A STUDY ON THE COAGULATION FACTOR XIII AND OTHER HAEMOSTATIC MARKERS IN NEUROSURGICAL PATIENTS

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aPTT	Activated partial thromboplastin time
AT	Antithrombin
BBB	Blood brain barrier
CRP	C-reactive protein
CT	Computerized tomography
DM	Diabetes mellitus
EDTA	Disodium ethylene diaminetetra-acetic acid
ELISA	Enzyme linked immunoassay
FXIII	Factor XIII
FDP	Fibrin degradation product
PAI-1	Plasminogen activator inhibitor-1
PC	Protein C
PS	Protein S
PT	Prothrombin time
t-PA	Tissue plasminogen activator
u-PA	Urokinase-type plasminogen activator
TAFI	Thrombin activatable fibrinolysis inhibitor
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TM	Thrombomodulin
vWF	von Willebrand factor

ABSTRAK

Keabnormalan pada sistem hemostasis menyebabkan komplikasi pendarahan atau penghasilan darah beku yang abnormal. Ini boleh menyebabkan kecacatan yang teruk atau membawa kematian kepada pesakit neurosurgeri. Oleh itu, sistem hemostasis yang berfungsi secara optima merupakan faktor penentu bagi keselamatan sesuatu pembedahan otak. Ujian koagulasi rutin memang efektif dalam mengesan sebarang kekurangan yang melibatkan penghasilan fibrin. Namun, kekuatan sesuatu gumpalan darah (fibrin) amat bergantung kepada faktor tiga belas (faktor XIII); yang mana ia berfungsi dalam penghasilan jalinan antara monomer fibrin dan juga menambahkan ketahanan fibrin terhadap proses fibrinolisis. Sehingga kini, aktiviti faktor XIII tidak diuji secara rutin sebelum sesuatu pembedahan itu dijalankan. Oleh itu, objektif kajian ini adalah untuk menentukan kaitan klinikal antara aktiviti faktor XIII serta penanda-penanda hemostasis yang lain dalam penghasilan komplikasi pendarahan (hematoma) selepas pembedahan bagi pesakit neurosurgeri.

Pesakit neurosurgeri yang menjalani pembedahan intracranium dari bulan Januari, 2008 hingga Jun 2009 terlibat dalam kajian ini. Darah pesakit tersebut akan diambil sebelum dan selepas pembedahan bagi ujian bilangan platlet, PT/INR, aPTT, paras fibrinogen serta aktiviti faktor XIII, antithrombin dan PAI-1. Imej CT otak selepas prosedur akan menentukan kehadiran atau tidak hematoma yang terjadi akibat prosedur tersebut. Berdasarkan imej CT tersebut, pesakit dikategorikan kepada tiga kumpulan iaitu hematoma signifikan, hematoma yang tak signifikan dan tiada hematoma. Software SPSS

Inc. versi 12.0 digunakan untuk analisis statistikal dan nilai kemungkinan <0.05 dikira signifikan.

84 orang pesakit terlibat dalam kajian ini. 51(60.7%) orang pesakit tidak mendapat komplikasi hematoma, 28(33.3%) pesakit mendapat hematoma yang tak signifikan dan 5(6%) orang mendapat hematoma yang signifikan. Bilangan platelet, PT/INR dan aktiviti faktor XIII selepas pembedahan menunjukkan penurunan yang signifikan berbanding bacaan sebelum pembedahan. . Platelet dan antithrombin merupakan 2 parameter yang berkait dengan risiko untuk mendapat hematoma selepas pembedahan. Manakala, pesakit yang mengalami kombinasi aktiviti faktor XIII serta platlet atau antithrombin yang rendah mempunyai risiko yang lebih tinggi untuk mendapat komplikasi hematoma.

Kajian ini membuktikan bahawa kepincangan dalam beberapa jenis parameter hemostasis, memainkan peranan yang penting dalam pembentukan hematoma pada pesakit surgeri neuro. Sebagai kesimpulan, pengawasan parameter koagulasi iaitu bilangan platelet dan aktiviti factor XIII serta antithrombin ketika dan selepas pembedahan kranium boleh membantu pakar bedah neuro untuk membuat jangkaan mengenai risiko seseorang pesakit untuk mendapat pendarahan atau hematoma selepas pembedahan.. Hasil dari penemuan ini, kajian tentang kelebihan penggunaan produk darah seperti platlet, antithrombin dan faktor XIII harus dipertimbangkan pada masa hadapan.

ABSTRACT

Abnormalities of haemostatic system can lead to bleeding complication or thromboembolic events which can cause severe impairment or even death of neurosurgical patients. Therefore, an optimal functional integrity of the hemostatic system is a prerequisite for the safe performance of neurosurgical procedures. To monitor the individual coagulation capacity of each patient, standard tests are effective to detect deficiencies involving the generation of fibrin. However, fibrin clot strength depends primarily on coagulation factor XIII, which cross-links fibrin monomers and enhances clot resistance against fibrinolysis. Currently, factor XIII activity levels are not routinely screened prior to surgical interventions and no effective screening test available to detect deficiency state. Therefore, the objective of this prospective study was to determine the clinical relevance of perioperative factor XIII activity levels and other haemostatic markers with postoperative haematoma formation in neurosurgical patients.

Neurosurgical patients who required intracranial surgery from January 2008 until Jun 2009 were admitted to the study. All included patients had their blood taken at preoperatively and within 24 hours postsurgical periods for platelet count, PT/INR, aPTT, fibrinogen, factor XIII, antithrombin and PAI-1 levels. Intracranial surgeries were proceeded according to the standard neurosurgical techniques. All operated patients had their CT brain scan done after the surgery. The post operative CT brain scan determined the presence or absence of postoperative haematoma. Based upon these CT brain findings, patients were subclassified into 3 groups; significant haematoma, insignificant

haematoma and no haematoma groups. The commercially available software SPSS Inc, version 12.0 was used in statistical analysis and probability values < 0.05 were considered significant.

A total of 84 patients were enrolled in this study. Fifty one patients did not have postoperative haematoma, 28 patients developed insignificant postoperative haematoma and 5 patients did have significant postoperative haematoma. Haematological tests for platelet count, PT/INR and factor XIII activity levels showed significant differences between pre and postoperative values. However, only postoperative platelet count was turned out to be significant and related to postoperative haematoma formation. Low platelet and antithrombin levels were the only two parameters that had significant association with increased risk for postoperative haematoma. Interestingly, combination of low platelets count and low factor XIII activity levels contribute a significantly higher odd ratio of having postoperative bleeding complications. Similarly, combination of low postoperative antithrombin and factor XIII activities also increased the odds of developing haematoma.

This study proved that presence of multiple defects in haemostatic markers, play an important role in the formation of significant postoperative haematoma for neurosurgical patients. In fact, five patients who developed significant postoperative haematoma did have low values for some of these markers. Platelet count, factor XIII and antithrombin activities were hardly monitored during intracranial surgery. These haemostatic markers tend to get compromised after complex cranial surgery leading to bleeding complication.

The results of this study indicated that few coagulation markers which are platelet count, factor XIII and antithrombin activities might serve to improve management in patients with intracranial surgery. Monitoring these parameters prior or post intracranial surgery, will allow the neurosurgeon to evaluate the risk of haematoma following the surgery. Studies in the usefulness of blood products like platelet, antithrombin and factor XIII concentrates could be considered in future based on these findings.

Chapter 1

Introduction

1.0 HAEMOSTATIC SYSTEM

Postoperative haematoma is a common complication of surgical procedures. However, this is rather dramatic after brain surgery and is frequently associated with severe neurological morbidity or even mortality (Gerlach *et al.,* 2002). The functional integrity of the haemostatic system is a prerequisite for the safe performance of neurosurgical procedures. After intracranial operative procedures, changes of plasmatic haemostasis may occur and impose a different risk with postoperative bleed or thrombotic complications. Assessment with haemostatic markers preoperative and postoperatively may improve the risk and prevent morbidity as well as mortality related to the surgical interventions.

Factor XIII is of physiological importance in hemostasis, especially in patients undergoing surgery. It catalyses the enzymatic cross-linking of fibrin monomers into stable polymers and protects polymers from plasmatic and non-specific degradation. Factor XIII is a protein transglutaminase found in both platelets and plasma, being activated at the end of the clotting cascade to convert the noncovalent fibrin polymer into a covalently cross-linked structure with increased tensile strength and resistance to fibrinolysis. While inherited factor XIII deficiency is a well known disorder and often leads to severe bleeding (in particular intracranial haemorrhage) by early childhood, the presence of an acquired factor XIII deficiency remains a doubtful entity particularly its clinical significance for neurosurgical cases.

There has been little knowledge on the haemostatic changes after neurosurgical procedure particularly for factor XIII. A study by (Menges *et al.,* 1994) found a decrease plasma factor XIII activity under 40% was associated with a latent clotting activity induced by a thrombin generation. A higher risk of rebleeding after an initial intracranial bleeding event was also observed. They proposed the necessity of substituting factor XIII in such cases to minimize risk the risk of rebleedings. Gerlach and collegues found the relative risk of developing a postoperative haematoma in neurosurgical patients with postoperative factor XIII activity under 60% (Gerlach *et al.,* 2002). Another study by Teich and colleagues, found factor XIII activity of below 70% was associated with severe haemorrhage for epilepsy surgery (Teich *et al.,* 2004).

Diagnosing factor XIII deficiency or dysfunctional protein is important and so far no screening method that can point to this disorder. The use of manual clot lysis is crude and could only detect very severe deficiency state. Therefore, factor XIII deficiency could only be diagnosed by factor XIII assay (a specialized test). Finding the correlation of defective factor XIII with neurosurgical intervention could prevent postoperative rebleeding and able to institute proper management. Other coagulation proteins also play an important role in surgical haemostasis and many of them could be easily screened for deficiency states by routine APTT (activated partial thromboplastin time) and PT (prothrombin time) screening tests as well as by doing fibrinogen levels. Abnormalities in platelet, fibrinogen, PT and APTT further increased the risk of postoperative haematoma in neurosurgical patients.

1.1 POSTSURGICAL HAEMATOMA

Postsurgical haematoma after brain surgery is disastrous and often associated with severe neurological impairment or even death (Palmer *et al.,* 1994). The cranium is a rigid box containing vital organ, the brain. The cranial volume is therefore fixed in the adults. Additional increase in cranial volume causes raise in intracranial pressure once the compensatory mechanisms failed. High intracranial pressure will lead to herniation syndrome and eventually death. The pathological increase in cranial volume is commonly due to haematoma or brain swelling. Other causes of increased cranial volume are brain tumours, brain abscess , hydrocephalus and etc. Intracranial haematomas include extradural, subdural , subarachnoid , intracerebral and intraventricular haemorrhage. Conditions that normally give rise to acute problem with raised intracranial pressure are extradural, subdural , intracerebral and intraventricular haemorrhages.

The incidence of postoperative intracranial haematoma is 4.3 % (Gerlach *et al.,* 2002). The postoperative intracranial haematoma can be clinically classified as significant haematoma which required urgent resurgery to evacuate or drain the haematoma. The insignificant haematoma refers to haematoma which do not require surgery. Postoperative neurosurgical haematoma can be the results of poor surgical technique, poor controlled of perioperative conditions and patient's factors. Most of these abnormalities can be detected and prevented by performing routine coagulation tests, obtained a good medical history and conducting the operation by a good and well trained neurosurgeon.

1.2 BLOOD COAGULATION AND ROLE OF FACTOR XIII

Recently, coagulopathy has been a subject of interest for neurosurgeons. Haemostatic abnormalities should be detected preoperative, intraoperative and postoperatively. This is extremely important to gain good surgical outcomes. Patients who had postoperative haematoma after brain surgery suffer from severe neurological impairment or death (Palmer *et al.*, 1994). A study reported an increased risk of postoperative haemorrhage after intracranial surgery in patients with decreased factor XIII activity (Gerlach *et al.*, 2002). Deficiency in factor XIII activity levels may cause postoperative haematoma via abnormal clot formation secondary to poor cross-links of fibrin monomers. It is likely that a stringent haemostatic function is required in brain surgery compared to other sites.

Coagulation factor XIII is a heterotetramer consisting of A subunit (factor XIIIA) and B subunit. The A-subunit contains the catalytic (active) domain while the B-subunit serves as carrier and regulatory protein. Factor XIII is a plasma 'transglutaminase' essential for normal haemostasis in the last step of the coagulation cascade (figure 1.1). Factor XIII catalyses intermolecular cross-linking reactions between fibrin monomers, α_2-plasmin inhibitor and fibronectin. These reactions will increase the mechanical strength of the fibrin clot and give resistance to proteolytic degradation and also enhance the assembly of the extracellular matrix. Deficient in factor XIII active component (factor XIIIA subunit) may lead to haemorrhagic complications or impaired wound healing. These were reported by (Gerlach *et al.*, 2002), which bound an association between postoperative haemorrhage after intracranial surgery and decreased factor XIII activity level. (Koseki-

Kuno *et al.*, 2003) also reported factor XIIIA subunit-deficient mice tend to develop severe spontaneous uterine haemorrhages. (Schroeder *et al.*, 2006) reported factor XIIIA-subunit mutations leading to congenital factor XIII deficiency and bleeding disorder.

A study on clot strength by (Chandler *et al.*, 2001) after cardiopulmonary bypass surgery demonstrated that a postoperative bleeding at 2 hour was inversely correlated with platelet count and factor XIIIA. Maintenance of adequate platelet counts and factor XIIIA levels at the end of cardiopulmonary bypass may play role in maintaining clot strength and reducing blood loss. The importance of factor XIII in cardiac surgery to prevent postoperative rebleeding was also demonstrated by (Godje *et al.*, 2006). Coagulation factor XIII administered during surgery reduces postoperative rebleeding after coronary surgery with extracorporeal circulation. The authors recommended measurement of plasma levels of factor XIII and administration of factor XIII concentrates in patients with low levels of factor XIII to reduce postoperative blood loss.

Beside fibrin clot stabilization, factor XIII has been found to reduce endothelial permeability independently of its role in the coagulation cascade (Schroeder *et al.*, 2006). Thus, decreased factor XIII may also influence the blood brain barrier (BBB) integrity, especially in patients with brain neoplasms with respect to brain oedema or post operative bleeding complication. Perilesional oedema for intracranial lesion is believed to be due to impairment in BBB and was classified as vasogenic oedema. Factor XIII deficiency may play a role in stabilizing the BBB and hence its deficiency may cause focal brain oedema and subjected to blood vessel wall damage and its consequences.

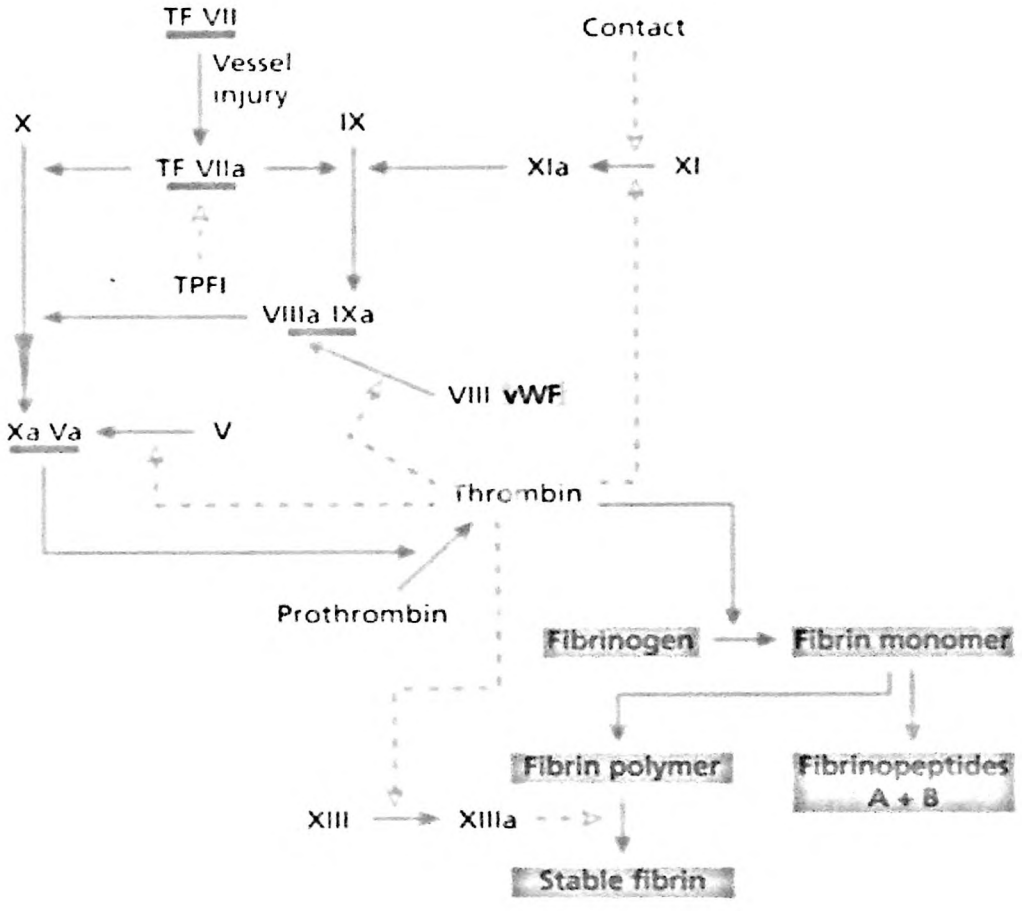

Figure 1.1: Pathway of blood coagulation and role of factor XIII in stabilizing the fibrin clot. (Adapted from Hoffbrand *et al.*, 2006).

1.3 OVERVIEW OF HAEMOSTASIS

The haemostatic system is a vital protective mechanism that is responsible for preventing blood loss by sealing sites of injury in the vascular system. However, this system must also be controlled so that the blood loss does not coagulate within the vasculature and restrict the normal blood flow. In other words, the integrity of the haemostatic systems depends upon equilibrium between coagulation activation, inhibition and fibrinolytic activity. And this complex process of haemostasis is determined by the interaction of endothelial and subendothelial cells, platelets, leucocytes, coagulation factors and coagulation inhibitors.

1.3.1 Coagulation system

The three major steps of the coagulation process are the initiation, amplification and propagation phases. Vascular injury leads to intravascular exposure of tissue factor (TF). Tissue factor binds circulating factor VII to form TF-FVII complexes. This initial step results in activation of factor X and IX on TF-bearing cells (initiation phase). Platelets are localized to the site of injury by adhesion to the subendothelial matrix mediated by interaction between collagen, von Willebrand factor (vWF) and GP1b receptors on the surface of platelets. During the initiation phase, activated factor X (FXa) generates small amount of thrombin, which is not high enough to produce a haemostatic sufficient fibrin clot, but leads to an activation of platelets and further enzymatic coagulation factors (factor XI, VIII and V) (amplication phase).

Activated platelets release thromboxane and their granules contents (ADP, serotonin, vWF, calcium and coagulation factors), which results in activation and aggregation of further platelets. Platelets also alter their surface by expressing negative charged phospholipids to facilitate calcium-mediated coagulation factor binding. The further activation of coagulation factors and the subsequent thrombin generation takes place on the surface of activated platelets (propagation phase). Thrombin itself potentiates its generation by activation of factor XI, VIII, and V, which results in a thrombin burst, sufficient to cleave fibrinogen and activate factor XIII as well as thrombin activatable fibrinolysis inhibitor (TAFI). Soluble fibrin monomers polymerize and are cross-linked by FXIII. Thereby fibrin and platelets form a stable clot that is anchored at the extracellular matrix due to the cross-linking of fibrin with adhesive protein (Gerlach *et al.*, 2009). The steps in the coagulation cascade are simplified in figure 1.2.

Fibrinogen (also called factor I) is a 340KDa glycoprotein synthesized in the liver hepatocytes and also in megakaryocytes. Fibrinogen is the main structural protein in the blood responsible for the formation of clots. It exists as a dimer of three polypeptide chain; the alpha, beta, and gamma are linked through 29 disulphide bonds. Fibrinogen has a trinodal structure, central nodules termed the E domain, while the two distal nodules termed D domain. Fibrinogen is proteolytically cleaved at the amino terminus of the alpha and beta chains releasing fibrinopeptides A and B and converted to fibrin monomer by thrombin. The resultant fibrin monomers non-covalently assemble into protofibrils by DE contacts on neighbouring fibrin molecules. Contacts are also established lengthwise between adjacent D domains (DD contacts) leading to lateral aggregation. Fibrin is then

cross linked by FXIII to form a clot. FXIII stabilizes fibrin further by incorporation of the fibrinolysis inhibitors alpha-2-antiplasmin and TAFI (thrombin activatable fibrinolysis inhibitor) and binding to several adhesive proteins of various cells (Muzbek *et al.*, 2008).

The fibrinogen concentration in blood plasma is 1.5 - 4g/L (normally measured by Clauss method). Higher levels are, amongst others, associated with cardiovascular disease (>4.6g/L) (Lang *et al.*, 2009). It may be elevated in any form of inflammation, as it is an acute phase protein. Congenital deficiency (afibrinogenaemia) or disturbed function of fibrinogen has been described in a few cases. It can lead to either bleeding manifestation, thromboembolic complications or is clinically without pathological findings. Acquired deficiency is found after haemodilution, blood loses and/or consumption such as in trauma patients, during some phases of disseminated intravascular coagulation (DIC) and also in sepsis. In patients with fibrinogen deficiency, the correction of bleeding is possible by infusion of fresh frozen plasma (FFP), cryoprecipitate (a fibrinogen rich plasma fraction) or by fibrinogen concentrates. There is increasing evidence that correction of fibrinogen deficiency or fibrinogen polymerization disorders is very important in patients with bleeding (Fries *et al.*, 2009).

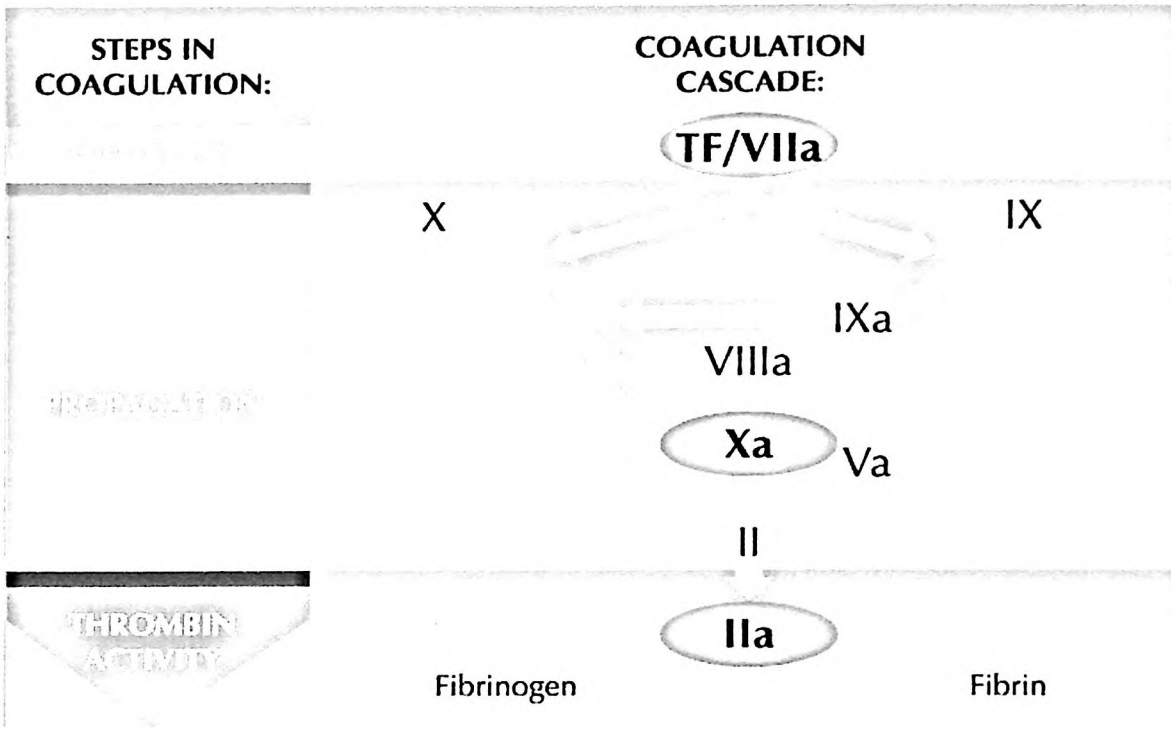

Figure 1.2: Steps in coagulation cascade. The initiation of coagulation is triggered by the tissue factor/factor VII complex, which activates factor IX and X. Activated factor IX (IXa) propagates coagulation by activating FX in a reaction that utilizes activated factorVII (aFVII) as a cofactor. aFXa with aFVa as a cofactor converts prothrombin (FII) to thrombin(IIa). Thrombin then converts fibrinogen to fibrin. (Adapted from (Weitz *et al,* 2004)

1.3.2 Fibrinolytic system

Fibrin formation occurs as a consequence of coagulation, inflammation and tissue repair. When no longer needed, fibrin must be removed so that normal vessel structure and function can be restored. The fibrinolytic system is responsible for dissolving clots and

maintaining a patent vascular system. With close regulation and control, the fibrinolytic system acts locally at sites of fibrin accumulation without causing systemic effects. Fibrin formation essentially initiates fibrinolysis. When clotting begins, plasminogen binds to fibrin throughout the clot. Tissue plasminogen (t-PA) also binds to fibrin, which increases its enzymatic activity so it can efficiently convert plasminogen to plasmin. Plasmin induced degradation of fibrin is a stepwise process that results in the formation of soluble degradation products. Fibrin fibrils are degraded to oligomers that consist of D- and E-fragments of fibrin in various combinations. The fragments decrease in size as the fibrinolyic continues. The oligomers are further degraded to DDE- fragments and eventually to a D-dimer (Doolittle, 1981).

The system is inhibited by the action of plasminogen activator inhibitors (PAIs) and by the plasmin inhibitor, α2-antiplasmin. PAI-1 is the primary inhibitor of t-PA and urokinase-type plasminogen activator (u-PA), while α2-antiplasmin is the physiological inhibitor of plasmin (figure 1.3). Together, these two inhibitors constitute a powerful, negative regulatory system for controlling the formation and activity of plasmin. The plasminogen activators, tissue type plasminogen activator (t-PA) and urokinase type plasminogen activator (u-PA), preferentially activate plasminogen on the surface of fibrin. When associated with fibrin surface, plasmin is protected from rapid inhibition by α2-antiplasmin so that efficient degradation of a fibrin clot may occur. If free plasmin leaks into the circulation, these plasmin molecules are rapidly inactivated by α2-antiplasmin. Thus the activity of this potentially dangerous proteolytic enzyme is localized to the fibrin clot.

Several inhibitors control and modulate the fibrinolytic system to prevent fibrinolysis. Most belong to a family of serine-protese inhibitors called serpins. The primary regulators are plasminogen activator inhibitors (PAIs) with PAI-1 is the most important. PAI-1 appears to be the primary physiological inhibitor of t-PA. PAI-1 is produced by endothelial cells, hepatocytes, smooth muscle cells and adipocytes. In plasma, PAI-1 acts as an acute phase reactant and behaves in similar fashion as C-reactive protein. Elevated PAI-1 levels result in a decrease in t-PA activation of plasminogen and a shift in the haemostatic balance towards hypercoagubality. Deficiency of PAI-1 results in a serious bleeding disorder due to unregulated and excessive fibrinolysis.

PAI-1 is found in the circulation, largely in association with platelets. It is also secreted by endothelial cells. Inflammatory cytokines, tumour necrosis factor and lipoproteins are important factors in regulating vascular PAI-1 expression. Both t-PA and PAI-1 show circadian rhythm, with the highest PAI-1 activity and the lowest t-PA activity in the morning. The concept that impaired fibrinolysis is a risk factor for future vascular disease, is strongly supported by numerous studies. PAI-1 activity is regulated by age; and older populations tend to have lower PAI-1 levels (Yarnell *et al.,* 2000). Elevated PAI-1 activity in stroke patients has also been reported, and this might reflect an endogenous predisposition to thrombogenesis. Majority of the circulating PAI-1 is contributed to by adipose tissue. Thus obese subjects have higher levels of PAI-1. Basal PAI-1 concentration in plasma is low at 0.5 nmol/L, of which at least 80% is in complex with tPA or uPA. As a consequence of considerable diurnal variation of PAI activity, the blood collection time should be standardized.

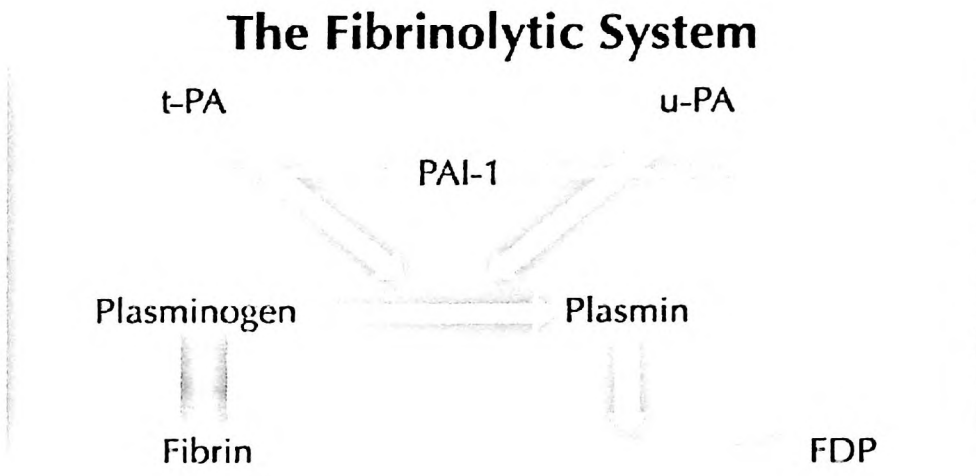

Figure 1.3: Plasmin induced degradation of fibrin. Fibrin localizes plasminogen to the surface of the thrombus, where plasminogen is activated to plasmin by t-PA and u-PA. These enzymes are regulated by PAI-1. Plasmin is responsible for the degradation of fibrin degradation products. The thin line indicates inhibition. (Adapted from Lee *et al.*, 2003)

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1.3.3 Natural inhibitors of coagulation system

The coagulation pathway is also regulated via three inhibitory systems. Tissue factor pathway inhibitor (TFPI) targets the initiation of coagulation, antithrombin blocks thrombin generation and thrombin activity, and protein C inhibits the propagation of coagulation, as shown in figure1.4. Tissue factor pathway inhibitor (TFPI) blocks FVIIa in a two step fashion. TFPI fist binds and inactivates FXa, and the TFPI/Xa complex then inactivates FVIIa bound to tissue factor. Circulating levels of TFPI are low, suggesting that the system is design to block uncontrolled activation of coagulation by the FVIIa/tissue factor (TF) complex, while allowing propagation of coagulation by intrinsic tenase. Propagation of coagulation is ensured because thrombin activates FXI on the platelet surface, where it is poised to activate platelet-bound FXI.

The most important direct inhibition mechanism of coagulation is neutralization of thrombin and factor Xa by antithrombin (AT). Antihrombin is synthesized in the liver. It was described as a protein required for antithrombotic activity of plasma (Seegers *et al.,* 1954). Antithrombin inhibits multiple components of the extrinsic, intrinsic and common pathways, including factors IIa, XIIa, XIa, Xa and kalikrein, but not FVIIa and FVa. Arginine-rich centers in AT react with the serine center of serine proteases. Complex formation between AT and its target enzymes is enhanced by heparin, which bind to lysyl residues on AT, making the critical arginine residue more available for interaction with thrombin.

The resting plasma concentration of AT is approximately 110-140mg/L, with serum half life of 36-48 hours (Mammen *et al.,* 1998). Acute inflammation, as a response to severe infection or trauma, results in systemic activation of coagulation system (Levi *et al.,* 2003, Esmon *et al.,* 1997). Cytokines have been shown to play an important mediatory role through the activation of the tissue factor VIIa (extrinsic pathway) (Osterud *et al.,* 2001), with subsequent consumption of anticoagulation factors including AT. Several studies have reported low AT levels, not only in patient with sepsis but in other groups of patients admitted to the intensive care unit (Wilson *et al.,* 1996, Rannuci *et al.,* 2005).

Patterns of AT levels in various groups of surgical ICU patients have been reported (Boldt *et al.,* 2000) but the possible association of AT levels with outcome was not characterized in that study due to the small number of patients studied. A potential role for AT levels as a predictor of outcome in patients with septic shock has been suggested (Mammen, 1998), and low AT levels have been associated with an increased risk of infections and death in patient after trauma (Wilson *et al.,* 1996). The availability of AT concentration and its used to treat Antithrombin deficiency has a role in treating patients with disseminated intravascular coagulopathy. In this study, we investigated the AT levels in neurosurgical patients and its association with post surgical hematoma.

Protein C (PC) is a vitamin-K dependent protein that is synthesized primarily in the liver and kidneys. Design to regulate thrombin generation, the PC pathway is initiated when thrombin binds to thrombomodulin(TM), its receptor on the endothelial cell surface. This forms the thrombin/TM complex which then activates PC by cleaving a single arginine

residue. The binding of thrombin to TM results in a ~ 1000-fold increase in the activation of protein C to activated protein C (APC). APC functions as an anticoagulant by proteolytically degrading and inactivating FVa and FVIIIa, thereby attenuating thrombin generation (Esmon, 1992). Degradation of FVa by APC is markedly enhanced by protein S (PS). PS is expressed in many tissues, including liver, endothelium and brain. The principle anticoagulant activity of PS is the inhibition of thrombin generation by acting as a cofactor to APC in the degradation of FVa and FVIIIA and independently inhibiting the prothrombinase-and FX activating complex. To date, several factors correlated with levels of natural anticoagulants have been identified: age, gender, race, Diabetes mellitus, dyslipidaemia and smoking (Rodeghiero *et al.,* 1996). Although deficiencies in natural anticoagulants are responsible for up to 20% of nontraumatic venous thrombosis, arterial thrombosis has not been prominently associated with the deficiencies in these factors (Tatsulimak *et al.,* 1995)

It has been suggested that the processes of the haemostatic system are controlled in a dynamic balance with the capacity to initiate repair of vascular lesions without interfering with the patency of vascular tree. Events which activate the haemostatic system may therefore affect this balance. Haemostatic imbalance may occur when one or more systems are overwhelmed or exhausted.

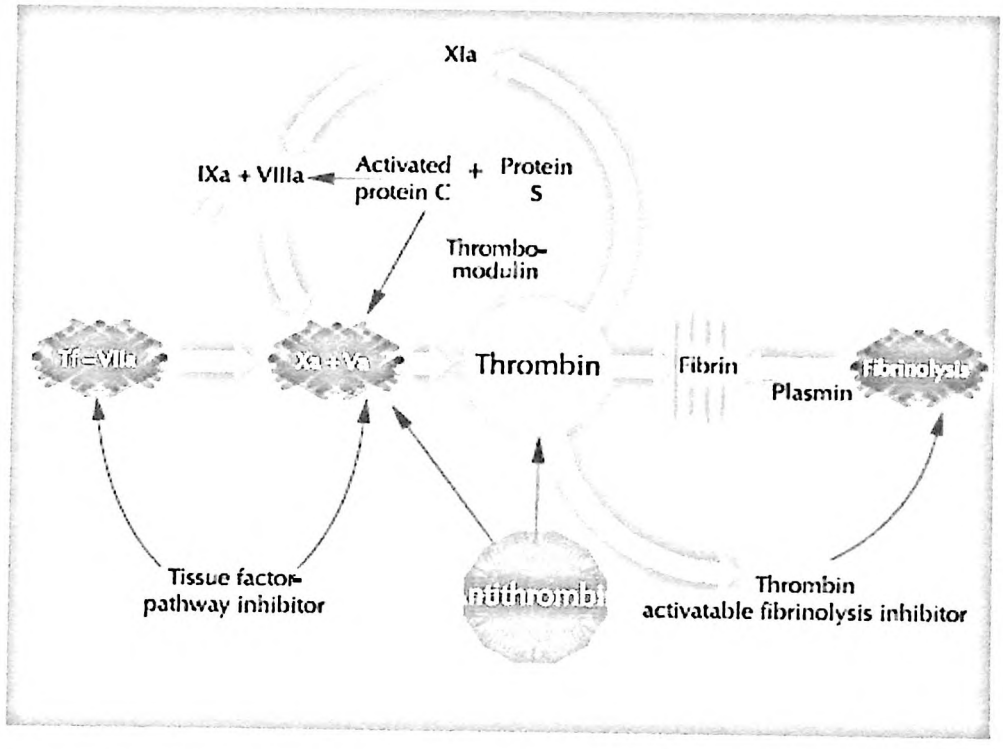

Figure 1.4: schematic diagram of regulation of coagulation. Formation of a clot is highly regulated by natural anticoagulant mechanisms that confine the haemostatic process to the site of injury in the vessel. Thin lines indicate inactivation. (Adapted from Neishem *et al.*, 2003)

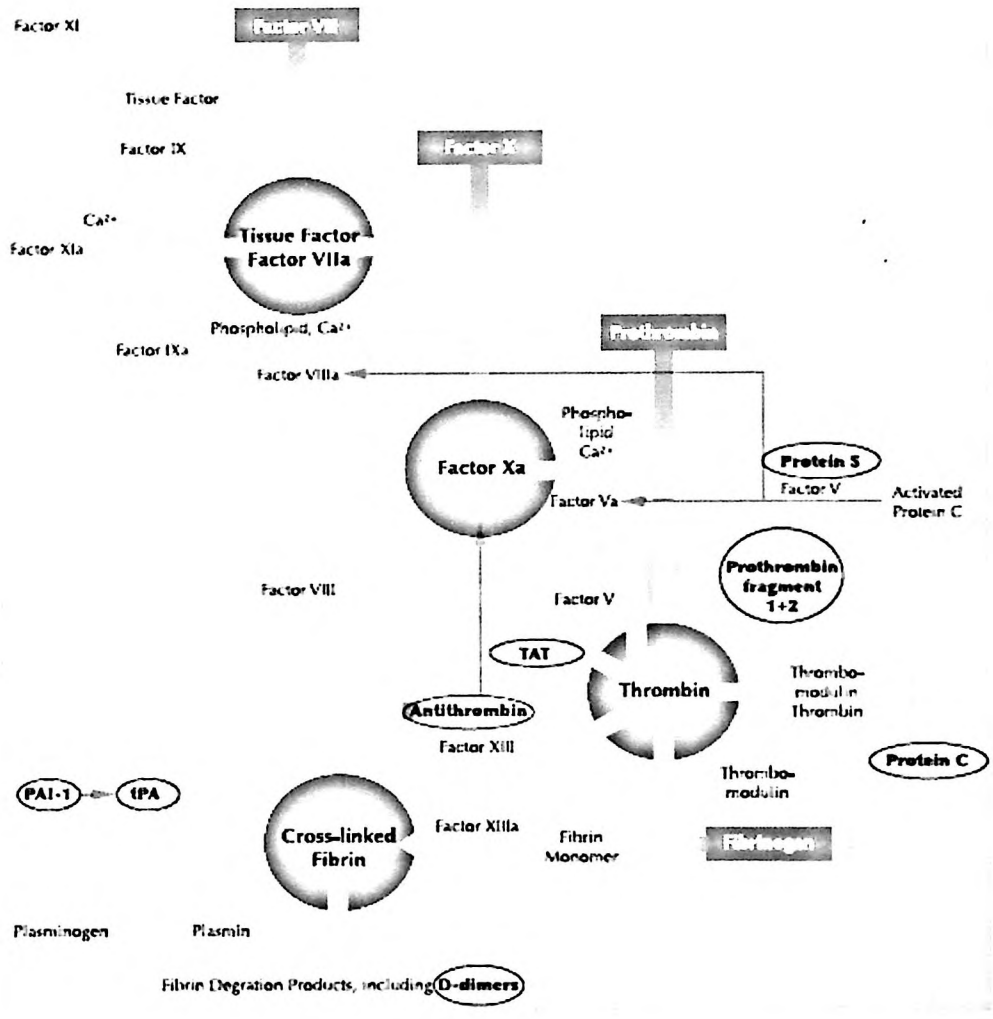

Figure 1.5: Factors of the coagulation and fibrinolysis systems and their interactions. Thin lines indicate inhibition/inactivation. Markers assessing coagulation and fibrinolysis are marked as ovals. (Adapted from Godsland., 2000).

1.4 ROUTINE SCREENING TESTS OF COAGULATION SYSTEM IN NEUROSURGICAL PATIENTS.

The coagulation system is previously divided into the contact activation pathway (formerly known as the intrinsic pathway) and the tissue factor pathway (formerly known as extrinsic pathway) . Although the division of the coagulation system into these two pathways failed to explain in vivo coagulation process , it is reasonable on the basis of ex-vivo testing methods. So normal laboratory data of standard coagulation tests neither exclude a perioperative bleeding complication nor guaranty sufficient haemostasis. However, up to now, they are parts of the routine preoperative work up in neurosurgical patients. Figure 1.6 shows the classical plasmatic coagulation model, which is helpful for routine screening of coagulation perturbations. The routine coagulation testing usually includes prothrombin time (PT), partial thromboplastin time (PTT), fibrinogen and platelet count.

A routine coagulation test to assess the extrinsic pathway is the prothrombin time (PT). It is performed by measuring the time it takes to form a clot after adding calcium and a tissue extract to plasma. A normal PT usually indicates normal levels of coagulation factor FVII, V, X and II and fibrinogen. A prolonged PT (or an elevated INR) may indicate coagulation factor deficiency (FII, FV, FVII, FX and fibrinogen), impaired hepathic synthesis, Vitamin K deficiency, anticoagulant therapy or dilutional respectively consumption coagulopathy.

The partial thromboplastin time (PTT) screens for intrinsic pathway and assess all coagulation factors except for factor X. A prolonged PTT can be due to coagulation factor deficiency (FII, FV, FVIII [hemophilia A, vonWillebrand syndrome], FIX [Haemophilia B], FX, FXI, FXII, fibrinogen, prekallikrein, high molecular weight kininogen [HK]), treatment with heparin or direct thrombin inhibitor (hirudin, argatroban), impaired hepatic synthesis, Vitamin K deficiency, dilutional respectively consumption coagulopathy or antiphospholipid syndrome.

Unfortunately standard coagulation tests leave a diagnostic gap (PTT and PT can be normal), especially for vonWillerbrand syndrome as the most common haemostatic disorders, acquired or hereditary coagulation FXIII deficiency and platelet dysfunction. Particularly, platelet dysfunction mediated by antiplatelet drugs (like aspirin or clopidogrel) cannot be detected by platelet count, but rather by a detailed clinical history. In cases of a positive history, further test with respect to disorders of primary haemostasis should be done. Therefore platelet function analysis should be performed in cases of emergency neurosurgery to verify or exclude antiplatelet drug effects. This is particularly important in cases of intracranial bleeding and unknown patient's history and medication.

There are no generally accepted absolute limits for routine coagulation tests defined by scientific evidence, which definitely permit or decline a neurosurgical procedure, neither from a medical nor from a legal aspect. However, elective procedures should be scheduled only with normal test results. If abnormalities are detected, an intensive laboratory work up is recommended and patients should be referred for consultation by

haemostaseologist even if this results in the postponing of the operation (Gerlach *et al,* 2009)

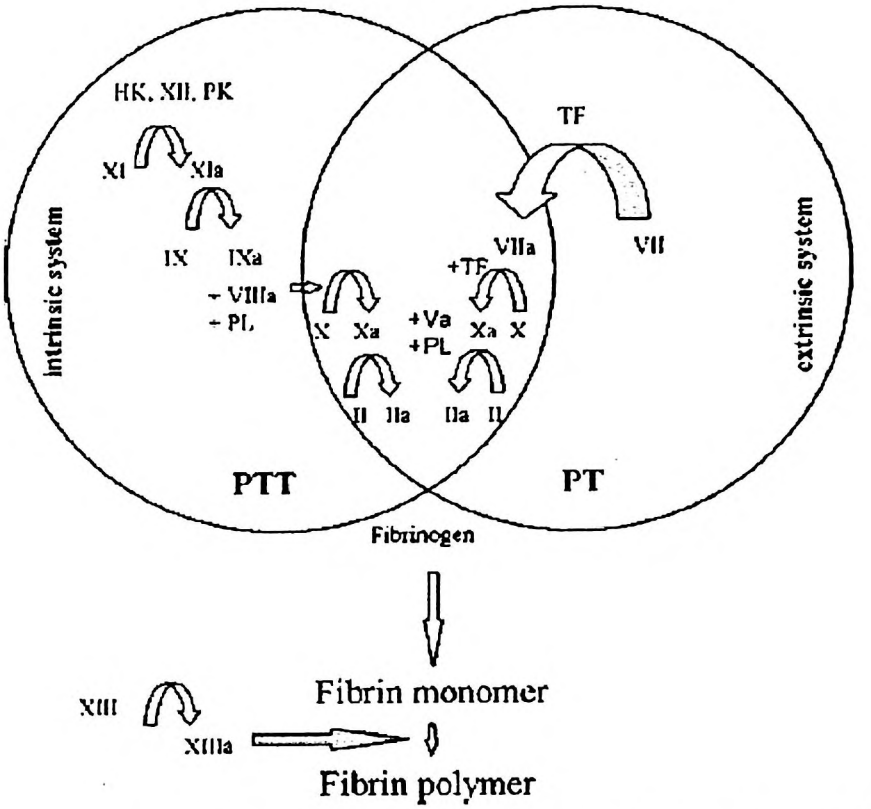

Figure 1.6: The classic model of coagulation activation as a simplified flowchart showing the order of factor activation (indicated by the arrows). (Adapted from Gerlach *et al.,* 2009)

1.5 BRAIN INSULTS AND ITS INTERFERENCE WITH COAGULATION SYSTEM

The brain is one of the richest sources of tissue factor (TF) in the human organs. It was described that a brain tissue injection caused disseminated intravascular coagulation more than 150 years ago. The consequence of this is that the source of TF in laboratory

determination of prothrombin time is an extract of rabbit brain tissue. Investigations of coagulation and fibrinolysis activity of the brain tissue in the sixties confirmed high tissue factor activity in brain (Astrup, 1965), and high fibrinolytic activity in highly vascularized brain tissue, such as choroid plexus and meninges (Takashima *et al.,* 1969). The primary source of TF are astrocytes (Flossel *et al.,* 1995) where as t-PA has been found in cerebral capillaries (Zlokovic *et al.,* 1995), which confirms fibrinolytic activities in brain tissue.

The presence of high concentration of blood coagulation and fibrinolytic activators in brain tissue lead to pathological intravascular activation of coagulation and fibrinolysis, particularly after brain tissue destruction induced by various aetiological insults. The blood clotting abnormalities have been widely described after head trauma. Patients with severe head injury have significantly higher TF concentration compared to moderate head injury (Pathak *et al.,* 2005). Brain contusion induces the destruction of brain tissue, while secondary haematomas compress the brain tissue and can cause cerebral ischaemia with secondary brain damage. These abnormalities have induced the activation of the coagulation factors and fibrinolysis. Brain tumour surgery can complicate with blood coagulation and fibrinolytic disorders. The pathogenesis of these disorders is complex, and includes various factors (Antovic *et al.,* 1998). (Goh *et al.,* 1999) described about 50% of tPA expression in meningiomas compared to glioblastomas, while (Sawaya *et al.,* 1993) reported that benign tumours contain three times more tPA compared to malignant tumours. However in the later study, no correlation of tissue tPA to plasma tPA was found.

In patients with brain tumours, an increased concentration of PAI-1 has been observed. This increased is more significant in patients with brain metastasis and in high grade gliomas (Rao *et al.,* 1993).On the other hand, the neurosurgical intervention itself causes the destruction of brain tissue and activation of coagulation system. The total fibrinolytic activity was decreased by about 15% in patients with malignant tumour, with a decrease in tPA and increased in protein C (Sawaya *et al.,* 1992). Tissue extracts of different brain tumours lead to a variable inhibition of plasmin. Patients with glioblastomas and intracerebral metastasis had significantly higher plasma tissue factor pathway inhibitor (TFPI) concentrations, which might reflect a compensatory mechanism to a general procoagulatoric activation in patients having this tumours. Taken together, these findings indicate that brain tumours and injured brain themselves have direct influence on coagulation and fibrinolysis. This could be an explanation for the common occurrence of intratumoural thrombosed vessels in glioblastomas and the high incidence of systemic thromboembolic complications such as pulmonary emboli due to an associated procoagulatoric state in neurosurgical patients (Gerlach *et al.,* 2009).

1.5.1 Bleeding complication

Hemorrhage inside the cranium can be disastrous to the neurosurgical patients. Monro-kellie doctrine stated that any increase in intracranial volume would lead to an increase in intracranial pressure. Untreated raised in intracranial pressure would finally lead to brain ischaemia and herniation. Bleeding after intracranial surgery therefore can lead to poor patient outcome. Besides herniation which could lead to death of the patient, haematoma