J Haematol*. 2006;134(2):227-232.

12. McKenna R, Bachmann F, Miro-Quesada M. Thrombo-embolism in patients with abnormally short activated partial thromboplastin time. *Thromb Haemost*. 1977;38(4):1576-1583.

13. Mcmichael MA, Smith SA. Viscoelastic coagulation testing: Technology, applications, and limitations. *Vet Clin Pathol*. 2011;40(2):140-153.

14. Reinhofer M, Brauer M, Franke U, Barz D, Marx G, Losche W. The value of rotation thromboelastometry to monitor disturbed perioperative haemostasis and bleeding risk in patients with cardiopulmonary bypass. *Blood Coagul Fibrinolysis*. 2008;19(3):212-219.

15. Rugeri L, Levrat A, David JS, et al. Diagnosis of early coagulation abnormalities in trauma patients by rotation thrombelastography. *J Thromb Haemost*. 2007;5(2):289-295.

16. Anderson L, Quasim I, Soutar R, Steven M, Macfie A, Korte W. An audit of red cell and blood product use after the institution of thromboelastometry in a cardiac intensive care unit. *Transfus Med*. 2006;16(1):31-39.

17. Shore-Lesserson L, Manspeizer HE, DePerio M, Francis S, Vela-Cantos F, Ergin MA. Thromboelastography-guided transfusion algorithm reduces transfusions in complex cardiac surgery. *Anesth Analg*. 1999;88(2):312-319.

18. Luddington RJ. Thrombelastography / thromboelastometry. *Clin Lab Haem*. 2005;27:81-90.

19. Cerutti E, Stratta C, Romagnoli R, et al. Thromboelastogram monitoring in the perioperative period of hepatectomy for adult living liver donation. *Liver Transplant Off Publ Am Assoc Study Liver Dis Int Liver Transplant Soc*. 2004;10(2):289-294.

20. O'Donnell J, Riddell A, Owens D, et al. Role of the Thrombelastograph as an adjunctive test in thrombophilia screening. *Blood Coagul Fibrinolysis*. 2004;15(3):207-211.

21. Mccrath DJ, Cerboni E, Frumento RJ, Hirsh AL, Bennett-guerrero E. Thromboelastography Maximum Amplitude Predicts Postoperative Thrombotic Complications Including Myocardial Infarction. 2005:1576-1583.

22. Papa ML, Capasso F, Pudore L, et al. ThrombOelastographic profiles as a tool for thrombotic risk in digestive tract cancer. 2007.

23. Levrat A, Gros A, Rugeri L, et al. Evaluation of rotation thrombelastography for the diagnosis of hyperfibrinolysis in trauma patients. 2008;100(6):792-797.

24. Myung S, Park, Wenjun Z, Martini, Michael A, Dubick, Salinas, Jose, Butenas, Saulius, Kheirabadi, Bijan S, Pusateri, Anthony E, Wang, JingJing, Vos, Jeffrey A, Guymon, Charles H, Wolf, Steven E, Mann, Kenneth G, Holcomb JB. NIH Public Access. *J Trauma*. 2009;67(2):266-276.

25. Massaro, Allie M, Doerfler, Sean, Nawalinski, Kelsey, Michel, Bernard, Driscoll, Nicolette, Ju, Connie, Patel, Hiren, Quattrone, Francis, Frangos, Suzanne, Maloney-Wilensky, Eileen, Grady, Michael Sean, Stein, Sherman C, Kasner, Scott E, Kumar MA. Thromboelastography Defines Late Hypercoagulability After TBI : A Pilot Study. *Neurocrit Care*. 2015;22(1):45-51.

26. Teodoro L, Nascimento B, Rizoli S. Thrombelastography (TEG W): practical considerations on its clinical use in trauma resuscitation. *Scand J Trauma*. 2013;21(29):1-8.

27. Preisman S, Kogan A, Itzkovsky K, Leikin G, Raanani E. Modified thromboelastography evaluation of platelet dysfunction in patients undergoing coronary artery surgery. *Eur J Cardiothorac Surg*. 2010;37(6):1367-1374.

28. Arias-Morales CE, Stoicea N, Gonzalez-Zacarias AA, et al. Revisiting blood

transfusion and predictors of outcome in cardiac surgery patients: a concise perspective. *F1000Research*. 2017;6.

29. Kremke M, Hansen MK, Christensen S, Tang M, Andreasen JJ, Jakobsen C-J. The association between platelet transfusion and adverse outcomes after coronary artery bypass surgery. *Eur J Cardio-Thoracic Surg*. 2015;48(5):e102-e109.

30. Ferraris VA, Ferraris SP, Saha SP, et al. Perioperative blood transfusion and blood conservation in cardiac surgery: the Society of Thoracic Surgeons and The Society of Cardiovascular Anesthesiologists clinical practice guideline. *Ann Thorac Surg*. 2007;83(5 Suppl):S27-86.

31. Kawano-castillo J, Ward E, Elliott A, et al. Thrombelastography Detects Possible Coagulation Disturbance in Patients With Intracerebral Hemorrhage With Hematoma Enlargement. 2014:683-689.

32. Ramchand P, Frangos S, Quattrone F, et al. Thromboelastography Determines A Transient Late Hypercoagulable State After Aneurysmal Subarachnoid Hemorrhage (P5.121). *Neurol* . 2014;82(10 Supplement). http://www.neurology.org/content/82/10_Supplement/P5.121.abstract.

33. Frontera JA, Aledort L, Gordon E, et al. Early platelet activation, inflammation and acute brain injury after a subarachnoid hemorrhage: a pilot study. *J Thromb Haemost*. 2012;10(4):711-713.

34. Davis PK, Musunuru H, Walsh M, et al. Platelet Dysfunction is an Early Marker for Traumatic Brain Injury-Induced Coagulopathy. *Neurocrit Care*. 2013;(18):201-208.

35. Windeløv NA, Welling K, Ostrowski SR. The prognostic value of thrombelastography in identifying neurosurgical patients with worse prognosis. *Blood Coagul Fibrinolysis*. 2011;(22):416-419.

36. Manoel, Airton Leonardo de Oliveira, Neto, Antonio Capone, Veigas, Precilla V, Rizoli S. Traumatic Brain Injury Associated Coagulopathy. *Neurocrit Care*. 2015;(22):34-44.

37. Yao X, Dong Q, Song Y, Wang Y, Deng Y. Thrombelastography Maximal Clot Strength Could Predict One-Year Functional Outcome in Patients with Ischemic Stroke. *Cerebrovasc Dis*. 2014;38:182-190.

38. Rugeri L, Levrat A, David JS, Delecroix E, Floccard B, Gros A. Diagnosis of early coagulation abnormalities in trauma patients by rotation thrombelastography. *J Thromb Haemost*. 2007;5:289-295.

39. Alexander DC, Butt WW, Best JD, Donath SM, Monagle PT, Shekerdemian LS. Correlation of thromboelastography with standard tests of anticoagulation in paediatric patients receiving extracorporeal life support. *Thromb Res*. 2010;125(5):387-392.

40. Camenzind V, Bombeli T, Seifert B, et al. Citrate Storage Affects Thrombelastograph ® Analysis. *Anesthesiology*. 2000;92:1242-1249.

41. Rajwal, Sanjay, Richards, Mike, O'Meara M. The use of recalcified citrated whole blood – a pragmatic approach for thromboelastography in children. *Pediatr Anesth*. 2004;14:656-660.

42. Zambruni A, Thalheimer U, Leandro G, Perry D, Burroughs AK. Thromboelastography with citrated blood: comparability with native blood, stability of citrate storage and effect of repeated sampling. *Blood Coagul Fibrinolysis*. 2004;15(1):103-107.

43. Trousseau A. Phlegmasia ad. Clinique Medicale of the Hotel-Dieu of Paris. 1865:654-712. https://archive.org/details/cliniquemdicaled01trou.

44. Blom JW, Vanderschoot JPM, Oostindier MJ, Osanto S, van der Meer FJM, Rosendaal FR. Incidence of venous thrombosis in a large cohort of 66,329 cancer

patients: results of a record linkage study. *J Thromb Haemost*. 2006;4(3):529-535.

45. Blom JW, Doggen CJM, Osanto S, Rosendaal FR. Malignancies, prothrombotic mutations, and the risk of venous thrombosis. *JAMA*. 2005;293(6):715-722.

46. Walker AJ, Card TR, West J, Crooks C, Grainge MJ. Incidence of venous thromboembolism in patients with cancer - a cohort study using linked United Kingdom databases. *Eur J Cancer*. 2013;49(6):1404-1413.

47. Rosendaal FR. Risk factors for venous thrombotic disease. *Thromb Haemost*. 1999;82(2):610-619.

48. Heit JA, Silverstein MD, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ 3rd. Risk factors for deep vein thrombosis and pulmonary embolism: a population-based case-control study. *Arch Intern Med*. 2000;160(6):809-815.

49. Arkel YS. Thrombosis and cancer. *Semin Oncol*. 2000;27(3):362-374.

50. Petterson TM, Marks RS, Ashrani AA, Bailey KR, Heit JA. Risk of site-speci fi c cancer in incident venous thromboembolism : A population-based study ☆. *Thromb Res*. 2015;135:472-478.

51. Horsted F, West J, Grainge MJ. Risk of venous thromboembolism in patients with cancer: a systematic review and meta-analysis. *PLoS Med*. 2012;9(7):e1001275.

52. D'Asti E, Fang Y, Rak J. Brain neoplasms and coagulation-lessons from heterogeneity. *Rambam Maimonides Med J*. 2014;5(4):e0030.

53. Magnus N, D'Asti E, Garnier D, Meehan B, Rak J. Brain neoplasms and coagulation. *Semin Thromb Hemost*. 2013;39(8):881-895.

54. Zadeh G, Koushan K, Baoping Q, Shannon P, Guha A. Role of angiopoietin-2 in regulating growth and vascularity of astrocytomas. *J Oncol*. 2010;2010:659231.

55. Hardee ME, Zagzag D. Mechanisms of glioma-associated neovascularization. *Am J Pathol*. 2012;181(4):1126-1141.

56. Zhang J, Jiang R, Liu L, Watkins T, Zhang F, Dong J. Traumatic Brain Injury-Associated Coagulopathy. *J Neurotrauma*. 2012;29(17):2597-2605.

57. Yu JL, May L, Lhotak V, et al. Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. *Blood*. 2005;105(4):1734-1741.

58. Wang J-G, Geddings JE, Aleman MM, et al. Tumor-derived tissue factor activates coagulation and enhances thrombosis in a mouse xenograft model of human pancreatic cancer. *Blood*. 2012;119(23):5543-5552.

59. Jenkins EO, Schiff D, Mackman N, Key NS. Venous thromboembolism in malignant gliomas. *J Thromb Haemost*. 2010;8(2):221-227.

60. Ilhan A, Gartner W, Neziri D, et al. Angiogenic factors in plasma of brain tumour patients. *Anticancer Res*. 2009;29(2):731-736.

61. Singh VP, Jain D, Mohan R, Bhatia R, Bhargava M. Haemostatic abnormalities in brain tumours. *Acta Neurochir (Wien)*. 1990;102(3-4):103-107.

62. Prasad KS, Sharma BS, Marwaha N, Sarode RS, Kak VK. Haemostatic derangement in patients with intracranial tumours. *Br J Neurosurg*. 1994;8(6):695-702.

63. Goh KY, Tsoi WC, Feng CS, Wickham N, Poon WS. Haemostatic changes during surgery for primary brain tumours. *J Neurol Neurosurg Psychiatry*. 1997;63(3):334-338.

64. Hamilton MG, Hull RD, Pineo GF. Venous thromboembolism in neurosurgery and neurology patients: a review. *Neurosurgery*. 1994;34(2):280-96; discussion 296.

65. Joffe SN. Incidence Of postoperative deep vein thrombosis in neurosurgical patients. *J Neurosurg*. 1975;42(4):201-203.

66. Kimmell KT, Walter KA. Risk factors for venous thromboembolism in patients undergoing craniotomy for neoplastic disease. *J Neurooncol*. 2014;120(3):567-573.

67. Semrad TJ, O'Donnell R, Wun T, et al. Epidemiology of venous thromboembolism in 9489 patients with malignant glioma. *J Neurosurg*. 2007;106(4):601-608.

68. Smith TR, Nanney AD 3rd, Lall RRR, et al. Development of venous thromboembolism (VTE) in patients undergoing surgery for brain tumors: results from a single center over a 10 year period. *J Clin Neurosci Off J Neurosurg Soc Australas*. 2015;22(3):519-525.

69. Khaldi A, Helo N, Schneck MJ, Origitano TC. Venous thromboembolism: deep venous thrombosis and pulmonary embolism in a neurosurgical population. *J Neurosurg*. 2011;114(1):40-46.

70. Rolston, John D, Han, Seunggu J, Bloch, Orin, Parsa AT. What clinical factors predict the incidence of deep venous thrombosis and pulmonary embolism in neurosurgical patients? *J Neurosurg*. 2014;121(October):908-918.

71. Lee AYY, Levine MN. Venous thromboembolism and cancer: Risks and outcomes. *Circulation*. 2003;107(SUPPL. 23).

72. Khorana AA. Venous Thromboembolism and Prognosis in Cancer. *Thromb Res*. 2010;125(6):490-493.

73. Gross CP, Galusha DH, Krumholz HM. The Impact of Venous Thromboembolism on Risk of Death or Hemorrhage in Older Cancer Patients. *J Gen Intern Med*. 2007;22(3):321-326.

74. van Korlaar IM, Vossen CY, Rosendaal FR, et al. The impact of venous

thrombosis on quality of life. *Thromb Res*. 2004;114(1):11-18.

75. Takai S, Akagi M, Crawford B, et al. Economic Impact of Venous Thromboembolism Following Major Orthopaedic Surgery in Japan. *Value Heal Reg Issues*. 2013;2(1):81-86.

76. Hamilton MG, Yee WH, Hull RD, Ghali WA. Venous thromboembolism prophylaxis in patients undergoing cranial neurosurgery: A systematic review and meta-analysis. *Neurosurgery*. 2011;68(3):571-581.

77. Collen JF, Jackson JL, Shorr AF, Moores LK. Prevention of venous thromboembolism in neurosurgery: A metaanalysis. *Chest*. 2008;134(2):237-249.

78. Salmaggi A, Simonetti G, Trevisan E, et al. Perioperative thromboprophylaxis in patients with craniotomy for brain tumours: A systematic review. *J Neurooncol*. 2013;113(2):293-303.

79. Jo JT, Schiff D, Perry JR. Thrombosis in brain tumors. *Semin Thromb Hemost*. 2014;40(3):325-331.

80. Agnelli G, Piovella F, Buoncristiani P, et al. Enoxaparin plus compression stockings compared with compression stockings alone in the prevention of venous thromboembolism after elective neurosurgery. *N Engl J Med*. 1998;339(2):80-85.

81. Vukovich TC, Gabriel A, Schaefer B, Veitl M, Matula C, Spiss CK. Hemostasis activation in patients undergoing brain tumor surgery. *J Neurosurg*. 1997;87:508-511.

82. Abrahams JM, Torchia MB, McGarvey M, Putt M, Baranov D, Sinson GP. Perioperative assessment of coagulability in neurosurgical patients using thromboelastography. *Surg Neurol*. 2002;58(1):5-11.

83. Kunio NR, Differding JA, Watson KM, Stucke RS, Schreiber MA.

Thrombelastography-identified coagulopathy is associated with increased morbidity and mortality after traumatic brain injury. *Am J Surg*. 2012;203(5):584-588.

84. Brohi K, Cohen MJ, Ganter MT, et al. Acute Coagulopathy of Trauma: Hypoperfusion Induces Systemic Anticoagulation and Hyperfibrinolysis. *J Trauma Inj Infect Crit Care*. 2008;64(5):1211-1217.

85. Schreiber MA, Differding J, Thorborg P, Mayberry JC, Mullins RJ. Hypercoagulability is most prevalent early after injury and in female patients. *J Trauma*. 2005;58(3):471-475.

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I express my sincere and deepest gratitude to my guide, Dr. Manikandan S, Professor, Department of Anaesthesiology, SCTIMST who ploughed through several preliminary versions of my text, making critical suggestions, and posing challenging questions. His expertise, invaluable guidance, constant encouragement, affectionate attitude, understanding, patience and healthy criticism has added considerably to my experience. Without his constant inspiration, it would have not been possible to complete this study.

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I also express my sincere thanks to the honorable members of the Institute Ethics committee and Technical Advisory committee for thoroughly reviewing my thesis work in its primitive stages and giving me valuable inputs. I am grateful to them for their kind approval of my research protocol.

Technical Advisory Committee (Clinical Studies)

SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES & TECHNOLOGY
THIRUVANANTHAPURAM – 695011, INDIA

TAC Registration No: SCT-/S/2015/411 **Date:** 27.01.2016

Project title: Evaluation of coagulation profile in supratentorial primary brain tumors in the perioperative period using Thromboelastography.

Principal Investigator:
Name: Dr. Gautham N S Degree: MBBS, MD. Senior Resident, Neuroanesthesia, Department of Anaesthesiology, SCTIMST
Co-Principal Investigator(s)
Name: Dr. Manikandan. S Degree : MBBS, MD,PDCC Additional Professor, , Neuroanaesthesiology Division, Department of Anaesthesiology, SCTIMST

Members who participated in the TAC meeting on 08/01/2016
Dr. Rupa Sreedhar (Chairperson)
Dr. Thomas Koshy
Dr. Lissy K Krishnan
Dr. Sylaja P.N
Dr. Krishnamoorthy KM
Dr. Biju Soman
Dr. Bejoy Thomas
Dr. Syam. K
Dr. K. Shivakumar (Member Secretary)

Dr. Thomas Koshy, Dr. Syam K, Dr. Bejoy Thomas and Dr. Sylaja.P.N stayed away from the proceedings when the projects in which they are involved (# 406, 415, 410, 413, 424, 426) as investigators were discussed.

Risk Classification of the project (Minimum/ Moderate/ High): Minimum

Requirement of DSMB: No

Recommended members of DSMB: Not applicable

Recommendations of TAC:
Recommended for consideration of IEC.

The PI may note that there can be no additions / alterations in the documents approved by TAC when they are submitted to the IEC.

Signature of the Member Secretary, TAC (Clinical Studies)

Note for IEC
Copy of the investigator's responses to questions/suggestions from TAC is attached (Appendix-1).

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान
तिरुवनन्तपुरम-695011, केरल, भारत
SREE CHITRA TIRUNAL INSTITUTE FOR MEDICAL SCIENCES AND TECHNOLOGY
THIRUVANANTHAPURAM - 695011, INDIA
(An Institute of National Importance under Govt. of India)

Institutional Ethics Committee
(IEC Regn No. ECR/189/Inst/KL/2013)

SCT/IEC/852/FEBRUARY-2016

29.03.2016

Dr. Gautham NS
Senior Resident
Department of Anaesthesiology
SCTIMST, Thiruvananthapuram

Dear Dr. Gautham,

The Institutional Ethics Committee reviewed and discussed your application to conduct the study entitled "EVALUATION OF COAGULATION PROFILE IN SUPRATENTORIAL PRIMARY BRAIN TUMORS IN THE PERIOPERATIVE PERIOD USING THROMBOELASTOGRAPHY" (IEC/852) on 20th February, 2016.

The following documents were reviewed:

Original submission

1. *Covering letter addressed to the Chairman, IEC, SCTIMST with check list*
2. *Project Proposal*
3. *IEC Application Form*
4. *TAC Approval Letter*
5. *Informed Consent Form in English and Malayalam*
6. *CV of the PI & Co-PI*

Revised submission

1. *Covering letter addressed to the Chairman, IEC, SCTIMST dated 17.03.2016 with check list*
2. *Project Proposal*
3. *IEC Application Form*
4. *TAC Approval Letter*
5. *Modified Informed Consent Form in Malayalam*
6. *Informed Consent Form in English*
7. *CV of the PI & Co-PI*

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The following members of the Ethics Committee were present at the meeting held on 20th February, 2016 at G. Parthasarathi Board Room, AMCHSS, SCTIMST

SL. No.	Member Name	Highest Degree	Gender	Scientific /Non Scientific	Affiliation with Institution(s)
1.	Justice Gopinathan. P.S	BSc. LLB	Male	Legal Expert (Chairperson)	No
2.	Dr. Asha Kishore	MD, DM	Female	Clinician (Neurologist)	Yes
3.	Shri. O.S. Neelakandan Nair	BE	Male	Engineer	Yes
4.	Dr. Meenu Hariharan	DM	Female	Clinician (Gastro-Enterologist)	No
5.	Dr. Rema M. N	MD	Female	Pharmacologist	No
6.	Dr. V. Raman Kutty	MPH(Harvard) MPhil, MD	Male	Public Health	Yes
7.	Dr. K R S Krishnan	ME, PhD	Male	Biomedical Scientist/Engineer	No
8.	Dr. Kala Kesavan. P	MD	Female	Pharmacologist	No
9.	Dr. Christina George	MD	Female	Psychiatrist	No
10.	Dr. Mala Ramanathan	MSc, PhD, MA	Female	Ethicist/Social Scientist (Member Secretary)	Yes

IEC Decision

The IEC approved the conduct of the study in the present form.

Remarks:
The Institutional Ethics Committee expects to be informed about the progress of the study, any SAE occurring in the course of the study, any changes in the protocol and patient information/informed consent and asks to be provided a copy of the final report.

There was no member of the study team who participated in voting / decision making process. The ethics committee is organized and operated according to the requirements of Good Clinical Practice and the requirements of the Indian Council of Medical Research (ICMR).

Sincerely,

Mala Ramanathan
Member Secretary, IEC

Consent Forms

PATIENT INFORMATION FORM

Title of the study: "Evaluation of coagulation profile in primary brain tumors in the perioperative period using Thromboelastography"

Name of the Investigators: Dr. Gautham N S, Dr. Manikandan S.

You are being requested to participate in a study to evaluate the coagulation abnormalities in primary brain tumors using Thromboelastography. Thromboelastography is an investigation which provides a global assessment of the coagulation system. Using this test, various parameters which identify the interplay between different components of the coagulation system can be obtained. A small quantity of blood withdrawn from the veins is required for testing using Thromboelastography. Thromboelastography will be done at multiple points during the disease and treatment to assess the coagulation abnormalities at different times.

Who will be included in this study?

We are planning to include 30 patients from our institute. Patients aged between 30 and 60 years, who are posted for surgery for supratentorial primary brain tumors, with no coexisting illness, haematological abnormalities, bleeding or coagulation disorders, or on any antiplatelet or anticoagulant drugs will be included. We will not recruit if the patient or patient relative is not willing to participate in the study. We will also not include if the subject has had a recurrent tumor, prolonged preoperative immobility, had a massive blood loss or massive transfusion intraoperatively, or is mechanically ventilated and therefore anticipated to have prolonged post-operative immobility.

If you take part what will you have to do?

If you agree to participate in this study, your demographic data will be recorded, followed by certain baseline investigations which are done as a part of routine pre-operative work up. Thromboelastography will be done preoperatively and post operatively at 24 hrs, 48 hrs and 72 hrs. The parameters thus obtained will be analyzed to study the coagulation profile in the perioperative period.

Does Thromboelastography pose any substantial health risk?

Thromboelastography is an investigation which involves subjecting a small sample of whole blood to the process of coagulation in a test tube, and studying the mechanics of coagulation using computer software. As it only requires a small quantity(3-5ml) of blood to perform the test, it is not expected to pose any health risk.

Can you withdraw from this study after it starts?

Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way. In addition, if you experience any serious side effects or your condition worsens, the study will be stopped and you may be given additional treatment.

What will happen if you develop any study related injury?

We do not expect any injury to happen to you but if you do develop any side effects or problems due to the study, these will be treated at no cost to you. However, we will be unable to provide any monetary compensation for the same.

.Will you have to pay for the study?

As the study is funded, you will not be expected to make any payments for Thromboelastography. However, all other investigations that are recorded in the study as baseline parameters will not be covered by the funding. They are also a part of routine anaesthetic work up as per our hospital protocol. Therefore, they will be charged as per hospital policy.

.What happens after the study is over?

This study will be conducted till 72 hours after the surgery. You will be treated as per protocol for all neurosurgical patients during and after the study. There will be no change in protocol for management following enrolment in this study.

Will your personal details be kept confidential?

The results of this study will be published in a medical journal but you will not be identified by name in any publication or presentation of results. However, your medical notes may be reviewed by people associated with the study, without your additional permission, should you decide to participate in this study.

If at any time you experience any problems, or if you have any further questions, please ask,

Dr. Gautham N S (principal investigator), nsgautham@sctimst.ac.in or mobile :09037229792

CONSENT FORM

Title of the study: "Evaluation of coagulation profile in primary brain tumors in the perioperative period using Thromboelastography"

Participant's name: **Age (in years)**:

I_____, son/daughter/husband/wife/----------of _____ declare that (Please tick boxes)

- I have read the above information provide to me regarding the study: [ ]
- I have clarified any doubts that I had. [ ]
- I also understand that my participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my usual treatment or my legal rights [ ]
- I understand that the study staff and institutional ethics committee members will not need my permission to look at my health records even if I withdraw from the trial. I agree to this access [ ]
- I understand that my identity will not be revealed in any information released to third parties or published [ ]
- I voluntarily agree to take part in this study [ ]
- I have been provided with the contact numbers of the principle investigator, in case I want to know more about the study and participants rights [ ].
- I received a copy of this signed consent form [ ]

Name: Signature: Date:

Name of witness: Signature: Date:

Relation to participant:

Person Obtaining Consent

I attest that the requirements for informed consent for the medical research project described in this form have been satisfied. I have discussed the research project with the participant and explained to him or her in nontechnical terms all of the information contained in this informed consent form, including any risks and adverse reactions that may reasonably be expected to occur. I further certify that I encouraged the participant to ask questions and that all questions asked were answered.

Name:

Signature:

Date:

Place: SCTIMST, Thiruvananthapuram

Proforma

Demographic data:

Number	
Age (Years)	
Sex	
Weight (Kg)	
Diagnosis	
Procedure	
Duration of surgery (mins)	

Baseline parameters:

Type of Tumor	
Size of Tumor (cm)	

Coagulation parameters:

Parameter	Pre Op	Post Op
Platelet count (lakhs/ml)		
Prothrombin time(PT) (sec)		
INR		
Activated Partial Thromboplastin Time (aPTT) (sec)		

Perioperative parameters:

Blood loss (ml)	
RBC transfusion (ml)	
FFP transfusion (ml)	
Platelet transfusion (ml)	
Post-Operative ICH (0/1)	
DVT (0/1)	
PE (0/1)	

Thromboelastography parameters:

Parameter	**Pre Op (T0)**	**24 hrs PO (T1)**	**48 hrs PO (T2)**	**72 hrs PO (T3)**
R Time (min)				
K Time (min)				
α angle (°)				
MA (mm)				
CI				
Ly30 (%)				

Key to Master chart

Pre Op PT	: Pre-Operative Prothrombin Time
Pre Op INR	: Pre-Operative International Normalized Ratio
Pre Op aPTT	: Pre-Operative Activated Partial Thromboplastin Time
Pre Op Platelet	: Pre-Operative Platelet Count
Post Op Platelet	: Post-Operative Platelet count
Post Op PT	: Post-Operative Prothrombin Time
Post Op INR	: Post-Operative International Normalized Ratio
Post Op aPTT	: Post-Operative Activated Partial Thromboplastin Time
R	: R Time
K	: K Time
Alpha	: α angle
MA	: Maximum Amplitude
CI	: Coagulation Index
Ly30	: Area under TEG curve at 30 minutes
Post Op ICH	: Intracranial Hematoma on Post op CT scan. 0 indicates no ICH, 1 indicates presence of ICH
DVT	: Deep Vein Thrombosis. 0 indicates no DVT, 1 indicates presence of DVT
PE	: Pulmonary Embolism. 0 indicates no PE, 1 indicates presence of PE

Number	Age (Yrs)	Sex	Weight (Kg)	Diagnosis	PT Pre Op (Sec)	INR Pre OP	aPTT Pre Op (sec)	Platelet Pre Op (cells/cu mm)	Platelet Post Op (cells/cu mm)	PT Post Op (sec)	INR Post Op	aPTT Post Op (sec)	Size of tumor(cm)	R at T0 (min)	R at T1 (min)	R at T2 (min)	R at T3 (min)	K at T0 (min)	K at T1 (min)	K at T2 (min)	K at T3 (min)	Alpha angle at T0 (Degree)	Alpha angle at T1 (Degree)	Alpha angle at T2 (Degree)	Alpha angle at T3 (Degree)	MA at T0 (mm)	MA at T1 (mm)	MA at T2 (mm)	MA at T3 (mm)	CI at T0	CI at T1	CI at T2	CI at T3	LY30 at T0 (%)	LY30 at T1 (%)	LY30 at T2 (%)	LY30 at T3 (%)	Bleeding (ml)	RBC transfusion (ml)	FFP transfusion (ml)	Platelet transfusion (ml)	Bleeding in post op CT	DVT	PE
1	30	M	63	Left Frontal glioma	15.6	1.06	35.6	379000	311000	15.2	1.02	35.2	6	6.4	5.7	5.3	3.1	3.4	2.6	2	1.3	50	56.5	66.9	71.4	57.5	52.7	60.1	74.9	-2.41	-1.75	0.36	4.28	1.6	1.2	0.7	5.9	500	0	0	0	0	0	0
2	41	M	67	Left Parietal meningioma	14.7	0.94	36	325000	248000	15.2	1.04	38	3.8	4.2	5.5	4.3	5.5	2.6	1.8	1	1.5	56.2	67.1	69.5	69.1	56.9	61.7	67.4	66.5	-0.28	0.59	2.44	1.44	1.2	1.3	1.1	0.5	1000	600	0	0	0	0	0
3	49	M	58	Left Parietal glioma	15.2	1.08	39.4	347000	312000	15.1	1.07	39	4.2	4.8	5.2	3.5	2.9	1.8	1.4	1	1	62.3	68.3	72.6	75.4	59.4	65.3	69.2	74.6	0.40	1.47	3.45	4.79	0	0.8	0.7	1.3	600	0	0	0	0	0	0
4	30	M	67	Left Frontal glioma	15.5	1.09	38	416000	325000	16	1.1	40	6	4.9	5.6	4.4	4	1.5	1.6	2	1.3	69.1	68.2	66.2	68.4	60.4	64.2	67.8	70.3	1.09	0.99	2.06	2.91	2.9	1.5	0	0	400	0	0	0	0	0	0
5	51	F	65	Left Sphenoidal meningioma	15.4	1.09	36	367000	298000	15.8	1.1	38	6	4.6	3.5	3	2.4	2.3	2.4	2	1.1	62.3	60	67.1	73.3	57.2	58.1	63.2	67.6	0.07	0.69	2.40	4.07	0	0.1	0.9	0.3	800	400	0	0	0	0	0
6	42	F	62	Left Frontal glioma	15.4	0.96	32.9	345000	253000	14	1.09	28.1	6	4.7	4.7	2.9	2.5	2.6	2	1	1.2	58.1	63.1	70.5	63.6	62.7	57.9	56.4	66.4	0.25	0.27	2.04	3.08	3.8	3	5.4	0	600	0	0	0	0	0	0
7	39	M	65	Left Frontoparietal glioma	15.8	1.12	40	372000	308000	15.4	1.07	39	4.5	3.9	3.8	4.8	4.8	2.4	1.7	2	1.4	60.1	67.8	68.9	69.5	47.6	50.5	69.2	75.3	-0.85	0.42	2.18	3.04	0	2.5	4.6	0.1	400	0	0	0	0	0	0
8	44	F	68	Left Petroclival meningioma	14.8	1	29	401000	315000	14.6	0.98	29.4	5.5	4.8	6.1	4.8	4.7	2.6	1.8	1	1.1	40.9	66	72.3	71.2	74.7	71	76.3	81.3	0.35	1.25	3.42	4.09	4.2	3.4	2.1	2.1	1600	800	400	0	0	0	0
9	50	M	70	Left Frontal gioma	16.1	1.13	39	298000	312000	15.7	1.06	38.9	5.5	4.8	5.5	2.8	2.6	2.7	1.4	2	1.5	53.8	72.1	69.3	71.3	51.7	59.5	64	69.8	-1.53	0.85	2.91	3.90	2.5	0	0	0.3	400	0	0	0	0	0	0
10	52	M	69	Left Parietal meningioma	16.3	0.98	35.8	328000	286000	16.1	1.01	35.4	5	4.8	4.1	4.8	4.8	1.2	1.7	1	1.7	57.5	65.9	67.5	72.4	58.1	60.9	78.1	76.6	0.10	1.35	3.23	3.31	0	0	0.6	0.5	500	0	0	0	0	0	0
11	34	M	72	Left Temporal glioma	16.8	1.16	39.3	317000	299000	16.4	1.16	38	4.8	5.8	4	4.2	4.3	2.4	1.1	1	1.2	58.4	71.6	72.1	73.8	61.3	68	78.8	79.4	-0.54	2.94	4.10	4.27	0.5	0.2	0.3	0.4	900	200	0	0	0	0	0
12	42	F	63	Right Frontal glioma	15.8	1.07	26.4	298000	206000	17.8	1.24	35.5	10	3.9	5.1	3.7	3.9	1.7	1.9	2	1.4	66.8	66.4	69.3	69.2	66.1	66.9	70.3	72.1	2.19	1.40	3.02	3.22	0	0	0.4	0.5	1000	200	0	0	0	0	0
13	30	F	65	Right Frontal glioma	14.1	0.94	24.6	387000	317000	14.6	1.02	27	5.5	3.4	3.8	3.4	3.6	4.8	1.1	1	1.4	43.2	69.6	69.4	72.1	44.9	80.4	80.2	84.1	-3.04	4.44	4.59	5.10	0.2	0	0.4	4.6	900	200	0	0	0	0	0
14	44	M	66	Left Frontoparietal glioma	14.6	0.94	32.2	324000	278000	14.2	0.97	33.4	4.2	3	5	4.2	3.5	3.4	2.7	1	1.1	51.4	58.2	67.2	71.3	41.5	49.2	58.6	67.9	-2.05	-1.63	1.22	3.23	5.5	2.3	0.4	0.4	600	0	0	0	0	0	0
15	35	M	62	Left Frontal glioma	14.7	0.98	27.9	363000	310000	14.3	0.98	28.8	4.5	9	5.5	3.2	4.2	2.3	2.2	2	1.7	34.3	63.1	64.3	67.3	58.6	65.5	78.6	73.8	-4.75	0.60	3.91	2.97	0.7	0	0	1	600	0	0	0	0	0	0
16	57	M	69	Right Parasagittal meningioma	14.6	0.95	33.9	365000	325000	14.3	0.98	34.6	5	6.2	6.6	4.3	3.9	2.7	2.9	2	1.6	60.4	58.3	69.4	63.5	67.3	54.6	68.4	76.8	-0.03	-2.08	2.22	3.28	1.3	0.5	1.5	2.1	500	0	0	0	0	0	0
17	53	M	68	Right Parasagittal meningioma	15.6	1.06	37.6	398000	340000	15.3	1.03	38.8	4.5	6	6.2	3.2	5.2	2.8	2.9	1	1.8	56.2	58.1	75.9	64.8	60.8	58.7	61.4	71.8	-1.05	-1.33	3.02	1.85	2.2	0.2	0.1	1.3	1200	600	400	100	0	0	0
18	39	F	64	Left Parietal glioblastoma multiformae	15.2	1.04	33.9	423000	310000	15.3	1.04	33.8	4	4.5	1.7	1.8	4.8	1.6	2.5	1	2	67.8	59.7	60.2	68.4	71.5	39.5	65.6	70.1	2.57	-0.48	3.14	2.10	0	2.4	0.5	0.9	200	0	0	0	0	0	0
19	38	M	72	Right Frontal glioma	14.3	0.94	34.6	376000	310000	14.6	1.02	36.4	5.3	4.1	7.6	5.5	3.5	2.1	5	3	1.6	62.4	43.9	53.8	73.6	58.8	59.3	57.3	73.4	0.68	-4.04	-1.30	3.89	2.3	0.5	2.3	1.2	400	0	0	0	0	0	0
20	40	M	73	Left Planum Sphenoidale meningioma	15.2	1.03	34.3	412000	353000	14.9	0.98	36.1	5.6	5.8	5.8	5.9	3.1	3	3.7	2	1.6	33.2	40.9	57.6	48.9	59.1	76.6	64.8	73.2	-2.95	-0.49	-0.20	2.26	0	0.2	4.9	3.6	700	0	0	0	0	0	0
21	45	M	70	Right Frontal glioma	14.1	0.94	37.6	378000	335000	14.4	1.01	35.4	6.7	5.4	5.5	4.2	3.8	3.3	2.4	3	1.9	58.4	61.7	64.8	72.1	56.3	63.2	69.2	69.6	-1.23	0.14	1.88	3.01	0	0	0	0	600	0	0	0	0	0	0
22	42	F	68	Left Parietal meningioma	15.6	1.06	38.7	392000	364000	15.2	1.03	36.2	5.8	6.1	5.3	4.7	4.9	2.9	2.8	3	2.1	62.3	65.3	68.7	72.6	59.8	63.4	66.9	78.9	-0.81	0.41	1.57	3.39	2.1	0.5	0.3	0.8	1100	400	200	0	0	0	0
23	53	F	62	Left Parasagittal meningioma	15.1	1.02	38.1	403000	381000	15.4	1.04	39.6	7.3	4.8	4.6	3.2	3.9	2.2	2.3	2	1.5	58.5	61.2	66.3	73.4	63.4	65.3	73.9	71.8	0.45	0.98	3.52	3.46	0.5	0	0.6	0	800	200	0	0	0	0	0
24	36	F	65	Right Frontal glioma	14.4	0.98	38.3	394000	351000	14.6	0.98	37.5	4.5	5.1	5.3	4.2	3.9	2.3	2.1	3	2.4	54.6	59.3	67.5	74.6	67.3	64.9	63.6	68.9	0.40	0.41	1.40	2.86	0	0	0	0	600	0	0	0	0	0	0
25	48	M	68	Right Parietal meningioma	15.3	1.03	37.8	365000	357000	15.2	1.02	38.1	5.6	4.8	5.2	4.3	3.7	2.9	2.5	2	1.8	61.7	60.4	68.3	69.2	59.4	57.4	65.8	64.3	-0.06	-0.51	1.85	2.24	0.3	2.1	1.5	1.2	800	400	0	0	0	0	0