THROMBOELASTOGRAPHY IN THE ASSESSMENT OF COAGULATION CHANGES IN PERIPHERAL VASCULAR DISEASE

V. K. Shankar

A thesis submitted in partial fulfilment of the requirements of Oxford Brookes University for the degree of Master of Philosophy

In collaboration with Nuffield Department of Surgery, John Radcliffe Hospital, Oxford

January 2010

ABSTRACT

Studies up to date in the western literature involving changes in coagulation in patients with peripheral vascular disease(PVD) have consistently reported activation of coagulation based on measurement of raised levels of markers of activation of coagulation(clot formation), decreased fibrinolysis (breakdown of fibrin), platelet activation leading to increased aggregation and defective endothelial (arterial wall) function in patients with PVD that leads to thrombosis in arterial circulation thus designating this group of patients as 'hypercoagulable.' Interestingly these patients are not deemed as such in clinical practice as the routine coagulation tests(RCTs) currently employed do not identify these abnormalities. The overall purpose of the research described in this thesis was to identify changes in coagulation in patients with peripheral vascular disease using Thromboelastography (TEG), a technique which exploits the visco-elastic properties of a blood clot as it forms, retracts or lyses (breaks down) using a small volume of whole blood.

Validation of TEG technique employing citrated whole blood (CWB) and heparinase modified (hepTEG) methods and standardisation of analysis to overcome variability in TEG analysis, blood sample instability due to storage times was carried out. TEG analysis after sample storage between 1-2 hours showed no significant inter or intra sample assay variability confirming excellent reproducibility((p= NS; reliability coefficients (Alpha) greater than 0.9 for R time, K time, Angle, Maximal Amplitude (MA) and Coagulation Index(CI)). hepTEG method confirmed complete reversal of heparin effect in blood samples obtained from participants following heparin administration.(R

time: 9.16+/- 1.68 Vs 8.90 +/- 1.48 p=NS; K time: 2.98+/- 0.87 Vs 3.20 +/- 1.05 p=NS; Angle: 56.86+/- 6.39 Vs 56.90 +/- 5.30 p=NS; MA 56.34+/- 6.39 Vs 56.79+/-6.23 p=NS; CI: 0.59+/-1.19 Vs 0.88+/-1.00 p=NS). 50 age matched controls to obtain reference values for comparison and documentation of any significant changes in TEG parameters due to age, gender anaesthesia and surgery was completed. Preparation /induction of anaesthesia led to a significant trend towards activation of coagulation in all age groups and gender (Angle 56.23 +/-0.89, CI 54.42, 58.04 Vs 58.21+/- 0.83, CI 56.54, 59.88;p=0.003 and MA 56.40 +/- 0.79 CI 54.80, 58.01 Vs 59.36 +/- 0.89, CI 57.56, 61.15; p=0.003 and CI 0.71 +/- 0.14, CI 0.42, 1.00 Vs 1.35 +/- 0.17, CI 1.00, 1.69;p=0.003)

Surgical stimulus led to a further activation of coagulation that followed induction of anaesthesia (Angle: 58.21+/- 0.83, CI 56.54, 59.88 Vs 60.98 +/- 0.69, CI 59.59, 62.37; p=0.001). TEG parameter values obtained from healthy controls aged over 45 are used as reference values for this study.

TEG showed no significant differences in TEG parameters when samples obtained from an upper limb artery and a vein were analysed. However when samples from the main lower limb artery (common femoral artery/CFA) and the major vein that drains the same limb (common femoral vein/CFV) in patients with symptomatic PVD, TEG identified significant activation of coagulation in samples obtained from the vein that drains an ischaemic limb (decrease in R time ($p<0.05$), an increase in MA ($p<0.05$) and an increase in CI ($p<0.002$)). This interesting finding led to a hypothesis that ischaemic tissue has a prominent role in the activation of coagulation observed in

patients with PVD. To test this hypothesis, common femoral venous and arterial samples from 30 patients with symptomatic PVD were analysed using TEG. TEG identified significant activation of coagulation in samples obtained from an artery downstream (CFA) when compared to those obtained from an artery that is proximal (Aorta). This change towards hypercoagulation was also found to be positively related to the degree of narrowing or stenotic disease (quantified using angiography) between these two sampling points(ΔR, $r=0.442$, $p<0.05$ /Δ MA, $r=0.379$, $p<0.05$ / ΔCI $r=0.429$, $p<0.05$). A significant positive relationship in between degree of ischaemia (ABPI) and difference in TEG parameter values in between arterial and venous blood samples obtained from an ischaemic limb (ABPI) on that side (ΔCI v ABPI $r = -0.427$ $p<0.05$, ΔMA v ABPI $r =-0.370$ $p<0.05$) was also found in this study. These findings suggest activation of coagulation as the blood flows down an atherosclerotic vessel and in combination with the observed changes due to the presence of peripheral ischemia tissue suggested that the hypercoagulability observed in PVD may have its origins in the ishaemic limb itself.

To clarify the role of non-ionic contrast media (NICM) in the context of conflicting findings regarding its thrombogenic potential especially in patients with PVD undergoing angiography, aortic blood samples(n=30) were obtained before and after injection of NICM. Heparinase modified TEG analysis showed that there was no activation of coagulation immediately after NICM exposure and in fact there was a significant trend towards hypocoagulation in contrast to the published reports of increased

thrombogenicity after NICM exposure (increase in R time (time to fibrin formation) (CI 7.8,10.18 minutes) (p=0.036), in K time (dynamics of clot formation) (CI 2.2,2.8 minutes) (p=0.028), and a reduction in Angle (decreased acceleration of fibrin build up) (CI 53.10,62.7 degrees) (p=0.013),MA (reduced ultimate clot strength) (CI 54.5,62.7 mm) (p=0.013) and (CI) (decreased overall coagulation status) (0.31,1.95) (p=0.032)). This study also showed that despite this significant reduction in the activation of coagulation after NICM exposure, PVD patients were consistently procoagulant when compared to age- matched controls(n=30) who were not exposed to NICM (R time: p=0.029/K time: p=0.001/Angle: p=0.003/MA: p=0.020 and CI: p=0.014)

Patients with ischaemic heart disease, a consequence of significant coronary artery atherosclerosis have reduced amounts of naturally occurring anticoagulant substances like heparan, heparan-sulphate proteoglycan and endogenous heparin. Since patients with PVD exhibit similar pathology in addition to the presence of peripheral ischaemic tissue, to identify any similar defect in PVD, blood samples from patients with symptomatic PVD(n=28) and age matched control subjects were analysed using hepTEG. Heparinase modified TEG analysis identified for the first time, heparinase sensitive heparin-like activity in peripheral venous samples in patients with PVD (R time 7.50 $\pm$ 0.44 min / CI 6.54, 8.46 Vs 7.17 $\pm$ 0.40 min / CI 6.30, 8.05 / p=0.041). Endogenous heparin-like activity is found to be reduced in PVD and this reduction also correlated with the degree of peripheral ischaemia

(ABPI) (correlation coefficient: abpi: 1.000 / change in R time 0.350 / p=0.021)

TEG analysis of samples from 30 patients with aortic aneurysmal disease and 14 aortic occlusive disease undergoing revascularisation and 30 controls were carried out using hepTEG method. Baseline TEG parameter values in the aneurysm group showed significant activation of coagulation when compared to the controls (R time (p=0.001); K time (p=0.008) and (CI) (p=0.047)). Following release of the aortic cross-clamp a significant trend towards activation of coagulation was noted in the aneurysm group and a similar but pronounced activation of coagulation was noted in the occlusive group, confirming activation of coagulation following reperfusion of ischaemic tissues (R time (p=0.042); K time (p=0.043) MA (p= 0.034) and CI (p=0.026)). Further TEG analysis during postoperative period showed that both these patient groups exhibit sustained periods of hypercoagulability. In addition to these findings hepTEG method further revealed underlying hypercoagulability, despite exogenous heparin administration, confirming presence and activation of non- AT-III dependent pathways of activation of coagulation in these patients.

TEG analysis of blood samples from 86 patients admitted with symptoms peripheral ischaemia requiring revascularisation surgery or amputation were carried out and TEG parameter values obtained from control group was used for comparison. Serial blood sampling (n=13) at various stages of surgery(baseline, before and after anaesthesia, before commencement of surgery before clamp of lower limb artery, before release of clamp, after release of

clamp, 3, 6, 12, 24, 48, 72 hrs, 7 days and 6 weeks after surgery) for CWB TEG and hepTEG methods were carried out. Baseline TEG showed significant activation of coagulation in PVD when compared to age matched controls (R time (p=0.003) Angle (p=0.016) MA (p=0.002) and CI (p=0.002). Following revascularisation there was a significant decrease in the time to fibrin formation and a significant increase in the maximum strength of clot suggesting increased platelet-fibrinogen and the overall coagulation status of the blood.(R time (p=0.026), MA (p=0.004) and CI (p=0.002)). These findings confirmed that ischaemic limb promoted activation of coagulation especially after revascularisation. This study demonstrated significant activation of coagulation before, during and after revascularisation procedures for critical limb ischaemia. TEG analysis also confirmed a prothrombotic state after revascularisation (MA (p=0.013) and CI (p=0.032)) that is shown to be associated with development of any post-operative thrombotic ischaemic event.

ACKNOWLEDGEMENTS

I would like to thank the following for their supervision and support during this project:

Linda Hands (Nuffield Department of Surgery, John Radcliffe Hospital)
Ashok Handa (Nuffield Department of Surgery, John Radcliffe Hospital)
Helen Dawes (School of Life Sciences, Oxford Brookes University)
Prof. David Beadle (School of Life Sciences, Oxford Brookes University)

I also thank the administration team at the Nuffield Department of Surgery, John Radcliffe Hospital for their practical support.

GLOSSARY OF ABBREVIATIONS (listed alphabetically)

AAA	Abdominal aortic aneurysm
ABPI	Ankle/Brachial Pressure Index
Alpha	Angle
APC	Activated Protein C
aPTT	Activated Prothrombin Time
AT	Antithrombin
BMI	Body Mass Index
CI	Coagulation Index
CPB	Cardio- Pulmonary Bypass
CRP	C - reactive protein
CVA	Cerebro Vascular Accident
D-D	D dimers
DVT	Deep Vein Thrombosis
EA	Epidural Anaesthesia
EVAR	Endovascular Aneurysm Repair
FDP	Fibrin degradation products
FIB	Fibrinogen
FPA	Fibrinopeptide A
GA	General Anesthesia
GAG	Glcosaminoglycan
Hct	Haematocrit
HRT	Hormone Replacement Therapy

HSPG	Heparan Sulphate Proteoglycan
IC	Intermittent Claudication
ICM	Ionic Contrast Media
K	Coagulation time
LA	Local Anaesthesia
LDL	Low Density Lipoprotein
MA	Maximal Amplitude
MI	Myocardial Infarction
NICM	Non-ionic Contrast Media
OCP	Oral contraceptive pill
PAI-1	Plasminogen activator Inhibitor-1
PAOD	Peripheral Arterial Occlusive Disease
PT	Prothrombin Time
PVD	Peripheral vascular Disease
R	Reaction time
RP	Rest Pain
SFMC	Soluble fibrin monomer complexes
SMC	Smooth Muscle Cells
TAT	Thrombin-Antithrombin Complex
TEG	Thromboelastography
TF	Tissue factor
TFPI	Tissue Factor Pathway Inhibition
Tx A2	Thromboxane A2

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

CHAPTER 1 - INTRODUCTION

1.1 - COAGULATION AND FIBRINOLYSIS

1.1.1 Coagulation

Haemostasis is one of the basic mechanisms of homeostasis. It ensures minimisation of blood loss from the circulation in the event of trauma whilst maintaining patency of the vascular tree. In combination with platelets, the coagulation pathway acts to rapidly seal any breach in the circulation. The fibrinolytic pathway acts to lyse clot and therefore functions as a check on coagulation, limiting the extension of clot formation and maintaining vessel patency. A number of endogenous 'natural anti-coagulants' also exist which further moderate the action of the clotting cascade[1]. As well as trauma, other factors are known to stimulate these mechanisms. Inflammation and ischaemia have both been demonstrated to be pro-thrombotic. The end point of the coagulation pathway (Figure 1.1) is the conversion of fibrinogen to fibrin and the formation of a fibrin clot. This is mediated by the serial conversion of pro-enzymes or factors(Table 1.1) into their active forms which, together with various co-factors, in turn catalyse the activation of the next pro-enzyme in the chain[2]. This cascade, along with several positive feedback loops, result in a magnification of the response to the original stimulus. Two pathways in coagulation, intrinsic and extrinsic have been established in vitro[3].

Figure 1.1 - The Coagulation Pathway

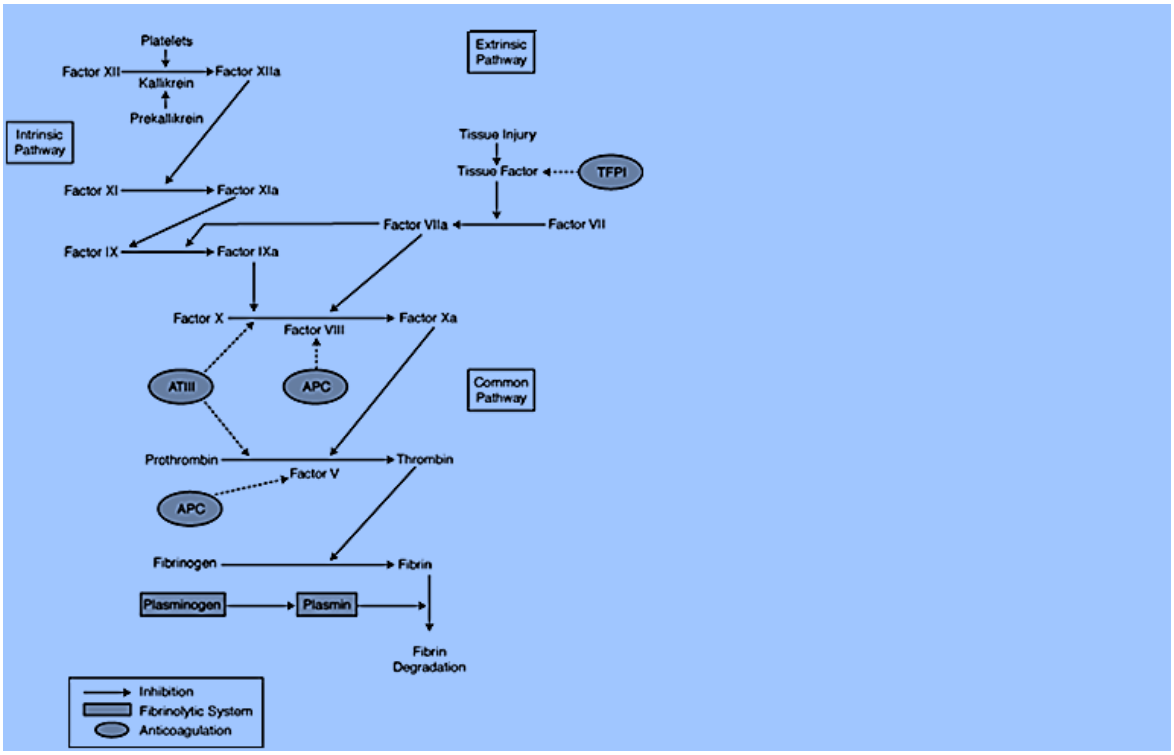


Table 1.1 Coagulation Factors

Factor Number or Name	Synonym	Purpose
I	Fibrinogen	A precursor of fibrin
II	Prothrombin	A precursor of thrombin, which converts fibrinogen to fibrin; activates factors V, VIII, XI, and XIII; and binds to thrombomodulin to activate protein C Is vitamin K–dependent
V	Proaccelerin	Is activated to factor Va, which is a cofactor for the enzyme factor Xa in the factor Xa/Va/phospholipid complex that cleaves prothrombin to thrombin.
VII	Proconvertin	Binds to tissue factor and is then activated to form the enzymatic component of the factor VIIa/tissue factor complex that activates factors IX and X. Is vitamin K–dependent
VIII	Antihemophilic globulin	Is activated to factor VIIIa, which is a cofactor for the enzyme factor IXa in the factor IXa/VIIIa/phospholipid complex that activates factor X Factor VIIIa is inactivated by activated protein C in complex with protein S (as is factor Va) Is a large cofactor protein (as is factor V) Circulates in plasma bound to von Willebrand's factor multimers.
IX	Christmas factor	Is activated to factor IXa, which is the enzyme of the factor. IXa/VIIIa/phospholipid complex that activates factor X. Is vitamin K–dependent
X	Stuart-Prower factor	Is activated to factor Xa, which is the enzyme of the factor Xa/Va/phospholipid complex that cleaves prothrombin to thrombin Is vitamin K–dependent.
XI	Plasma thromboplastin antecedent	Is activated to factor XIa, which activates factor IX in a reaction requiring Ca^{2+} ions
Prekallikrein	Fletcher factor	Participates in a reciprocal reaction in which it is activated to kallikrein by

		factor XIIa As kallikrein, catalyzes further activation of factor XII to factor XIIa Circulates as a biomolecular complex with high mol wt kininogen
High mol wt kininogen	Fitzgerald factor	Circulates as a bimolecular complex with prekallikrein
XII	Hageman factor	When activated to factor XIIa by surface contact, kallikrein, or other factors, activates prekallikrein and factor XI, triggering the intrinsic coagulation pathway in vitro
XIII	Fibrin stabilizing factor	When activated by thrombin, catalyzes formation of peptide bonds between adjacent fibrin monomers, thus strengthening and stabilizing the fibrin clot
Protein C	—	Is activated by thrombin bound to thrombomodulin; then proteolyses and destroys (in the presence of protein S and phospholipid) the cofactor activity of factors VIIIa and Va Is vitamin K–dependent
Protein S	—	Circulates in plasma as free protein S and as protein S bound to C4b-binding protein of the complement system Functions in its free form as a cofactor for activated protein C Is vitamin K–dependent
Cell surface factors		
Tissue factor	Tissue thromboplastin	Is a lipoprotein that is constitutively present on the membrane of certain tissue cells, including perivascular fibroblasts, boundary epithelial cells (eg, epithelial cells of the skin, amnion, and GI and GU tracts), and glial cells of the nervous system May also develop in pathologic states on activated monocytes and macrophages and on activated vascular endothelium Is present on some tumour cells Binds factor VIIa, which initiates the extrinsic coagulation pathway
Procoagulant	—	Acidic phospholipid (primarily

phospholipid		phosphatidyl serine) present on the surface of activated platelets and other tissue cells Is a component of the factor IXa/VIIIa/phospholipid activator of factor X and of the factor Xa/Va/phospholipid activator of prothrombin Functions as the lipid moiety of tissue factor
Thrombomodulin	—	Is an endothelial cell surface binding site for thrombin, which, when bound to thrombomodulin, activates protein C.

1.1.2 The Intrinsic Pathway

The Intrinsic Pathway is initiated by plasma coming into contact with negatively charged surfaces in association with kallikrein. This causes the activation of clotting Factor XII to its' active form, XIIa. XIIa, together with high molecular weight kininogen (HMWK) and thrombin, catalyses the conversion of Factor XI to XIa. XIa catalyses its' own production, thereby providing positive feedback for this activation. Factor XIa, with calcium ions as co-factor, catalyses the conversion of Factor IX to IXa. Thrombin catalyses the conversion of Factor VIII to VIIIa and this then combines with IXa on membrane surfaces to form the tenase complex which further acts via the common pathway. Calcium ions are again a co-factor for this reaction. Although this pathway is well recognised in vitro, it has in fact been demonstrated that XII, pre-kallikrein and HMWK deficiencies have no bleeding phenotypes, suggesting they are not required for haemostasis, though they may be involved in fibrin formation during inflammation and

wound healing. The von Willebrand factor acts as a carrier for Factor VIII, prolonging it's' half-life and protecting it from inactivation by activated protein C.

1.1.3 The Extrinsic Pathway

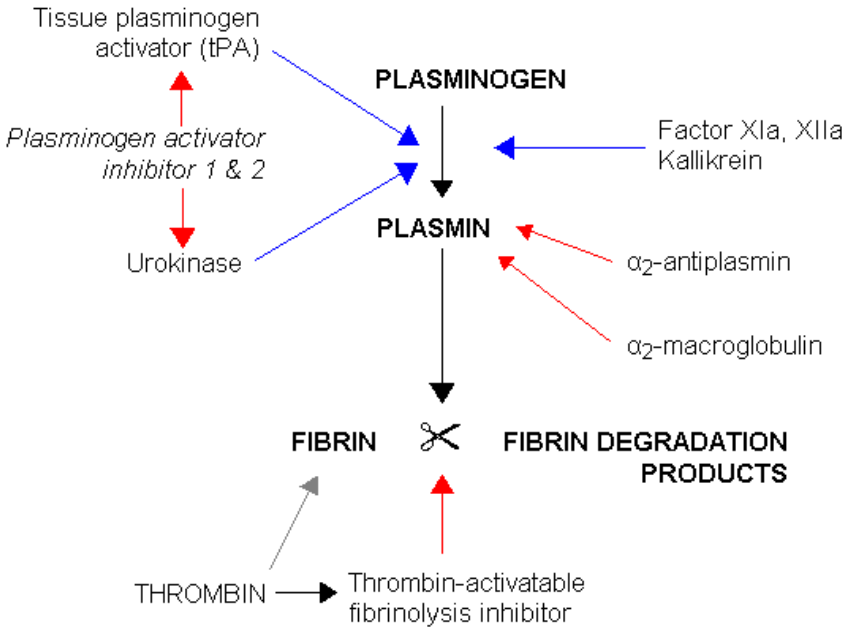
The Extrinsic Pathway is initiated when plasma comes into contact with thromboplastin (also known as tissue factor), a receptor ubiquitous in extra vascular tissues. This catalyses the conversion of Factor VII to VIIa which then combines with thromboplastin in a calcium ion dependant reaction. VIIa, VIIa/thromboplastin and Xa all exert positive feedback on the conversion of VII to its' active form. The VIIa/thromboplastin molecule also stimulates the activation of IX in the Extrinsic Pathway. Activated Factor VIIa is present in low concentrations (10-100 pm) in normal plasma. The formation of the IXa/VIIIa (tenase) and VIIa/thromboplastin complexes allow the reactions of the Common Pathway to proceed as both these compounds, with calcium ions as co-factor, catalyse the conversion of Factor X to Xa. The activation of Factor V is catalysed by the presence of thrombin. Factors Va and Xa then combine in the presence of calcium ions to form the molecule known as prothrombinase. Prothrombinase catalyses the conversion of prothrombin to thrombin, with co-factor calcium, and thrombin then catalyses the conversion of fibrinogen to fibrin plus by-products fibrinopeptides A and B. The activation of Factors X and V, and the combination of their active forms, take place on the surface membranes of platelets or endothelial cells.

The terminal event in the coagulation pathways is the conversion of fibrinogen to fibrin by the action of thrombin. Thrombin acts to cleave fibrinogen and the cleaved portions being referred to as fibrinopeptide A and B respectively) thus forming the fibrin monomer which is then able to polymerise. The fibrin polymers are initially held together by weak non-covalent bonds but the action of factor XIII results in the formation of a stable fibrin polymer bound by covalent bonds.

1.1.4 The Fibrinolytic Pathway

The fibrinolytic system (Figure 1.2) acts to lyse fibrin clot formed by the processes stated above. Plasmin catalyses the breakdown of fibrin into its degradation products. The inactive molecule plasminogen is converted to its active form plasmin by the cleavage of a single peptide bond. This may occur via an intrinsic pathway (by the action of factors XIa, XIIa, kallikrein and HMWK) or by the action of specific activators. Two plasminogen activators are released from endothelial cells into the plasma, tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA). They catalyse the cleavage of plasminogen to plasmin. Plasmin, Xa and kallikrein promote the cleavage of single chain u-PA and t-PA into their much more active two-chain forms (tcu-PA). Single chain u-PA (scu-PA) has relatively little proteolytic activity in solution but this is enhanced by the presence of fibrin. Two chain u-PA is unaffected by the presence of fibrin. Both forms of t-PA show considerably enhanced activity when bound to fibrin. Inhibition of fibrinolysis may occur at the level of the plasminogen activators or by action on plasmin itself.

Figure 1.2 - The Fibrinolytic Pathway

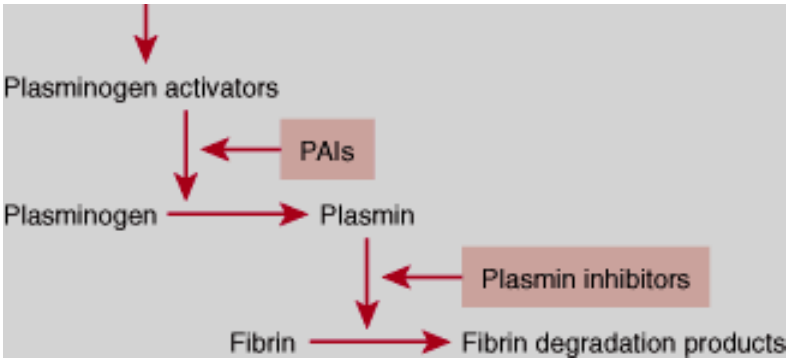


1.1.5 Regulation of Fibrinolysis

Fibrinolysis is regulated by plasminogen activator inhibitors (PAIs) and plasmin inhibitors that slow fibrinolysis (Figure 1.3). PAI-1, the most important PAI, inactivates tPA and urokinase and is released from vascular endothelial cells and activated platelets. The primary plasmin inhibitor is α_2-antiplasmin, which quickly inactivates free plasmin escaping from clots. Some α_2-antiplasmin is also cross-linked to fibrin by the action of factor XIIIa during clotting. This may prevent excessive plasmin activity within clots. tPA and

urokinase are rapidly cleared by the liver, which is another mechanism of preventing excessive fibrinolysis.

Figure 1.3 - Regulation of Fibrinolysis



1.1.6 Natural Anticoagulant Mechanisms

There are 3 major anti-coagulant systems occurring naturally in normal plasma[4]. The glycoprotein antithrombin III acts by combining with the proteases of the coagulation cascade in the ratio 1:1 to form inactive complexes. This process is the major mechanism for the inhibition of thrombin and Factors Xa and XIa, but is slow in the absence of heparin, which accelerates the reaction by a factor of a thousand. AT-III and heparin only inhibit factor VIIa when it is complexed with thromboplastin. The anti-coagulant tissue factor pathway inhibitor (TFPI) acts by combining with Xa. The resultant complex combines with the VIIa/Thromboplastin complex in the presence of calcium ions, thereby inactivating it. The action of the third anti-coagulant, protein C differs from the other two in that it inhibits two of

the non-proteolytic co-factors, Va and VIIIa. Thrombin binds to thrombomodulin, a transmembrane receptor present on vascular endothelial cells. The resultant complex catalyses the activation of protein C on the membrane surfaces of endothelial cells and platelets, activated protein C (APC), in the presence of the co-factor protein S, catalyses the conversion of Factors Va and VIIIa to their inactive forms. APC is itself inhibited in the plasma by protein C inhibitor (PAI-3). In addition, 60% of protein S is normally complexed to the acute phase protein C4b-binding protein and is not functionally active. The proportion of bound protein S may increase in the presence of inflammation, reducing it's' activity further. The mechanism of action of PAI-2 is uncertain. It may only be relevant in pregnancy[5]. At the level of plasmin itself, molecule α2-anti-plasmin combines with plasmin in the ratio of 1:1 to form an inactive compound and thereby inhibits its action.

1.1.7 The Role of the Platelet

In the event of vascular trauma, circulating platelets adhere to the affected area and aggregate to form a platelet clot that helps plugs the breach in the vessel wall. This constitutes a vital component of haemostasis[6]. However, they also perform an important role in the processes of coagulation, fibrinolysis and anti-coagulation. The platelet surface membrane provides a highly catalytic surface on which many of the reactions of the coagulation pathway take place. In particular, the platelet membrane strongly binds both fibrinogen and insoluble fibrin, promoting the conversion of fibrinogen to fibrin and stabilising the fibrin clot. Platelets are also a source of many of the factors affecting coagulation and fibrinolysis, including Factors V, XI and

XII, fibrinogen, HMWK, protein S and PAI-1. Conversely, the components of the clotting cascade have an effect on platelet function. Thrombin is the most potent activator of fibrinogen binding, platelet aggregation and clot retraction.

1.1.8 The Role of the Endothelium

The endothelium is a critical component of the haemostatic system. Under disease and injury-free conditions, the endothelium is antithrombotic, helping to maintain blood in its fluid form. Conversely, when the endothelium is disrupted for any reason, it becomes an integral component in the response to this[7].

1.1.8.1 Functions of Uninjured Endothelium

The uninjured endothelium helps to prevent intravascular blood coagulation by effects on the clotting cascade, platelet function and the fibrinolytic system[8]. Vascular endothelial cells produce a number of heparinoid substances, such as heparan and dermatan sulphate. These are catalysts for the inactivation of factors XIa, Xa, XIa and Thrombin by inhibitors such as antithrombin, resulting in an anti-coagulant effect on the luminal surface. The endothelial surface is also involved in the action of the natural anticoagulants Protein C and Protein S. Protein S is produced by the vascular endothelium, as is thrombomodulin (a surface receptor). When thrombin is generated, it combines with the thrombomodulin receptor. The resultant complex activates Protein C which, when bound to Protein S, cleaves factors Va and VIIIa, preventing further thrombin generation.

Protein C also promotes fibrinolysis by inhibition of PAI-3[9]. Tissue factor pathway inhibitor is also synthesized by the endothelium. This is thought to inhibit the activation of factor X by the Tissue Factor-factor VIIa complex at the start of the clotting cascade[10].

Adherence of platelets to the vascular endothelium is vital for effective haemostasis, but this is inhibited in normal vessels. The endothelium produces prostacyclin and nitric oxide (NO), both of which are powerful inhibitors of platelet adhesion to the vascular wall, in addition to their effects on vascular smooth muscle tone. Prostacyclin production (by the action of cyclooxygenases) is stimulated by the presence of thrombin while NO production is stimulated by a number of cytokines, including tumour necrosis factor[11].

Vascular endothelial cells promote fibrinolysis by producing plasminogen activators. The most important is t-PA, secreted in response to thrombin, histamine, vasopressin and others, but urokinase can also be produced as part of an inflammatory response. The plasminogen activation inhibitors (PAIs) are also produced by the vascular endothelium, as well as by platelets[12].

1.1.8.2 Haemostatic Function of Injured Endothelium

When the vascular wall is disrupted, either by injury or disease processes, procoagulant properties that are normally suppressed or dormant, over-ride its natural antithrombotic state. The presence of tissue factor initiates the clotting cascade by interaction with factor VII and is the major mechanism of

coagulation initiation. Tissue factor expression is known to be induced by a wide range of stimuli, including cytokines (IL-1, TNF), infective organisms and their lipopolysaccharide endotoxins, homocysteine and thrombin. Injury to the endothelium also produces a reduction in fibrinolysis, further heightening the procoagulant state[13]. It has been demonstrated that treatment of cultured vascular endothelial cells with IL-1 results in reduced t-PA secretion and a concomitant increase in PAI-1 secretion with the balance between the two shifting towards reduced fibrinolytic activity[14].

1.1.9 Inflammation and Coagulation

The presence of atherosclerotic disease is accompanied by a low grade inflammatory response. Many of the acute phase proteins that are produced as a part of this response have an effect on blood coagulation, such as fibrinogen. In addition, some inflammatory mediators such as IL-1, IL-6 and tumour necrosis factor (TNF) appear to have an effect on haemostasis. The role of IL-6 in activation of coagulation has been studied in humans and apes, using both endotoxin and recombinant IL-6.

1.1.9.1 Interleukin 6 (IL-6)

Interleukin 6 (IL-6) is an inflammatory cytokine, forming part of the acute phase response to infection or injury. It has effects on a wide range of systems, including aspects of coagulation and haemostasis. IL-6 can be produced by many different cell types, both immune and immune accessory cells and non-immune cells, after appropriate stimulation. These include monocytes and macrophages, fibroblasts, endothelial cells, vascular

smooth muscle cells as well as several tumour cell lines. The action of IL-6 is mediated via a transmembrane receptor which is not involved in signal transduction itself, but instead acts on another transmembrane receptor, gp130. A soluble form of the IL-6 receptor, consisting of the extracellular portion of the receptor, also exists. IL-6 is able to activate the gp130 receptor via this soluble receptor. This enables IL-6 to act on many cells that do not carry transmembrane IL-6 receptors, facilitating its action on such a wide variety of cell types.

The association between inflammation and atherosclerotic disease is well recognised. Given its central role in the inflammatory response, it is therefore not surprising that IL-6 has been implicated in cardiovascular and peripheral vascular disease. It has previously been demonstrated that IL-6 is produced by both normal and aneurysmal endothelial cells and IL-6 gene transcripts have been demonstrated in atherosclerotic lesions. Seino et al[15] examined levels of IL-6 mRNA in atherosclerotic lesions from the arteries of patients undergoing revascularisation procedures. These were found to be 10 to 40 times higher than levels in non-atherosclerotic arteries. In addition, these transcripts were found to be in the thickened intimal layer of the lesion. Rus et al[16] similarly found elevated IL-6 levels in fibrous plaques when compared to normal intima. This would suggest that the increased levels of IL-6 seen in the circulation in arterial disease arises from the atherosclerotic plaques themselves, in which there is low grade inflammation, rather than being a marker of a systemic inflammatory response. A similar picture is seen in aneurysmal disease as well.

Szekanecz et al[17] demonstrated significantly elevated concentrations of IL-6 in the culture supernatants of explants of abdominal aortic aneurysm (AAA) when compared to normal aortic explants. However, levels were also significantly greater than those from aorta affected by occlusive arterial disease. Shteinberg et al[18] similarly found elevated IL-6 concentrations in aortic wall from AAAs in comparison to aortic occlusive disease. Interleukin-6 may also exert part of its effect in vascular disease by influencing fibrinogen levels. As stated above, IL-6 is a powerful component of the acute phase response. Fibrinogen is one of the products of this response. IL-6 is a direct stimulant for increased hepatic production of fibrinogen, which in turn affects changes in blood by increasing the viscosity.

To summarise, coagulation is an intricate and a dynamic process that maintains haemostasis by regulating coagulation and fibrinolysis. The role of platelets, natural anticoagulant mechanisms and vascular factors are vital to this.

1.2 PERIPHERAL VASCULAR DISEASE

1.2.1 Introduction

Peripheral vascular disease is a common condition throughout the world. Several large population studies have examined the prevalence and incidence of both symptomatic and asymptomatic disease. These have indicated a prevalence of almost nil below the age of 40 years, increasing to between 1% and 2% for those aged 40 to 60 years and rising sharply with age beyond this. Prevalence of 20% or more has been reported for subjects over 75 years. Annual incidence rates also rise with increasing age, with reported incidences of 0.2-0.3% in subjects between 45 and 55 years and 0.5% for 55-65 year olds. The presence of peripheral vascular disease correlates strongly with the presence of coronary vascular disease, cerebro-vascular disease and diabetes. A significant number of patients with intermittent claudication die within 5 years from the time of presentation. Several studies have demonstrated increased risk of death, from all causes and from cardiovascular disease, in patients with PVD compared to age and sex matched controls without the condition. A study of the population of Framingham[19] in Massachusetts between 1949 and 1963 found a relative risk of death from all causes of 1.9 in men with PVD and 2.3 for women. The relative risks of death for those with co-existent angina pectoris was 2.8 (men) and 5.2 (women). The Whitehall Study[20] of 18,388 male civil servants aged 40-64 years between 1967 and 1969 found that those with probable intermittent claudication, on the basis of the London School of Hygiene and Tropical Medicine Questionnaire, had relative risks of death from all causes

of 1.72 and 2.69 from cardiovascular death. The Speedwell Study's[21] examination of 2348 men aged 45-59 years between 1979 and 1982 showed that those with a diagnosis of PVD on the basis of the same questionnaire had a relative risk of death of 3.8 from all causes and 4.4 from all circulatory causes, after adjustment for age and smoking. Similar relative risks of death to these are found in other studies from the United States, Scandinavia and the United Kingdom. Population studies have demonstrated a strong association between the presence of peripheral vascular disease and an increased risk of death, with a predominance of death from cardiovascular disease.

1.2.2 Presentation

Atherosclerosis underlies most peripheral arterial disease. Although a majority of patients are asymptomatic at the time of diagnosis, a percentage of patients with PVD will present with intermittent claudication, characterized by cramping pain in the legs with exertion that is relieved with rest[22], which will worsen in approximately 10-20% and progress to critical limb ischemia. Narrowed vessels that cannot supply sufficient blood flow to exercising leg muscles may cause intermittent claudication, which is brought on by exercise and relieved by rest. As vessel narrowing increases, critical limb ischemia can develop when the blood flow does not meet the metabolic demands of tissue at rest. Critical limb ischemia[23] is defined not only by the clinical presentation but also by an objective measurement of impaired blood flow.

Critical limb ischemia include either one of the following (1) more than two weeks of recurrent foot pain at rest that requires regular use of analgesics and is associated with an ankle systolic pressure of 50 mm Hg or less, or a toe systolic pressure of 30 mm Hg or less, or (2) a non-healing wound or gangrene of the foot or toes, with similar hemodynamic measurements.

While critical limb ischemia may be due to an acute condition such as an embolus or thrombosis, most cases are the progressive result of a chronic condition, most commonly atherosclerosis.

1.2.3 Pathophysiology of Peripheral Vascular Disease

Plaques tend to localize at the bifurcations or proximal segments of large and medium-size arteries. The femoral and popliteal arteries are affected in 80% to 90% of symptomatic PVD patients, the tibial and peroneal arteries in 40% to 50%, and the aortoiliac arteries in 30%. Single or multiple arterial stenoses produce impaired hemodynamics at the tissue level in patients with PVD[24]. Arterial stenoses, end result of significant peripheral atherosclerosis lead to alterations in the distal pressures available to affected muscle groups and to blood flow. Atherosclerosis involves several highly interrelated processes, including lipid disturbances, platelet activation, thrombosis, endothelial dysfunction, inflammation, oxidative stress, vascular smooth cell activation, altered matrix metabolism, remodelling, and genetic factors. There are seven stages of development of an atherosclerotic plaque[25]. First LDL moves into the subendothelium and is oxidized by macrophage and SMCs (1 and 2). Release of growth factors and cytokines attracts additional monocytes (3 and

4). Foam cell accumulation and SMC proliferation result in growth of the plaque (6, 7, and 8).This sequence is shown schematically in Figure. 1.2.1.

Figure 1.2.1 -The 7 Stages of Development of an Atherosclerotic Plaque

Source: Libby P. Inflammation in atherosclerosis. Nature. 2002; 420: 868–874.

1.2.4 Risk Factors

Risk factors play an important role in initiating and accelerating the complex process of atherosclerosis. Major risk factors for vascular disease are smoking, diabetes mellitus, dyslipidaemia, and hypertension[26].

Smoking

Smoking is associated with a marked twofold increased risk for cerebrovascular and fourfold increased risk for peripheral vascular disease[27]. The number of pack-years is associated with global atherosclerotic disease severity, including increased incidence of myocardial infarction[28], higher risk of stroke, and an increased risk of amputation, peripheral graft occlusion, and death.

Diabetes

Diabetes, predominately type 2, is a clear risk factor for stroke, particularly ischaemic stroke, with prospective studies reporting up to a threefold increase of the relative risk and a more severe stroke-related disability and mortality. Although diabetes has been found to be associated with angiographically demonstratable extracranial carotid and basilar artery occlusion, few reports have clarified the influence of diabetes on large vessel cranial atherosclerosis. Diabetes is one of the strongest predictors for PVD (twofold increase of relative risk of intermittent claudication) and its associated complications, including higher amputation (up to 10-fold increase) and mortality rates[29].

Dyslipidaemia

Several epidemiologic studies have clearly shown that total hypercholesterolaemia is among the most important risk factors for PVD[30]. Independent lipidaemic risk factors include elevated levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglycerides, and lipoprotein (a). Elevated levels of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein (a-1), the major protein component of HDL-C, seem to confer a protective effect. Low HDL-C, high triglycerides, and more recently, high lipoprotein (a) levels have been also associated with an increased risk of MI and stroke.

Hypertension

Hypertension is associated with all forms of cardiovascular disease and the associated mortality increases progressively and linearly with increasing blood pressure levels[31].

Predisposing risk factors

Predisposing risk factors for atherosclerosis are those that confer their risk through conventional factors and through potentially independent effects. Those that are *nonmodifiable* include advanced age, gender (male sex; postmenopausal women), family history and genetics, and race (eg, African Americans) and ethnicity[32]. Those that are *modifiable* include overweight and obesity, physical inactivity, insulin resistance (even without diabetes), and socioeconomic-behavioral factors such as social isolation, depression, type A personality, work, and family stress.

Conditional risk factors

Conditional risk factors have an association with an increased risk of cardiovascular disease, although their independent contribution is not well documented. They include homocysteine, C-reactive protein (CRP), fibrinogen, lipoprotein (a), and hypertriglyceridemia.

Homocysteine

Reports from prospective and retrospective studies suggest that elevated homocysteine levels are an independent risk factor for vascular disease[33] associated with a mildly increased risk of CHD, stroke, PD, and venous thromboembolism.

C-reactive protein

Observational and prospective studies have consistently reported that elevated CRP serum levels definitely have prognostic value for cardiovascular events and death[34]. To date, CRP is the only inflammatory marker used in clinical practice, but it needs to be emphasized that the predictive value of CRP can be estimated only through high-precision assays for high-sensitivity CRP. It is within these lower, previously “normal” ranges that the high-sensitivity CRP levels seem to have predictive abilities for cardiovascular events.

Fibrinogen

Several prospective studies have associated fibrinogen levels and subsequent cardiovascular disease risk[35], particularly for coronary heart disease and cerebrovascular disease. Some have advocated that fibrinogen may have equal effectiveness as total cholesterol in predicting future risk. However, whether elevated fibrinogen levels are a cause or consequence of atherosclerosis remains unclear.

Inflammatory markers

Apart from CRP and fibrinogen, several other inflammatory markers have been associated with atherosclerosis and cardiovascular disease[36]. Serum amyloid A (an apolipoprotein associated with HDL-C), high white blood cell count, cytokines (interleukins 6 and 18, tumor necrosis factor-α [TNF-α], monocyte chemotactic protein-1 [MCP-1]), endothelial adhesion molecules (intercellular adhesion molecule 1, P-selectin, etc), circulating soluble CD40

ligand (an immune mediator), protease-activated receptors (PARs), and lipoprotein-associated phospholipase A2 have been all associated with PVD.

Infection

Several infectious agents have been associated with the formation of atherosclerotic plaque and its complications. Of bacteria, *Chlamydia pneumoniae*, a human respiratory pathogen, has been most strongly associated with cardiovascular disease, particularly with acute myocardial infarction (MI), cerebrovascular symptoms, PVD, and abdominal aortic aneurysm expansion[37]. However, the lack of standardized methods of testing for *C pneumoniae* infection led to conflicting findings. Currently, the presence of *C pneumoniae* in atherosclerotic vascular tissues merely suggests an association; it does not establish an etiologic relationship.

Haemostatic factors and hypercoagulable states

There is now sufficient evidence that correlates, apart from homocysteine and fibrinogen, other hemostatic factors and hypercoagulable states with atherosclerosis. D-dimers, plasminogen activator inhibitor activity, von Willebrand factor, and procoagulant factor VII are independent risk factors for CHD, PVD, and stroke[38]. Antiphospholipid antibodies such as lupus anticoagulant and anticardiolipin antibodies are also associated with vascular damage and have been advocated as independent risk factors for PVD. No therapeutic interventions have yet been proposed.

Creatinine, urate, and microalbuminuria

Renal impairment is associated with an increased risk of death in advanced PVD, irrespective of the presence of hypertension and diabetes. Hyperuricemia is associated with worse functional status of the peripheral circulation in hypertensive patients. Microalbuminuria is gaining recognition as a marker of an atherogenic milieu and cardiovascular disease. The presence, but not magnitude of albuminuria, is an important risk factor for PVD in diabetic but not in non-diabetic subjects[39].

1.2.5 Management

The consensus document produced by the Trans -Atlantic intersociety TASC working group[40] outlines the guidelines for management of patients with symptomatic PVD.

1. Clinical history and physical examination, including the coronary and cerebral circulations.
2. Hematologic and biochemical tests—complete blood count, platelet count, fasting blood glucose, hemoglobin A_K, and creatinine levels, fasting lipid profile, and urinalysis (for glycosuria and proteinuria)
3. Resting electrocardiogram.
4. Ankle or toe pressure measurement or other objective measures of severity of ischemia.
5. Imaging of lower limb arteries in patients considered for endovascular or surgical intervention.
6. Duplex scan of carotid arteries—should be considered for select patients at high risk.
7. A more detailed coronary assessment may be performed in select patients for whom coronary ischemic symptoms would otherwise merit

such an assessment if CLI were not present (such coronary assessments should generally not impede associated CLI care).

1.2.5.1 Diagnosis of PVD

Symptomatic PVD can be diagnosed by the presence typical symptoms of claudication or pain at rest and calculating the ankle-brachial Pressure index (ABPI). The ABPI is the ratio of systolic blood pressure measured at the ankle by a Doppler ultrasound device to the higher systolic blood pressure at the brachial arteries.

The estimation of ABPI is helpful because of the following:

1. Most PVD patients are asymptomatic or have atypical leg symptoms.
2. Among symptomatic patients, 11% to 33% report classic intermittent claudication, and up to one third fail to communicate their symptoms to their health care provider.
3. Those with asymptomatic PVD have a risk of cardiovascular events (e.g., myocardial infarction, stroke, cardiovascular mortality) comparable with that of patients with symptomatic coronary artery disease.
4. The severity of PVD (as determined by a lower ABI value or presentation with symptomatic disease) predicts limb outcome and patient survival.
5. The ABPI is an accurate measure (ABPI less than 0.9 has a sensitivity of 95% and specificity of 100% in detecting PVD) that is noninvasive,

inexpensive, office-based, and can be reliably performed by primary care providers and other health care personnel.

Based on these characteristics, the most recent American College of Cardiology–American Heart Association (ACC/AHA) guidelines advocate ABPI measurement for high-risk individuals. It is hoped that such strategy will facilitate early detection and institution of management strategies (e.g., risk factor modification, exercise rehabilitation, limb revascularization) that will help prevent disability, decrease cardiovascular events, and lower early mortality.

The shortcomings of the ABPI test include the potential to miss mild proximal disease of the aorta and iliac arteries (also referred to as inflow disease) in those with well-developed collaterals and those with significant medial artery calcification. Thus, an exercise ABPI should be determined when the resting ABPI value is normal if the pretest probability of PVD is high. Other diagnostic tests (e.g., pulse volume recording, duplex ultrasonography, magnetic resonance imaging, and computed tomography angiography) are recommended for those with calcified vessels (e.g., older individuals, those with long-standing diabetes or end-stage renal disease) suspected of having PVD but with a resting ABPI value of more than 1.3. Conventional contrast-mediated arteriography rarely is needed to establish the diagnosis of PVD; it is largely limited to patients being considered for revascularisation procedures.

1.2.5.2 Treatment

The goals of PVD management are limb salvage, symptom relief, improving functional status, and preventing cardiovascular events (acute myocardial infarction [MI], stroke, and vascular death). All PVD patients require intensive cardiovascular risk reduction and should be referred to a supervised exercise program. Limb revascularization procedures are offered to select patients. Indications for limb revascularisation include acute limb ischemia, critical limb ischemia, or lifestyle-limiting claudication. Options include endovascular / percutaneous or surgical bypass graft repairs to treat occluded arterial segments.

To summarise, PVD is a major manifestation of atherosclerosis and is associated with significant cardiovascular morbidity, limb loss and death.

1.3 COAGULATION CHANGES IN PERIPHERAL VASCULAR DISEASE

1.3.1 Coagulation in Peripheral Vascular Disease

Patients with peripheral arterial occlusive disease (PAOD) also known as peripheral vascular disease (PVD) are found to be hypercoagulable. They manifest an increased risk of developing thrombotic events for varying reasons. Virchow's triad[41] of reduced blood flow, increased coagulability and tissue wall damage promotes the occurrence of intravascular thrombosis. The formation and progression of atherosclerotic plaques and intra-arterial thrombosis are both mechanisms that may result in narrowing or occlusion of arteries seen in cardiovascular and peripheral vascular disease.

1.3.2 Fibrinogen

Fibrinogen metabolism has been shown to be abnormal in both symptomatic and asymptomatic peripheral vascular disease in a number of studies, and correlations have been demonstrated between disease severity and fibrinogen levels. Fibrinogen is also an acute phase protein, whose concentration is increased in the event of trauma, inflammation or infection. Dormandy et al[42] measured fibrinogen levels in 126 patients with intermittent claudication. Sixty percent of the patients had fibrinogen levels above the normal upper limit of 4 g/l. In addition, there was a significant correlation between plasma fibrinogen and disease progression. Plasma fibrinogen also correlated strongly with plasma viscosity. Lowe et al[43] reported the results of the Edinburgh Artery Study with respect to a number

of coagulation factors, including fibrinogen, in 1993. In this study, 1592 men and women were drawn at random from general practices in Edinburgh and screened for both symptomatic and asymptomatic peripheral vascular disease. Patients were assessed for PVD using the WHO questionnaire on intermittent claudication and medical examination, including assessment of ankle-brachial pressure indices and a reactive hyperaemia test. Plasma fibrinogen levels were assessed in a sample of peripheral venous blood by a thrombin-clotting turbidometric method. Patients with both intermittent claudication and major asymptomatic disease had levels of plasma fibrinogen significantly greater than in patients with minor asymptomatic disease or controls. There was also found to be a strong inverse correlation when plasma fibrinogen was related separately to ABPI and this correlation was much more marked in men than in women. Woodburn et al[44] reported similar results in 219 patients with stable intermittent claudication: venous plasma fibrinogen levels were significantly elevated in patients when compared to age-matched controls and there was a strong correlation between severity of disease, assessed by angiographic scoring, and fibrinogen. However, multivariate analysis showed that only fibrin degradation products were independently associated with angiographic severity of disease. Lassila et al[45] also showed a relation between fibrinogen levels and severity of PVD, as measured by ABPIs, in 409 stable claudicants. Smith et al[46] followed 607 patients with intermittent claudication in Edinburgh for 6 years and related the incidence of vascular events to haemostatic factors. The main outcomes observed were stroke (both fatal

and non-fatal), myocardial infarction and cardiac death. They observed a significantly higher median level of fibrinogen in those who suffered a vascular event than those who did not. There is also some evidence that raised fibrinogen levels are related to the patency of bypass grafts. Wiseman et al[47] investigated 157 patients undergoing saphenous vein femoropopliteal bypass grafting. At one year, 113 grafts remained patent and 44 had occluded. Plasma fibrinogen concentration was identified as the strongest predictor of graft occlusion, followed by smoking. Hamer et al[48] demonstrated a significant difference between fibrinogen levels in patients with patent or occluded femoropopliteal or aorto-iliac segments after reconstructive surgery when followed up for more than 12 months. Lee et al, in an analysis of data from the Edinburgh Artery Study[49], found that fibrinogen levels in diabetics with peripheral arterial disease were significantly raised compared to diabetics without evidence of PVD. When adjustment for fibrinogen, VWf, t-PA and D-dimers were made, the odds ratio for PVD was reduced from 1.45 to 1.11, suggesting that haemostatic factors, including fibrinogen, may be partly responsible for the increased risk for PVD observed among diabetics. Fibrinogen is one of the acute phase proteins synthesised in the liver in response to stimuli such as trauma, inflammation or infection. This response has been demonstrated in the presence of peripheral vascular disease. Stuart et al[50] showed elevated levels of the acute phase proteins fibrinogen, antithrombin III, factor VIII and serum globulin, as well as high platelet count and activity and neutrophilia, in 40 patients with peripheral arterial disease compared to 29 healthy

controls.

It has also been demonstrated that inflammatory markers such as interleukins (IL) 1 and 6 and tumour necrosis factor alpha are elevated both locally and systemically in the presence of vascular disease, suggesting that an inflammatory reaction is taking place in the atherosclerotic plaque. It has been postulated that elevated levels of these cytokines, particularly IL-6, stimulate the synthesis of fibrinogen in the liver and thus the hyperfibrinogenaemia seen in vascular disease. While the relationship between fibrinogen and vascular disease has been most completely characterised, an association between a number of the other components of the haemostatic system and vascular disease has also been investigated.

1.3.3 Markers of Activation of Coagulation in PVD

Aside from increased fibrinogen levels, raised levels of fibrin degradation products, in particular D-dimers, have been demonstrated in a number of studies. There is also good evidence of abnormal coagulation activation (as witnessed by abnormal levels of thrombin-antithrombin complexes (TAT) and Prothrombin fragments F1.2), and of impaired fibrinolysis in peripheral vascular disease. The high levels of von Willebrand factor seen in PVD also suggest an endothelial element to the abnormal coagulation seen in the disease process. Cortellaro et al., reporting on the PLAT study[51], Lassila et al[52], Fowkes et al[53], Trifilleti et al[54], Woodburn et al[55], De Buyzere et al[56] and Lee et al[57] all report a statistically significant association between the

presence of both symptomatic and asymptomatic peripheral vascular disease and raised levels of fibrin degradation products (FDPs). As with fibrinogen, Lassila et al[17] and Woodburn et al[20] demonstrated a significant correlation between the severity of the disease, as indicated by both ankle-brachial pressure indices and the angiographic extent of disease, and FDPs. Lee et al[49]. demonstrated a strong inverse correlation between FDPs and ABPIs in both symptomatic and asymptomatic PVD sufferers in the Edinburgh Artery Study. The significance of this was reduced after adjustment for risk factors including smoking, but remained significant in men. This was also the case after adjustment for fibrinogen concentrations. Raised FDP levels have also been shown to be related to the progression of PVD and to the risk of cardiovascular events. Fowkes et al[18] showed a significant independent correlation between cross-linked fibrin degradation products and progression of PVD (as assessed by serial ABPIs) in 617 claudicants. There was also a strong relationship between FDPs and (combined fatal and non-fatal) cardiac events during a 1 year follow-up period. Boneu et al[58] showed a relationship between D-dimers and the risk of a vascular event at 2 years follow-up in 324 claudicants, but this relationship became non-significant on multivariate analysis. Woodburn et al[59] measured FDP levels before and 16 weeks after resolution of critical limb ischaemia but found that, unlike fibrinogen, there was no significant change after revascularisation.

Further evidence of activation of the coagulation system comes from studies of TAT complex and Prothrombin F1.2, indicators of thrombin

production. De Buyzere et al[60] demonstrated that levels of TAT and F1.2 were significantly elevated in 34 patients with Fontaine[61] stage II PVD when compared to age and sex-matched controls. These factors were not altered by treadmill testing. Similar findings were reported by Herren et al[62] in 22 patients with similar disease when compared to 13 controls. Strano et al[63] were also able to show raised levels of TAT in 103 PVD patients when compared to controls. Lassila et al[17] demonstrated that TAT levels were progressively associated with the angiographic and clinical severity of PVD in a similar relationship to that seen with FDPs. Boneu et al[23] demonstrated that high levels of TAT complexes were independently predictive of vascular events in the 324 claudicants studied. These data suggest that there is increased activity of the coagulation system in patients with PVD with a resultant increase in thrombin formation. However, the subsequent role of the thrombin formed is less clear. In the coagulation cascade, thrombin cleaves fibrinogen to form fibrin and the protein fibrinopeptide A (FPA). FPA is thus a good marker of the action of thrombin on fibrinogen. However, there is disagreement in the literature over the relationship between FPA levels and vascular disease. Lassila et al[17] found elevated levels of FPA in 60% of their patients with stable claudication and FPA correlated significantly with fibrinogen and TAT levels. Fowkes et al[19]demonstrated a significant correlation between urinary FPA levels and the risk of fatal (but not non-fatal) coronary events in 617 claudicants. Lee et al[14] demonstrated a significant independent correlation between urinary FPA levels and the risk of intermittent claudication in the same patients. However, neither

Herren et al[28] nor Lowe et al[8] were able to demonstrate a relationship between urinary and plasma FPA and the presence of peripheral vascular disease, although the former did demonstrate relationships between F1.2, TAT and PVD. This may have been related to insensitivity of the assays used as the quantities of FPA are very small and it has a half-life of only around 4 minutes in plasma. High levels of D-dimer, a product of the degradation of fibrin suggests that there is increased fibrin formation in patients with PVD. How much of this due to the excess formation of thrombin in these patients and to what extent this excess is negated by formation of a complex with anti-thrombin remains unclear.

1.3.4 Fibrinolysis in PVD

As has been stated above, there is much evidence of activation of the coagulation system in the patient with vascular disease. However, there are indications that the fibrinolytic system is also deranged. Plasmin catalyses the cleavage of fibrin to FDPs in the plasma. It is itself formed from the inactive precursor plasminogen by the action of plasminogen activators, t-PA and u-PA, a process which is weak in the absence of fibrin but is greatly enhanced in the presence of excess fibrin. The action of these catalysts is opposed by the plasminogen activator inhibitors PAI-1 and PAI-2, though the former is much more significant. Studies have demonstrated abnormalities in both plasminogen activation and its inhibition in the vascular patient. In the PRIME study, Scarabin et al[64] conducted a prospective study of 10,500 men between 50-59 years old from France and Northern Ireland. Patients with coronary arterial disease or peripheral

arterial disease showed significantly elevated levels of PAI-1, though this association was not seen in men with symptomatic cerebro-vascular disease. Elevated PAI-1 was also associated with diabetes mellitus, smoking, alcohol intake, higher body mass index, increasing waist: hip ratio and plasma triglyceride levels. De Buyzere et al[28] and Trifiletti et al[65]also demonstrated significantly elevated levels of PAI-1 in PVD patients. In the former, a similar relationship was seen for t-PA antigen levels. They found no difference in t-PA antigen levels in patients versus controls. Phillipp et al[66] reporting on 46 patients in the Arterial Disease Multiple Intervention Trial, also found PAI-1 to be significantly elevated in patients but multivariate analysis showed this not to be independent of other confounding factors. Boneu et al[14] showed that high PAI-1 levels correlated with subsequent vascular events in claudicants. Roller et al[67] demonstrated significantly elevated levels of PAI-1 antigen and activity in PVD patients. PAI-1 activity at 24 and 48 hours after percutaneous transluminal angioplasty also correlated with late re-stenosis at 6 months. An elevated t-PA antigen level was also seen in PVD patients in this study, but no relationship to re-stenosis was seen. Killewich et al[68] demonstrated significantly elevated PAI-1 levels in patients with both mild and severe intermittent claudication, when compared to controls. Low t-PA activity was seen in patients in the severe claudication group when compared to the mild claudication and control groups, as seen in other studies. Levels of t-PA antigen also correlated inversely with pain-free walking time. The difference between t-PA activity and antigen levels is because the assay of

antigen measures both free plasma t-PA and that bound to PAI-1 in active complexes. A finding of high antigen levels but low activity would thus be consistent with high PAI-1 production, partly neutralised by combination with t-PA. Lee et al[69] found a correlation between severity of peripheral arterial disease, as measured by ABPIs, and t-PA antigen.

1.3.5 Von Willebrand Factor

A few studies have examined the relationship between Von Willebrand factor and vascular disease, as it is a possible marker of endothelial disease. Woodburn et al[70] found significantly higher vWF levels in patients with PVD. The levels were significantly reduced 4 months after revascularisation, though still elevated when compared to controls. Lee et al[49] found a correlation between vWF levels and severity of PVD, but only in men. The ADMIT study did not detect any difference in vWF levels between patients and controls. Cortellaro et al[71] found a significant correlation between vascular events and elevated levels of vWF antigen in patients with angina pectoris, but not in patients with peripheral vascular disease. Relationships between some other elements of the coagulation system and vascular disease have also been examined in few studies. Low levels of Protein C and Protein S have been demonstrated in PVD patients by Strano et al[72] reported an increased risk of vascular events in PVD patients associated with low Protein C levels. Trifiletti et al[73] demonstrated significantly elevated plasma thrombomodulin levels in PVD patients which may be a marker of endothelial damage and promoter of thrombosis. Boneu

et al[74] found no association between the level of soluble thrombomodulin and the risk of vascular events in claudicants.

To summarise, a large body of evidence now exists in the literature that, patients with peripheral vascular disease exhibit abnormalities in a number of elements of the coagulation and fibrinolytic systems. Changes are also seen in PVD patients after reconstructive surgery, though not with a complete return to normal values. There is also clear evidence of activation of the coagulation system, as witnessed by increased thrombin formation (raised TAT and F1.2 levels) and fibrinogen turnover (elevated FDPs). Conversely, there is also good evidence for defective fibrinolysis in these patients. Several studies demonstrate high PAI-1 activity and t-PA antigen levels, as well as low t-PA activity suggesting an overall shift in the balance of factors affecting fibrinolysis towards decreased fibrinolysis.

Available evidence point towards a hypercoagulable state in patients with peripheral vascular disease, activation of coagulation and impairment of fibrinolysis contributes to this. Whether this hypercoagulable state contributes to the formation and progression of PVD, or is merely a result or reflection of it remains unclear.

1.4 THROMBOELASTOGRAPHY (TEG)

1.4.1 History and Principles of Thromboelastography (TEG)

Helmut Hartert, a physicist from Germany, first described the Thromboelastograph in 1948 and it has been utilised as a research tool since then, though it has only been applied to clinical situations in the past two decades.

The end point of blood clotting is the formation of a stable clot that is capable of limiting blood loss from a damaged vessel. The production of this clot requires the correct functioning of many different components involving clotting factors, platelets and other blood cells and the vascular endothelium. A similarly complex process governs the dissolution of the clot. A defect in one or more of these components, or in the interactions between them, will have an adverse effect on the body's ability to bring about haemostasis effectively. A wide range of tests are available to both researchers and clinicians examining blood clotting. However, these are usually only able to examine one component of clot formation and it is often unclear what impact a specific defect will have on clotting overall. In addition, many tests are time-consuming with blood samples needing to be separated into cellular and non-cellular components before testing. This is of particular concern in the clinical setting. The coagulation profile of a critically ill patient often changes very rapidly so that information from laboratory-based tests may be obsolete by the time it is received. In addition, while current laboratory tests may give information about the rate of clot formation, they tell nothing about the quality or stability of the clot. It

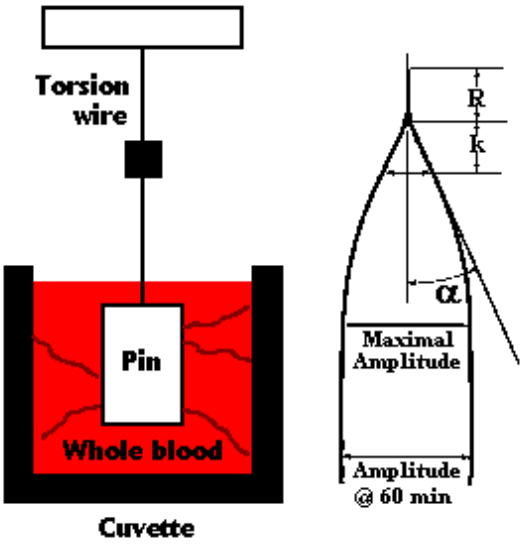
is clear that there is a place for a global test of blood clotting that may be performed rapidly and at the point of care.

Thromboelastography (TEG)[75] measures the visco-elastic properties of a blood clot and thereby gives information about the combined function of all the factors involved in the process of clot formation and its subsequent dissolution. This is in contrast to routinely available coagulation tests, which assess only isolated stages of this process. This technique is a global test of coagulation that can be performed on a small sample of blood and can be performed repeatedly as a point of care testing method to monitor changes in coagulation of blood.

1.4.2 Technique of TEG

Thromboelastography (TEG) fulfils many of the criteria required of a global test of blood clotting. TEG principle is based on the changes in the physical properties during and after the process of formation of a blood clot. The TEG apparatus consists of a cup or a cuvette heated to 37°C containing 0.36 ml of whole blood. If the blood has been preserved with citrate, then it may be recalcified by the addition of 0.15 ml of calcium chloride. The cup moves through 40-44 degrees of rotation every ten seconds in an oscillating pattern. Into this cup of blood is lowered a pin which is suspended from a torsion wire, connected to a transducer (Figure.1.4.1).

Figure 1.4.1 Principles of Thromboelastography



When the blood is in its liquid phase, there is no coupling between the pin and the movement of the cup and a flat trace is produced. However, as clot begins to form, the pin begins to be dragged along with the cup, a motion which increases as the clot progresses. The motion of the pin is converted by the transducer into a trace either on paper or, more usually now, onto a computer. The TEG is effectively measuring the shear modulus of the clot. The TEG, like many conventional tests of clotting, will measure the time it takes for clot to first develop. This is known as *r* (reaction time) and is taken as the time from the start of the test to when the TEG tracing reaches 2mm in amplitude. The normal range for this is 6-8 minutes. The *r* value represents initial fibrin strand formation. Whereas this marks the end point for most conventional tests, the TEG goes on to measure the rate of clot formation. The *K* value or clot formation time is from the *r* time until the tracing reaches an amplitude of 20mm and indicates the time taken for the

clot to attain an arbitrarily chosen (but constant) degree of visco-elasticity. This is dependent on the degree of fibrin formation and cross-linking. The normal value for this is between 3 and 6 minutes. The Alpha angle is the angle of the tracing between the *r* and *K* values and denotes the rate of clot formation. This is normally between 50 and 60 degrees. The Maximum Amplitude (MA) of the trace is indicative of the peak strength that the clot attains and is usually between 50 and 60 mm. Once the MA has been reached, the clot will begin to weaken as the process of fibrinolysis begins to outpace that of coagulation. This is also measured by the TEG. The A_{60} is the amplitude of the tracing 60 minutes after MA is attained. It is therefore a measure of clot lysis and has a normal range of MA-5mm. The CL30 and CL60 are estimates of the proportion of the clot that has been lysed 30 and 60 minutes respectively after MA has been reached. A typical TEG trace for a normal blood sample is shown in Figure.1.4 2. The key measurements of the TEG are combined to produce the Coagulation Index (CI). This single value gives an overall picture of the coagulation for a given sample and allows different samples to be compared easily. It is derived by the following formula:

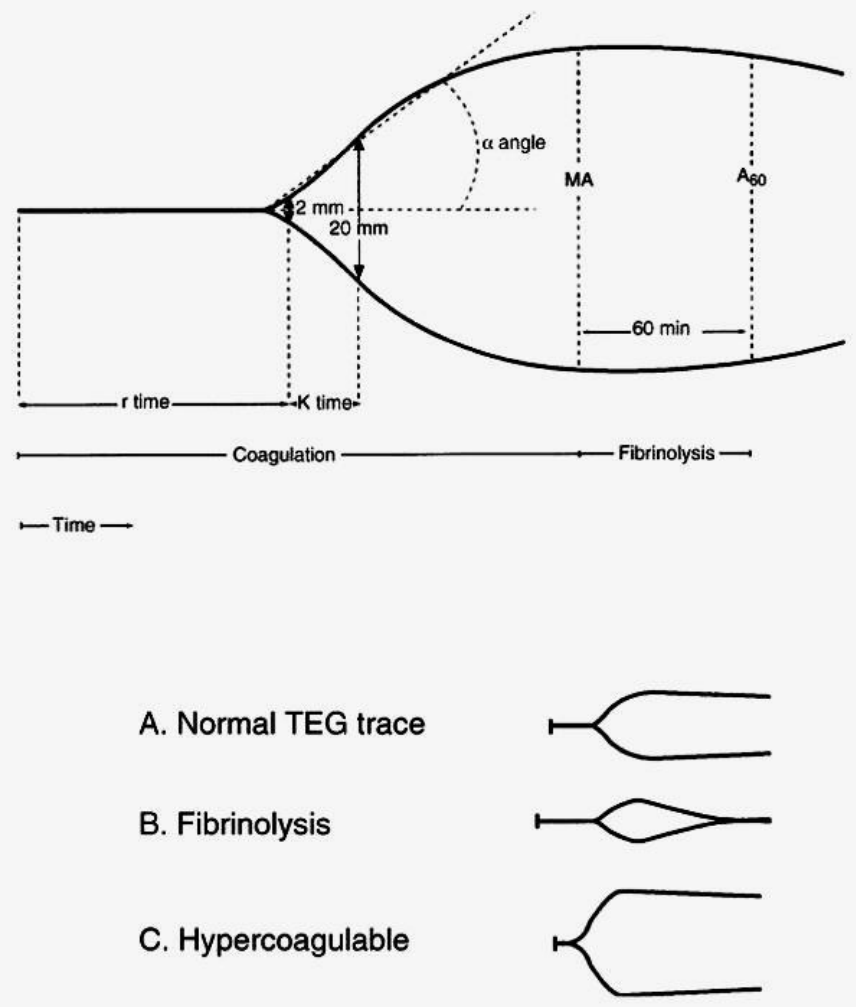
Coagulation Index = 0.1227*r* + 0.0092*K* + 0.1655MA - 0.0241α angle - 5.0220.[76]

1.4.3 Interpretation of a TEG Analysis

Correlation between TEG and the established tests of coagulation[77] is poor because of the interactions between different pathways that may affect

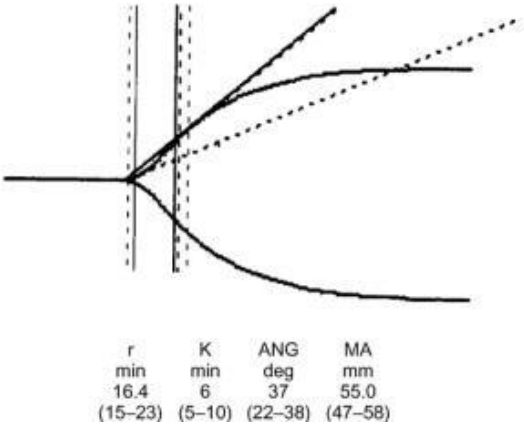
overall clotting. However, different TEG variables are disproportionately affected by defects in particular components of the coagulation system. The initiation and early rate of fibrin strand formation is related to the intrinsic pathway and it is therefore defects in this that predominantly affect the *r* time. Deficiencies in coagulation factors, heparinisation or hypofibrinogenaemia may all cause the *r* time to be increased, while it may be shortened in conditions that cause hypercoagulability[78]. The *K* time reflects continued clot formation and its cross-linking and is affected by the functioning of intrinsic clotting factors, fibrinogen and platelets. The (Alpha) α angle is affected predominantly by fibrinogen and platelet activity and will be reduced in the presence of thrombocytopaenia or hypofibrinogenaemia. The MA is affected most markedly by any abnormality in the number or functional competence of platelets. Some defects of haemostasis do not directly affect clot strength or stability and thus do not manifest themselves at all in the TEG trace[79]. The commonest of such defects is the effect of low-dose aspirin on platelets. Because aspirin has little effect on thrombin-induced platelet aggregation it does not affect clot integrity or the TEG trace[80]. Similarly, the reduced adhesion of platelets to the endothelium that is characteristic of chronic renal failure does not affect the physical properties of the clot that is tested by TEG and thus does not affect the trace.

Figure 1.4.2 Thromboelastography Trace



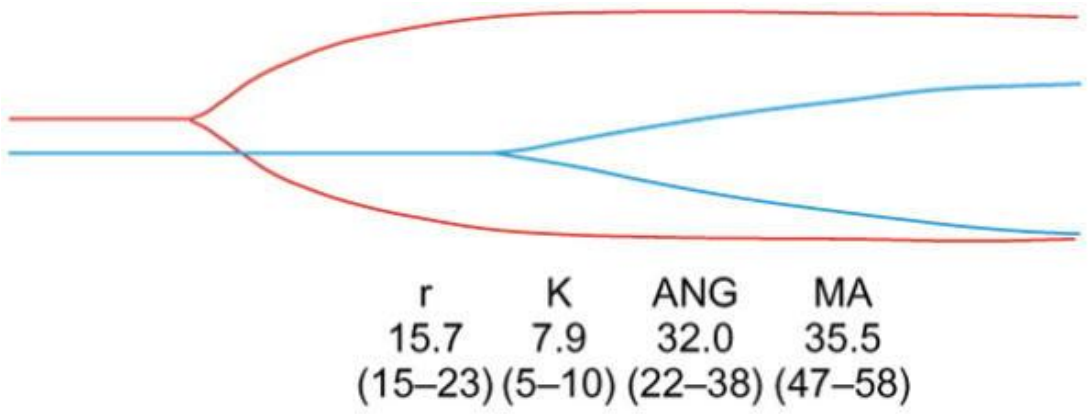
A schematic of a thrombelastograph (TEG(R)) trace showing the r time, K time, alpha ([alpha]) angle, maximum amplitude (MA) and A60 (see text). In comparison with a normal trace (A), fibrinolysis (B) is associated with a prolonged r time, reduced MA and a reduced A60, while hypercoagulability (C) is associated with a short r time and an increased MA.

Figure 1.4.3 TEG Analyser and a Computer Generated TEG Trace



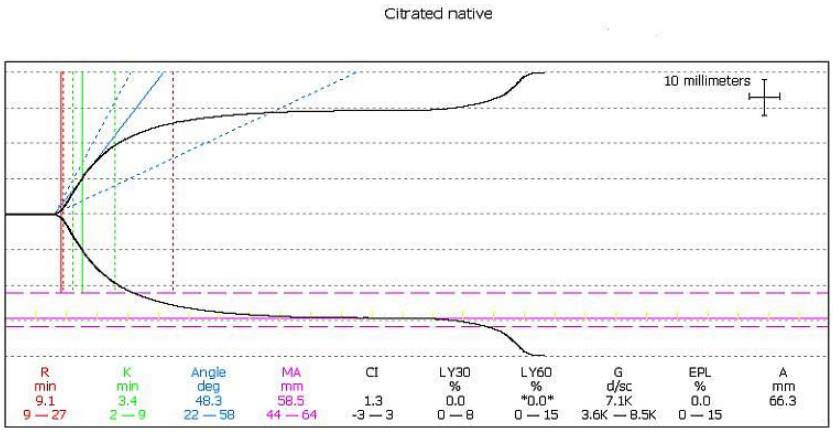
Thrombelastograph(R) 5000 coagulation analyser (courtesy Medicell Ltd.) (Figure 1.4.3) and a normal trace generated by computerized software (courtesy of Dr Sue Mallet, Royal Free Hospital). Parameters are given together with a normal range in parentheses (Figure 1.4.5).

Figure 1.4.4 Heparinase Modified TEG (hepTEG)



This two-channel, two-colour thrombelastograph trace (Figure 1.4.4) was generated by simultaneous recordings from a standard cup and a heparinase-coated cup at the reperfusion phase of orthoptic liver transplantation (courtesy of Dr. Sue Mallet, Royal Free Hospital). The native sample (blue trace) initially generates a straight-line trace indicating the reperfusion coagulopathy. The improvement that occurs when engraftment occurs is indicated by a widening in the amplitude of the trace. Comparison with the trace generated from the heparinized cup (red trace) enables the contribution of heparin to the coagulopathy to be assessed. The reduced maximum amplitude (MA) seen in the red trace from the heparinase cup is indicative of a persistent underlying coagulopathy in this patient, unrelated to heparin administration.

Figure 1.4.5 Normal Citrated Whole Blood TEG Trace



Key: R, Reaction time (time to initial fibrin formation);*K*, dynamics of clot formation; *Angle*, acceleration of fibrin buildup and cross-linking (clot strengthening); *MA*, maximum amplitude (ultimate clot strength); *CI*, coagulation index (overall coagulation status); *LY30*, percent lysis at 30 minutes after MA;*LY60*, percent lysis at 60 minutes after MA; *G*, clot firmness as shear elastic modulus; *EPL*, estimated percent lysis; *A*, current amplitude

1.4.4 Current Applications of TEG

Thromboelastography has not become widely accepted as a test of coagulation in clinical practice, but its use is well established in some specialities, most notably liver transplantation and cardiac surgery that use cardiopulmonary bypass[81]. Patients undergoing orthotopic liver transplantation are usually thrombocytopaenic and deficient in clotting factors due to their underlying liver disease and are therefore in a

hypocoagulable state prior to surgery. During the course of transplantation severe fibrinolysis causes this hypocoagulable state to deepen rapidly to the extent that no clot is formed in the period shortly after the transplanted organ is reperfused. Kang et al [82]demonstrated that the patient's coagulation state could be rapidly monitored in the operating theatre using TEG and that this resulted in reduced blood loss in 66 patients undergoing liver transplantation. The specific therapy instituted for coagulopathies in these patients could be guided by the TEG results. For example, clotting factors (in the form of fresh frozen plasma) if the *r* time was prolonged or platelets if the MA was low. This directed therapy ensured that the most appropriate blood product was given for the defect present and avoided the unnecessary use of others. McNicol et al[83] demonstrated reduced blood loss and transfusion requirements in 75 patients undergoing liver transplantation whose coagulation was monitored using TEG. Harding et al[84] demonstrated that the use of heparinase-coated cups in TEG allowed the presence of heparin-like substances to be allowed for in the monitoring of coagulopathies of patients undergoing liver transplantation. Many of the defects of haemostasis seen in these patients are also evident in those with liver disease such as cirrhosis. Chau et al[85] and Papatheodoridis et al[86] successfully applied TEG in the management of these patients. Similar problems of hypocoagulability present themselves during cardiac surgery in which cardiopulmonary bypass is employed. Patients on bypass are usually heparinised and this is commonly reversed with protamine sulphate at the end of the procedure. In addition, cardiopulmonary bypass in itself seems to

produce abnormalities of haemostasis though it is not known whether this is due to bypass, the effects of haemodilution or some other cause. Because these changes in coagulation are rapid and unpredictable, the use of TEG has been shown to be valuable during and after cardiac surgery[87]. Spiess et al[88] showed that TEG was a better indicator of postoperative bleeding and the need for re-operation than conventional clotting tests after cardiac surgery. They found that transfusion with fresh frozen plasma was not indicated by the TEG in any of the 92% of patients who received it after cardiac surgery. Of the patients who were given platelet transfusions, 60% did so unnecessarily.

Thromboelastography has also been suggested in a wide range of other applications. These include the monitoring of oral anti-coagulation therapy[89], the management of snake bite[90] victims (many of which are powerful natural anti-coagulants) and the prediction of bleeding in patients after renal biopsy and transplantation[91]. TEG has been shown to identify functional abnormalities of factors involved in coagulation even if their concentration is in the normal range and it is a sensitive, specific and rapid way to assess coagulation[92.] TEG can detect activation of coagulation in patients undergoing general surgical[93,] abdominal aortic[94] and neuro- surgical procedures[95] and TEG guided transfusion protocols have been shown to reduce blood and blood product usage in patients undergoing cardiac bypass procedures[96.] TEG in patients undergoing abdominal aortic aneurysm repair can identify post- operative hypercoagulability[13], monitor the adequacy of anticoagulation (heparinisation) during peripheral vascular reconstruction

procedures[97] and assess platelet function in vascular patients[98.] TEG has been shown to reduce the consumption of blood and blood products in the treatment of massive haemorrhage[99] complicating open cardiac procedures and is now routinely employed as point of care testing method in cardiac surgical departments. TEG is now routinely employed to identify hypercoagulation following neurosurgery, in major trauma and in liver transplantation to guide administration of blood components, coagulation factors and platelets. The sensitivity and specificity of this technique has been shown to be particularly good when compared to routine tests of coagulation, anti-Xa levels, bleeding time, thrombin time and APTT/PT ratio estimations[100] especially in the management of coagulopathy.

The application of TEG in the management of patients with peripheral vascular disease (occlusive) has not been described. However, many abnormalities of coagulation are encountered in these patients. The majority will have been on long-term anti-platelet therapy. Many will be anti-coagulated with heparin during surgery and the surgeon may choose to reverse this with protamine sulphate at the end of the procedure. It is known that a large proportion of these patients have an underlying hypercoagulable state, although the reasons for this have not been fully elucidated. Some will undergo procedures of long duration with large requirements for intravenous fluid replacement which may also compromise coagulation. The combination of these many different factors can make it difficult to assess and correct a patient's overall coagulation state accurately. Yet it is obviously important to do this to prevent bleeding from

vascular anastamoses. TEG may provide the means to do this rapidly in the operating theatre or ward setting.

1.4.5 Citrate Storage of Blood Samples and TEG

TEG analysis of fresh whole blood sample is performed within 6 minutes of collection. This is not always possible as TEG analysers are not readily available currently in our hospitals. Hence citration and analysis of samples collected are carried out after storage and recalcification to obtain reliable TEG parameter values. TEG assay of citrated blood samples exhibit variability and are unstable for a period between 0-30 minutes after citration. The range provided by the manufacturer is wide, makes no allowance for age or sex, and may need to be modified because of local variations in practice. Rajwal et al[101] found that there was significant difference between TEG parameters for fresh native whole blood and citrated whole blood and recommended establishment of a specific range for citrated whole blood for usage in clinical practice. Dormandy et al[102] suggested a formal standard operating procedure for citrated whole blood TEG (Figure.1.4.5) that takes in to consideration the initial period of instability. Camenzind et al[103]looking in to the effects of citrate storage on TEG showed that the parameters were different in recalcified, citrated blood samples compared with native blood. The observed changes were progressive in samples during 0-30 min. of storage but were stable thereafter and the authors recommend analysis after a citrate storage period between 1- 8 hours for reliable TEG results.

1.4.6 The Use of Heparinase in TEG (hepTEG)

This technique employs modified TEG cups containing heparinase-I, an enzyme obtained from *Flavobacterium heparinum* which selectively breaks down the GAGs, heparin and heparan-sulphate[11] to identify heparin-like activity in a given blood sample. R time represents such anticoagulant activity in a given sample of blood. Heparinase has been shown to neutralise heparin more effectively than protamine in blood samples obtained during cardiopulmonary bypass[104]. This method has been shown to be both sensitive and specific for the detection of changes due to heparin activity when compared with conventional tests of anti-Xa activity, activated partial thromboplastin time and activated coagulation time[105]. The technique is used in cardiac surgery to check the extent of heparin reversal by protamine in postoperative patients[106] and to detect increased endogenous heparin activity following orthotopic liver transplantation.[9]

SUMMARY OF INTRODUCTION

Consistent findings of activation of coagulation and impairment of fibrinolysis confirm a hypercoagulable state in patients with peripheral vascular disease. Whether this hypercoagulable state contributes to the formation and progression of PVD, or is merely a result or reflection of it remains unclear.

Atherosclerosis and inflammation along with contributions from activated platelets and defective endothelial function lead to formation of atherosclerotic plaques. Symptomatic PVD patients incur higher risk of cardiovascular morbidity, limb loss and death.

TEG technique though initially used as a research tool has now been proven to be a global test of coagulation that gives comprehensive and reliable information regarding the status of coagulation using a small volume of blood sample and is now routinely used to assess coagulation in patients undergoing cardiac surgery and liver transplantation.

AIMS OF THE THESIS AND PLAN OF INVESTIGATION

AIMS OF THE THESIS AND PLAN OF INVESTIGATION

The purpose of the research described in this thesis is:

1. To investigate changes in coagulation using TEG in healthy individuals undergoing elective surgical procedures and to obtain reference TEG parameter values to act as controls.

2. To identify any effect of peripheral atherosclerosis and ischaemia on coagulation in patients with symptomatic PVD.

3. To identify and document any significant changes in coagulation in patients with symptomatic PVD undergoing treatment using TEG.

OVERVIEW OF INVESTIGATION

1. Validation and standardisation of TEG methods
2. Investigation of the effects of age, gender and anaesthesia on coagulation using TEG - control group study
3. Investigation of the effects of sampling site, peripheral atherosclerosis and peripheral ischaemia on TEG
4. Investigation of the thrombogenic potential of non-ionic contrast media in PVD using TEG.
5. Identification of endogenous heparin-like activity in PVD using TEG. Investigation of changes in coagulation changes in aortic aneurysmal disease using TEG.
6. Investigation of TEG changes in patients with PVD undergoing surgery.

CHAPTER 2

MATERIALS AND METHODS

CHAPTER 2 - MATERIALS AND METHODS

This project was approved by the local ethics committee and registered with the Oxford Radcliffe Hospitals Trust.

All patients and healthy controls were recruited at John Radcliffe Hospitals after an informed consent.

2.1 - VALIDATION AND STANDARDISATION OF TEG METHODS

2.1.1 Patients and Participants

15 patients undergoing abdominal aortic aneurysm repair or peripheral vascular surgery consented to participate in the study.

2.1.2 Blood Sampling

Manspeizer et al[107] in their study involving arterial and venous samples from 40 cardiac surgical patients, yielding 134 pairs for comparison demonstrated that when arterial blood samples were analysed using TEG, values reflecting stronger (larger maximum amplitude) and faster (shorter reaction time and K value, wider alpha angle) clot formation were obtained. However twenty-nine comparisons (control) were between arterial and arterial samples and were not significantly different. Thrombelastograph (TEG) values obtained from venous blood samples were different from values obtained from arterial blood samples in their study. The results suggested that users of TEG coagulation analysers should be consistent with the site of blood sampling given the potential differences obtained in cardiac surgery patients. Hence this

validation study sought to identify any such differences in the study group (patients with PVD) by obtaining peripheral venous blood samples (n=15) and radial artery samples (n=15) for analysis using TEG.

Radial artery samples were obtained by aspiration from an arterial line placed prior to commencement of surgery. The samples were obtained before and after administration of IV heparin 5000 IU. These samples were used for validation of heparinase coated cups and to confirm reversal of heparin effect by heparinase[108] during TEG analysis.

Camenzind et al[109], in their study involving citrated venous samples for TEG analysis showed that TEG parameters were found to be stable and reliable when analysed after a period of delay with citrate storage between 1-8 hours. Hsu et al[110] in their study involving orthopaedic patients and TEG showed evidence for activation of coagulation when a tourniquet was used to obtain peripheral venous samples for TEG analysis. Hence in this study, peripheral venous samples were obtained following venupuncture without application of a tourniquet were used for validation of TEG analysis. Blood samples were analysed after 1-2 hours of citrate storage using Thromboelastograpy(TEG) for this study as it was in the recommended time frame after citration, allowed collection of blood samples at closely timed sampling points and for logistical reasons including the fact that the TEG analyser was located far from where the samples were collected. To assess TEG assay variability, each sample analysed was divided in to 3 and each one assayed separately.

2.1.3 TEG Analysis

The TEG apparatus is ensured that its temperature is at 37°C. A plain plastic disposable cuvette or a cup is used for analysis of blood samples. Heparinase coated cups were used for samples collected after exposure to heparin administration. Once the cup or a cuvette is in place, recalcification of citrated samples is done by adding 20 microlitres of calcium chloride to the pipetted blood (360 microlitres) and a pin connected by a torsion wire which in turn is connected to a computer is lowered in to the sample and analysis is commenced and TEG trace starts. Five TEG parameters, R time, K time, Angle, MA and CI are used in this study.

2.1.4 Statistics

To assess TEG assay variability, TEG parameters values obtained (each sample which was analysed divided in to 3 and each one assayed separately) were tested for intra–assay variability and the reliability coefficients were also calculated.

Validation of the use of heparinase cups by TEG analysis of blood samples was done by comparing TEG parameter values obtained before and after heparin administration using Mann-Witney test.

2.2 - INVESTIGATION OF THE EFFECTS OF AGE, GENDER AND ANAESTHESIA ON COAGULATION USING TEG - CONTROL GROUP STUDY

2.2.1 Subjects

Subjects undergoing elective general surgical, orthopaedic and gynaecological procedures were recruited prospectively for this study. (Table 2.2.1)

Exclusion criteria included all patients with any malignancy, a known coagulation disorder, chronic liver disease, chronic renal failure, previous history of DVT, patients on OC pills, HRT, pregnancy and those with symptoms of peripheral vascular disease or an ABPI (Ankle/Brachial Pressure Index) of < 0.9.

The participants did not receive any exogenous heparin prior to surgery (day surgery procedures).

Subjects were recruited based on age, gender and menopausal status (n=50). Groups included 1) men aged 35-65 years (n=10), 2) men aged > 65 years (n=10), 3) pre-menopausal women aged 35-50 years (n=10), 4) postmenopausal women aged 50-65 years (n=10) and 5) women aged > 65 years (n=10).

Table 2.2.1 Demographic and Vascular Risk Factor Details of the Participants

Group (gender / age / yrs)	Men 35-65	Men > 65 yrs	Women 35-50yrs	Women 50-65 yrs	Women > 65 yrs
Number(n= 50)	10	10	10	10	10
Age(median/range)	43.6 +/- 6.3	71.1+/- 4.4	46.7 +/- 4.6	62 +/- 1.2	68.2 +/- 1.4
ABPI(Mean/SD)	1.1+/- 0.10	0.92 +/- 0.12	1.1+/- 0.24	1.0 +/-0.28	0.96 +/- 0.17
BMI (Kg/m2)	25.06 +/- 2.5	23.1+/- 2.4	23.4 +/- 3.6	22.2 +/- 2.2	23.9 +/- 2.7
DM	-	2	-	1	1
Hypertension	1	3	-	1	3
Hyperlipidaemia	2	2	-	1	2
Prev AMI	-	1	-	-	1
Prev CVA	-	1	-	-	1
Prev DVT	-	-	-	-	-
Abd.Aortic aneurysm	-	1	-	-	-
Antiplatelet use	2	4	-	1	3
Statin intake	2	2	-	2	3
Current smoking	2	1	1	2	-

Key: ABPI: Ankle/Brachial Pressure Index; BMI: Basal Metabolic Index; DM: diabetes mellitus; AMI: acute Myocardial infarction; CVA: cerebro vascular event; DVT: deep vein thrombosis. Hypertension defined as any blood pressure measurement above 140/80.

2.2.2 Blood Sampling

Peripheral venous blood samples were obtained at three different time points.

Time points for sampling were 1)10-15 minutes before induction of anaesthesia (epidural (n=10), general (n=30) or epidural+ general anaesthesia (n=10)), 2) 5-10 minutes after induction of anaesthesia (on table / before skin incision) and 3) at 20-30 minutes after start of surgery.

Samples were obtained from the ante-cubital vein without tourniquet to minimise activation of the coagulation cascade during sample collection.

Samples were analysed after a delay of 1-2 hours of citrate storage prior recalcification. 5 TEG parameters (R, K, Angle ($\propto$), MA and CI) were used in this study.

2.2.3 Statistical Methods

TEG values for samples obtained at baseline (pre-induction of anaesthesia), post anaesthesia (prior to commencement of surgery) and at 30 minutes of surgery were compared. To account for three sampling points, a Bonferroni-corrected value of $P<0.017$(obtained by dividing 0.05 by 3) was used to identify statistical significance. TEG data within groups were analysed using Wilcoxon Signed Rank Test. Significant differences in TEG parameters between groups was sought using Mann-Witney U test. Data are presented as mean +/- SD and all p values are two-tailed.

2.3 - INVESTIGATION OF THE EFFECTS OF SAMPLING SITE, PERIPHERAL ATHEROSCLEROSIS AND PERIPHERAL ISCHAEMIA ON TEG

2.3.1 Aim of Study

To measure changes in coagulability of blood as it circulates from the aorta through an ischaemic leg to the common femoral vein using Thromboelastography® (TEG).

To identify any relationship between changes in TEG parameters and angiographic severity of atherosclerotic disease in the intervening vessels.

To identify any relationship between changes in TEG parameters as blood flows from common femoral artery to vein and the degree of ischaemia in that limb.

2.3.2 Subjects

30 sequential patients over the age of 40yr who were undergoing transfemoral angiography for lower limb peripheral vascular disease via a retrograde femoral puncture and who agreed to participate in the study were recruited.

Demographic data, the coexistence of other disease and smoking habits were all recorded. The ankle/brachial pressure index (ABPI) in both legs was measured and that in the symptomatically less affected leg (see later) used as a measure of the severity of ischaemia for the purposes of this study. Details are given in Table 2.3.1.

All patients underwent duplex study of the lower limb veins bilaterally to rule out deep vein thrombosis (DVT) before angiography.

Exclusion criteria for participants included the presence of DVT, liver impairment, a known hypercoagulable state (excluding PAOD) and the presence of any malignancy. Any patients taking Warfarin had this medication stopped 3 days before angiography.

2.3.3 Blood Sampling and Analysis

Blood samples were obtained from the common femoral vein (Sample V) on the same side as the intended arterial puncture, by separate percutaneous puncture, after infiltration of the subcutaneous tissue with local anaesthetic (lignocaine 1%).

The radiology protocol dictated that this puncture was on the side of the less symptomatic leg. A sample was then taken from the ipsilateral common femoral artery (Sample A) via the angiography sheath and then from the abdominal aorta (Sample Ao) via the catheter prior to injection of contrast material.

Samples were analysed after a delay of 1-2 hours of citrate storage prior recalcification. 5 TEG parameters (R, K, Angle ($\propto$), MA and CI) were used in this study.

2.3.4 Assessment of Disease Severity

Arteriography findings were interpreted by a vascular radiologist blinded to the TEG results. Atherosclerotic disease in the aorto-iliac segment on the same side as the artery puncture was reported as maximum percentage (%)

diameter stenosis and graded as $<$ 50% (minimal), 50 – 70%(moderate), and $>$70%(severe). Infrainguinal disease was graded in 4 segments (Superficial femoral artery /popliteal artery/Trifurcation / Crural vessels) as $<$ 50% or $\geq$ 50% stenosis at any point. A score of 0 for $<$ 50% and 1 for $\geq$ 50% was given and the total score for infrainguinal disease calculated for the puncture side limb of each patient.

Table 2.3.1 Demographic and Risk Factor Details of Patients

Risk factors	**Number**
Anti hypertensive therapy	26/30
Diabetes	11/30
Smoking (Current)	16/30
Hyperlipidaemia	19/30
Previous AMI (Acute Myocardial Infarction)	7/30
Previous CVA (Cerebrovascular accident)	4/30
Previous DVT	2/30
Previous peripheral vascular surgery	13/30
Aortic Aneurysmal Disease	4/30
Anti-platelet therapy	12/30
Anticoagulant therapy	4/30
(Warfarin stopped 3 days prior to angiography)	
Symptoms	
Short distance claudication	7/30
Rest pain	9/30
Tissue loss	14/30
Mean ankle/brachial pressure indices (ABPI)	
Puncture side limb	0.74 ± 0.15
Opposite (more symptomatic) limb	0.50 ± 0.15
Angiographic severity of Iliac disease in the puncture side limb (%)	
Stenosis of ≥ 70% (severe)	8/30
Stenosis of 50-70% (moderate)	10/30
Stenosis of < 50% (minimal)	12/30
Total angiographic score for Infra-inguinal disease (Median ± SD)	2.00 ± 0.81

2.3.5 Statistical Methods

TEG and patient group data were analysed using Wilcoxon Signed Rank Test, Spearman`s correlation test and descriptive analyses (SPSS version 11.00) including mean and standard deviation values for the TEG parameters, and frequency of distribution of vascular risk factors.

2.4 - INVESTIGATION OF THE THROMBOGENIC POTENTIAL OF NON-IONIC CONTRAST MEDIA IN PVD USING TEG

2.4.1 Patients

30 sequential patients over the age of 40yr who were undergoing transfemoral angiography for lower limb peripheral vascular disease via a retrograde femoral puncture and who agreed to participate in the study were recruited. Details are given in Table 2.4.1.

Exclusion criteria for participants included the presence of liver impairment, a known hypercoagulable state including DVT (excluding PAOD) and the presence of any malignancy. All patients underwent duplex study of the lower limb veins bilaterally to rule out deep vein thrombosis (DVT) before angiography. Any patients taking Warfarin had this medication stopped 3 days before angiography.

2.4.2 Controls

30 age matched healthy control subjects who were admitted to undergo elective day surgery (details given in Table 2.2.1.) were also recruited to obtain peripheral venous samples for comparisons with the study group.

2.4.3 Blood Sampling

Blood samples were obtained from the abdominal aorta (Sample Ao) via the angiography catheter before heparin flush and contrast injection and within two minutes after injection of the last bolus of contrast medium. The contrast medium used was Iohexol, a non-ionic contrast medium (NICM) (Omnipaque

350,350mg iodine ml^{-1}, Nycomed Amersham, Chesham, UK) and the total volume ranged between 80 and120 ml.

Table 2.4.1 Demographic and Risk Factor Details of Patients

Risk factors	
Anti hypertensive therapy	26/30
Diabetes	11/30
Smoking (Current)	16/30
Hyperlipidaemia	19/30
Previous AMI (Acute Myocardial Infarction)	7/30
Previous CVA (Cerebrovascular accident)	4/30
Previous DVT	2/30
Previous peripheral vascular surgery	13/30
Aortic Aneurysmal Disease	4/30
Anti-platelet therapy	12/30
Anticoagulant therapy	4/30
(Warfarin stopped 3 days prior to angiography)	
Symptoms	
Short distance claudication	8/30
Rest pain	9/30
Tissue loss	13/30
Ankle/brachial pressure indices (ABPI)	
(Mean±SD)	
Puncture side limb	0.74 ± 0.15
Opposite (more symptomatic) limb	0.50 ± 0.15

2.4.4 Analysis of Blood Samples

Citrated tubes were used for storage of blood samples prior to analysis. Samples were analysed after 1-2 hours of citrate storage using Thromboelastograpy (TEG), to identify changes in the coagulability of blood samples.

Samples collected were analysed using heparinase-modified cups to counter any residual heparin activity in the blood from previous exogenous administration and heparin use during the angiography procedure itself.

5 TEG parameters (R, K, Angle ($\propto$), MA and CI) were used in this study.

2.4.5 Statistical Methods

TEG and patient group data were analysed using Wilcoxon Signed Rank Test, and descriptive analyses (SPSS version 11.00) including mean and standard deviation values for the TEG parameters, and frequency of distribution of vascular risk factors.

2.5 - IDENTIFICATION OF ENDOGENOUS HEPARIN-LIKE ACTIVITY IN PVD USING THROMBOELASTOGRAPHY

2.5.1 Objective

To measure endogenous heparin like activity in patients with PAOD and to identify its relationship to the severity of disease and to determine whether replacement of heparin restored coagulability to normal, if so, excluding AT deficiency rather than heparin deficiency as a cause of coagulation abnormalities.

2.5.2 Subjects

Patients

Sequential patients presenting to the vascular outpatient department with claudication or rest pain due to atherosclerotic disease were asked to participate. 14 patients with intermittent claudication and 14 patients with advanced peripheral vascular disease presenting with rest pain were recruited.

Controls

15 subjects over 45 years with no symptoms of PAOD and ABPI $>$ 0.9 and no history of cardiovascular / cerebrovascular disease or malignant disease were recruited from amongst general surgical patients.

Control and patient group demographic and vascular risk factor details are given in Table 2.5.1.

Part 1:

Native and heparinase modified Thromboelastography were performed on citrated peripheral venous samples to measure heparin-like anticoagulant activity.

Part 2:

Heparin equivalent to that found in control subjects was added to patient samples and baseline (non- heparinase modified) TEG repeated.

15 patients with symptoms of PAOD including short distance claudication (n=4) and rest pain (n=11), admitted for peripheral vascular reconstruction procedures, were also recruited.

No patient in any of the groups received any form of exogenous heparin or other anticoagulation agent prior to this study within at least the previous two weeks.

2.5.3 Blood Sampling

Samples were collected from the ante-cubital vein without tourniquet to avoid activation of the coagulation cascade. Samples were stored in citrated tubes for1-2 hours and were analysed after recalcification using both native and heparinase modified TEG, a method that gives better reproducibility than immediate analysis of fresh sample. 5 TEG parameters (R, K, Angle ($\propto$), MA and CI) were used in this study.

2.5.4 Statistical Methods

TEG data within groups were analysed using Wilcoxon Signed Rank Test and significant differences in TEG data in between groups sought using a Mann-Witney U test. Any relationship between TEG variables and subject variables were identified using Spearman`s correlation test. A p value of $<$ 0.05 was considered significant (two-tailed).

Table 2.5.1 Control and Patient Group Demographic and Vascular Risk Factor Details

Group	**Control**	**IC**	**RP**
Number of participants	15	14	14
Gender	2F/13M	4F/10M	2F/12M
Age (Median/Range)	60Yr (49, 74)	66Yr (56,80)	67.5 Yr (54,84)
Ankle/brachial pressure indices (Mean±SD)	1.04 ± 0.11	0.69 ± 0.09	0.45 ± 0.08
Anti hypertensive therapy	4/15	6/14	8/14
Diabetes	2/15	3/14	3/14
Smoking (Current)	3/15	4/14	2/14
Hyperlipidaemia (on treatment)	1/15	3/14	3/14
Previous MI (Myocardial Infarction)	0/15	0/14	1/14
Previous CVA (Cerebrovascular accident)	0/15	0/14	1/14
Previous history of DVT	0/15	0/14	0 /14
Previous peripheral vascular surgery	0/15	0/14	3/14
Aortic aneurysmal disease	0/15	0/14	1/14
Anti-platelet therapy	4/15	5/14	9/14

Key: IC - Intermittent Claudication; RP – Rest pain; DVT- Deep Vein Thrombosis; SD - standard deviation

Table 2.5.2 Demographic and Vascular Risk Factor Details of Patients with PAOD

Number of participants	n= 15
Gender	5F/10M
Age (Median/Range)	62Yr (56, 84)
Mean ankle/brachial pressure indices (ABPI)	0.56 $\pm$ 0.04
Anti hypertensive therapy	9/15
Diabetes	4/15
Smoking (Current)	2/15
Hyperlipidaemia (on treatment)	1/15
Previous AMI (Acute Myocardial Infarction)	2/15
Previous CVA (Cerebrovascular accident)	3/15
Previous history of DVT	0/15
Previous peripheral vascular surgery	3/15
Aortic aneurysmal disease	1/15
Anti-platelet therapy	8/15

2.6 - INVESTIGATION OF CHANGES IN COAGULATION CHANGES IN AORTIC ANEURYSMAL DISEASE USING TEG

2.6.1 Objective

To identify changes in coagulation during elective non-ruptured abdominal aortic aneurysm repair and in those undergoing reconstruction procedures for occlusive aortic disease using Thromboelastography (TEG)

2.6.2 Patients

30 sequential patients undergoing elective infra-renal abdominal aortic aneurysm repair (AAA group) and 14 patients who underwent reconstruction procedure for occlusive aortic disease (Occ group) and 30 age matched subjects (control group) undergoing elective day case surgical procedures who agreed to participate in the study were recruited(Tables 2.6.1, 2.6.2 and 2.6. 3). All patients underwent duplex study of the lower limb veins bilaterally to rule out deep vein thrombosis (DVT).

Exclusion criteria for participants included the presence of DVT, liver impairment, a known hypercoagulable state (excluding PAOD) and the presence of any malignancy. Any patients taking Warfarin had this medication stopped 3 days before surgery.

Demographic, clinical data, routine coagulation tests, platelet count, aortic cross-clamping time and any thrombotic event recorded. dose of aspirin in the 7 days prior to surgery time since last dose of SC Heparin, Anaesthetic

technique (GA, Epidural),Fluids given intravenously in the perioperative period, Transfusion of blood and blood products, Timing and dose of any intravenous heparin, Timing of operative stages (clamp time, duration of surgery etc) and Core body temperature.

Table 2.6.1 Vascular Risk Factors/ Control Group (n=30)

Gender	12F/18M
Age	60 ± 8.5 Yr
Mean ankle/brachial pressure indices (ABPI)	1.10 ± 0.11
Anti hypertensive therapy	4/30
Diabetes	2/30
Smoking (Current)	3/30
Hyperlipidaemia	3/30
Previous AMI (Acute Myocardial Infarction)	0/30
Previous CVA (Cerebrovascular accident)	0/30
Previous DVT	0/30
Previous peripheral vascular surgery	0/30
Aortic Aneurysmal Disease	0/30
Anti-platelet therapy	12/30
Anticoagulant therapy	0/30

Table 2.6.2 Vascular Risk Factors/ Aortic Occlusive Group (n=14)

Gender	4F/10M
Age (Median)	66.5 ± 6.9 Yr
Mean ankle/brachial pressure indices (ABPI)	0.69±0.9
Anti hypertensive therapy	6/14
Diabetes	3/14
Smoking (Current)	6/14
Hyperlipidaemia	6/14
Previous AMI (Acute Myocardial Infarction)	2/14
Previous CVA (Cerebrovascular accident)	1/14
Previous DVT	0/14
Previous peripheral vascular surgery	4/14
Aortic Aneurysmal Disease	0/14
Anti-platelet therapy	8/14
Anticoagulant therapy	0/14

Table 2.6.3 Vascular Risk Factors / Aortic Aneurysm Group (n=30)

Gender	5F/25M
Age (Median)	69.5 ± 6.0 Yr
Mean ankle/brachial pressure indices (ABPI)	0.89±0.2
Anti hypertensive therapy	16/30
Diabetes	6/30
Smoking (Current)	4/30
Hyperlipidaemia	6/30
Previous AMI (Acute Myocardial Infarction)	2/30
Previous CVA (Cerebrovascular accident)	1/30
Previous DVT	0/30
Previous peripheral vascular surgery	1/30
Anti-platelet therapy	18/30
Anticoagulant therapy	2/30

2.6.3 Blood Sampling and Analysis

Peripheral venous blood samples were collected 1) Before(S1) and after anaesthetic induction(S2), 2) Before aortic clamp release(S3) and 5 minutes after release of clamp(S4), 3) At the end of surgery(S5), 4) 1 Hour after arrival in intensive care unit(S6),5) 6 hours post operatively(S6), 6) 24 hours(S7), 3 days(S8), and (S9)7days postoperatively.

Peripheral venous samples collected in citrated tubes were used for storage of blood samples prior to analysis. Samples collected were analysed using heparinase-modified cups to counter any residual heparin activity in the blood from previous exogenous administration and heparin use during surgery. Native citrated whole blood TEG and heparinase modified TEG performed after storage of between 1-2 hours prior to analysis. 5 TEG parameters (R, K, Angle ($\propto$), MA and CI) were used in this study.

2.6.4 Statistical Methods

TEG and patient group data were analysed using Wilcoxon Signed Rank Test, and descriptive analyses (SPSS version 15.00) including mean and standard deviation values for the TEG parameters, and frequency of distribution of vascular risk factors.

2.7- INVESTIGATION OF TEG CHANGES IN PATIENTS WITH PVD UNDERGOING SURGERY

2.7.1 Objectives

This study looked at TEG detectable abnormalities of coagulation in patients with leg ischaemia presenting for surgery. Identification of any significant changes in coagulation during revascularization or amputation and investigation of any relationship between baseline TEG profile and any post-operative thrombotic event (MI, CVA, DVT, graft occlusion) and identification of any significant relationship between TEG changes during surgery and any post-operative thrombotic event was also carried out.

2.7.2 Patients

86 sequential patients undergoing peripheral vascular reconstruction and amputation for symptomatic PVD and 50 age matched subjects (control group) undergoing elective day case surgical procedures who agreed to participate in the study were recruited. Exclusion criteria for participants included the presence of DVT, liver impairment, a known hypercoagulable state (excluding PVD) and the presence of any malignancy. Any patients taking Warfarin had this medication stopped 3 days before surgery. Patient and control group details are given in Table 2.7.1. and 2.7.2.

2.7.2.1. Initial assessment

Clinical data, baseline ECG, ankle/brachial pressure index (severity of ischaemia) blood sample for TEG, FBC, haematocrit estimations were

obtained. Duplex ultrasound study of deep veins both legs to assess preoperative state of deep veins (ie +/-DVT) was completed in all patients.

2.7.3 Blood Sample Collection and Analysis

Blood samples (6 ml from arm vein or arterial line (intraoperatively) as it is easily accessible, least traumatic and shown in the earlier validation experiments to be not significantly different to venous samples) were obtained for TEG, platelet count and haematocrit measurements. Samples were obtained immediately before and after anaesthetic induction, immediately before and at the end of arterial clamping, 5 minutes after release of clamps (in the case of vascular reconstruction), at the end of surgery, 30 minutes after surgery, at 6 hours after surgery, 24 hours after surgery, 3 days after surgery, 7 days after surgery and 6 weeks after surgery.

Samples were analysed after a delay of 1-2 hours of citrate storage prior recalcification. 5 TEG parameters (R, K, Angle ($\propto$), MA and CI) were used in this study.

2.7.4 Operative Variables

Dose of aspirin in the 7 days prior to surgery; time since last dose of sc Heparin; anaesthetic technique (GA (drugs recorded), Epidural); fluids given intravenously in the perioperative period (including blood and blood products); timing and dose of any intravenous heparin; timing of operative stages (initial dissection, clamping etc); nature of any implant.

2.7.5 Detection of Thrombotic Events

Bilateral leg vein Duplex study 3 and 7days and 6 weeks post operatively to detect DVT and 12 lead ECG in theatre recovery with serum troponin measurement if any clinical or ECG evidence of new ischaemia, 12 lead ECG prior to discharge and clinical assessment, 12 lead ECG at 6 week follow up were done to record any evidence of myocardial ischaemia following surgery. Occurrence of stroke was recorded after clinical assessment during postoperative period and at 6 week follow-up after surgery. Duplex scans were done at 7 days and 6 weeks post operatively to pick up graft occlusion following surgery.

2.7.6 Statistics

TEG and patient group data were analysed using Wilcoxon Signed Rank Test, and descriptive analyses (SPSS version 11.00) including mean and standard deviation values for the TEG parameters, and frequency of distribution of vascular risk factors.

Any relationship between TEG variables and subject variables were identified using Spearman`s correlation test. A p value of < 0.05 was considered significant (two-tailed).

Table 2.7.1 Vascular Risk Factor Details of Patient Group (n=86)

Gender (M / F)	62/24
Age	70.1 +/- 8.4
Risk Factors	
Anti hypertensive therapy	74/86
Diabetes	36/86
Smoking (current)	28/86
Hyperlipidaemia	55/86
Previous AMI (Acute Myocardial Infarction)	21/86
Previous CVA (Cerebrovascular accident)	16/86
Previous DVT	5/86
Previous peripheral vascular surgery	10/86
Aortic Aneurysmal Disease	12/86
Anti-platelet therapy	67/86
Anticoagulant therapy	5/86
Symptoms	
Short distance claudication	16/86
Rest pain	31/86

Table 2.7.2 Risk Factor Details of Control Group (n=50)

Gender	22F/28M
Age	60-85
Mean ankle/brachial pressure indices (ABPI)	1.10-0.11
Anti hypertensive therapy	7/50
Diabetes	2/50
Smoking (current)	3/50
Hyperlipidaemia	4/50
Previous AMI (Acute Myocardial Infarction)	0/50
Previous CVA (Cerebrovascular accident)	0/50
Previous DVT	0/50
Previous peripheral vascular surgery	0/50
Aortic Aneurysmal Disease	0/50
Anti-platelet therapy	14/50
Anticoagulant therapy	0/50

CHAPTER 3

RESULTS

CHAPTER 3 – RESULTS

3.1 - VALIDATION AND STANDARDISATION OF TEG METHODS

3.1.1 Reliability of Citrate TEG Method

TEG parameters were found to be stable and reliable when analysed after a period of delay with citrate storage between 1-8 hours. To assess TEG assay variability, 15 peripheral venous blood samples collected from patients with vascular disease undergoing abdominal aortic aneurysm/peripheral vascular reconstruction procedures. Citrated tubes were used for storage of blood samples prior to analysis. Samples were analysed after a delay of 1-2 hours of citrate storage prior recalcification to obtain reliable results. Each sample was analysed divided in to 3 and each one assayed separately. The intra–assay variability was excellent. (p= NS) and the reliability coefficients (Alpha) were greater than 0.9 for all parameters studied(R time, K time, Angle, MA and CI). Results of this part of the validation study are given in Tables 3.1.1 to 3.1.10.

3.1.2 Validation of Heparinase Modified TEG

Validation of the use of heparinase cups for TEG analysis of blood samples was again done using the samples obtained from 15 patients undergoing aortic aneurysm repair or peripheral vascular reconstruction procedures. The samples were taken from a radial artery line before and 2 minutes after heparin (5000 I.U) administration via a central vein. No significant differences

(Mann-Witney) in TEG parameters between samples analysed before and after heparin administration using heparinise coated cups were found thus confirming complete reversal of exogenous heparin. Results are given in Tables. 3.1.11 to 3.1.17.

VALIDATION OF CITRATE TEG AND HEPARINASE MODIFIED TEG METHODS

Table 3.1.1 Intra-assay results -citrated (n=15) - TEG parameter- R time

R1	R2	R3
13.00	13.40	13.00
11.50	11.40	11.50
6.00	6.00	6.00
9.10	9.10	9.10
9.40	9.40	9.40
11.00	11.10	11.00
9.00	9.00	9.10
9.50	9.00	9.50
8.40	8.40	8.40
9.80	9.80	9.80
7.50	7.50	7.50
12.00	11.80	12.00
8.40	8.40	8.40
8.50	8.40	8.50
9.40	9.30	9.40

Table 3.1.2 Intra-assay results –citrated - TEG parameter- K time (K)

K1	K2	K3
3.00	3.00	3.20
4.90	4.80	4.90
2.80	2.80	2.80
2.40	2.40	2.40
5.50	5.50	5.50
3.00	2.80	2.80
2.00	2.00	2.00
6.00	6.00	5.80
3.00	3.00	3.00
2.80	2.50	2.50
3.80	3.80	3.80
5.10	5.10	5.10
4.00	4.00	4.10
2.00	2.00	2.00
2.70	2.70	2.80

Table 3.1.3 Intra-assay results -citrated TEG parameter- Angle (A)

A1	A2	A3
55.00	55.00	55.00
52.50	52.50	52.50
64.00	63.80	64.00
52.70	52.70	53.00
63.00	63.40	63.40
44.40	44.40	44.40
62.50	62.50	62.50
65.60	65.60	65.60
55.50	55.50	55.50
48.00	49.00	48.00
46.00	46.00	46.00
43.00	44.00	43.00
52.00	52.10	52.00
44.00	45.00	44.00
56.00	56.00	56.00

Table 3.1.4 Intra-assay results -citrated- TEG parameter- Maximal Amplitude (MA)

MA1	MA2	MA3
50.80	50.80	52.00
57.70	57.70	57.70
54.60	54.60	54.60
59.00	58.00	58.00
49.00	48.00	49.00
61.50	61.50	61.50
46.60	47.40	46.60
55.00	55.00	55.00
63.50	63.50	63.50
56.00	56.20	56.00
54.00	54.00	54.00
53.00	53.00	53.10
55.00	55.00	56.00
54.60	54.60	54.60
50.80	50.80	50.80

Table 3.1.5 Intra-assay results -citrated samples- TEG parameter- Coagulation Index (CI)

CI1	CI2	CI3
-1.07	-1.07	-1.07
.53	.53	.53
1.05	1.05	1.05
1.12	1.12	1.12
-.64	-.64	-.64
1.44	1.44	1.44
-.98	-.98	-.98
.28	.28	.28
2.15	2.15	2.15
.73	.73	.73
1.04	1.04	1.04
-.13	-.13	-.13
.84	.84	.84
.91	.91	.91
-.22	-.22	-.22

SUMMARY OF RESULTS FOR INTRA- ASSAY TESTS

Table 3.1.6 Intra- assay TEG parameter values- R time

	R1	R2	R3
n =	15	15	15
Mean	9.5000	9.4667	9.5067
Std. Deviation	1.79165	1.83874	1.78984
Variance	3.210	3.381	3.204
Range	7.00	7.40	7.00
Reliability(Alpha)	.949		

Table 3.1.7 Intra- assay TEG parameter values- K time

	K1	K2	K3
n =	15	15	15
Mean	3.5333	3.4933	3.5133
Std. Deviation	1.28656	1.30026	1.27552
Variance	1.655	1.691	1.627
Range	4.00	4.00	3.80
Reliability(Alpha)	.932		

Table 3.1.8 Intra- assay TEG parameter values- Angle

	A1	A2	A3
n =	15	15	15
Mean	53.6133	53.8333	53.6600
Std. Deviation	7.62476	7.40537	7.65831
Variance	58.137	54.840	58.650
Range	22.60	21.60	22.60
Reliability(Alpha)	.944		

Table 3.1.9 Intra- assay TEG parameter values Maximal Amplitude (MA)

	MA1	MA2	MA3
n =	15	15	15
Mean	54.7400	54.6733	54.8267
Std. Deviation	4.51138	4.45605	4.39537
Variance	20.353	19.856	19.319
Range	16.90	16.10	16.90
Reliability (Alpha)	0.902		

Table 3.1.10 Intra- assay TEG parameter values Coagulation Index (CI)

	CI1	CI2	CI3
n =	15	15	15
Mean	0.4706	0.4890	0.4766
Std. Deviation	0.91986	0.91386	0.92006
Variance	0.846	0.859	0.846
Range	3.22	3.29	3.18
Reliability(Alpha)	0.921		

Results of Validation of Heparinase Modified TEG Method

Table 3.1.11 Native TEG (before addition of heparin) and Heparinase modified TEG (after addition of heparin) - R time

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of R is the same across categories of heparin.	Independent-Samples Mann-Whitney U Test	.618	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 3.1.12 Native TEG (before addition of heparin) and Heparinase modified TEG (after addition of heparin) - K time

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of K is the same across categories of heparin.	Independent-Samples Mann-Whitney U Test	.632	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 3.1.13 Native TEG (before addition of heparin) and Heparinase modified TEG (after addition of heparin) – Angle

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of A is the same across categories of heparin.	Independent-Samples Mann-Whitney U Test	.884	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 3.1.14 Native TEG (before addition of heparin) and Heparinase modified TEG (after addition of heparin) – Maximal Amplitude (MA)

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of MA is the same across categories of heparin.	Independent-Samples Mann-Whitney U Test	.803	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 3.1.15 Native TEG (before addition of heparin) and Heparinase modified TEG (after addition of heparin) – coagulation Index

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of CI is the same across categories of heparin.	Independent-Samples Mann-Whitney U Test	.520	Retain the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 3.1.16 TEG Parameter values- Native TEG method

	R1	K1	A1	MA1	CI1
n=	15	15	15	15	15
Mean	9.1600	2.9867	56.8667	56.3467	.5986
Std. Deviation	1.68387	.87901	5.95107	6.39574	1.19367
Range	5.60	3.10	20.10	19.30	4.79

Key:
R1- R time before exposure to heparin
K1- K time before exposure to heparin
A1- Angle before exposure to heparin
MA1- Maximal Amplitude before exposure to heparin
CI1- Coagulation Index before exposure to heparin.

Table 3.1.17 TEG Parameter values- heparinase modified TEG method

	R2	K2	A2	MA2	CI2
n=	15	15	15	15	15
Mean	8.9000	3.2133	56.9067	56.7933	.8845
Std. Deviation	1.48276	1.05551	5.30316	6.23097	1.00841
Range	5.50	2.90	17.60	22.20	3.98

Key:
R2- R time after exposure to heparin
K2- K time after exposure to heparin
A2- Angle after exposure to heparin
MA2- Maximal amplitude after exposure to heparin
CI1- Coagulation Index after exposure to heparin.

3.2 - INVESTIGATION OF THE EFFECTS OF AGE, GENDER AND ANAESTHESIA ON COAGULATION USING TEG - CONTROL GROUP STUDY

Fifty patents were enrolled in the study. Age, gender, BMI and vascular risk factor details are given in Table 2.2.1. No patient was on warfarin or received any form of an exogenous heparin in at least 2 weeks prior to participation in this study.

Baseline platelet, haematocrit, coagulation profile and fibrinogen level, renal function (creatinine), anaesthesia, type and volume of intravenous fluids administered during anaesthesia and surgical procedure details are given in Table 3.2.1.

Baseline (pre-induction of anaesthesia) routine haematological tests (Haemoglobin, Platelet count, Haematocrit, PT, aPTT and Fibrinogen levels) were within normal range and there were no significant differences between, within and in between men and women. There were no significant associations between the baseline tests, baseline TEG parameter values and BMI identified in this study.

TEG showed significant reduction in the K time values indicating more rapid clot formation in women compared to men (2.76 +/- 1.58 (Mean+/- SD), CI 2.43, 3.08 Vs 3.44 +/-1.20, CI 2.88, 4.00; p=0.024). This difference was due to a significant decrease in K time among pre-menopausal women. Amongst women TEG identified that K time was reduced significantly in pre-menopausal women when compared to post-menopausal women (2.39 +/-

0.77, CI 1.83, 2.94 Vs 3.42 +/- 0.28, CI 2.77, 4.06; p=0.009). No significant associations between BMI and baseline haematological tests and TEG parameters were identified in this study.

Induction of anaesthesia led to significant increases in Angle (or Alpha,α) (acceleration of fibrin build up and cross-linking (clot strengthening) pre-induction(56.23 +/-0.89, CI 54.42, 58.04 Vs post induction (58.21+/-0.83, CI 56.54, 59.88;p=0.003) and MA (ultimate clot strength) Pre-induction (56.40 +/- 0.79 CI 54.80, 58.01) Vs post induction (59.36 +/- 0.89, CI 57.56, 61.15; p=0.003) leading to a significant increase in the overall coagulation status CI (Coagulation Index)Pre induction (0.71 +/- 0.14, CI 0.42, 1.00) Vs post induction (1.35 +/- 0.17, CI 1.00, 1.69;p=0.003) of blood. These TEG parameter values are given Table.3.2.2. Changes in TEG during anaesthesia and surgery are presented in Figures.3.2.1 and 3.2.2.

No significant differences in coagulation were seen between individual groups who underwent different modalities of anaesthesia (general, epidural and general + epidural anaesthesia).

There was a significant further increase in Angle, (post induction 58.21+/-0.83, CI 56.54, 59.88) Vs at 30 minutes of surgery (60.98 +/- 0.69, CI 59.59, 62.37; p=0.001) that suggested further activation of fibrin build-up and polymerisation observed after induction of anaesthesia during surgery. No significant associations between IV fluid type (0.9% Normal saline and Hartmann`s) or the volume (145+/-20 ml (mean/SD), CI 70+/-50, 180+/-50

ml) and rates (50-120/hr) at which they were administered and TEG parameters were found in this study.

Among demographic vascular risk factors no significant associations were found between the use of low dose aspirin (n=9), statin intake (n=6) or any anti-hypertensive therapy (n=7) and TEG parameters were found.

Table 3.2.1 Baseline Haematological, Anaesthesia and Surgical Procedure Details (n=50)

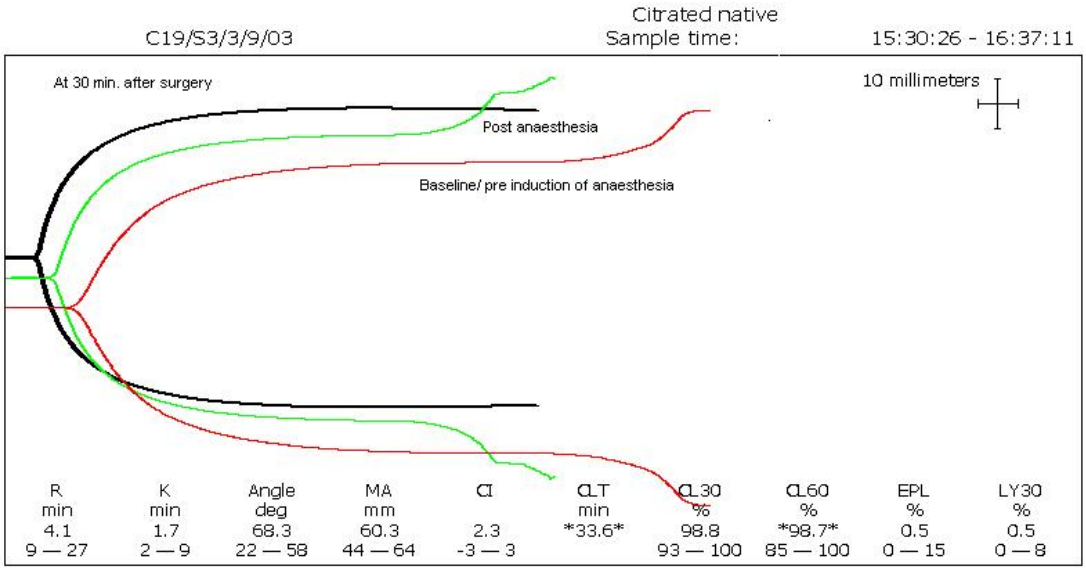
Group	Men 35-65 (n=10)	Men > 65 yr (n=10)	Women 35-50yr (n=10)	Women 50-65yr (n=10)	Women > 65 yr (n=10)
Haematology Haematocrit	0.42+/-.03 (0.40,0.45)	0.42+/-.04 (0.39,0.45)	0.41+/-.02 (0.39,0.44)	0.41+/-.04 (0.38,0.42)	0.43+/-.03 (0.41,0.45)
Platelet count	229+/-59 (187,272)	271+/-47 (236, 305)	239+/- 54 (200,278)	238+/-70 (188, 288)	267+/-67 (219, 315)
PT	10.94+/-0.12 (10.88,11.02)	10.46+/-0.47 (10.14,10.82)	10.71+/-0.42 (10.71,11.01)	10.72+/-0.34 (10.48,10.97)	10.70+/-0.29 (10.49,10.91)
aPTT	30.10+/-3.44 (27.63,32.56)	29.50+/-4.11 (26.55,32.44)	30.30+/-3.56 (27.75,32.84)	30.00+/-3.94 (27.17,32.82)	28.00+/-2.99 (26.75,31.04)
Fibrinogen	241.60+/-29.30 (228.11,284.69)	251.10+/-41.10 (228.11,284.69)	239.50+/-24.65 (281.33,357.67)	247.50+/-23.60 (248.99,318.41)	257.80+/-24.65 (239.98,321.02)
Creatinine	94.04+/-28.03 (68.06,86.06)	96.04+/-35.03 (66.02,73.05)	99.04+/-29.02 (72.00,88.02)	92.04+/-28.03 (63.06,80.04)	94.14+/-32.08 (66.02,83.06)
Laparoscopic .Cholecystectomy (n=9)	2	2	3	2	-
Open hernia repair(n=11)	4	3	-	2	2
Lump excision (n=6)	2	1	-	2	1
Urological procedures (n=8	2	3	-	1	2
Gynaecological procedures (n=5)	-	-	2	1	2
Thyroid surgery (n=2)			1	1	-
Colorectal surgery (n=3)	2		-	1	
ENT surgery (n=2)	1		-	1	
Orthopaedic surgery (n=4)		2		1	1
Anaesthesia Epidural (n=10)	2	1	3	2	2
General (n=30)	6	7	5	6	6
Epidural+ General (n=10)	2	4	-	1	3

Table 3.2.2 TEG (Citrated, Native) Values at Baseline, Post Induction of Anaesthesia and at 30 Minutes after Commencement of Surgery

TEG parameter	Baseline	Post induction of anaesthesia	At 30 minutes after start of surgery	Baseline Vs Post induction of anaesthesia (p value)	Post induction of anaesthesia Vs 30 minutes after start of surgery (p value)
R time(minutes)	9.38+/-1.70	8.58+/-1.82	8.19+/-1.92	0.046	0.102
K time (minutes)	3.27+/-1.11	2.96+/-1.05	2.57+/-0.94	0.078	0.142
Angle(α) (degrees)	56.23+/-6.35	58.21+/-5.87	60.98+/-4.88	0.003	0.001
MA (mm)	56.40+/-5.64	59.36+/-6.32	62.99+/-8.48	0.003	0.022
CI	0.71+/-1.02	1.35+/-1.21	1.97+/-1.55	0.003	0.018

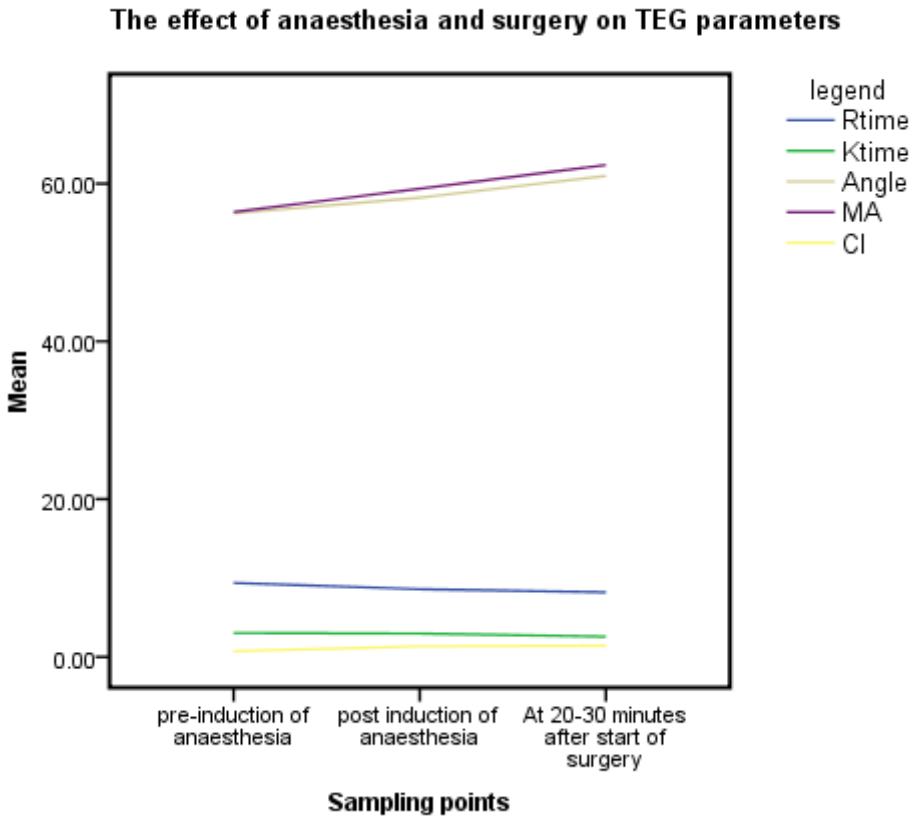
(Key: p values <0.017 considered significant (after Bonferroni correction) as multiple comparisons were made between groups and sampling points in this study.)

Figure 3.2.1 Citrated Whole Blood TEG Showing Changes in Coagulation during Surgery



Key: R, Reaction time (time to initial fibrin formation); *K*, dynamics of clot formation; *Angle*, acceleration of fibrin build up and cross-linking (clot strengthening); *MA*, maximum amplitude (ultimate clot strength); *CI*, coagulation index (overall coagulation status); *LY30*, percent lysis at 30 minutes after MA; *LY60*, percent lysis at 60 minutes after MA; *G*, clot firmness as shear elastic modulus; *EPL*, estimated percent lysis; *A*, current amplitude

Figure 3.2.2 TEG Changes during Anaesthesia and Surgery



3.3 - INVESTIGATION OF THE EFFECTS OF SAMPLING SITE, PERIPHERAL ATHEROSCLEROSIS AND PERIPHERAL ISCHAEMIA ON TEG

The 30 patients (median age 72 Years ± 9.6, 21male/9 female) in this study underwent transfemoral angiography for a range of ischaemic symptoms including intermittent claudication (8), rest pain (9) and ischaemic tissue loss (13). Further details are given in Table. 2.2.1.

55% of common femoral arterial punctures were on the left side. Angiographic scoring for disease severity is given in Table. 2.2.1. An overview of sampling and TEG results were given in Figure.3.3.1.

When comparing common femoral artery samples with aortic samples (Table 3.3.1) there was a decrease in R (time to onset of clotting -reflecting platelet activity) ($p<0.05$) (Figure. 3.3.2), an increase in MA (clot strength) ($p<0.05$) (Figure.3.3.3) and an increase in CI (coagulation index)($p<0.002$)(Figure.3.3.4) consistent with an increase in coagulability due to both platelet activation and increased thrombin generation as blood flowed down the iliac arteries. These changes in TEG parameters also correlated with the severity of atherosclerotic disease in the ipsilateral aorto- iliac segment (ΔR, $r=0.442$,$p<0.05$ /Δ MA, $r=0.379$, $p<0.05$ / ΔCI $r=0.429$, $p<0.05$).

TEG parameters showed significant differences in R $p<0.05$), Angle (measure of clot development) ($p<0.05$), (MA) ($p<0.005$) and CI ($p<0.001$) between common femoral arterial and venous samples confirming that

venous samples were more coagulable in this group of patients, again due to increased platelet activation and thrombin production (Table 3.3.2.). This difference in coagulation between the arterial and venous ends of the circulation in the puncture side limb correlated inversely with degree of ischaemia (ABPI) on that side (ΔCI v ABPI r = -0.427 $p<0.05$, ΔMA v ABPI r =-0.370 $p<0.05$) (Figure 3.3.5. and Figure.3.3.6.). The change in MA across the limb circulation also correlated with the angiographic score for infra-inguinal disease (ΔMA r=0.459,$p<0.05$) (Figure.3.3.7.) but none of the other TEG parameters did so.

Table 3.3.1 TEG Changes and Aorto-Iliac Disease

	Mean difference between Aortic & Femoral samples (Mean/CI)	**Correlation with Iliac disease**(r, p)
R	-0.44 ± 3.1(-1.6,.72)	r = .443 p = 0.014
K	0.08 ± 1.67(-0.54,.7)	r = .151 p = 0.151
Angle	2.08 ± 12.3(-2.5,6.6)	r = .218 p = 0.248
MA	3.1 ± 9.97(-0.62,6.8)	r = .379 p = 0.039
CI	0.75 ± 1.42(0.22,1.2)	r = .429 p = 0.018

Figure 3.3.1 Overview of Sampling Sites and TEG Results

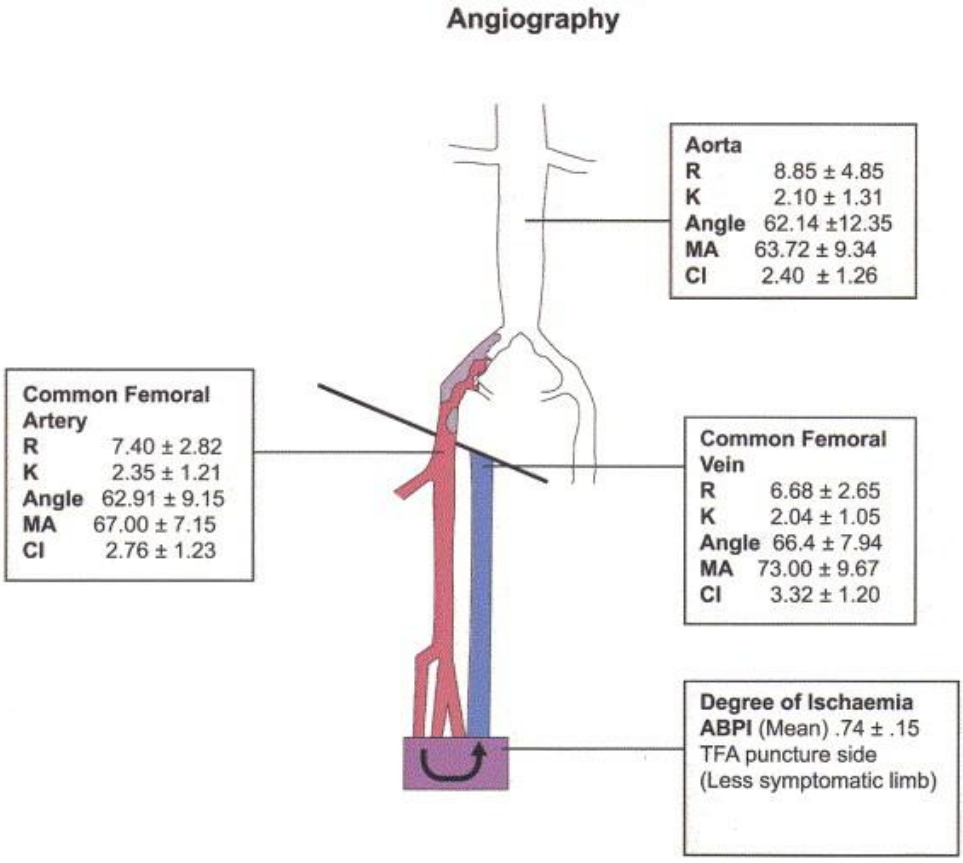


Figure 3.3.2 Severity of Iliac Disease and Change in Time to Fibrin Formation

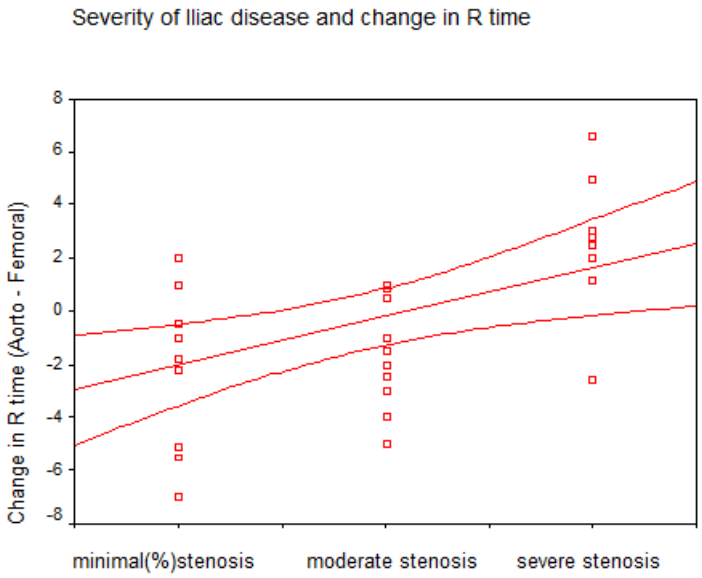


Figure 3.3.3 Severity of iliac Disease and Change in Ultimate Clot Strength

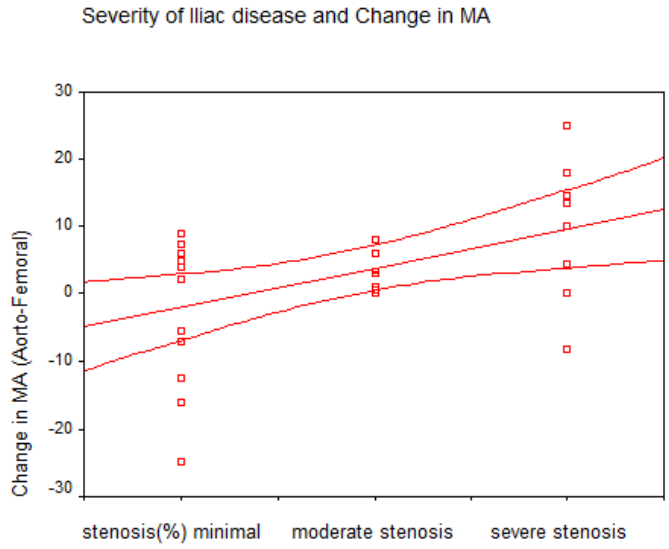


Figure 3.3.4 Severity of Iliac Disease and Change in Overall Coagulation Status

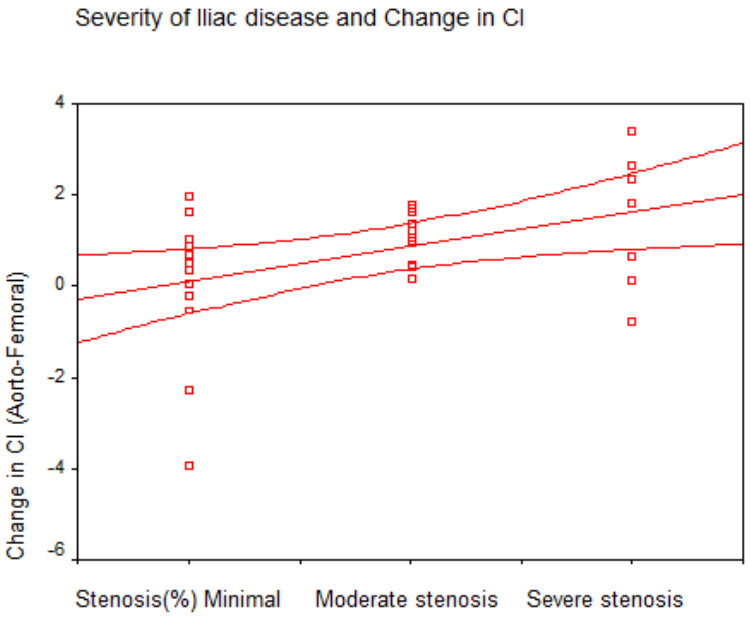


Table 3.3.2 Correlation between Changes in TEG Parameters between CFA and Vein and ABPI

	Mean difference across (A-V segment (Mean/CI)	**Correlation with ABPI** (r, p)
R	-0.91 ± 0.43 (-1.7, -.02)	r = -0.370 p=0.074
K	-0.30 ± 0.15 (-.62,.01)	r = 0.129 p=0.496
Angle	3.53 ± 1.49 (0.47,6.5)	r = -0.209 p=0.268
MA	3.81 ± 1.04 (1.6,5.9)	r = -0.370 p=0.044
CI	0.76 ± 0.19 (.36,1.16)	r = -0.427 p= 0.018

Figure 3.3.5 Relationship between Peripheral Ischaemia and Overall Coagulation Status

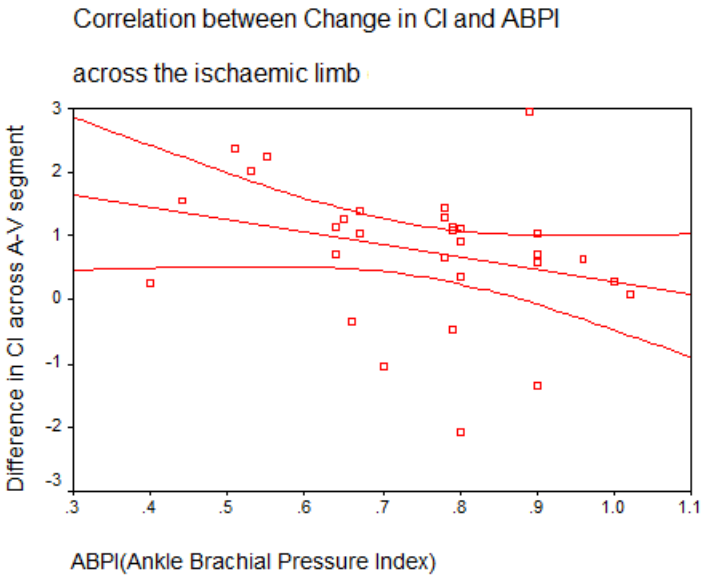


Figure 3.3.6 Relationship between Severity of Peripheral Ischaemia and Ultimate Clot Strength

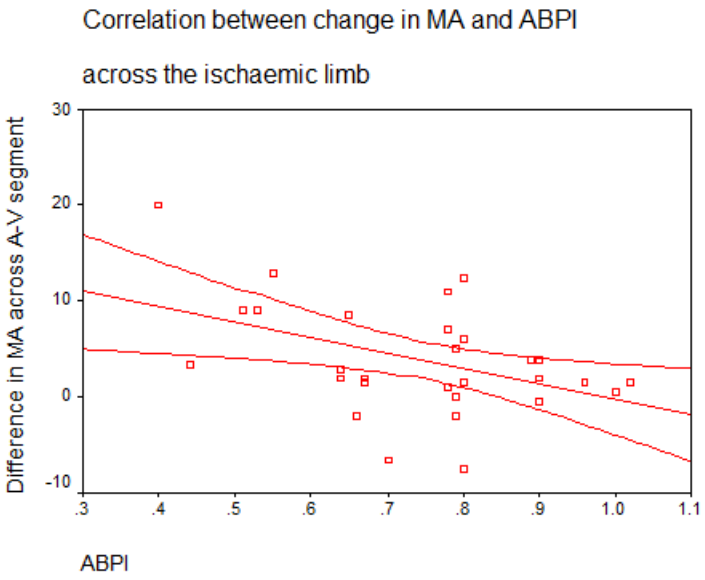
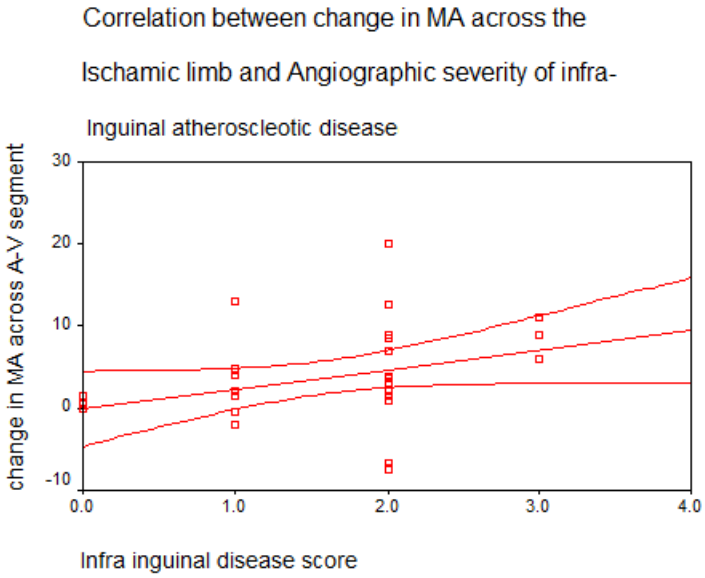


Figure 3.3.7 Angiographic Severity and its Relation to Change in Ultimate Clot Strength across Arterio-Venous Segment of an Ischaemic Limb



3.4 - INVESTIGATION OF THE THROMBOGENIC POTENTIAL OF NON-IONIC CONTRAST MEDIUM IN PVD USING TEG

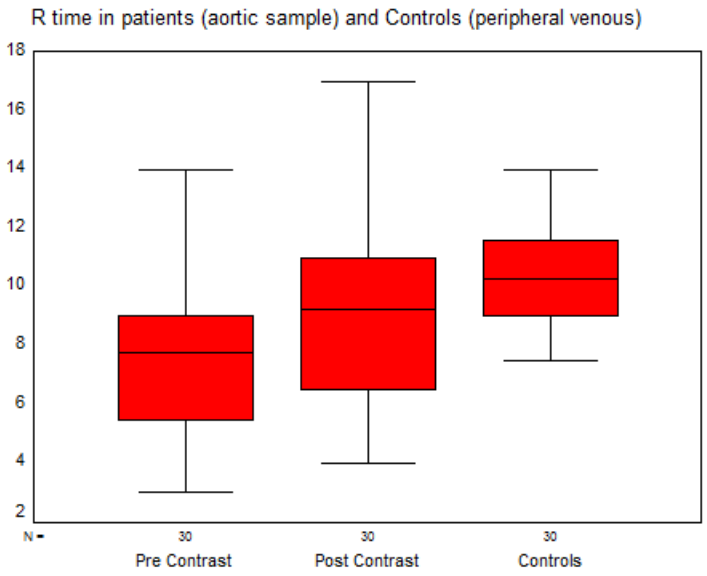
The 30 patients (median age 72 years (range 54-92) 21male/9 female) in this study underwent transfemoral angiography for a range of ischaemic symptoms including intermittent claudication (n=8), rest pain (n=9) and ischaemic tissue loss (n=13). More details of the patients are given in Table 2.4.1. 55% of common femoral arterial punctures were on the left side. Median age of control subjects was 62 years (range 50-82).

TEG tracings of samples taken from the aorta after injection of Iohexol showed a significant increase in R time (time to fibrin formation) (CI 7.8,10.18 minutes) (p=0.036), in K time (dynamics of clot formation) (CI 2.2,2.8 minutes) (p=0.028), and a reduction in Angle (decreased acceleration of fibrin build up) (CI 53.10,62.7 degrees) (p=0.013), Maximal amplitude (MA)(reduced ultimate clot strength) (CI 54.5,62.7 mm) (p=0.013) and Coagulation Index (CI) (decreased overall coagulation status) (0.31,1.95) (p=0.032), (Figure 3.4.2-6) demonstrating a significant reduction in the coagulability of aortic blood samples taken after the last bolus of contrast medium (NICM) but prior to any intervention procedure. This reduction in the coagulation status suggests that there is at least a temporary reduction in the coagulability of blood during angiography in patients with PAOD when a NICM (Iohexol) is used. Despite this reduction in the coagulability of aortic samples after exposure to NICM, patients with PAOD remain

hypercoagulable when compared to age matched controls whose results are also shown in Fig 2-6.

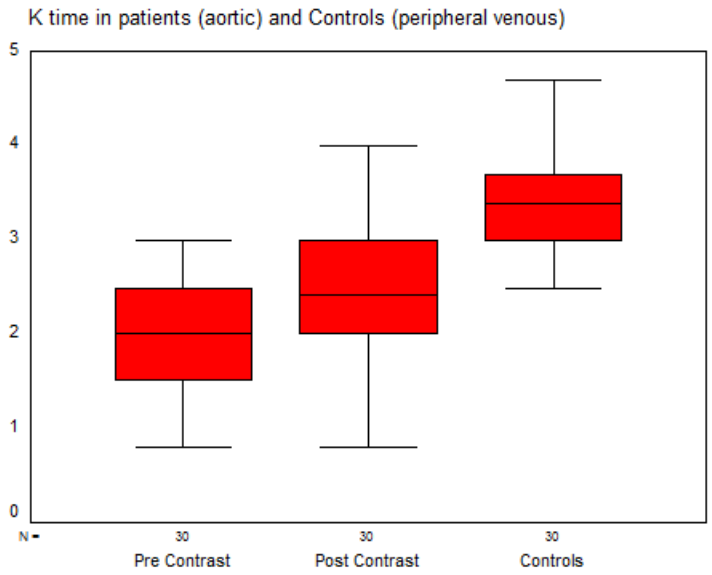
These changes in TEG parameters suggest that the local effect of NICM is a reduction of coagulation activity rather than the activation suggested by some previous studies and in patients with peripheral vascular disease iohexol, a NICM leads to no further increase in their blood coagulability during peripheral diagnostic angiography.

Figure 3.4.1 R time and NICM



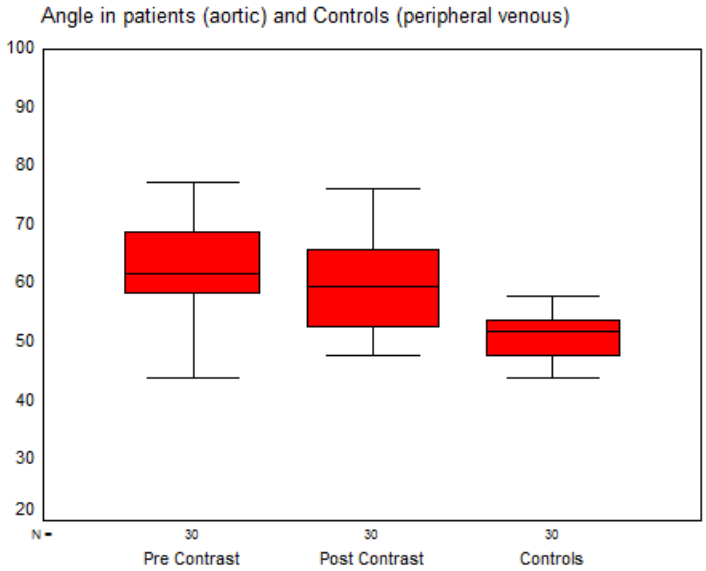
P values: Pre Vs Post NICM (p= 0.018); Post NICM Vs Controls (p=0.029)

Figure 3.4.2 K time and NICM



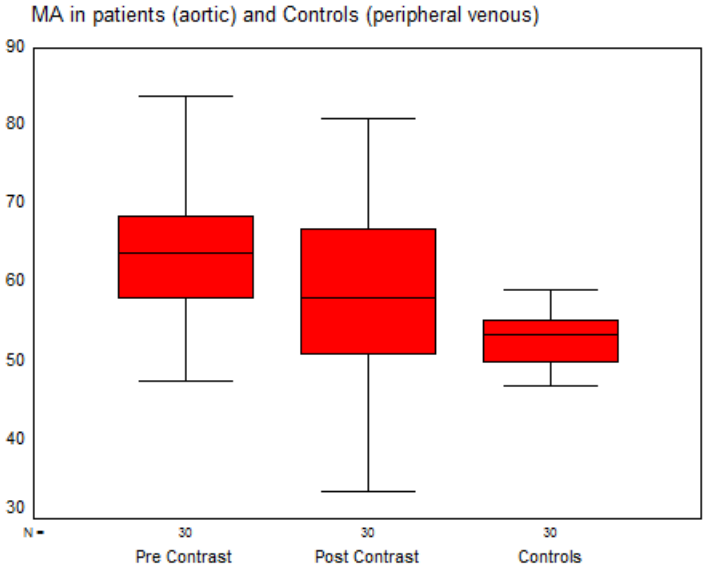
P values: Pre Vs Post NICM in patients (p= 0.028); Post NICM Vs Controls (p=0.001)

Figure 3.4.3 Angle and NICM



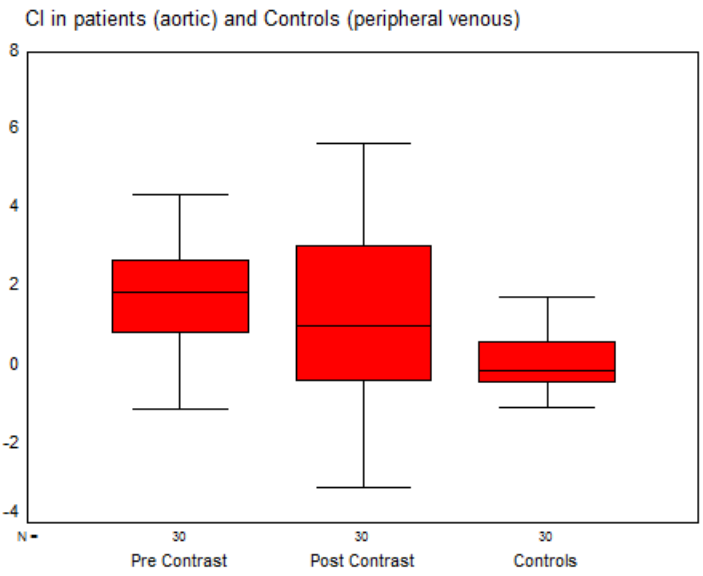
P values: Angle: Pre Vs Post NICM in patients (p= 0.013); Post NICM Vs Controls (p=0.003)

Figure 3.4.4 MA and NICM



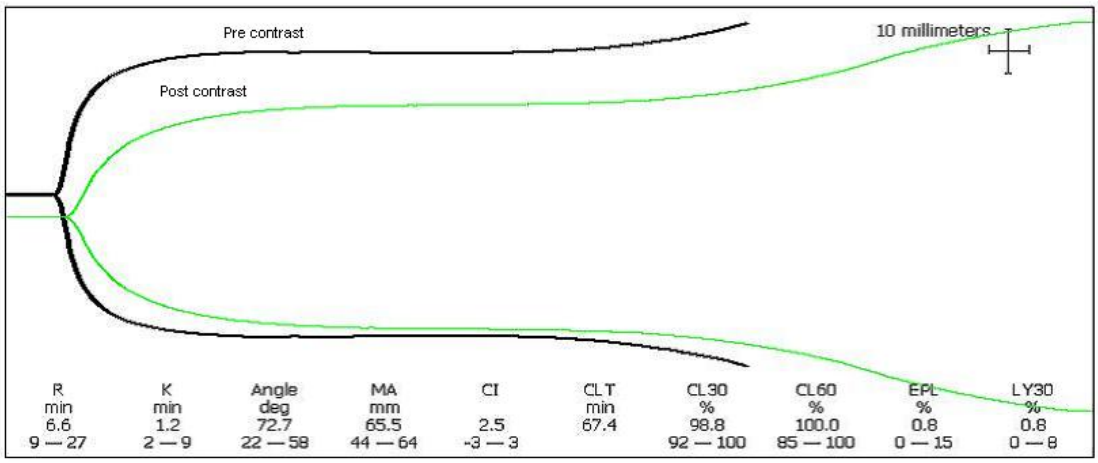
P values: MA: Pre Vs Post NICM in patients (p= 0.018) Post NICM Vs Controls (p=0.020)

Figure 3.4.5 CI and NICM



P values: CI: Pre Vs Post NICM in patients (p= 0.032); Post NICM Vs Controls (p=0.014)

Figure 3.4.6 Effect of Non-Ionic Contrast Medium (NICM) on Coagulability of Aortic Blood Samples Shown by TEG



Key: R, Reaction time (time to initial fibrin formation);*K*, dynamics of clot formation; *Angle*, acceleration of fibrin buildup and cross-linking (clot strengthening); *MA*, maximum amplitude (ultimate clot strength); *CI*, coagulation index (overall coagulation status); *LY30*, percent lysis at 30 minutes after MA;*LY60*, percent lysis at 60 minutes after MA; *G*, clot firmness as shear elastic modulus; *EPL*, estimated percent lysis; *A*, current amplitude

3.5 - IDENTIFICATION OF ENDOGENOUS HEPARIN-LIKE ACTIVITY IN PVD USING TEG

Part 1

ABPI measurements of patients and controls are shown in Tables 2.5.1 and 2.5.2.

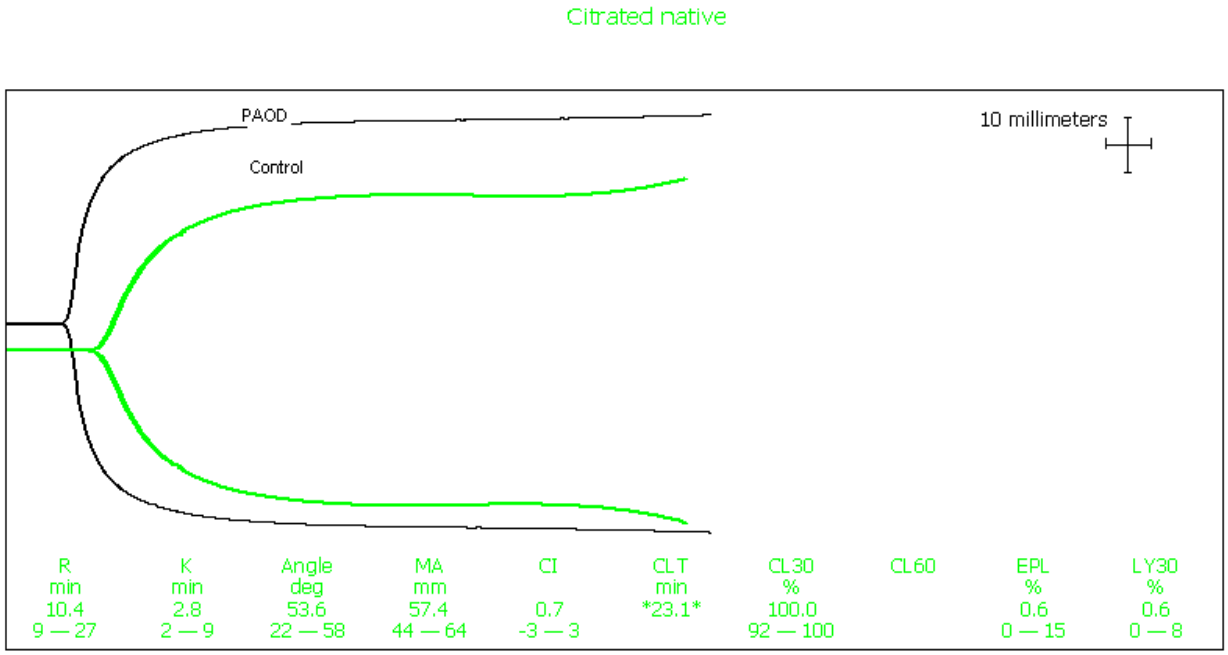
The baseline TEG parameters showed a significant increase in the coagulability of blood samples from patients with symptoms of PAOD compared to the control group (Figure3.5.1) (shortened R time (6.53 ± 0.40 min / CI 5.69,7.36 Vs 9.40 ± 0.44 min / CI 8.40,10.36 / p=0.004), K time (1.92± 0.15 min / CI 1.69,2.36 Vs 2.88 ± 0.16 min / CI 2.52,3.23 / p=0.011), increased Angle (64.88± 1.71 degrees / CI 61.37,68.39 Vs 56.23± 1.26 degrees / CI 53.51, 58.95 p=0.008), increased MA (66.80± 1.57 / CI 63.57,70.02 Vs 58.07± 1.28 / CI 55.30, 60. 83 / p=0.033) and increased CI (2.26 ± 0.24 / CI 1.76,2.77 Vs 1.06 ± 0.23 / CI 0.56,1.57 p=0.009).

In the control group heparinase modified TEG showed a significant decrease in the R time (9.40 ± 0.44 min / CI 8.40,10.36 Vs 8.62 ± 0.37 min / CI 7.82, 9.42 / p=0.002), K time (2.88 ± 0.16 min / CI 2.52,3.23 Vs 2.59 ± 0.11 min / CI 2.33,2.84 / p=0.021), Angle (56.23± 1.26 degrees / CI 53.51, 58.95 Vs 57.72± 1.25 degrees / CI 55.03, 60.41/ p=0.038) and CI (1.06 ± 0.23 / CI 0.56,1.57 Vs 1.20 ± 0.26 / CI 0.63, 1.78 p=0.011) (Table 3.5.3) when compared to the native TEG confirming heparinase-1 sensitive endogenous heparin-like activity (Figure 3.5.2).

The same method revealed a significant reduction in R time (7.50 ± 0.44 min / CI 6.54,8.46 Vs 7.17 ± 0.40 min / CI 6.30, 8.05 / p=0.041) suggesting the presence of a detectable heparinase-1 sensitive endogenous heparin-like activity, in patients with intermittent claudication but not in those with rest pain and tissue loss (Figure3.5.3). Tables 3.5.4, 3.5.5 and 3.5.6.

A significant negative relationship between change in the R time and the severity of disease (ABPI) (correlation coefficient: abpi: 1.000 / change in R time 0.350 / p=0.021) was identified in patients with PAOD suggesting reduced endogenous heparin-like activity with advanced peripheral vascular disease. (Figure 3.5.4)

Figure 3.5.1 TEG Comparison between Baseline Venous Sample from Control Subjects and Patients with PAOD



Key: R, Reaction time (time to initial fibrin formation);*K*, dynamics of clot formation; *Angle*, acceleration of fibrin buildup and cross-linking (clot strengthening); *MA*, maximum amplitude (ultimate clot strength); *CI*, coagulation index (overall coagulation status); *LY30*, percent lysis at 30 minutes after MA;*LY60*, percent lysis at 60 minutes after MA; *G*, clot firmness as shear elastic modulus; *EPL*, estimated percent lysis; *A*, current amplitude

Figure 3.5.2 Heparin-like Activity in Controls

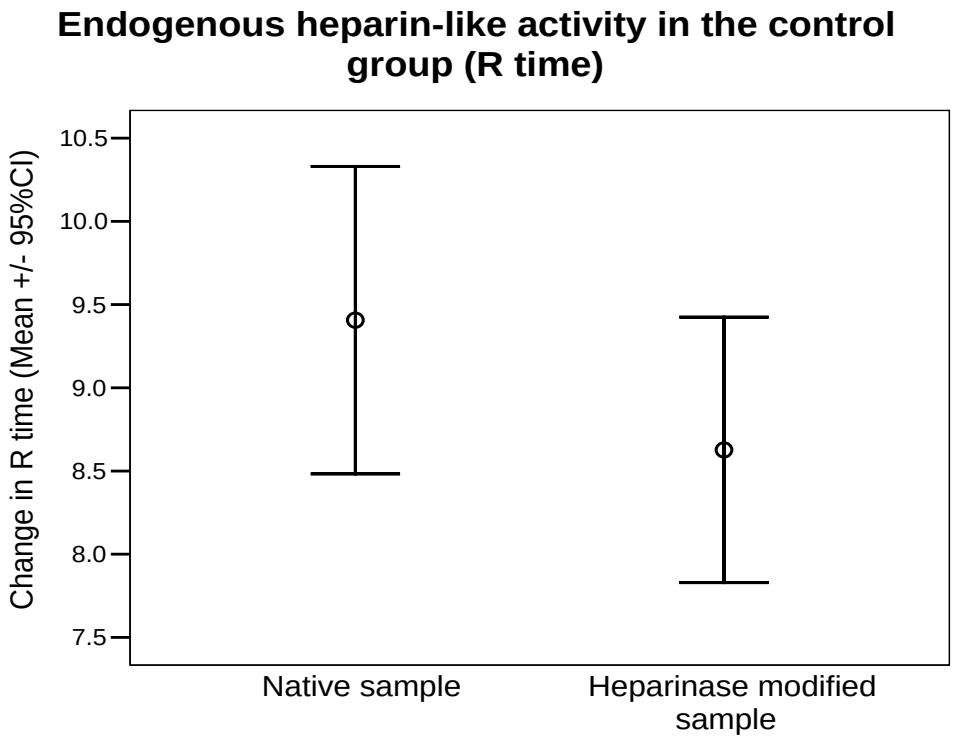


Figure 3.5.3 Endogenous Heparin-like Activity in Patients with PVD

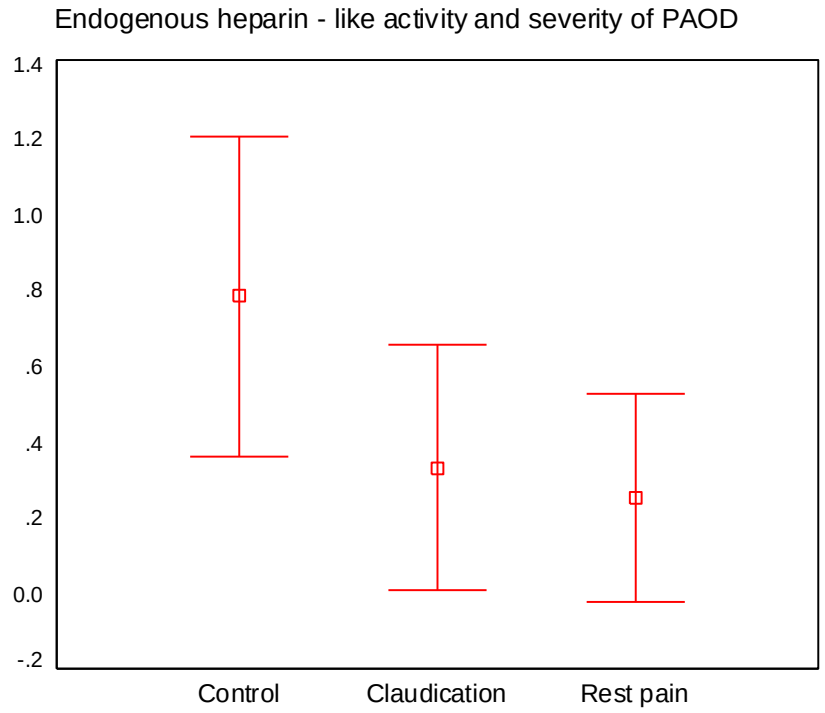
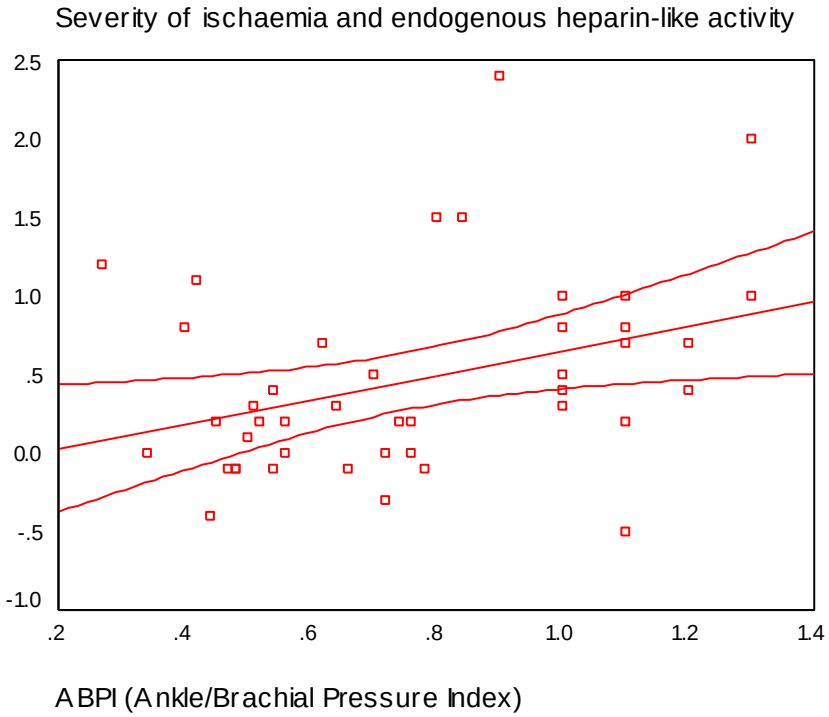


Figure 3.5.4 Relationship between Heparin-like Activity (R time) and Degree of Peripheral Ischaemia (ABPI)

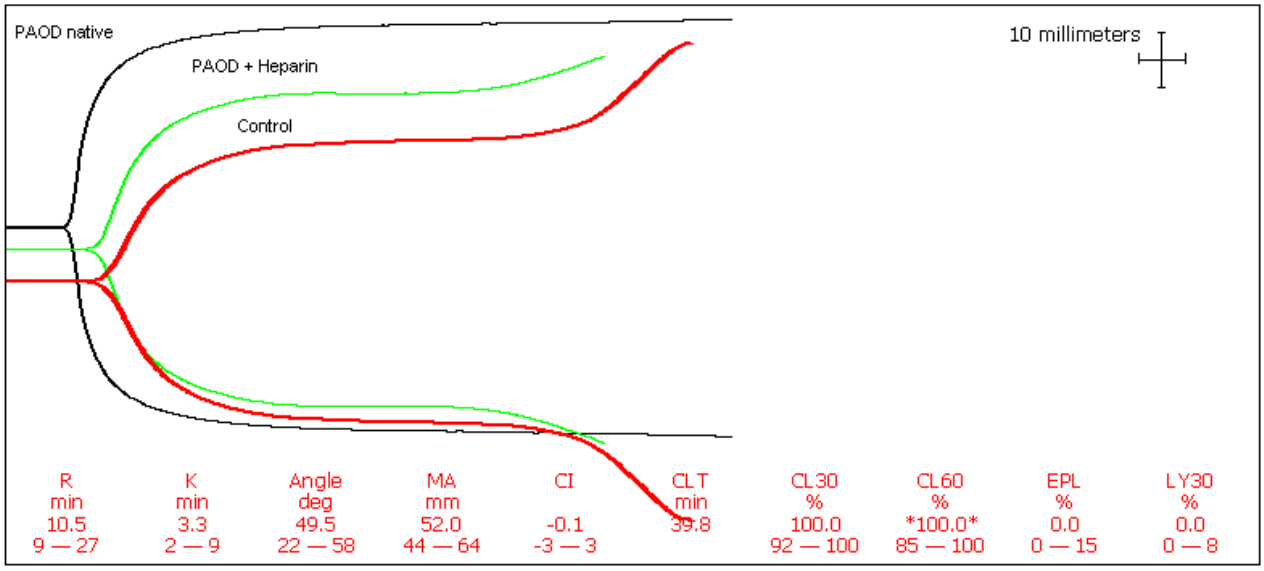


Part 2

When unfractionated heparin (0.1 I.U/ml) equivalent to the level of endogenous heparin found in normal human plasma[5], was added to the blood samples from patients with symptoms of PAOD, TEG showed significant increases in the R time (5.84 ± 0.39 min / CI 4.98,6.69 Vs 8.33 ± 0.72 min / CI 6.77, 9.89 / p=0.002) and K time (1.84 ± 0.17 min / CI 1.45,2.22 Vs 2.96 ± 0.36 min / CI 2.16, 3.75 p=0.001) and reductions in Angle (66.41± 2.00 degrees / CI 62.10, 70.72 Vs 58.12± 2.22 degrees / CI 53.35, 62.90/ p=0.001), MA (65.94± 2.42 degrees / CI 60.75, 71.14 Vs 61.11±2.45 / CI

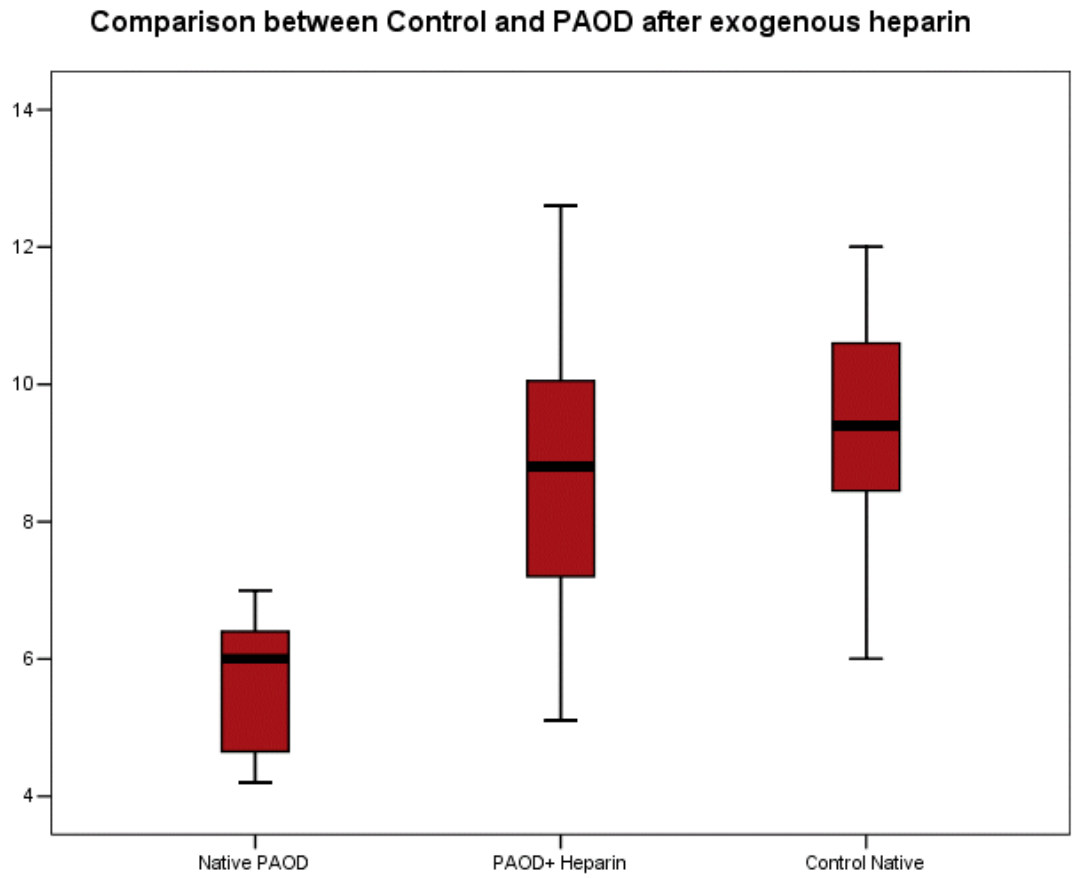
55.83, 66.38/ p=0.001) and CI (2.89 ± 0.32 / CI 2.19,3.59 Vs 1.72 ± 0.32 / CI 0.97, 2.47 p=0.001) (Table 7) restoring coagulability to that of control subjects (Figure 3.5.5).

Figure 3.5.5 TEG changes following addition of exogenous heparin (0.1 I.U/ml) to venous blood samples from patients with PAOD



Key: R, Reaction time (time to initial fibrin formation);*K*, dynamics of clot formation; *Angle*, acceleration of fibrin buildup and cross-linking (clot strengthening); *MA*, maximum amplitude (ultimate clot strength); *CI*, coagulation index (overall coagulation status); *LY30*, percent lysis at 30 minutes after MA;*LY60*, percent lysis at 60 minutes after MA; *G*, clot firmness as shear elastic modulus; *EPL*, estimated percent lysis; *A*, current amplitude

Figure 3.5.6 Heparin Effect in PVD



No significant effects of antiplatelet agents on the TEG parameters in any group and no significant difference in endogenous heparin-like activity on TEG in between those with evidence of coexistent systemic atherosclerotic disease (previous AMI/CVA) (n=7) compared to those without (n= 25). (Table 3.5.1 and 3.5.2) was identified amongst the study groups.

Table 3.5.1 Differences in TEG parameters between native and heparinase modified methods in the control group. (Mean ± SD/ CI)

TEG parameters	Native TEG	Heparinase modified TEG	Change in TEG from native to heparinase modified method	p value
R	9.40 ± 0.43 (8.48,10.33)	8.62 ± 0.37 (7.82,9.42)	0.78 ± 0.18 (0.39,1.16 min)	0.002
K	2.88 ± 0.16 (2.52,3.23)	2.59 ± 0.11 (2.33,2.84)	0.28 ± 0.11 (0.04, 0.52 min)	0.021
Angle(alpha)	56.23 ± 1.26 (53.51,58.95)	57.72 ± 1.25 (55.03,60.41)	-1.49 ± 0.65 (-2.90,-0.08 deg)	0.038
MA	58.07 ± 1.28 (55.30,60.83)	58.33 ± 1.35 (55.42,61.23)	-0.26 ± 0.43 (-1.18, 0.66 mm)	0.277
CI	1.06 ± 0.23 (0.56,1.57)	1.20 ± 0.26 (0.63,1.78)	-0.14 ± 0.06 (-0.274, -0.007)	0.011

(Key: R- Reaction time/ K- coagulation time/ Angle-acceleration of clot formation/ MA-Maximal Amplitude/ CI- Coagulation Index. SD-Standard deviation/ CI- 95%Confidence Interval)

Table 3.5.2 Heparin-like activity (R time) in study groups

R time (heparin like activity)	Control	Intermittent Claudication	Rest pain
Native TEG	9.40 ± 0.43 (8.48,10.33 min)	7.50 ± 0.44 (6.52,8.42 min)	5.55 ± 0.58 (4.29,6.81)
Heparinase modified TEG	8.62 ± 0.37 (7.82,9.42 min)	7.17 ± 0.40 (6.30,8.05 min)	5.30 ± 0.51 (4.18, 6.42)
Change in R time	0.78 ± 0.18 (0.39,1.16 min)	0.32 ± 0.156 (0.0041,0.653min)	0.250 ± 0.126 (-0.022,0.522)
p value	0.002	0.034	0.070

Table 3.5.3 Heparinase Sensitive Anticoagulant Activity in Study Groups

a) Intermittent claudication group

TEG parameters	Native TEG	Heparinase modified TEG	Change in TEG parameter.	p value
R	7.50 ± 0.44 (6.54,8.46)	7.17 ± 0.40 (6.30,8.05)	0.32 ± 0.15 (0.004,0.65)	0.034
K	2.14 ± 0.24 (1.61,2.66)	1.87 ± 0.24 (1.34,2.41)	-0.14 ± 0.69 (0.16,0.13)	0.255
Angle(alpha)	66.52 ± 2.71 (60.64,72.39)	65.47 ± 2.74 (59.54,71.40)	1.05 ± 0.81 (-0.70,2.80)	0.148
MA	64.17 ± 2.37 (59.04,69.30)	64.81 ± 2.45 (59.51,70.11)	-0.64 ± 0.78 (-2.33, 1.04)	0.379
CI	2.21 ± 0.42 (1.29,3.12)	2.40 ± 0.42 (1.49,3.31)	-0.19 ± 0.14 (-0.49, 0.11)	0.198

Table 3.5.4 Heparinase-1 Sensitive Anticoagulant Activity in Study Groups

b) Rest Pain Group

TEG parameters	Native TEG	Heparinase modified TEG	Change in TEG parameter.	p value
R	5.55 ± 0.58 (4.29,6.81)	5.30 ± 0.51 (4.18,6.42)	0.25 ± 0.12 (-0.02,0.12)	0.070
K	1.71 ±0.19 (1.29,2.13)	1.70 ± 0.20 (1.26,2.13)	0.014± 0.069 (-0.13, 0.16)	0.878
Angle(alpha)	63.25 ± 2.08 (58.74,67.75)	67.64 ± 1.83 (63.68,71.59)	-4.39 ± 1.60 (-7.87, -0.91)	0.022
MA	69.42 ± 1.88 (65.34,73.50)	75.57 ± 2.09 (71.04,80.09)	-6.14 ± 2.14 (-10.78, -1.50)	0.008
CI	2.32 ± 0.27 (1.73,2.91)	2.89 ± 0.28 (2.27,3.51)	0.25 ± 0.12 (-0.22, 0.52)	0.109

Table 3.5.5 Differences in TEG Parameters between Native and Heparinase Modified Methods in Study Groups

R time (minutes)

Group	native	Heparinase modified	Change in R time	p value
Controls	9.40 ± 0.43 (8.48,10.33)	8.62 ± 0.37 (7.82,9.42)	0.78 ± 0.18 (0.39,1.16)	0.002
Claudication	7.50 ± 0.44 (6.54,8.46)	7.17 ± 0.40 (6.30,8.05)	0.32 ± 0.156 (0.004,0.65)	0.034
Rest pain	5.55 ± 0.58 (4.29,6.81)	5.30 ± 0.51 (4.18, 6.42)	0.25 ± 0.12 (-0.02,0.52)	0.070

K time (minutes)

Group	native	Heparinase modified	Change K time	p value
Controls	2.88 ± 0.16 (2.52,3.23)	2.59 ± 0.11 (2.33,2.84)	0.28 ± 0.11 (0.04, 0.52)	0.021
Claudication	2.14 ± 0.24 (1.61,2.66)	1.87 ± 0.24 (1.34,2.41)	-0.14 ± 0.69 (0.16,0.13)	0.255
Rest pain	1.71 ±0.19 (1.29,2.13)	1.70 ± 0.20 (1.26,2.13)	0.014± 0.069 (-0.13, 0.16)	0.878

Angle (degrees)

Group	native	Heparinase modified	Change in Angle	p value
Controls	56.23 ± 1.26 (53.51,58.95)	57.72 ± 1.25 (55.03,60.41)	-1.49 ± 0.65 (-2.90,-0.08)	0.038
Claudication	66.52 ± 2.71 (60.64,72.39)	64.81 ± 2.45 (59.51,70.11)	1.05 ± 0.81 (-0.70,2.80)	0.148
Rest pain	63.25 ± 2.08 (58.74,67.75)	67.64 ± 1.83 (63.68,71.59)	-4.39 ± 1.60 (-7.87, -0.91)	0.022

MA (millimeters)

Group	native	Heparinase modified	Change in MA	p value
Controls	58.07 ± 1.28 (55.30,60.83)	58.33 ± 1.35 (55.42,61.23)	-0.26 ± 0.43 (-1.18, 0.66)	0.277
Claudication	64.17 ± 2.37 (59.04,69.30)	64.81 ± 2.45 (59.51,70.11)	-0.64 ± 0.78 (-2.33, 1.04)	0.379
Rest pain	69.42 ± 1.88 (65.34,73.50)	75.57 ± 2.09 (71.04,80.09)	-6.14 ± 2.14 (-10.78,-1.50)	0.008

CI

Group	native	Heparinase modified	Change in CI	p value
Controls	1.06 ± 0.23 (0.56,1.57)	1.20 ± 0.26 (0.63,1.78)	-0.14 ± 0.06 (-0.27, 0.00)	0.011
Claudication	2.21 ± 0.42 (1.29,3.12)	2.40 ± 0.42 (1.49,3.31)	-0.19 ± 0.14 (-0.49, 0.11)	0.198
Rest pain	2.32 ± 0.27 (1.73,2.91)	2.89 ± 0.28 (2.27,3.51)	0.25 ± 0.12 (-0.22, 0.52)	0.109

Table 3.5.6 Differences in Native TEG Parameters before and after Addition of 0.1 I.U/Ml of Unfractionated Heparin in Patient Group (Mean ± SD/ CI)

TEG parameters	Native TEG	Native + Heparin TEG	Change in TEG after heparin	p value
R	5.84 ± 1.54 (4.98,6.69)	8.33 ± 3.81 (6.77,9.89)	2.49 ± 2.78 (0.95, 4.03 min)	0.002
K	1.84 ± 0.69 (1.45,2.22)	2.96 ± 1.43 (2.16,3.75)	1.12 ± 1.38 (0.04, 0.52 min)	0.001
Angle(alpha)	66.41 ± 7.78 (62.10,70.72)	58.12 ± 8.62 (53.35,62.90)	-8.28 ± 6.32 (-11.78,-4.78 deg)	0.001
MA	65.94 ± 9.39 (60.74,71.14)	61.11 ± 9.52 (55.83,66.38)	-4.83 ± 4.70 (-7.44, -2.22 mm)	0.001
CI	1.72 ± 1.26 (2.19,2.59)	1.72 ± 1.35 (0.97,2.47)	-1.17 ± 0.94 (-1.69, -0.065)	0.001

3.6 - INVESTIGATION OF CHANGES IN COAGULATION CHANGES IN AORTIC ANEURYSMAL DISEASE USING TEG

Demographic, vascular risk factor details of the patients and the control are given in Tables 2.6.1 - 3.

Baseline TEG analyses showed significant reduction in AAA group when compared to the control group in R time (p=0.001); K time (p=0.008) and a significant increase in the overall coagulation status, coagulation Index (CI) (p=0.047)(Figures 3.6.1, 3.6.2, 3.6.4 and 3.6.5). No significant differences in base line TEG parameters were found in between the AAA group and aortic occlusive group.

TEG identified significant increase in R time (p=0.042), K time (p=0.037) and reductions in MA (p=0.044) and CI (p=0.007) in the AAA group in aortic pre - clamp release samples when compared to the samples obtained from control subjects during surgery(S3). No such significant differences found in between the AAA group and the aortic occlusive group at the same sampling point (Figure 3.6.6).

Abdominal aortic cross clamp release led to a further significant decrease in the R time (p=0.042); K time (p=0.043) and significant increases in the MA (p= 0.034) and CI (p=0.026) suggesting further activation of coagulation following release of aortic cross clamp in the AAA group. No significant relationship between clamp time and TEG parameters were identified in both AAA and occlusive groups.

Significant increases in MA (p=0.042); Angle (p=0.0026) and CI (p=0.044) in the post op samples obtained on Day 3 was found in the aortic aneurysm

group but not in the occlusive group. No significant differences in coagulation patterns between the AAA group and the aortic occlusive disease group were found in the post op samples obtained at days 3 and 7. 3 episodes of myocardial ischaemia (troponin positive), one acute myocardial infarction and I below knee DVT were diagnosed in the AAA group and 2 MI and 1CVA (TIA) episode and 2 DVTs were diagnosed in the aortic occlusive group. No significant relationships between these ischaemic, thrombotic events and TEG (baseline/post op) variables were found in this study.

Figure 3.6.1 R time - groups

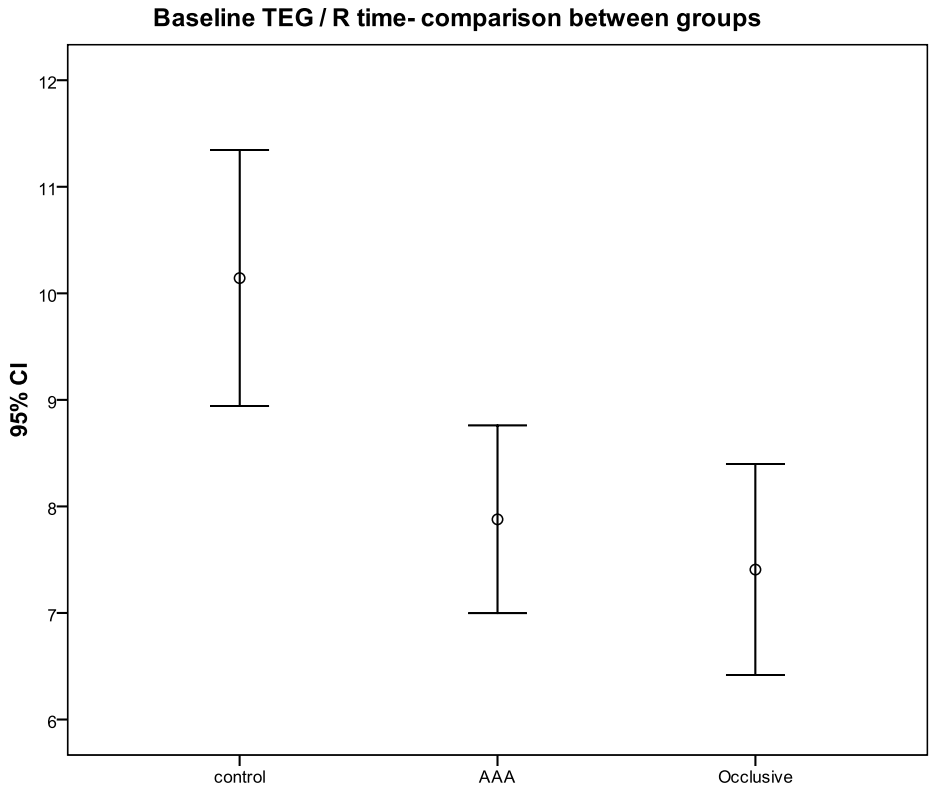


Figure 3.6.2 K time - groups

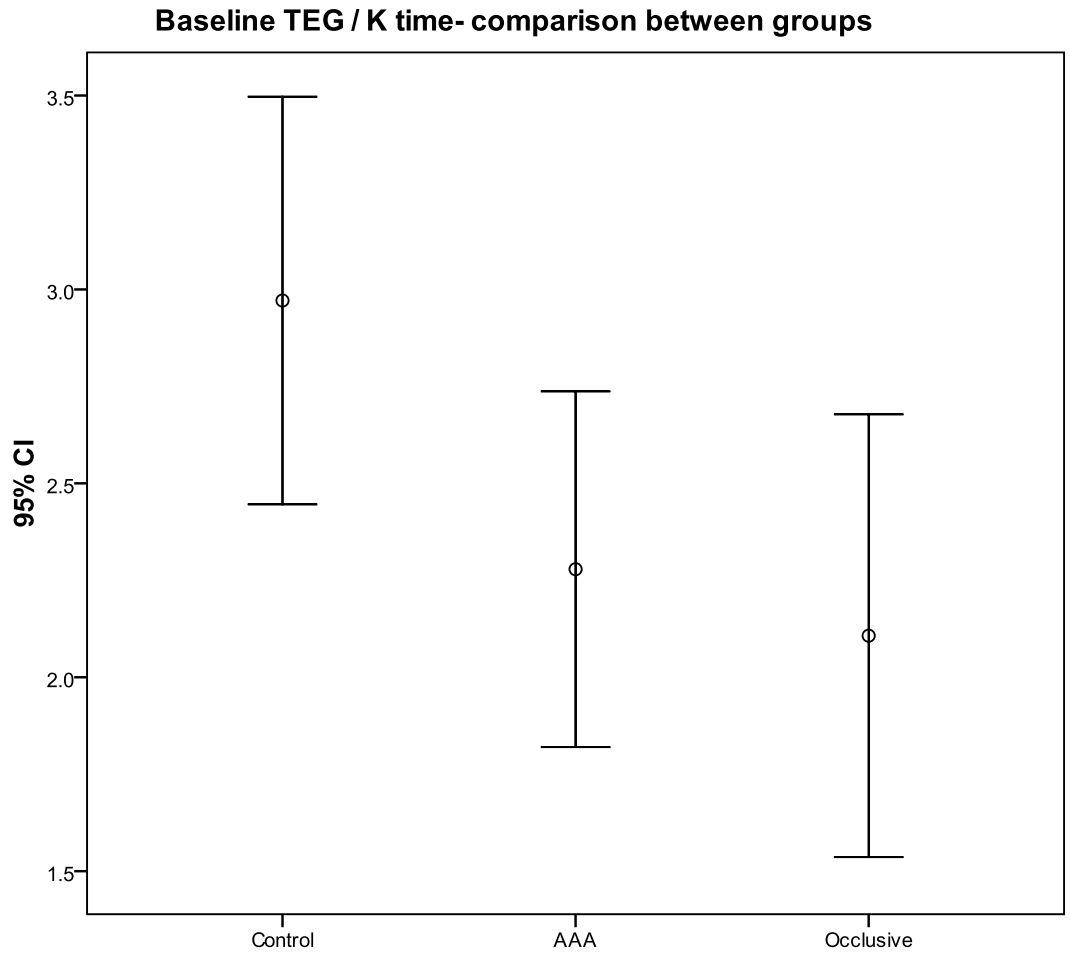


Figure 3.6.3 Angle - groups

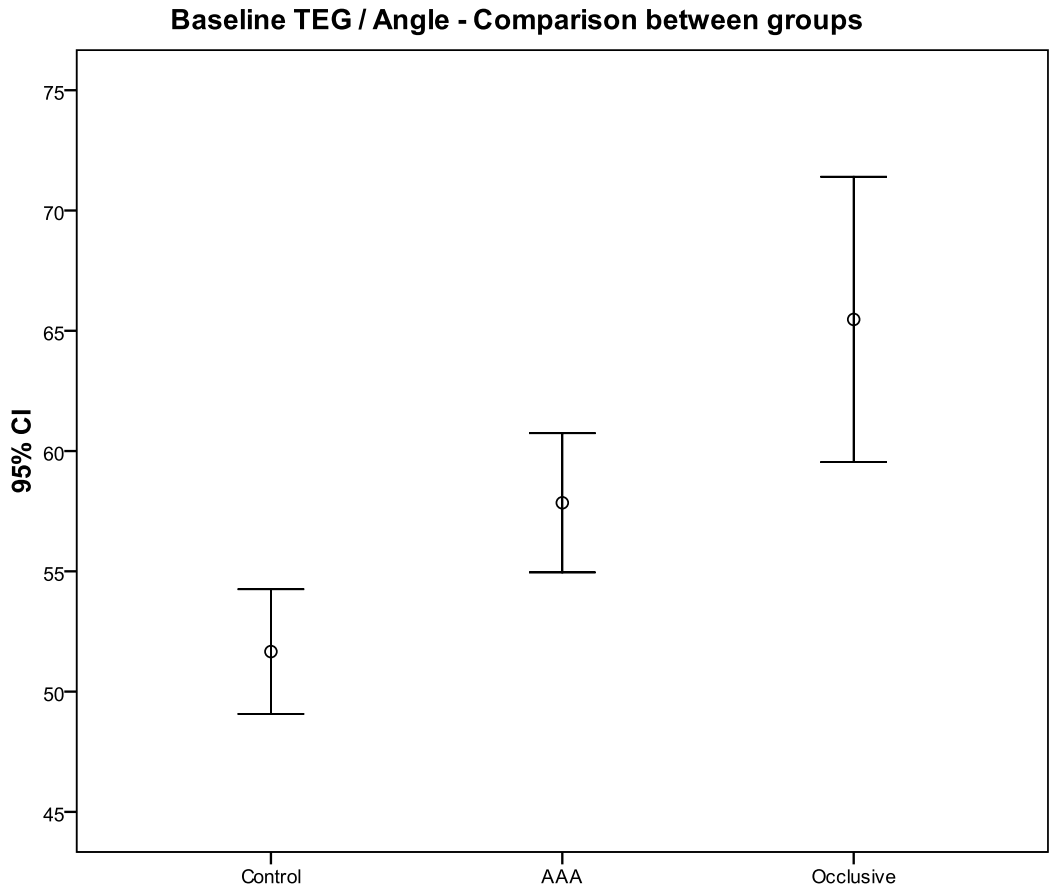


Figure 3.6.4 MA - groups

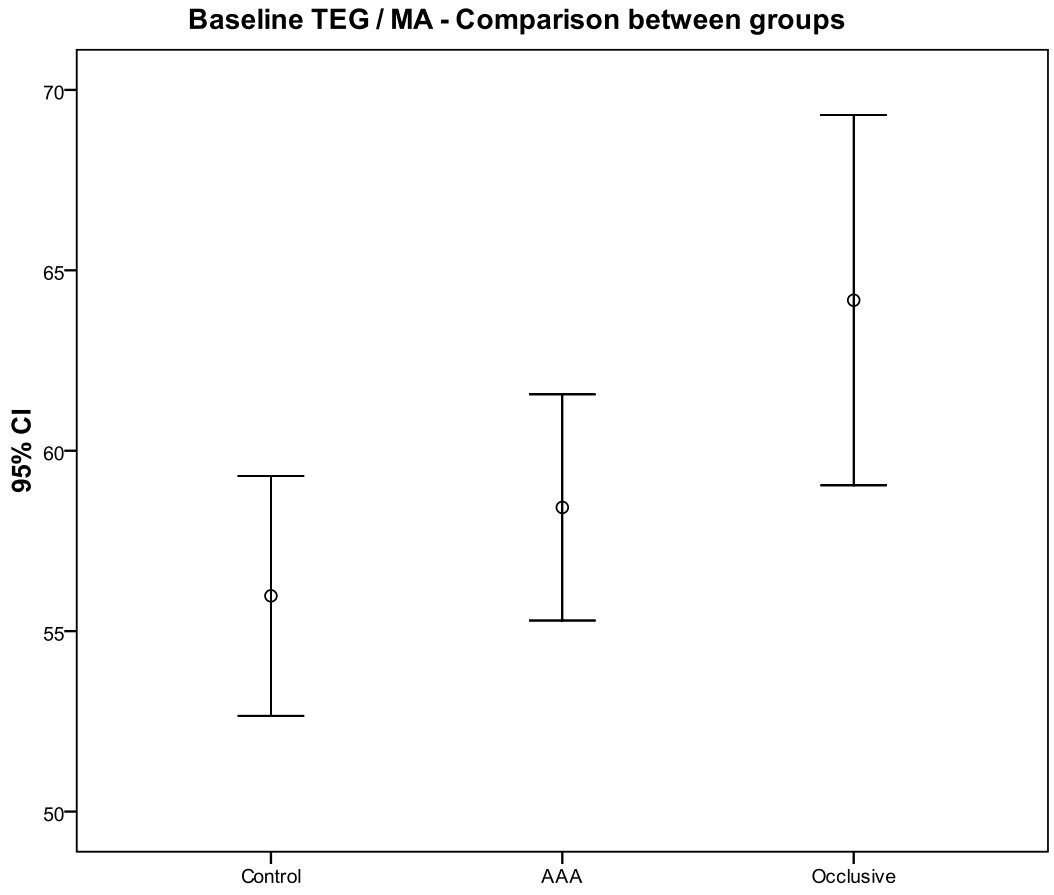


Figure 3.6.5 CI - groups

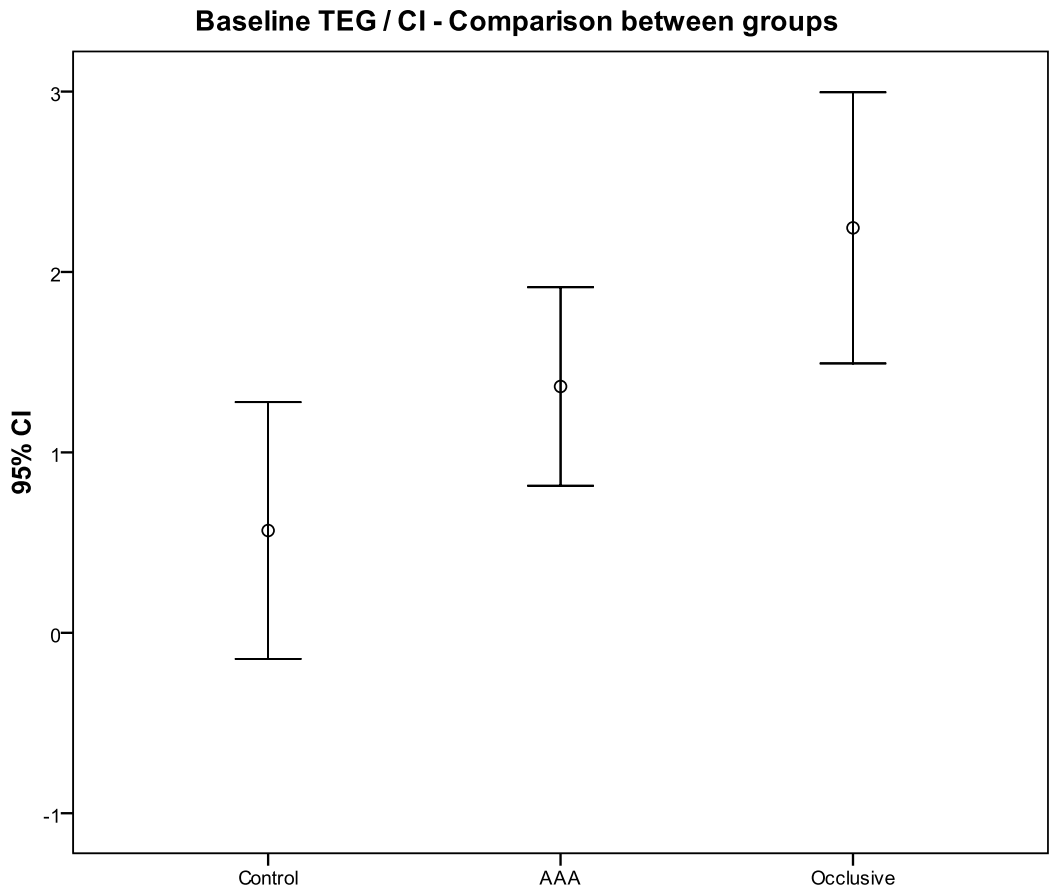
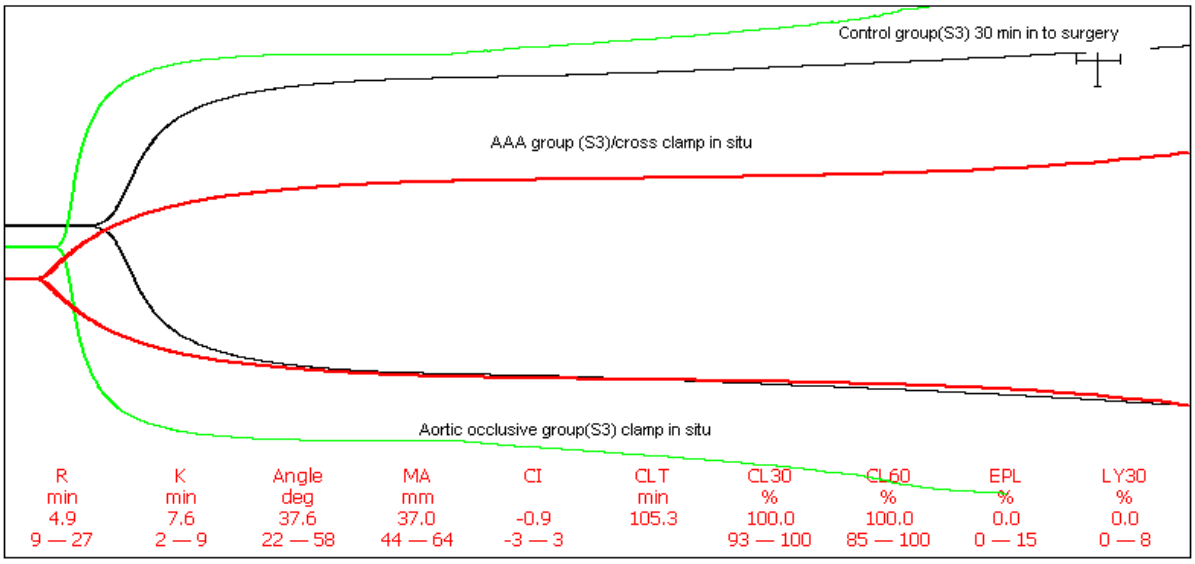


Figure 3.6.6 TEG Patterns during Aortic Cross Clamp



Key: R, Reaction time (time to initial fibrin formation);*K*, dynamics of clot formation; *Angle*, acceleration of fibrin buildup and cross-linking (clot strengthening); *MA*, maximum amplitude (ultimate clot strength); *CI*, coagulation index (overall coagulation status); *LY30*, percent lysis at 30 minutes after MA;*LY60*, percent lysis at 60 minutes after MA; *G*, clot firmness as shear elastic modulus; *EPL*, estimated percent lysis; *A*, current amplitude

3.7- TEG CHANGES DURING PERIPHERAL VASCULAR SURGERY

TEG analysis of citrated blood samples from symptomatic PVD patients undergoing surgery (Table.2.7.1) was carried out as per study plan outlined in section 2.7.

Baseline TEG analyses showed significant increase in R time (p=0.003) (Figure 3.7.1), Angle (p=0.016) (Figure.3.7.2.) MA (p=0.002) (Figure.3.7.3) and CI (p=0.002) (Figure.3.7.4.) when compared with control group confirming activation of coagulation in the PVD group at the outset.

Figure 3.7.1 PVD Vs Control group. R time

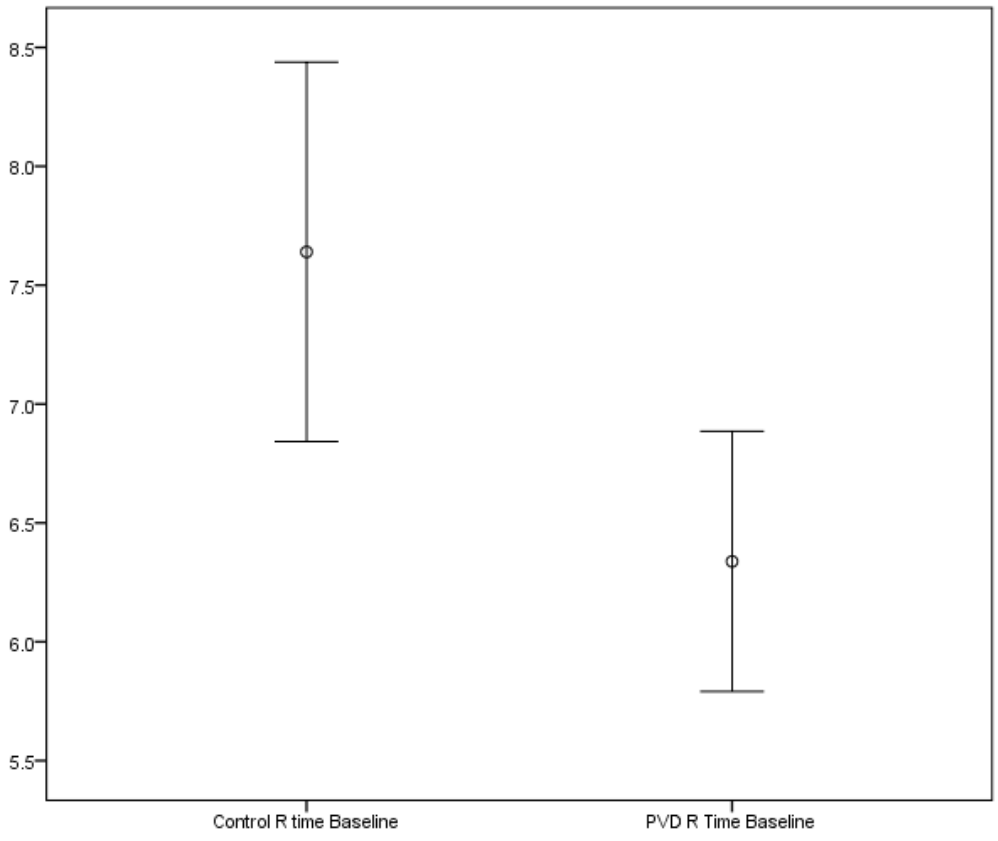


Figure 3.7.2 PVD Vs Control group (Angle)

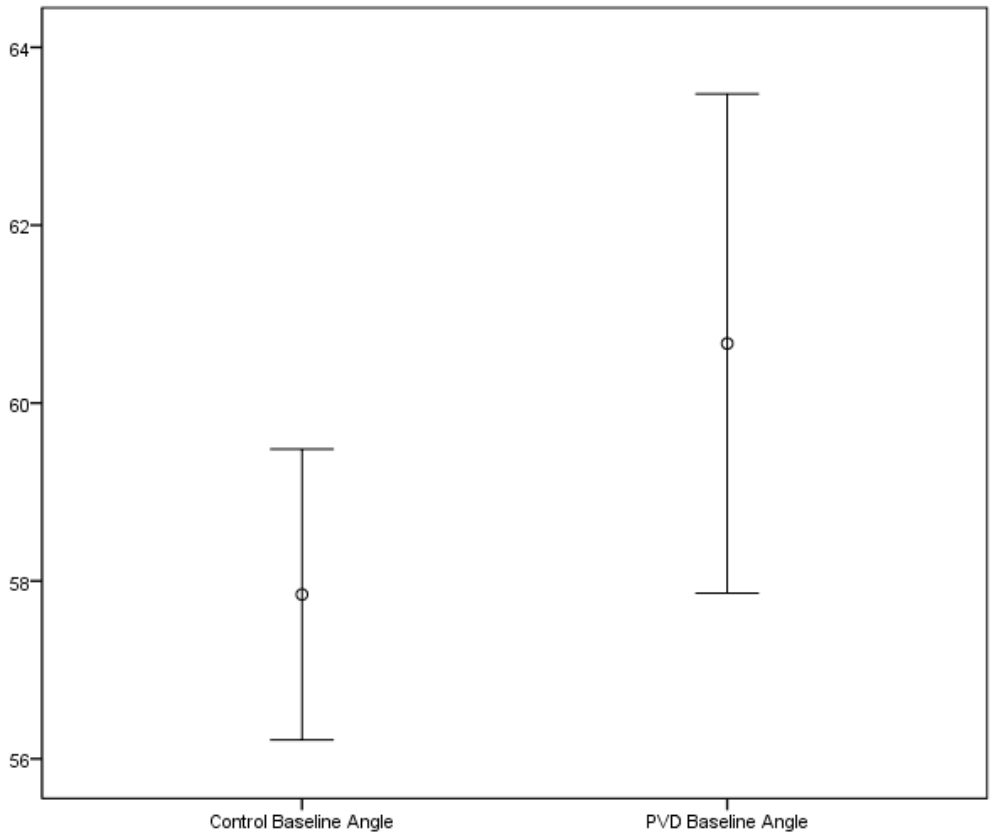


Figure 3.7.3 PVD vs Control group. MA (Maximal Amplitude)

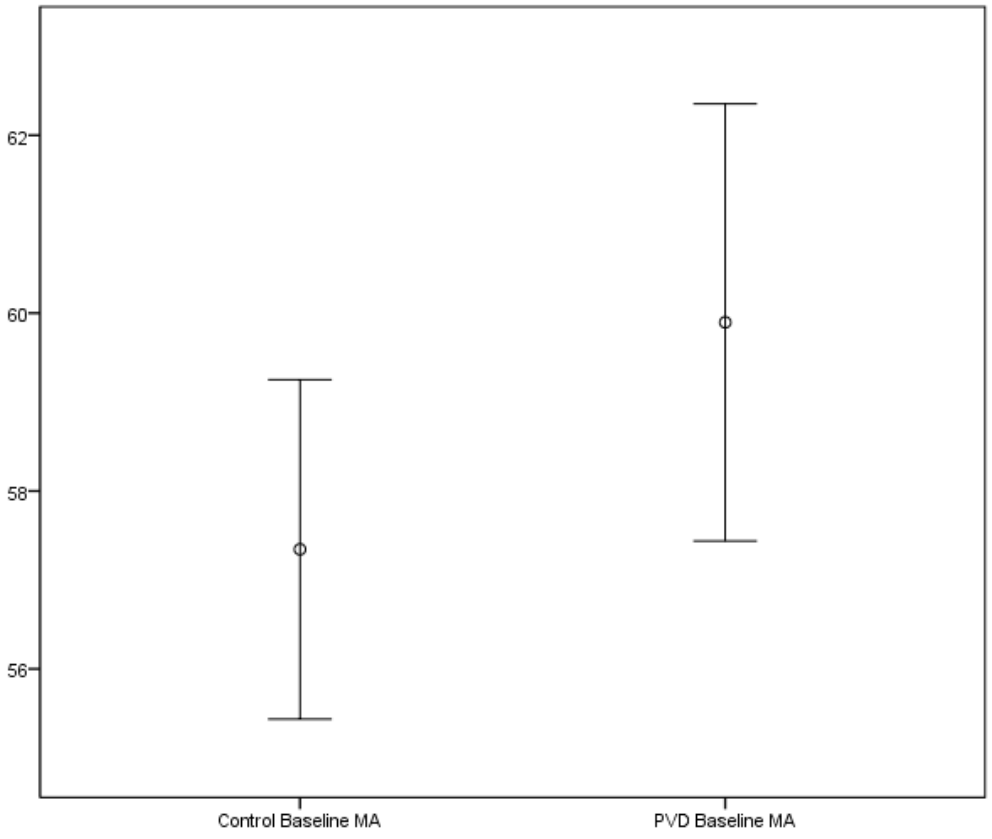
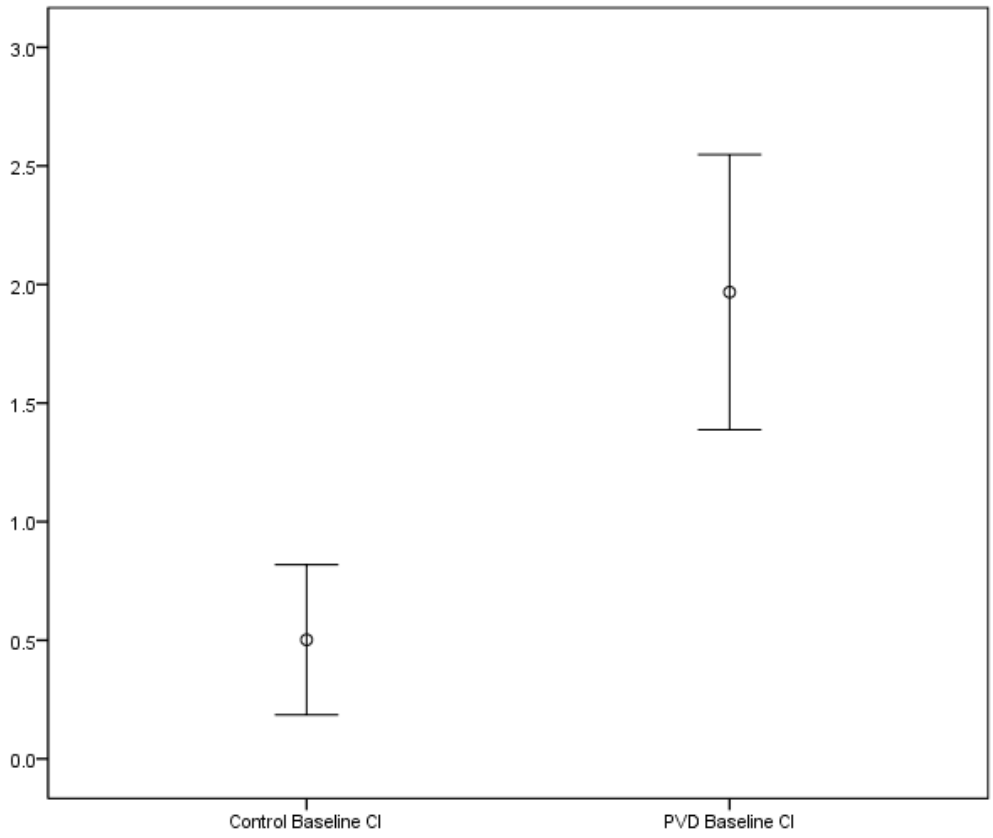


Table 3.7.4 PVD Vs Control group. Coagulation Index (CI)



Details of anaesthetic and surgical procedures involving patients with symptomatic PVD are given in Table.3.7.1.and Table.3.7.2.

Table 3.7.1 Vascular Surgical procedures

Surgery	Frequency	Percent	Valid Percent	Cumulative Percent
Above knee vein graft	8	9.3	9.3	9.3
Above knee PTFE graft	9	10.5	10.5	19.8
Below knee vein graft	7	8.1	8.1	27.9
Femoro-distal graft	9	10.5	10.5	38.4
Above knee Amputation	14	16.3	16.3	54.7
Below knee amputation	20	23.3	23.3	77.9
Femoro-Femoral crss over graft	6	7.0	7.0	84.9
Femoral endarterectomy	7	8.1	8.1	93.0
Aorto-bifemoral graft	4	4.7	4.7	97.7
Axillo-bifemoral graft	2	2.3	2.3	100.0
Total	86	100.0	100.0	

Key: PTFE: Poly Tetra Fluoro Ethylene.

Table 3.7.2 Anaesthetic details of PVD group

Anaesthesia	Frequency	Percent	Valid Percent	Cumulative Percent
GA	53	61.6	61.6	61.6
Epidural	29	33.7	33.7	95.3
Epidural+ Sedation	4	4.7	4.7	100.0
Total	86	100.0	100.0	

Key: GA: General anaesthesia

TEG parameter values obtained at sampling points outlined earlier are given in the Tables. 3.7.3 to Table.3.7.9.

TEG parameter Values

Table 3.7.3 Control group Baseline TEG parameter values

	Baseline R time	Baseline K time	Baseline Angle	Baseline MA	Baseline CI
n =	50	50	50	50	50
Mean	7.6400	3.0360	57.8480	57.3440	.5019
Std. Deviation	2.80866	1.05883	5.75160	6.71535	1.11371

Table 3.7.4 PVD group Baseline TEG parameter values

	PVD Baseline R time	PVD Baseline K time	PVD Baseline Angle	PVD Baseline MA	PVD Baseline CI
n =	86	86	86	86	86
Mean	6.3233	2.5209	61.9733	62.1360	1.5569
Std. Deviation	1.89400	1.10081	9.36726	8.99669	2.19672

Table 3.7.5 PVD group TEG- R time values

	n	Mean	Std. Deviation
r1	86	6.2849	1.99184
rr2	86	6.5163	2.12646
r3	86	6.9209	2.41379
r4	86	6.5721	2.13930
r5	86	7.0547	2.12258
r6	86	7.0128	2.58970
r7	86	7.5151	2.90276
r8	86	7.3512	2.11986
r9	86	7.0279	2.33675
r10	86	6.9430	2.62430
r11	86	7.1663	2.30200
r12	86	7.1186	2.15491

Key: r1:R time immediately before anaesthetic induction; r2: R time after anaesthetic induction; r3: R time immediately before arterial clamping;r4:R time at the end of arterial clamping; r5:R time 5 minutes after release of

clamps (in the case of vascular reconstruction); r6: R time at the end of surgery; r7:R time 30 minutes after surgery; r8:R time at 6 hours after surgery; r9:R time 24 hours after surgery; r10:R time 3 days after surgery; r11:R time 7 days after surgery; r12:R time 6 weeks after surgery.

Table 3.7.6 PVD group TEG- K time values

	n	Mean	Std. Deviation
k1	86	2.1849	.88922
k2	86	2.1326	.73283
k3	86	2.1721	.61331
k4	86	2.2302	.57334
k5	86	2.2570	.82769
k6	86	2.2930	.74420
k7	86	2.2198	.47819
k8	86	2.2302	.57334
k9	86	2.2302	.57334
k10	86	2.2267	.50746
k11	86	2.2674	.51505
k12	86	2.4523	.79004

Key: k1:K time immediately before anaesthetic induction; k2: K time after anaesthetic induction; k3: K time immediately before arterial clamping; k4:K time at the end of arterial clamping; k5:K time 5 minutes after release of clamps (in the case of vascular reconstruction); k6: K time at the end of surgery; k7:K time 30 minutes after surgery; k8:K time at 6 hours after surgery; k9:K time 24 hours after surgery; k10:K time 3 days after surgery; k11:K time 7 days after surgery; k12:K time 6 weeks after surgery.

Table 3.7.7 PVD group TEG - Angle values

	n	Mean	Std. Deviation
a1	86	66.3477	6.95890
a2	86	66.4953	6.72890
a3	86	66.4198	6.20396
a4	86	66.2337	7.36637
a5	86	65.6814	7.41470
a6	86	66.6733	7.58933
a7	86	66.2849	7.68878
a8	86	66.4953	6.72890
a9	86	66.4198	6.20396
a10	86	65.9349	6.18322
a11	86	66.2442	5.38378
a12	86	65.3395	6.16146

Key: a1:Angle immediately before anaesthetic induction; a2: Angle after anaesthetic induction; a3: Angle immediately before arterial clamping; a4: Angle at the end of arterial clamping; a5:Angle 5 minutes after release of clamps (in the case of vascular reconstruction); a6: Angle at the end of surgery; a7:Angle 30 minutes after surgery; a8:Angle at 6 hours after surgery; a9:Angle 24 hours after surgery; a10:Angle 3 days after surgery; a11:Angle 7 days after surgery; a12:Angle 6 weeks after surgery.

Table 3.7.8 PVD group TEG – Maximum Amplitude (MA) values

	n	Mean	Std. Deviation
ma1	86	67.7453	7.42466
ma2	86	74.1465	8.37798
ma3	86	72.6942	8.06342
ma4	86	74.2872	8.10543
ma5	86	72.5860	7.17832
ma6	86	71.9791	9.06683
ma7	86	74.0895	9.38093
ma8	86	72.3663	9.18146
ma9	86	73.6419	8.51956
ma10	86	71.1907	14.01948
ma11	86	71.7826	8.52378
ma12	86	74.0163	8.37556

Key: ma1:MA immediately before anaesthetic induction; ma2: MA after anaesthetic induction; ma3: MA immediately before arterial clamping; ma4: MA at the end of arterial clamping; ma5: MA 5 minutes after release of clamps (in the case of vascular reconstruction); ma6: MA at the end of surgery; ma7: MA 30 minutes after surgery; ma8: MA at 6 hours after surgery; ma9: MA 24 hours after surgery; ma10: MA 3 days after surgery; ma11: MA 7 days after surgery; ma12: MA 6 weeks after surgery.

Table 3.7.9 PVD group TEG – Coagulation Index (CI) values

	n	Mean	Std. Deviation
ci1	86	3.0823	1.24632
ci2	86	4.0818	1.41629
ci3	86	3.9452	1.55836
ci4	86	4.0580	1.40521
ci5	86	3.8304	1.29179
ci6	86	3.7604	1.32876
ci7	86	3.8485	1.82485
ci8	86	3.5841	1.57309
ci9	86	3.7992	1.41972
ci10	86	3.5237	2.47205
ci11	86	3.5393	1.44658
ci12	86	3.9452	1.55836

Key: ci1: CI immediately before anaesthetic induction; ci2: CI after anaesthetic induction; ci3: CI immediately before arterial clamping; ci4: CI at the end of arterial clamping; ci5: CI 5 minutes after release of clamps (in the case of vascular reconstruction); ci6: CI at the end of surgery; ci7: CI 30 minutes after surgery; ci8: CI at 6 hours after surgery; ci9: CI 24 hours after surgery; ci10: CI 3 days after surgery; ci11: CI 7 days after surgery; ci12: CI 6 weeks after surgery.

TEG parameters obtained at induction of anaesthesia, after the induction of anaesthesia and at 30 minutes after commencement of surgery obtained from control group were compared with the TEG parameters obtained from the PVD group undergoing surgery at corresponding sampling points. Significant reductions in R time (p=0.018), Maximal amplitude (p=.024) and CI (p=0.004) were noted in the PVD group after the induction of anaesthesia. No significant relationships between type of anaesthesia and TEG parameters were identified in this study. Amongst PVD subjects significant elevations in Angle (p=0.023) and MA (p=0.034) were found in patients with diabetes when compared to non-diabetics with PVD.

No significant relationships between time duration of starvation prior to surgery haematocrit, time from last dose of LMW heparin and type and volume of IV fluids administered and the TEG parameters were found. Routine haematological tests including baseline coagulation tests were within normal range for the PVD group at this sampling point. When TEG parameters of samples obtained at 30 minutes after commencement of surgery were compared, there were significant reductions in the R time (p=0.016) and significant increase in Angle (p=0.002) and MA (p=0.018) values were identified. No significant TEG changes due to the type of graft used were found in this study.

When pre and post revascularisation samples were analysed TEG identified significant increases in R time (p=0.026), Maximal amplitude (p=0.004) and Coagulation Index values (p=0.002) confirming activation of coagulation

following revascularisation of an ischaemic limb. This sharp rise in TEG values at corresponding sampling point was not in seen in the samples obtained from those who underwent major limb amputation for irreversible, non reconstructable peripheral ischaemia (p=0.146)

Further postoperative TEG analysis of samples obtained from PVD patients who underwent surgery showed consistent increase in Maximal Amplitude (p=0.013) and Coagulation Index (p=0.032) values when compared to the baseline values. No significant TEG parameter changes were identified between those who had amputation or revascularisation surgery up to 6 weeks following surgery.

DETECTION OF THROMBOTIC EVENTS

Table 3.7.10 Detection of Thrombotic Events

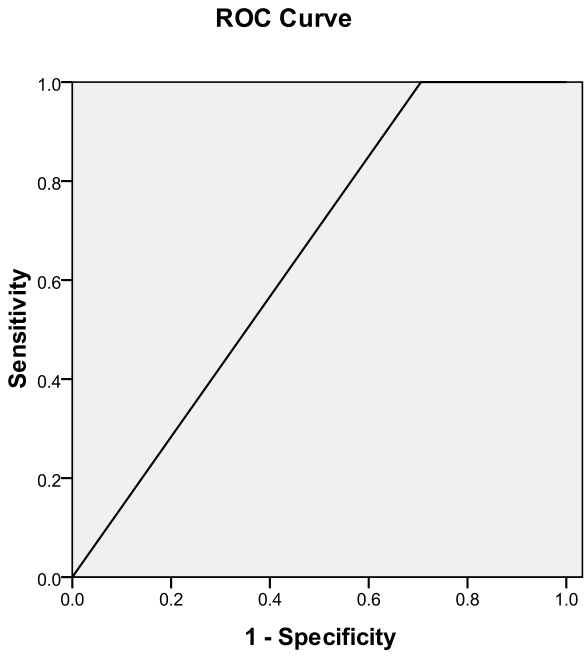
Post-operative thrombotic events

	Frequency	Percent	Valid Percent	Cumulative Percent
AMI	10	11.6	11.6	11.6
DVT	8	9.3	9.3	20.9
PE	2	2.3	2.3	23.3
TIA	6	7.0	7.0	30.2
Stroke	2	2.3	2.3	32.6
Grocc	4	4.7	4.7	37.2
Death	2	2.3	2.3	39.5
None	52	60.5	60.5	100.0
Total	86	100.0	100.0	

Key: AMI: acute myocardial ischaemia; DVT: Deep vein Thrombosis; PE: Pulmonary Embolism; TIA: Transient Ischaemic Attack; Grocc: Graft Occlusion.

Post-operative clinical and laboratory methods of detection of thrombotic events (details given in section 2.7) detailed above (Table 3.7.10) occurred in those with PVD who underwent revascularisation or amputation procedures. TEG profiles of PVD patients obtained at the baseline and during the course of surgery and in the post period were analysed. Among the TEG parameters an MA value of more than 60 at 72 hours after surgery is found to be related to occurrence of any thrombotic event. The ROC curve estimation value is 0.647 suggesting a significant association between raised MA value obtained after surgery (Day3) and any postoperative thrombotic event.

Figure 3.7.11 MA at Day 3 after Surgery and Post-Op Thrombotic Event



Further analysis of TEG parameter profiles obtained from PVD subjects during the postoperative period, identified no such associations to the occurrence of a thrombotic event. No significant relationship between these events and the routine haematological tests including platelet count and coagulation screening tests (aPTT and PT) were identified in this study.

3.8 - SUMMARY OF RESULTS

The summary of the results of the investigations detailed in this thesis is as follows:

This study confirmed that TEG analysis following citrate storage of 1-2 hours produce reliable and reproducible TEG parameter values. Heparinase modified TEG method can unmask underlying coagulation defect (hypercoagulation) when samples are analysed after administration of heparin.

This study found that blood increases in platelet and coagulation factor activity as it flows down the iliac vessel in proportion to the extent of iliac atherosclerotic disease. This study has also shown that blood circulating through an ischaemic limb increases in coagulability.

TEG analysis in this study has shown that the local effect of NICM is a reduction of coagulation activity rather than the activation suggested by some previous studies and in patients with peripheral vascular disease iohexol, a NICM leads to no further increase in their blood coagulability during peripheral diagnostic angiography.

Using heparinase modified method, this study has conclusively proven that patients with advanced PAOD exhibit reduced endogenous heparin activity and this reduced activity is related to the severity of peripheral ischaemia.

TEG analysis of blood samples from those with abdominal aortic aneurysm show activation of coagulation. Reperfusion after aortic aneurysm repair lead

to a hypercoagulable state that was consistent and sustained in the post-operative period.

TEG analysis of PVD subjects undergoing surgery confirmed the presence of a hypercoagulable state at the outset which further accelerates following revascularisation or amputation. This study has shown that raised values of one of the TEG parameters, MA (maximal amplitude) is significantly associated with the occurrence of any post-operative thrombotic event.

CHAPTER 4

DISCUSSION AND FUTURE PLANS

CHAPTER 4 - DISCUSSION AND FUTURE PLANS

4.1 - OVERVIEW

TEG method was used as a research tool when the concept was first promoted for use in identifying coagulation abnormalities in the late 70s. This technique has now been incorporated as an important bedside test in majority of intensive care units, in liver and pancreas transplant units and in the management of coagulation abnormalities that complicate major cardiac surgical procedures. This study used TEG technique to identify coagulation changes in patients with peripheral vascular disease. TEG method needs standardisation and needs modified methods to uncover underlying coagulation abnormality when there is evidence of exposure to exogenous anticoagulant administration i.e., heparin. Using heparinase modified TEG method this study looked in to natural endogenous heparin- like activity in patients with symptomatic PVD. Using TEG, this study also looked in to the coagulation changes in abdominal aortic aneurysmal disease focussing on the changes during the perioperative period. This study is based on the central premise that peripheral atherosclerosis and ischaemia has a role to play in coagulation. Local (ischaemic limb) and systemic blood sampling methods were used to identify significant changes due to severity of PVD following surgery and document any significant relationship between observed TEG changes and postoperative thrombotic events that occurred during the study period.

4.2 - VALIDATION AND STANDARDISATION OF TEG METHODS

This study has shown that reliable baseline TEG parameter for studies employing citrated whole blood TEG method can be obtained by recalcification and analysis after a minimum period of 1-2 hours of citrate storage following collection of a sample. This study has also confirmed that heparinase coated cups negate any heparin effect in a given blood sample and it can be used to carry out TEG analysis of blood samples obtained after exposure to any form of exogenous heparin to reveal underlying coagulation patterns.

Thromboelastography is a valuable tool in the management of coagulation defects in an acute care setting. TEG is capable of measuring global coagulation activity in a small sample of whole blood but is subject to variability due to many factors. Sample collection, its storage, time to analysis, and local variations in the availability of the analyser and personnel are some of the important factors. Citrate TEG method needs to be standardised for reliable, reproducible results. The range provide by the manufacturer is wide, makes no allowance for age or sex, and may need to be modified because of the above mentioned factors. TEG assay of citrated blood samples exhibit variability and has been shown to be unreliable when samples are analysed between 0 and 30 minutes after citration. Rajwal et al[111] found that there was significant difference between TEG parameters for fresh native whole blood and citrated whole blood and recommended

establishment of a specific range for citrated whole blood for usage in clinical practice. Dormandy et al[112] suggested a formal standard operating procedure for citrated whole blood TEG that takes in to consideration, the initial period of instability. Camenzind et al[113]looking in to the effects of citrate storage on TEG confirmed that the parameters were different in recalcified, citrated samples in comparison to native blood samples. The observed changes were progressive during 0-30 min. of storage but were stable thereafter and recommended analysis after a citrate storage period between 1- 8 hours for reliable TEG results. This study followed the above method and carried out TEG after a citrate storage period of 1-2 hours for all samples in an effort to obtain reliable results.

4.3 - INVESTIGATION OF THE EFFECTS OF AGE, GENDER AND ANAESTHESIA ON COAGULATION USING TEG – CONTROL GROUP STUDY

The main objective of this study is to obtain baseline values for citrate TEG method using blood samples obtained from a group of participants acting as controls for our study looking in to coagulation changes in patients undergoing during peripheral vascular surgical procedures and their relationship to postoperative thrombotic events. This study also looked at any significant changes in coagulation due to host factors (i.e., age, gender and BMI) and perioperative variables (induction of anaesthesia, IV fluid administration and surgery) towards coagulation during surgery.

This study found no significant relationships between baseline routine coagulation tests and TEG parameters from control subjects, which is not surprising as routine coagulation tests to do not take in to consideration the extrinsic component of coagulation cascade and the contribution from platelets, an important aspect of coagulation. Findings of this study are consistent with previous studies employing citrated TEG.

Various factors including, preoperative starvation, hydration, temperature, anxiety and stress related to undergoing a surgical procedure can influence coagulation. There is now convincing evidence that patients undergoing major surgical procedures exhibit hypercoagulability[114,115] albeit transient, during the perioperative phase. Several investigators have attempted to identify the factors that may be responsible for this hypercoagulable state observed during the perioperative period using Thromboelastography.

Among other factors stress, anxiety prior to surgery, starvation, dehydration[116], preparation for anaesthesia[117], iv fluid administration[118], anaesthesia[119] and surgical stimulus[120] are identified as possible reasons for the changes in coagulation observed during surgery.

This study did not identify any significant relationship between duration of starvation or body temperature and baseline TEG parameters. However, Gorton et al[121] in their study involving 20 women undergoing caesarean section found significant changes towards hypercoagulation on TEG. They attributed these changes in TEG parameters to events during the pre-induction phase of anaesthesia i.e., placing of venous cannula, period of waiting in an area next to the operative theatre and possibly due to increase in circulating catecholamines.

Bauer et al[122] (Normative Ageing Study) found significant positive correlations between increasing age and F1+2 levels, fibrinopeptide A and protein C activation peptide levels. Ofosu et al[123] reported significant increases in coagulation system proteins including markers of activation of coagulation resulting in high thrombotic risk with ageing. Boldt et al[124] using TEG, found significant activation of coagulation in elderly (>80 years) cardiac surgery patients compared to younger patients (< 60 years) prior to surgery. Boldt et al[125] again reported that elderly patients showed more prothrombin activation/thrombin generation and increased fibrinolytic activity prior to surgery (baseline) than younger patients among those undergoing major general surgical procedures. Yamamoto et al[126] in their review state that

increased levels of PAI-1, a principal inhibitor of fibrinolysis, found to increase significantly with age in several studies, could play a key role in the progression of cardiovascular disease in the elderly by promoting thrombosis and atherosclerosis. Age is also associated with abnormal platelet function and procoagulant activity[127] as its consequence and can lead to development of athero-thrombotic events.

No significant associations between age and TEG parameters were found in this study in contrast to studies involving ageing and coagulation and thrombotic risk where investigators have found altered platelet function, abnormal tissue factor pathway inhibition (TFPI),[128] with increasing age. These findings could well be due to the fact that very few (n=3) of the participants were over 80 years in this study.

Gorton et al[129] showed gender differences in coagulation using Thromboelastography with significant trend towards increasing whole blood coagulability from men through non-pregnant to pregnant women. Pre-menopausal women were found to be significantly more hypercoagulable following trauma when compared to men and TEG was sensitive in identifying this coagulation abnormality in the first 24 hours after trauma[130]. In post-menopausal women, the combination of ageing and oestrogen deficiency has been found to be significantly associated with a significant increase in the incidence of myocardial infarction, claudication and stroke compared to pre- menopausal women[131]. Koch et al[132] in a study involving outcomes after cardiac bypass surgery found that female gender is

associated with more complications predominantly due to activation of coagulation and fibrinolytic impairment especially after menopause leading to a significant increase in athero-thrombotic events.

The incidence of thrombotic events like myocardial infarction, stroke and deep vein thrombosis increases with age and it increases exponentially in women after menopause[133]. There is convincing evidence of activation of coagulation shown by significant increases in coagulation factors including thrombin, in elderly women compared to pre-menopausal women due to ageing and associated changes in vascular and haemostatic mechanisms related to oestrogen deficiency.[134] There are no useful markers or definitive tests of thrombotic tendency due to oestrogen related changes in coagulation and fibrinolytic mechanisms to identify individuals at risk. However, TEG has been shown to reflect changes associated with hormonal status of an individual especially in those on oestrogen containing oral contraceptive pills by identifying rapidity in fibrin formation and acceleration of increase in clot firmness among its users. The literature suggests that post- menopausal women exhibit increased coagulability compared to pre-menopausal women and men. Women in general, respond to trauma with significantly increased activation of coagulation than men.

Various studies have noted that obesity is associated with significant arterial and venous thrombotic events. Body mass index (BMI) is associated with increase in activation of coagulation factors, impaired fibrinolysis, defective endothelial function and increased levels of circulating tissue factor (TF)[135] leading to a prothrombotic state in patients with high BMI. Abnormal lipid

metabolism and hyperglycaemia are other significant abnormalities associated with this condition. Hyperlipoproteinaemia, which behaves like LDL (low-density lipoprotein) due its composition leads peroxidation resulting promotic inflammation that in turn leads to activation of coagulation and progression of atherosclerotic lesions. Abnormal glucose metabolism i.e., hyperglycaemia hyperinsulinaemia and insulin resistance have all been shown to promote activation of coagulation leading to a prothrombotic state in diabetes mellitus. Hyperglycaemia associated with increasing plasma level of fibrinogen, levels of activated factors VII, VIII, von Willebrand and X. This is also associated with significant increases in TAT complexes (thrombin-antithrombin) and soluble tissue factor levels irrespective of insulin levels. Hyperinsulinaemia on the other hand has been shown to inhibit fibrinolysis by decreasing plasminogen activator activity by increasing the PAI-1 levels irrespective of glucose levels. Insulin resistance is associated again with elevated PAI-1 inhibitor levels, increased levels of coagulation factors, endothelial dysfunction leading to suppression of synthesis of nitric oxide and prostacyclin and platelet activation resulting in high incidence of atherothrombotic events in these patients.[136]

The effect of intravenous fluids on coagulation has been a much-debated topic. Ruttmann et al[137] in their initial study found that when patients' whole blood samples were haemodiluted with 0.9% saline in vitro there was a significant increase in coagulability than when a colloidal solution (Haemacel) was used on TEG especially, the clot strength. Ruttmann et al[138] later in an in vivo study of haemodilution of normal blood reported a procoagulant effect;

possibly, by enhancement of thrombin formation due to low levels of AT-III was noted following haemodilution. They also reported that in vitro haemodilution-induced coagulation enhancement is attenuated, but not prevented, if AT III levels were maintained in the normal range in keeping with the concept of an antithrombin threshold preventing positive coagulation feedback into the intrinsic pathway. The same group also found that this hypercoagulable response following haemodilution was unaffected by Aspirin administration.[139] Boldt et al[140]reported a small but significant shift towards hypercoagulability immediately after surgery and five hours after the end of surgery in those undergoing major abdominal surgical procedures receiving intravascular volume replacement with crystalloids (Ringer's lactate (n=21) and 0.9% saline solution (n=21)) and found no significant differences in coagulation patterns in between those who received Ringer's lactate (18750+/-1890ml) and 0.9% saline solution (17990+/-1790ml). Gorton et al[18] also found no significant changes in coagulation on TEG after fluid administration (500ml of Gelofusine IV over 15 minutes) during the pre-induction phase.

Ng et al[141] after a randomised control study involving patients undergoing major hepato-biliary surgery found that replacement of 30% of blood volume with saline over 30 minutes(n=10) can induce a hypercoagulable state when compared to those who did not receive (n=10) any such replacement of blood volume. However Butwick et al[142] found no significant effects on coagulation following preloading with 1500 mL Ringer`s lactate using TEG and Ekseth et al[143] (n=12 patients) suggested that with crystalloids and

albumin, dilution had to exceed 50% before coagulation was impaired. Available evidence suggests that there is an increase in the coagulation activity when significant haemodilution takes place and that the effect may be more marked with saline solution than other IV fluids. TEG is a sensitive test for identifying subtle and early changes due to fluid administration especially haemodilution with various colloid and crystalloid solutions that are used during the induction phase of anaesthesia.

Significant changes in TEG parameters have been reported with administration various colloid, crystalloid infusions, blood and blood products during induction of anaesthesia and surgery. The predominant change observed so far in TEG patterns due to crystalloid administration was that of a temporary hypercoagulable pattern after haemodilution by rapid infusion of isotonic (0.9%) saline ranging between 30-50% of total blood volume. This study group did not receive such volumes and no significant relationships between the type of fluids (0.9% Normal saline and Hartmann`s) or the volume (145+/-20 ml (mean/SD), CI 70+/-50, 180+/-50 ml) and rates (50-120/hr) at which they were administered and TEG parameters were found in this study.

Anaesthetic techniques/agents (general/spinal/epidural/regional/local) have been shown to affect blood coagulability. Several investigators have used TEG to identify such changes in coagulation due to anaesthetic agents (inhalational, intravenous and local anaesthetic). Sharma et al[144] found significant reductions in r and k times in patients who received a general anaesthetic (n=15) when compared to those who had a spinal anaesthetic

(n=15) prior to caesarean section. General anaesthesia in combination with epidural analgesia has been shown to lead to fewer post op thrombotic events than general anaesthesia alone. Possible mechanism involved in this finding include a reduced response to stress during surgery and is associated with reduced cortisol and epinephrine levels in those who had combined general anaesthetic and epidural analgesia resulting in reduced athero-thrombotic cardiac events[145]. In addition, there was less platelet aggregation during surgery, in the GA+EPI group compared to GA alone[146]. Bupivacaine, a routinely used long acting (local / epidural) anaesthetic on TEG® lead to significant reductions in MA (Maximal Amplitude) values when used at 2.5µg/ml dosage in vitro.[147]. Kohrs et al[148] in another in vitro study reported that Bupivacaine inhibits thromboxane A2 (TX) signalling and suggested that it might partly explain the beneficial effects of epidural anaesthesia on postoperative thrombotic events. However, no consistent results were shown by other studies looking at the effects of local anaesthetic agents at varying dosages for epidural anaesthesia.[149] Benzon et al[150] in a randomised, double blind study found no significant differences in TEG® variables due to Bupivacaine on epidural Fentanyl epidural analgesia. Gibbs et al[151] found in 12 patients no significant effects on TEG variables due to Bupivacaine in clinically relevant doses (2.7µg ml). Niemi et al[152] found that I.V. regional anaesthesia (IVRA) with high i.v. lignocaine concentrations (median 144.4 micrograms ml-1 in cubital veins at the end of the tourniquet time) potentiated ischaemia-induced fibrinolysis activation during IVRA. Kohro et al[153] in an in vitro study (14 patients) again using TEG® reported that Propofol promoted

fibrinolysis. Law et al[154] in a prospective randomised study found no such coagulation abnormalities on TEG® due to propofol use and in between those who received propofol (n=19) or isoflurane (n=20).

This study has shown that significant activation of coagulation occurs following induction of anaesthesia. However, no significant relationship between the type anaesthesia (Epidural, Epidural +General or General) or the agents used and the TEG parameters considered in this study. Contrary to previous studies showing reduction in overall coagulability with Bupivacaine, a long acting local anaesthetic agent using TEG no such alterations in TEG were found when the agent is used for epidural anaesthesia (2.7µml-1ml) in this study. Sharma et al[155] in their study concluded that general anaesthetic is associated with accelerated coagulability when compared with spinal anaesthesia. No such significant difference in TEG parameters due to a specific anaesthetic method was identified in our study. Findings could well be due to the fact that these studies involved pregnant women who are about to undergo caesarean section and their overall coagulability is significantly increased at the outset and probably not an ideal group for comparison. The significant shift towards hypercoagulation after induction of anaesthesia seen in this study is more likely due to the cumulative effects of various factors including IV fluid usage, anaesthetic method and agents administered than any one of these factors on its own.

Various studies have shown activation of coagulation and fibrinolysis by measuring significant increases in markers of the same including Thrombin-antithrombin III complexes (TAT), soluble fibrin monomer complexes (SFMC), fibrin degradation products (FDP), D-dimers (D-D), fibrinogen (FIB)[156] in blood samples obtained immediately at the end of a surgical procedure and in another study involving patients undergoing total hip arthroplasty procedures, Prothrombin fragments 1+2 [157] on post op day 1. Platelet activation resulting in increased aggregation that extends up to 20 hours after completion of surgery completes a picture of an acquired hypercoagulable state following surgery[158] resulting in increased thrombotic risk during the same period. Stress response to surgery (both endocrine and metabolic), activation of tissue factor, endothelial dysfunction, associated stasis and alteration of constituents (viscosity) of blood (Virchow`s triad) due to preoperative factors (starvation, dehydration, IV fluid administration etc.,) are some of the possible causes for these alterations in coagulation observed during the peri-operative phase. Significant activation of coagulation and fibrinolysis occurs within the first 24 hours (18.1+/-6.4 hours) after trauma and as mentioned previously, more commonly seen in women[159]. Studies have shown that this increase in coagulability is decreased in laparoscopic procedures in comparison with open procedures,[160] increased activation of coagulation in older patients undergoing surgery than in younger patients[42], increased thrombo-embolic events in those undergoing surgery for malignancy[161] and a prolonged period of hypercoagulability (post op day 7) after major abdominal[162] and aortic[163] surgical procedures.

Various investigators have identified a period of hypercoagulability albeit transient in those undergoing major abdominal, vascular and cardiac surgical procedures. The significance of this finding lies in the fact that these patients were found to have normal coagulation pattern following baseline haematologic and routine coagulation tests carried out prior to commencement of a surgical procedure as in our study. Several studies have reported significant activation of coagulation in the postoperative period following major surgery identified by TEG but there were no specific information with regard to the contribution of individual events or factors involved during surgery.

This study has demonstrated that the hypercoagulable response that starts after the induction of anaesthesia continues at a higher level during surgery identified by a significant increase in Angle, representing acceleration of fibrin build up and cross-linking (clot strengthening). This phenomenon also appears to be independent of the level of complexity of surgical procedure involved with no significant differences in TEG parameters among patients who underwent various surgical procedures detailed in this study.

4.4 - INVESTIGATION OF THE EFFECTS OF SAMPLING SITE, PERIPHERAL ATHEROSCLEROSIS AND PERIPHERAL ISCHAEMIA ON TEG

For this study Thromboelastography (TEG)[164] was used to measure changes in platelet function and the coagulation cascade across the ischaemic limb and then sought to determine whether the changes in coagulability were caused by flow through an atherosclerotic vessel or through ischaemic tissue.

Peripheral venous blood samples demonstrate a systemic increase in coagulability in patients with peripheral arterial occlusive disease (PAOD) affecting the legs[165,166,167,168]. Fibrinogen[169], and products of the coagulation cascade including thrombin-antithrombin complexes and prothrombin F1+F2[170] as well as D- dimers[171], indicating increased thrombin synthesis and fibrinolysis, are found consistently raised in patients with PAOD. Ischaemic heart disease is associated with similar changes[172,173,174]. The mechanism responsible for these changes is not clear. Potential causes of activation of the coagulation cascade include contact between circulating blood and atherosclerosis in the vessel wall or passage of blood through ischaemic tissue. Circulating fibrinogen levels in PAOD correlate inversely with the mean ABPI [175] and with angiographic scoring[176]. The degree of coagulation activation, indicated by systemic levels of thrombin-antithrombin complex, increases with the severity of disease on angiography or duplex scanning.[11] Fibrinolytic activity, reflected in D-dimer levels, also correlates inversely with mean ABPI[12] and angiographic scoring[11]. It is therefore clearly

established that an ischaemic limb is associated with increased clotting activity but although Lassila et al[11] and Woodburn et al[12] showed a correlation between the level of activation and the extent of atherosclerosis, the latter also correlates with the degree of ischaemia and so it is still not clear which drives the increase in clotting activity.

This study has clearly shown that blood coagulability increases as it flows down an atherosclerotic artery and that the magnitude of that change relates to the severity of stenosis. It is likely that the changes in blood coagulability are caused either by exposure to atherosclerotic disease itself or possibly by the turbulence produced by a stenosis. It is unlikely that ischaemia plays a part in this situation. The changes recorded as blood flows through the ischaemic limb are more complex. Presumably the same mechanisms which induced a change in coagulability in the iliac segment also play a part in the changes within the limb. Sobel et al[177] demonstrated increased thrombin generation within the ischaemic limb by measuring fibrinopeptide A (FPA) levels that were consistently higher in venous samples than in the arterial inflow to an ischaemic limb. The same authors found no increase in β thromboglobulin and concluded that there was no activation of platelets across the ischaemic leg although other studies have found systemic evidence of platelet activation in such subjects[178].

This attempt to measure the extent of atherosclerotic disease in the infrainguinal vessels using angiographic scoring may seem like a simplistic approach but arteries which are very heavily diseased are likely to cause

diversion of flow into disease free collateral vessels; indeed blood will never be exposed to the most heavily diseased (occluded) arteries. This study did show a correlation between the increase in coagulability across a limb and the extent of ischaemia, measured by ABPI on that side. Thus flow through ischaemic tissue may promote coagulation but this cannot exclude the possibility that this relationship arises through the close association between the severity of ischaemia and the extent of atherosclerotic disease past which the blood must flow to the distal limb. The changes in coagulability could all be due to exposure to atherosclerotic disease.

ABPI was used as a measure of ischaemia in this study rather than as a measure of atherosclerotic disease in the limb because it reflects the perfusion pressure at ankle level and thus relates to the level of ischaemia. It depends on flow through collateral vessels as well as through stenosed arteries and is therefore not a measure simply of atherosclerotic disease. Presenting symptoms could have been used as a measure of ischaemia (ie asymptomatic v claudication v rest pain) in this study but in the less symptomatic leg (the limb in this study) any symptoms of claudication could have been masked by more severe disability in the other leg.

TEG was used to assess coagulation because its sensitivity and specificity compares well with routine coagulation tests[179,180] and it provides a measure of the overall coagulation status of a given blood sample rather than of individual clotting factors in isolation[181]. TEG analysis of citrated samples is more sensitive in the detection of procoagulant alterations than other

coagulation tests and is therefore ideal for the assessment of coagulation in patients with PVD.

This study has confirmed that blood becomes more coagulable as it traverses the ischaemic limb and has shown that platelet activation, as well as increased thrombin production, takes place within the limb. This study has clearly shown that blood coagulability increases as it flows down an atherosclerotic iliac artery and that the magnitude of that change relates to the severity of stenosis. Ischaemia is unlikely to play a part here and it is probable that the changes in blood coagulability are caused by the presence of atherosclerotic disease. This may be due to interaction with the atherosclerotic plaque but in the carotid and coronary arteries, where this has been extensively studied, enhanced coagulation is usually associated with 'unstable plaque' and ulceration. Another possible cause of coagulation activation in the iliac arteries might be the increased shear stress[182]associated with localised stenosis. This has been shown to induce platelet aggregation in both acute coronary syndromes[183]and chronic coronary artery disease[184]. It is possible that other steps in coagulation are also activated under such circumstances. The third possibility is that turbulence in blood flow over irregular plaque promotes coagulation although there is no direct evidence for this in the literature.

4.5 - INVESTIGATION OF THE THROMBOGENIC POTENTIAL OF NICM IN PVD USING TEG

Patients with PAOD are known to be systemically hypercoagulable and many of these patients undergo angiography or angioplasty and any changes in coagulability caused by injection of contrast medium may have major implications for the patient. This would be particularly important if contrast injection led to an increase in coagulability because it could promote thrombosis at the site of angioplasty and in the poorly perfused distal vessels of an ischaemic leg. This study looked at the changes in coagulation adjacent to the site of contrast injection/potential angioplasty to determine the magnitude of change locally.

There have been conflicting data from both *in vitro* and *in vivo* studies regarding the effect of non-ionic media (NICM). NICM have been shown to interact with haemostatic factors via platelet[185], endothelial[186], coagulation[187] and fibrinolytic[188] mechanisms to increase coagulability when mixed with blood *in vitro*. Comparisons with ionic contrast media (ICM) in these studies suggest that the NICM have a greater procoagulant effect. At least one prospective *in vivo* study[189], however, in which peripheral venous samples were taken for analysis before and after injection of contrast media in patients undergoing angiography for peripheral arterial occlusive disease (PAOD) and randomised to NICM or ICM, has shown no difference between the effects of the different contrast media.

Modern techniques have led to a reduction in thrombo-embolic episodes in patients undergoing diagnostic and therapeutic angiographic procedures and, in those undergoing angioplasty procedures, much improved patency rates.[190] Despite this positive trend there is anxiety among radiologists and in the cardio-vascular fraternity about the role of contrast medium in thrombo-embolic complications. Patients with coronary and peripheral vascular disease have an activated state of coagulation manifest by consistently elevated of markers of activation of platelet, endothelium and coagulation factor mechanisms. When these patients undergo angiographic procedures there is a potential risk of further activation of these coagulant mechanisms due to interactions with the contrast medium used and the endothelial injury following angioplasty.

Contrast agents and their effect on coagulation in patients undergoing diagnostic and therapeutic angiographic procedures has been a much debated topic. Various investigators have reported the pro and anti-coagulant effects of both ICM and NICM using *in vitro* and *in vivo* methods. Grines et al[191] found ICM caused less *in-vivo* thrombosis than NICM in a canine model of arterial injury. Hwang et al[192] suggested that the use of NICM increased the potential risk of thrombosis in patients undergoing coronary angioplasty procedures. Kopko et al[193] reported thrombin generation in peripheral venous blood after mixing with NICM in glass syringes and Robertson et al[194] reported clot formation in angiographic syringes containing NICM. Grollman et al[195] reported three cases in which clots formed during angiography with NICM and Fareed et al[196] in their

review also suggested that the potential for thrombotic complications is higher with non-ionic contrast agents based on generation of thrombin when blood is mixed with NICM in glass syringes and reported a procoagulant response on TEG profile[197] when NICM (Iopamidol) was mixed at a ratio of 1:80 (contrast media: blood). Gasperetti et al[198] reported an incidence of thrombosis up to 18% in patients undergoing coronary angioplasty when NICM was used compared to 4% in those where an ionic contrast agent was used. Davidson et al[199] however reported an incidence of thrombosis in 0.18% in 7230 consecutive patients who underwent cardiac catheterisations and found no difference in thrombotic events between ICM and NICM usage. This study included patients who were on heparin prior to catheterisation and the results were attributed to the effect of heparin. Recently Mukherjee et al[200] reported an increase in thrombin-antithrombin (TAT) and F_1 and F_2 in peripheral venous samples 30 minutes after Iohexol (NICM) use in 10 patients undergoing coronary angiography but no such increase in patients who underwent coronary angioplasty who also received heparin during the procedure suggesting that the thrombogenic potential of iohexol was neutralised by heparin. Contrast agents are also reported to affect the platelet aggregation and adhesion in various *in vitro* studies. Wiesel et al[201] found increased platelet adhesion *in vitro* on the luminal surface of a vascular graft and Grabowski et al[202] reported platelet adhesion on the endothelial surface in another *in vitro* model after exposure to NICM. Heptinsall et al[203] reported potentiation of platelet aggregation and degranulation with the use of NICM using flow cytometry and P-selectin measurements after mixing

contrast agents with peripheral venous samples. Brosstad et al[204] in their review stated that the observed discrepancies in contrast induced changes are due to the various *in vitro* tests being at best, comparable to stagnant flow conditions and suggested that satisfactory comparisons can only be made *in vivo* studies under dynamic flow conditions as in this study.

This study identified a global reduction in the coagulability of blood adjacent to the site of injection of NICM during routine diagnostic peripheral angiography. This should reflect the situation at the site of proposed angioplasty prior to starting the procedure, with mixing of contrast agent and circulating blood rather than the static situation studied in the *in vitro* studies discussed above. TEG also identifies overall changes in coagulation rather than changes in individual factors and so has more relevance to the clinical situation.

Results from this study shows that contrary to several published reports Iohexol, a NICM does not lead to activation of coagulation in patients undergoing diagnostic angiography but in fact reduces overall coagulability. The effect of contrast injection in the aorta, in a volume used for angiography, is to partly counteract that hypercoagulability associated with PAOD. Despite this reduction the overall coagulation status of patients with PAOD remains significantly elevated when compared to the baseline peripheral venous samples obtained from age matched control subjects ($p=0.014$). Peripheral venous samples from controls were used for comparisons as it is difficult ethically to justify invasive aortic sampling and the coagulation should be similar in healthy subjects irrespective of site of blood sampling.

The significant changes found in TEG parameters suggest that the local effect of NICM is a reduction of coagulation activity rather than the activation suggested by some previous studies and in patients with peripheral vascular disease iohexol (a NICM) leads to no further increase in their blood coagulability during peripheral diagnostic angiography.

4.6 - IDENTIFICATION OF ENDOGENOUS HEPARIN-LIKE ACTIVITY IN PVD USING TEG

This study for the first time showed that PVD is associated with reduced heparin- like activity in the blood and the magnitude of reduction relates to the severity of atherosclerotic disease measured by ABPI. This study demonstrated that heparinase modified TEG can identify endogenous heparin activity in both controls and patients with PAOD.

Patients with peripheral vascular disease are known to have systemic hypercoagulability. This study was based on a hypothesis that part of the heightened coagulant activity is related to reduced production of endogenous heparin-like substance in these patients. Long-term intermittent heparin therapy in non-anticoagulant doses has been shown to significantly reduce the cardiovascular deaths when compared to control groups in patients with angina[205] and intermittent exogenous heparin in non-anticoagulant doses has also been shown to reduce fibrinogen levels[206], an independent marker for cardiac event in patients with PVD.

Studies involving patients with PVD have shown improvement in pain free walking distances and maximum walking distances in those receiving heparin compared to platelet agents[207,208]. Tuman et al, [24] using the heparinase modified TEG method in 42 healthy volunteers, found no significant endogenous heparin-like activity in most of samples obtained from healthy volunteers analysed 3 minutes after collection and citration although they did

find some activity in a small group which they ascribed to contamination. Ideally blood samples should be analysed within 6 minutes after collection[209]. This is often not practicable and samples are usually citrated and analysed after a delay.

A recent study[210]looking in to the effects of citrate storage on TEG confirmed that TEG parameters were different in recalcified, citrated blood samples compared with native blood. The observed changes were progressive in samples during 0-30 min. storage but were stable thereafter and the authors recommend analysis after a citrate storage period between 1- 8 hours for reliable TEG results. We followed the above method and carried out native and heparinase modified TEG after a citrate storage period of 1-2 hours for all samples in an effort to obtain reliable results. This may explain the difference between our results and those of Tuman et al[211].

The reduction in heparin like activity is probably related to both reduced production in the arterial wall and increased heparin consumption. Studies of atherosclerotic human coronary arteries have shown a reduction in HSPG in the arterial wall with a proportional increase in chondroitin-6-sulphate and dermatan sulphate proportional to the increasing severity of atherosclerosis[212]. Heparan sulphate content in the intima of human arteries especially in atherosclerosis-prone regions such as coronary arteries, renal arteries, internal carotid artery at the level of the carotid sinus, abdominal aorta and stenotic iliac arterial segments have been shown to decrease with an increase in the severity of atherosclerotic lesions.[213] There may well be

increased consumption of heparin in PAOD in view of increased activity of coagulation cascade with a positive feedback from increased thrombin levels and reduced anti-thrombin levels shown to exist in patients with progressive peripheral vascular disease[21].

Heparin exerts its effects through two mechanisms, both of which requires binding of antithrombin, firstly to the pentasaccharide polymer within the AT structure that leads to a change in the protein that mediates its inhibition of factor Xa and secondly binding of thrombin (factor IIa) to heparin close to the pentasaccharide AT binding site. Formation of this complex between AT, thrombin and heparin results in inactivation of thrombin[214]. Thus the effects of heparin on coagulation are mediated through AT. We know that serum from patients with PAOD has reduced levels of AT. Lack of antithrombin activity may account for reduced measured heparin activity. Reduced "heparin-like" activity has obvious implications. Endogenous heparin and HSPGs found on the negatively charged[215] luminal surfaces of the endothelial cells are essential for inhibition of coagulation at the endothelial surface[216] and lipoprotein lipase function[217] both of which are defective in atherosclerotic vascular endothelium[218]. Circulating endogenous heparin and heparan sulphates in the proteoglycan layer of the intima subjacent to the lumen bind with and activate anti-thrombin to form an effective anticoagulant complex on the endothelial surface[11]. Endogenous heparin has also been shown to decrease micro thrombi formation at sites of endothelial injury[219]. Heparin has been shown to inhibit platelet activation following flow through vessels with a high and moderate shear stress by its action that is not mediated by its

antithrombin activity[220]. Heparin also prevents endothelial cell dysfunction following ischaemia by augmenting vasodilation mediated by endothelial derived relaxing factor by a mechanism independent of its anticoagulant effect.[221] These non-anticoagulant actions of heparin are of particular importance as both high shear and endothelial dysfunction following ischaemia are shown to exist in patients with PVD.

Patients with PVD manifest changes in haemostatic factors[222], and platelet function.[223,224] Studies of haemostatic factors and products of coagulation show a similar trend in both cardiac and PAOD patients suggesting activation of coagulation. Once patients get to a stage where endogenous heparin activity is significantly reduced then the obstructive effects of atherosclerosis on blood flow may be compounded by an increasing tendency to thrombosis within the downstream vessels. These changes in coagulation are particularly exaggerated during the perioperative period and can predispose PAOD patients to thrombotic events following revascularisation[225,226].

A variety of heparin related products have also been shown to inhibit vascular smooth muscle cell proliferation[227] and they play a regulatory role that alters the endothelial response after vascular injury. Indeed, exogenous heparin preparations have been shown to effectively suppress smooth muscle cell proliferation following injury to vascular endothelium[228]. Low molecular weight heparin (LMWH) has been shown to reduce neointimal hyperplasia in cultured human saphenous vein and the use of heparin in non-anticoagulant doses to achieve similar benefit in practical clinical situations has been suggested by the authors of the study[229]. Low levels of

endogenous heparin may allow excessive intimal hyperplasia at parts of trauma within the vascular system –either due to clinical intervention or in areas of turbulence within the circulation.[230]

Studies undertaken to check the clinical and haemostatic effects of small doses of LMWH (15,000 anti Xa units/day/SC) in patients with PAOD has shown an improvement in absolute claudication time and distance along with a significant increase in activated partial thromboplastin time (APTT) that remained within normal limits. A three month course of unfractionated heparin (12,500 I.U once daily) has also been shown to improve pain free walking distances to a greater extent than an anti-platelet agent, ticlopidine alone[231,232,233] in patients with PAOD.

This study provides supporting evidence for the use of low dose heparin in patients with PVD. This may be particularly important around the time of prolonged immobility and surgical intervention.

4.7 - INVESTIGATION OF CHANGES IN COAGULATION CHANGES IN AORTIC ANEURYSMAL DISEASE USING TEG

This study looked at coagulation changes in patients with non-ruptured abdominal aortic aneurysm (AAA) undergoing elective aneurysm repair. Bradbury et al[234] reported that preoperative haematological parameters were frequently found to be normal in this group of patients who later developed thrombocytopenia, significantly related to the duration of aortic cross-clamp, thrombocytosis and hyperfibrinogenaemia in the postoperative period. Similar changes were also seen in patients undergoing aortic surgery for occlusive disease. Gibbs et al[235] reported that when coagulation factors were measured preoperatively, and on days two, four, and six postoperatively it was found that there were no significant changes outside the normal range in prothrombin time, partial thromboplastin time, or thrombin clotting time. However, there were large increases in the procoagulants, fibrinogen, factor VIII coagulant, factor VIIIRag/von Willebrand factor, and in alpha 1-antitrypsin. Over the same time there were marked decreases in the naturally occurring anticoagulants, protein C and antithrombin III, and in alpha 2-macroglobulin. These changes implied that the patients were "hypercoagulable" in the postoperative period. The maximum changes in the procoagulants occurred on either postoperative day two or day four. The maximum changes in the natural anticoagulants occurred on postoperative day two. There were no significant changes in factor V, factor X, alpha 2-antiplasmin, or platelet aggregability. The timing of the changes coincided

with a period of high risk of perioperative myocardial infarction and other thrombotic events.

Holmberg et al[236] found that prothrombin fragment 1+2 (F1+2), thrombin-antithrombin complex (TAT) and soluble fibrin (SF) were elevated preoperatively in AAA patients. During aortic clamping all parameters increased significantly in cubital blood as well as in femoral blood and after aortic declamping F1+2 and TAT increased further. F1+2, TAT and SF were significantly higher in femoral than cubital blood. Postoperatively F1+2 and TAT returned to preoperative values, while SF still had a significantly higher level than preoperatively. The findings indicated that the coagulation system was strongly activated in the presence of an AAA with a further activation seen after surgery. The activity was still high, but on decline, one week postoperatively. Ischaemia and reperfusion of the lower part of the body were considered as major stimuli for thrombin generation and activity.

Holmberg et al[237] also found that F1+2, TAT, and D-dimer values in preop AAA patients were significantly higher compared to age matched controls and in the post-op period these parameters were significantly lower compared to preoperative period. The activity of the coagulation and fibrinolytic systems were shown to decrease after AAA surgery however, the activity was still found to be higher than in healthy age matched controls and the explanation may be that the thrombogenicity is lower in a vascular graft than in an aneurysmal sac but still higher than in a nonaneurysmal aorta. Adam et al[238] in their study involving ruptured and non-ruptured AAA

demonstrated that ruptured AAA repair is associated with inhibition of systemic fibrinolysis and intense thrombin generation. Similar changes were also seen in non-ruptured AAA but are of a lesser magnitude. They also concluded that the observed procoagulant state contributes to the microvascular and macrovascular thrombosis that leads to myocardial infarction, multiple organ failure, and thromboembolism. Skagius et al[239] indicated a state of activated coagulation in patients with a non-ruptured AAA that was intensified by rupture. They also reported normal fibrinolytic activities in patients with a non-ruptured AAA, but increased systemic fibrinolysis, as demonstrated by elevated tissue plasminogen antigen (tPAag) level, in patients with a ruptured AAA. The hyperfibrinolytic state was reinforced by shock in that study. However, they also found a relatively high incidence of thrombosis-related deaths, indicating a prothrombotic state instead of a hyperfibrinolytic state in patients presenting with shock following a ruptured AAA.

Patients with aortic aneurysm are in a chronic inflammatory state and a chronic hypercoagulable state. Preoperative thrombin-antithrombin complex (TAT), d-dimer, and cytokines are increased when compared with healthy subjects, indicating a hypercoagulable state. The hypercoagulable state and inflammatory process activate macrophages and leukocytes to release cytokines. These cytokines further induce hypercoagulability and an inflammatory reaction Thus, excessive activation of coagulation or severe inflammation can occur after aortic aneurysm surgery that leads to organ failure. Bown et al[240] in their study involving those undergoing elective

asymptomatic AAA repair, those with symptomatic but nonruptured AAA, and those with ruptured AAA reported 89% of the elective group, 92% of the emergency nonruptured (urgent) group, and 100% of the ruptured group developed systemic inflammatory response syndrome (SIRS). Multiorgan failure occurred in 3.8% of the elective group, 38% of the urgent group, and 64% of the ruptured AAA group.

Recent study by Wallinder et al[241] investigating haemostatic markers, Thrombin-antithrombin (TAT), prothrombin fragment 1+2 (F 1+2)--markers of thrombin generation, and von Willebrand factor antigen (vWFag) considered as a reliable marker of endothelial dysfunction, in patients with nonruptured abdominal aortic aneurysm with special regard to the influence of aneurysm size found high higher levels of TAT and D-dimer in patients with abdominal aortic aneurysm. The highest level of TAT and D-dimer were detected in patients with large compared to small AAA indicating a state of activated coagulation in patients with abdominal aortic aneurysm which is dependent on aneurysm size.

In a study conducted to determine activation of coagulation in patients undergoing open and endovascular infrarenal abdominal aortic aneurysm repair (EVAR), elevated markers of coagulation were measured in both groups. Fibrinopeptide A and D-dimer levels did not differ significantly between the groups but the levels of fibrin monomer and thrombin-antithrombin complex were significantly higher in patients undergoing EVAR reflecting increased thrombin activity and thrombin formation compared with

open surgery suggesting increased procoagulant activity in EVAR when compared with open surgery.

Gibbs et al[242] compared postoperative changes in procoagulant, anticoagulant, and antifibrinolytic factors in patients undergoing abdominal aortic surgery, carotid endarterectomy, and femoro-popliteal bypass. Significant increases in plasma fibrinogen and factor VIII coagulant levels were found following all three procedures. There were significant decreases in antithrombin III and protein C, and increases in thrombin-antithrombin complex levels were found in the abdominal aortic group only and no significant changes in type 1 plasminogen activator inhibitor levels following any of the groups. The results indicated that all three procedures are associated with an increased potential for thrombosis. However, patients undergoing abdominal aortic surgery are particularly at risk due to concurrent decreases in natural anticoagulant factors. Many studies have reported similar haemostatic abnormalities and observed thrombotic events and especially an increased incidence of myocardial infarction in those with AAA undergoing surgery. A prospective multi-centre study conducted by Joint Vascular Research Group[243] involving patients undergoing elective abdominal aortic aneurysm (AAA) surgery investigated the relationship between intraoperative intravenous heparinisation, blood loss during surgery and thrombotic complications reported that 5.7% of the non-heparin group but only 1.4% of the heparinised patients suffered a fatal perioperative myocardial infarction (MI); $p < 0.05$. All MI, including non-fatal events, affected 8.5% and 2% respectively ($p = 0.02$). Suggested intravenous

heparin, given before aortic cross clamping, can play an important prophylactic role against perioperative MI in relation to AAA surgery. The current practice of intravenous heparin prior to aortic cross clamping and subsequent administration of low molecular weight heparin in the postoperative period reflect the need to counteract the hypercoagulability and its consequences observed after AAA surgery.

Routine coagulation tests in patients undergoing uncomplicated abdominal aortic aneurysm repair do not reflect the sustained hypercoagulability observed and reported by various studies. These tests do not reflect well-documented abnormalities of platelet function and deranged fibrinolytic mechanisms especially in the postoperative phase where hypercoagulability can lead to thrombotic events involving critical vessels leading to increased morbidity and mortality. This hypercoagulability is not reflected by standard coagulation monitoring[244] and seems to be predominantly caused by increased platelet reactivity.

TEG technique involves coagulation and dissolution and provides the information lacking in standard tests of coagulation, i.e, the functional aspect of platelets, coagulation factors and the speed and extent of fibrinolysis in given blood sample within a reasonable short period of time that can be repeated to assess the effectiveness of the treatment. This is important as coagulopathy and massive bleeding complicating ruptured abdominal aneurysm surgery are the major causes of death in patients who present with shock following rupture of AAA.

TEG has been shown to reduce the consumption of blood and blood products in the treatment of massive haemorrhage[245] complicating open cardiac procedures and is now routinely employed as point of care testing method in cardiac surgical departments. TEG is now routinely employed to identify hypercoagulation following neurosurgery, in major trauma and liver transplantation to guide administration of blood components, coagulation factors and platelets. The sensitivity and specificity of this technique has been shown to be particularly good when compared to routine tests of coagulation, anti-Xa levels, bleeding time, thrombin time and APTT/PT ratio estimations especially in the management of coagulopathy.

Thrombelastography can identify hypercoagulability following abdominal aortic bypass surgery using a small volume whole blood sample. Modified TEG techniques, incorporating specific blockers to negate the effects of specific anticoagulant(heparin), platelet blocking agent(Aspirin, Absciximab) or a component of the coagulation cascade(Tissue factor) that can give accurate and sensitive assessment of the underlying coagulation pattern.

Gibbs et al[246] employing native whole blood thrombelastography performed preoperatively and on days 1, 2, and 3 postoperatively observed a significant decrease in r on day one ($P < 0.0001$), with concurrent increases in alpha ($P < 0.0001$) and MA ($P < 0.001$). On days 2 and 3 there were further increases in MA ($P < 0.0001$). These changes indicated enhanced procoagulant activity and progressive increases in maximum clot strength. Butler et al identified similar hypercoaculable pattern in recalcified whole blood in the samples

obtained during surgery and in the immediate postoperative period. Increased fibrinogen concentration was identified as the dominant factor in determining the post-operative changes shown by TEG. Mahla et al[247] tested the hypothesis that the parallel use of standard and abciximab-cytochalasin D-modified TEG can assess 7 days' postoperative hypercoagulability and can estimate the independent contribution of procoagulatory proteins and platelets and concluded that serial standard and modified thromboelastography can reveal prolonged postoperative hypercoagulability and the independent contribution of platelets and procoagulatory proteins to clot strength.

Gibbs et al[248] used TEG native whole blood TEG and Heparinase modified TEG to identify the effect of low-dose heparin on postoperative hypercoagulability in patients undergoing elective abdominal aortic surgery who received unfractionated heparin 5000IU bd SC commencing on the first postoperative day found significant decreases in r and K, and increases in alpha in the heparinase-modified TEGs postoperatively ($P < 0.01$). There were significant differences between the postoperative native whole blood and heparinase-modified TEGs for all TEG variables ($P < 0.01$). The results indicated that low-dose heparin reduces postoperative hypercoagulability following abdominal aortic surgery as assessed by TEG.

This study has shown that non-ruptured AAA and aortic occlusive disease are associated with activation of coagulation. TEG identified baseline hypercoagulation in these groups in the absence of any significant

abnormality in routine coagulation tests performed at the baseline. This study also showed a significant reduction in hypercoagulation during aortic cross clamping in AAA group but not in the occlusive group. This is an interesting finding as platelet sequestration and consumption of coagulation factors are shown to be responsible for this change in the coagulation observed in AAA patients without rupture and is reported to be more pronounced in those presenting with rupture and shock. Continued hypercoagulability after cross clamping in occlusive group is consistent with further activation of coagulation resulting from additional peripheral ischaemia over and above pre-existing critical limb ischaemia with or without tissue loss.

Significant increase in TEG parameters in the post-operative period observed in patients with non-ruptured AAA and in those with aortic occlusive disease undergoing reconstruction procedures is consistent with findings in many studies employing markers of activation of haemostasis and tests of fibrinolysis. TEG has shown significant and sustained activation of coagulation during surgery which is sustained for up to six weeks in the postoperative period. However, absence of any significant relationship between baseline TEG, haemostatic variables and observed thrombotic events in this study identified the reduced predictive value of TEG parameters used in this study.

4.8 - INVESTIGATION OF TEG CHANGES IN PATIENTS WITH PVD UNDERGOING SURGERY

Patients with peripheral arterial occlusive disease (PAOD) are at significantly increased risk of morbidity and mortality from cardiovascular and cerebrovascular events.[249] This is particularly the case in the early post-operative period. Myocardial infarction is the commonest life-threatening complication of major vascular surgery. Overt myocardial infarction occurs in 12% of patients following lower limb surgery[250], and in a recent study a significant rise in troponin occurred in 37%.[251] Abnormal platelet function has been implicated in the development and progression of atherosclerosis, as well as in the pathogenesis of acute cardiac ischaemic events.[252]. Brittenden et al[253] and others have shown that platelet activation is increased in patients with lower limb ischaemia suggesting an underlying pro-thrombotic state.[254] In addition to platelet activation, coagulation activation has been implicated in the increased incidence of ischaemic events in patients with PAOD.[255] Raised levels of various markers of coagulation have been shown to occur in patients with intermittent claudication such as D-dimer and thrombin-antithrombin complex (TAT)[256]. In addition, a number of studies have demonstrated the presence of a hypercoagulable state following lower limb revascularisation.[257],[258] Peripheral venous blood samples demonstrate a systemic increase in coagulability in patients with peripheral arterial occlusive (PAOD) affecting the legs,[259,260,261]. Fibrinogen[262] and products of the coagulation cascade including thrombin-antithrombin complexes and

prothrombin F1+F2[263] as well as D- dimers[264], indicating increased thrombin synthesis and fibrinolysis are consistently raised in patients with PAOD. Ischaemic heart disease is associated with similar changes[265,266,267]. It is also clearly established that an ischaemic limb is associated with increased clotting activity.

The primary aim of this study is to assess the effects of major arterial revascularization surgery on coagulation activation in patients with symptomatic peripheral vascular disease and to document any significant relationship to TEG changes observed during and after surgery

This study has shown that PVD subjects exhibit an heightened state of coagulation at the outset shown by significantly raised TEG parameter values consistent with activation of coagulation when compared to control subjects. Serial TEG analysis of subjects undergoing surgery has shown significant activation of coagulation during surgery but before revascularisation when compared to the control subjects. This study has shown further activation of coagulation following revascularisation of an ischaemic limb. After revascularisation, an on-going prothrombotic state lasting more than a week has been demonstrated in this study. This study also demonstrated significant activation of coagulation before, during and after revascularisation procedures for critical limb ischaemia. This has been observed despite the use of heparin and aspirin. These findings add support to the concept that arterial revascularisation surgery in patients with PAOD is associated with increased coagulation and platelet activation. It is unclear whether this is

related to an ischaemia-reperfusion injury or indeed if surgery in non-vascular patients may also induce these effects. This study did not show any significant changes in TEG values that can be attributed to the effect of confounding factors such as clamp time and type of surgical procedure.

Patients with PVD are at risk of developing a significant postoperative thrombotic event. This study has identified a significant association between MA, a measure of ultimate clot strength (a reflection of activation of platelets and coagulation factor; thrombin) in a given blood sample and occurrence of any postoperative thrombotic event.

Significant changes in TEG parameter values that were sustained during the postoperative phase up to 6 weeks is consistent with activation of platelet activity and coagulation factors in this group of patients. Interestingly there were no abnormalities in the routine coagulation tests during the same period in this study. This suggests that TEG is helpful in identifying such changes and that appropriate therapeutic interventions i.e., prolonged use of heparin outside immediate perioperative period can be helpful in reducing the incidence of thrombotic events in this group of patients.

4.9 - FINAL COMMENTS

To summarise, this prospective clinical study has confirmed that TEG can be used to monitor coagulation changes in patients with symptomatic peripheral vascular disease. For the first time this study has shown that the presence of a hypercoagulable state in PVD may have its origins in an ischaemic limb itself. While severe systemic atherosclerotic load and presence of ischaemic tissue can be the reasons for the activation of coagulation in patients with symptomatic PAOD, the same need not be the case with those with abdominal aortic aneurysmal disease. Routine coagulation tests often do not identify any haemostatic abnormality at the outset in patients with abdominal aortic aneurysm while tests for thrombophilia and fibrinolysis have consistently shown activation of coagulation and reduced inhibition of fibrinolysis. This pattern continues after surgery for a significant length of time and again routine coagulation tests again do not identify this sustained hypercoagulabiltiy. Period of ischaemia of lower limbs following aortic cross clamping during aortic surgery and presence of significant systemic atherosclerosis are logical explanations for the observed coagulation changes in these patients. However activation due to other factors such as stress related to surgery, administration of fluids, blood products, anaesthesia and surgery itself as in any non-vascular surgery cannot be discounted. This study also confirmed reduced endogenous heparin-like activity in PVD, a finding that provides evidence for use of low dose heparin in subjects with symptomatic ischaemia following surgery or prolonged periods of immobility.

Finally, this study has shown significant procoagulant changes during peripheral vascular surgery using Thromboelastography. These changes are significantly related to the occurrence of an ischaemic event in the postoperative period.

4.10 - FUTURE PLANS

This prospective clinical study using TEG has shown activation of coagulation during peripheral vascular surgery and has identified a significant positive relationship between postoperative coagulation status and occurrence of an ischaemic event.

Possible future research avenues include:

- Further studies using modified TEG methods, incorporating tissue factor blockade and aspirin blockade to identify specific defects in coagulation and platelet function in PVD.
- Further TEG studies involving postoperative PVD patients to assess if there is any significant reduction in postoperative ischaemic events as a result of current treatment guidelines that includes routine use of low molecular weight heparin in postoperative PVD patients following surgical revascularisation or amputation for critical limb ischaemia.

REFERENCES

[1] Senzolo M, Coppell J, Cholongitas E, Riddell A, Triantos CK, Perry D, Burroughs AK. The effects of glycosaminoglycans on coagulation: a thromboelastographic study. Blood Coagul Fibrinolysis. 2007;18(3):227.

[2] Bruce Bennett and Hamish M A Towler. HAEMOSTATIC RESPONSE TO TRAUMA. British Medical Bulletin (1985) 41 (3): 274-280.

[3] John P. Greer, Maxwell Myer Wintrobe. Chapter 20.Blood Coagulation and Fibrinolysis. Wintrobe's clinical hematology. 12th Edition.1999.

[4] Armando Tripodi, Massimo Primignani, Veena Chantarangkul, Alessandra Dell'Era, Marigrazia Clerici, Roberto de Franchis, Massimo Colombo§ and Pier Mannuccio Mannucci. Basic—Liver, Pancreas, and Biliary Tract. 10.1053j.Gastro.2009.08.045

[5]Reith A, Booth NA, Moore NR, Cruickshank DJ, Bennett B. Plasminogen activator inhibitors (PAI-1 and PAI-2) in normal pregnancies, pre-eclampsia and hydatidiform mole. Br J Obstet Gynaecol. 1993 Apr;100 (4):370-4.

[6] Davi, G, Patrono, C (2007) Platelet activation and atherothrombosis. N Engl J Med357, 2482-2494

[7] H. Rasche. Haemostasis and thrombosis: an overview. European Heart Journal Supplements (2001) **3** (Supplement Q), Q3–Q7

[8] Wu KK, Thiagarajan P. Role of endothelium in thrombosis and hemostasis. Annu Rev Med 1996; 47: 315–31.

[9] Rosenfeld SJ, Gralnick HR. Adhesive interactions in hemostasis. Acta Haematol 1997; 97: 118–25.

[10] Key NS, Bach RR. Tissue factor as a therapeutic target. Thromb Haemost 2001; 85: 375–6.

[11] Preissner KT, Nawroth PP, Kanse SM. Vascular protease receptors: integrating hemostasis and endothelial cell functions. J Pathol 2000; 190: 360–72.

[12] Rosenberg RD, Aird WC. Mechanism of disease: vascular-bedspecific hemostasis and hypercoagulable states. N Engl J Med 1999; 340: 1555–64.

[13] Turitt VT, Hall CL. Mechanical factors affecting hemostasis and thrombosis. Thromb Res 1998; 92(suppl 2): 25–32.

[14] Coleman RW, Hirsh J, Marder VJ, Clowes AW, George JN (editors). Hemostasis and thrombosis. Basic principals and clinical practice. Philadelphia: Lippincott Williams & Wilkins, 2001.

[15] Seino Y, Ikeda U, Ikeda M, Yamamoto K, Misawa Y, Hasegawa T, Kano S, Shimada K. Interleukin 6 gene transcripts are expressed in human atherosclerotic lesions. Cytokine. 1994 Jan; 6(1):87-91.

[16] Rus HG, Vlaicu R, Niculescu F. Interleukin-6 and interleukin-8 protein and gene expression in human arterial atherosclerotic wall. Atherosclerosis. 1996 Dec 20; 127(2):263-71.

[17] Szekanecz Z, Shah MR, Pearce WH, Koch AE. Human atherosclerotic abdominal aortic aneurysms produce interleukin (IL)-6 and interferon-gamma but not IL-2 and IL-4: the possible role for IL-6 and interferon-gamma in vascular inflammation. Agents Actions. 1994 Oct; 42(3-4):159-62.

[18] Shteinberg D, Halak M, Shapiro S, Kinarty A, Sobol E, Lahat N, Karmeli R. Abdominal aortic aneurysm and aortic occlusive disease: a comparison of risk factors and inflammatory response. Eur J Vasc Endovasc Surg. 2000 Nov; 20(5):462-5.

[19] D'Agostino RB, Kannel WB, Belanger AJ, Sytkowski PA.
Trends in CHD and risk factors at age 55-64 in the Framingham Study. Int J Epidemiol. 1989;18(3 Suppl 1):S67-72.

[20] Clarke R, Emberson J, Fletcher A, Breeze E, Marmot M, Shipley MJ. Life expectancy in relation to cardiovascular risk factors: 38 year follow-up of 19,000 men in the Whitehall study. BMJ. 2009 Sep 16;339:b3513. doi: 10.1136/bmj.b3513.

[21] Baker IA, Eastham R, Elwood PC, Etherington M, O'Brien JR, Sweetnam PM. Haemostatic factors associated with ischaemic heart disease in men aged 45 to 64 years. The Speedwell study. Br Heart J. 1982 May;47(5):490-4.

[22] Hirsch AT, Haskal ZJ, Hertzer NR *et al.:* ACC/AHA 2005 practice guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease): endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. *Circulation* 113(11), E463—E654 (2006).

[23] Weitz JI, Byrne J, Clagett GP *et al.:*Diagnosis and treatment of chronic arterial insufficiency of the lower extremities: a critical review. *Circulation* 94(11), 3026— 3049 (1996).

[24] Criqui MH, Fronek A, Barrett-Connor E, Klauber MR, Gabriel S, Goodman D: The prevalence of peripheral arterial disease in a defined population. *Circulation* 71(3), 510—515 (1985).

[25] Libby P. Inflammation in atherosclerosis. Nature. 2002; 420: 868–874.

[26] Bhatt DL, Steg PG, Ohman EM, *et al*.. International prevalence, recognition, and treatment of cardiovascular risk factors in outpatients with atherothrombosis. *JAMA* 2006;**295**:180–9

[27] Anthonisen NR, Skeans MA, Wise RA, *et al*.. The effects of a smoking cessation intervention on 14.5-year mortality: a randomized clinical trial. *Ann Intern Med* 2005;**142**:233–9.

[28] Wilson K, Gibson N, Willan A, *et al*.. Effect of smoking cessation on mortality after myocardial infarction: meta-analysis of cohort studies. *Arch Intern Med* 2000;**160**:939–44

[29] Nicolas W Shammas. Epidemiology, classification, and modifiable risk factors of peripheral arterial disease. Vasc Health Risk Manag. 2007 April; 3(2): 229–234.

[30] Gotto AM Jr. Low high-density lipoprotein cholesterol as a risk factor in coronary heart disease: a working group report. *Circulation* 2001;103:2213–2218.

[31] Neaton JD, Wentworth D, for the Multiple Risk Factor Intervention Trial Research Group. Serum cholesterol, blood pressure, cigarette smoking, and death from coronary heart disease: overall findings and differences by age for 316,099 white men. *Arch. Intern. Med*. 1992;152:56–64.

[32] Criqui MH: Peripheral arterial disease — epidemiological aspects. *Vasc. Med.* 6 (Suppl. 3), 3—7 (2001).

[33] Graham IM, Daly LE, Refsum HM, et al. Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project. JAMA. 1997; 277:1775-81

[34] I. Ikonomidis, K. Stamatelopoulos, J. Lekakis, G.D. Vamvakou and D.T. Kremastinos, Inflammatory and non-invasive vascular markers: The multimarker approach for risk stratification in coronary artery disease, *Atherosclerosis* **199** (2008), pp. 3–11

[35] HennekensCH. Increasing burden of cardiovascular disease: current knowledge and future directions for research on risk factors. Circulation. 1998;97:1095–1102.

[36] K. Stamatelopoulos I. Ikonomidis, J. Lekakis, G.D. Vamvakou and D.T. Kremastinos, Inflammatory and non-invasive vascular markers: The multimarker approach for risk stratification in coronary artery disease, *Atherosclerosis* **199** (2008), pp. 14–17.

[37] E.A. Kaperonis, C.D. Liapis, J.D. Kakisis, D. Dimitroulis, V.G. Papavassiliou and D. Perrea *et al.*, Inflammation and Chlamydia pneumoniae infection correlate with the severity of peripheral arterial disease, *Eur J Vasc Endovasc Surg* **31** (2006), pp. 509–515.

[38] I.J. Kullo and C.M. Ballantyne, Conditional risk factors for atherosclerosis, *Mayo Clin Proc* **80** (2005), pp. 219–230.

[39] P. Gaede, P. Vedel, N. Larsen, J.V. Jensen, H.H. Parving and O. Pedersen, Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes, *N Engl J Med* **348** (2003), pp. 383–393.

[40] CLI, critical limb ischemia. Adapted from Dormandy JA, Rutherford RB: Management of peripheral arterial disease (PAD). TASC Working Group. TransAtlantic Inter-Society Concensus (TASC). J Vasc Surg 2000;31(Suppl):S1-S296. © 2000.

[41] Virchow RLK. Cellular pathology as based upon PhysiolоIgical and Pathohistology. In: Virchow RLK, ed. Thrombosis and Emboli. Frankfurt: Meidinger Sohn & Co., 1856. Matzdorff AC, Bell WR (transl), ed. Canton: Science History Publications, 1998;5–11,110

[42] Dormandy J, Heeck L, Vig S. Predictors of early disease in the lower limbs. Semin Vasc Surg. 1999 Jun; 12(2):109-17.

[43] Lowe GD, Fowkes FG, Dawes J, Donnan PT, Lennie SE, Housley E. Blood viscosity, fibrinogen, and activation of coagulation and leukocytes in peripheral arterial disease and the normal population in the Edinburgh Artery Study. Circulation. 1993 Jun; 87(6):1915-20.

[44] Woodburn KR, Lowe GD, Rumley A, Love J, Pollock JG. Int Angiol. 1995 Dec; 14(4):346 Relation of haemostatic, fibrinolytic, and rheological variables to the angiographic extent of peripheral arterial occlusive disease. Int Angiol. 1995 Dec; 14(4):346-52.

[45] Lassila R, Peltonen S, Lepäntalo M, Saarinen O, Kauhanen P, Manninen V. Severity of peripheral atherosclerosis is associated with fibrinogen and degradation of cross-linked fibrin. Arterioscler Thromb. 1993 Dec; 13(12):1738-42.

[46] Smith FB, Lee AJ, Hau CM, Rumley A, Lowe GD, Fowkes FG. Plasma fibrinogen, haemostatic factors and prediction of peripheral arterial disease in the Edinburgh Artery Study. Blood Coagul Fibrinolysis. 2000 Jan; 11(1):43-50.

[47] Wiseman S, Kenchington G, Dain R, Marshall CE, McCollum CN, Greenhalgh RM, Powell JT. Influence of smoking and plasma factors on patency of femoropopliteal vein grafts.BMJ. 1989 Sep 9;299(6700):643-6.

[48] Glueck CJ, Glueck HI, Stroop D, Speirs J, Hamer T, Tracy T. Endogenous testosterone, fibrinolysis, and coronary heart disease risk in hyperlipidemic men. J Lab Clin Med. 1993 Oct; 122(4):412-20.

[49] Smith FB, Lee AJ, Hau CM, Rumley A, Lowe GD, Fowkes FG. Plasma fibrinogen, haemostatic factors and prediction of peripheral arterial disease in the Edinburgh Artery Study. Blood Coagul Fibrinolysis. 2000 Jan; 11(1):43-50.

[50] Stuart J, George AJ, Davies AJ, Aukland A, Hurlow RA. Haematological stress syndrome in atherosclerosis. J Clin Pathol. 1981 May; 34(5):464-7.

[51] Cortellaro M, Cofrancesco E, Boschetti C, Mussoni L, Donati MB, Catalano M, Gabrielli L, Lombardi B, Specchia G, Tavazzi L, et al. Association of increased fibrin turnover and defective fibrinolytic capacity with leg atherosclerosis. The PLAT Group. Thromb Haemost. 1994 Aug; 72(2):292-6.

[52] Mustonen P, Lepäntalo M, Lassila R. Physical exertion induces thrombin formation and fibrin degradation in patients with peripheral atherosclerosis. Arterioscler Thromb Vasc Biol. 1998 Feb; 18(2):244-9.

[53] Mustonen P, Lepäntalo M, Lassila R.Thrombin formation and fibrin degradation in patients with peripheral atherosclerosis. Arterioscler Thromb Vasc Biol. 1998 Feb; 18(2):244-9.

[54] Trifiletti A, Barbera N, Pizzoleo MA, Leone G, Lucifora S, Soraci S, Scamardi R, Pedullà M, Frisina N. Hemostatic disorders associated with arterial hypertension and peripheral arterial disease. J Cardiovasc Surg (Torino). 1995 Oct; 36(5):483-5.

[55]Woodburn KR, Rumley A, Lowe GD, Pollock JG. Fibrinogen and markers of fibrinolysis and endothelial damage following resolution of critical limb ischaemia. Eur J Vasc Endovasc Surg. 1995 Oct; 10(3):272-8.

[56] De Buyzere M, Philippé J, Duprez D, Baele G, Clement DL. Coagulation system activation and increase of D-dimer levels in peripheral arterial occlusive disease. Am J Hematol. 1993 Jun; 43(2):91-4.

[57] Tzoulaki I, Murray GD, Price JF, Smith FB, Lee AJ, Rumley A, Lowe GD, Fowkes FG. Hemostatic factors, inflammatory markers, and progressive peripheral atherosclerosis: the Edinburgh Artery Study. Am J Epidemiol. 2006 Feb 15; 163(4):334-41. Epub 2005 Dec 15.

[58] Boneu B, Leger P, Arnaud C. Haemostatic system activation and prediction of vascular events in patients presenting with stable peripheral arterial disease of moderate severity. Royat Study Group. Blood Coagul Fibrinolysis. 1998 Mar;9(2):129-35.

[59] Woodburn KR, Rumley A, Lowe GD, Pollock JG. Fibrinogen and markers of fibrinolysis and endothelial damage following resolution of critical limb ischaemia. Eur J Vasc Endovasc Surg. 1995 Oct; 10(3):272-8.

[60] De Buyzere M, Philippé J, Duprez D, Baele G, Clement DL. Coagulation system activation and increase of D-dimer levels in peripheral arterial occlusive disease. Am J Hematol. 1993 Jun; 43(2):91-4.

[61] Becker F.Exploration of arterial function with noninvasive technics. Results in chronic arterial occlusive disease of the lower limbs according to Leriche and Fontaine classification. Int Angiol. 1985 Jul-Sep; 4(3):311-22.

[62] Herren T, Stricker H, Haeberli A, Do DD, Straub PW. Fibrin formation and degradation in patients with arteriosclerotic disease. Circulation. 1994 Dec; 90(6):2679-86.

[63] Strano A, Hoppensteadt D, Walenga JM, Fareed J, Sabbá C, Berardi E, Allegra C, Carlizza A, Binaghi F, Fronteddu F, Del Guercio R, Del Guercio M, Pinto A, Alletto G, Nazzari M, Ferrari PA. Plasma levels of the molecular markers of coagulation and fibrinolysis in patients with peripheral arterial disease. Semin Thromb Hemost. 1996; 22 Suppl 1:35-40.

[64] Scarabin PY, Aillaud MF, Amouyel P, Evans A, Luc G, Ferrières J, Arveiler D, Juhan-Vague I. Associations of fibrinogen, factor VII and PAI-1 with baseline findings among 10,500 male participants in a prospective study of myocardial infarction—the PRIME Study. Prospective Epidemiological Study of Myocardial Infarction. Thromb Haemost. 1998 Nov; 80(5):749-56.

[65] Trifiletti A, Barbera N, Pizzoleo MA, Leone G, Lucifora S, Soraci S, Scamardi R, Pedullà M, Frisina N. Hemostatic disorders associated with arterial hypertension and peripheral arterial disease. J Cardiovasc Surg (Torino). 1995 Oct; 36(5):483-5.

[66] Philipp CS, Cisar LA, Kim HC, Wilson AC, Saidi P, Kostis JB. Association of hemostatic factors with peripheral vascular disease. Am Heart J. 1997 Nov;134(5 Pt 1):978-84.

[67] Roller RE, Janisch S, Carroll V, Kvas E, Pilger E, Binder BR, Wojta J. Changes in the fibrinolytic system in patients with peripheral arterial occlusive disease undergoing percutaneous transluminal angioplasty. Thromb Res. 1999 May 15; 94(4):241-7.

[68] Killewich LA, Gardner AW, Macko RF, Hanna DJ, Goldberg AP, Cox DK, Flinn WR. Progressive intermittent claudication is associated with impaired fibrinolysis. J Vasc Surg. 1998 Apr; 27(4):645-50.

[69] Lee AJ, Fowkes FG, Lowe GD, Rumley A. Fibrin D-dimer, haemostatic factors and peripheral arterial disease. Thromb Haemost. 1995 Sep; 74(3):828-32.

[70] Woodburn KR, Rumley A, Lowe GD, Pollock JG. Fibrinogen and markers of fibrinolysis and endothelial damage following resolution of critical limb ischaemia. Eur J Vasc Endovasc Surg. 1995 Oct; 10(3):272-8.

[71] Cortellaro M, Boschetti C, Cofrancesco E, Zanussi C, Catalano M, de Gaetano G, Gabrielli L, Lombardi B, Specchia G, Tavazzi L, et al. The PLAT Study: hemostatic function in relation to atherothrombotic ischemic events in vascular disease patients. Principal results. PLAT Study Group. Progetto Lombardo Atero-Trombosi (PLAT) Study Group. Arterioscler Thromb. 1992 Sep; 12(9):1063-70.

[72] Strano A, Hoppensteadt D, Walenga JM, Fareed J, Sabbá C, Berardi E, Allegra C, Carlizza A, Binaghi F, Fronteddu F, Del Guercio R, Del Guercio M, Pinto A, Alletto G, Nazzari M, Ferrari PA. Plasma levels of the molecular

markers of coagulation and fibrinolysis in patients with peripheral arterial disease. Semin Thromb Hemost. 1996; 22 Suppl 1:35-40.

[73] Trifiletti A, Barbera N, Pizzoleo MA, Leone G, Lucifora S, Soraci S, Scamardi R, Pedullà M, Frisina N. Hemostatic disorders associated with arterial hypertension and peripheral arterial disease. J Cardiovasc Surg (Torino). 1995 Oct; 36(5):483-5.

[74] Boneu B, Leger P, Arnaud C.Haemostatic system activation and prediction of vascular events in patients presenting with stable peripheral arterial disease of moderate severity. Royat Study Group. Blood Coagul Fibrinolysis. 1998 Mar; 9(2):129-35.

[75] Thromboelastograph® Haemostasis Analyser, Haemoscope Corporation, Niles IL USA.

[76] Cohen E, Caprini J, Zuckerman L, Vagher P and Robinson B. Evaluation of three methods used to identify accelerated coagulability.Thromb Res. 1977 Apr; 10(4): 587-604.

[77] Zuckerman.L, Cohen.E, Vagher J.P, Woodward E and Caprini.J.A. Comparison of Thromboelasrography® with Common Coagulation Tests. Thromb Haemostas (Stuttgart) 46(4) 752-756.

[78] Magruder C. Donaldson MD; Eileen T Matthews; Jane Hadjimichael; Fredercik and R.Rickles. Markers of thrombotic activity in arterial disease. Arch Surg 1987; 122:897-900.

[79] Leroy- Matheron C and Gouault-Heilmann M. Influence of conditions of blood sampling on coagulation activation markers (prothrombin fragment 1+2, thrombin-antithrombin complexes and D-dimers) measurements. Thromb Res 1994; 74:339-407.

[80] Whitten CW, Greilich PE. Thromboelastography: past, present, and future. Anesthesiology 2000;92:1223–5

[81] Essell.J.H, Martin.T.J, Salinas J, Thompson.J.M, and Smith.V.C. Comparison of Thromboelastography to Bleeding Time and Standard Coagulation Tests in Patients After Cardiopulmonary Bypass. Journal of cardiothoracic and Vascular Anaesthesia, Vol 7, No 4(August), 1993: pp410-415.

[82] Kang YG, Martin DJ, Marquez J, et al. "Intraoperative Changes in Blood Coagulation and [Thrombelastograph®] Monitoring in Liver Transplantation." *Anesthesia and Analgesia.* 1985. 64(9):888-896.

[83] McNicol PL, Liu G, Harley ID, McCall PR, Przybylowski GM, Bowkett J, Angus PW, Hardy KJ, Jones RM. Blood loss and transfusion requirements in liver transplantation: experience with the first 75 cases.

[84] Harding SA, Mallett SV, Peachey TD, Cox DJ.Use of heparinase modified thrombelastography in liver transplantation. Br J Anaesth. 1997 Feb;78(2):175-9.

[85]Chau TN, Chan YW, Patch D, Tokunaga S, Greenslade L, Burroughs AK..Thrombelastographic changes and early rebleeding in cirrhotic patients with variceal bleeding. Gut. 1998 Aug;43(2):267-71.

[86] Papatheodoridis GV, Patch D, Webster GJ, Brooker J, Barnes E, Burroughs AK. Infection and hemostasis in decompensated cirrhosis: a prospective study using thrombelastography. Hepatology. 1999 Apr;29(4):1085-90.

[87] 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 Feb;88(2):312-9

[88] Spiess BD, Gillies BS, Chandler W, Verrier E. Changes in transfusion therapy and reexploration rate after institution of a blood management program in cardiac surgical patients. J Cardiothorac Vasc Anesth. 1995 Apr;9(2):168-73.

[89]Shinoda T, Arakura H, Katakura M, Shirota T, Nakagawa S. Usefulness of thrombelastography for dosage monitoring of low molecular weight heparin and unfractionated heparin during hemodialysis. Artif Organs. 1990 Dec;14(6):413-5.

[90] Hadley GP, McGarr P, Mars M.The role of thromboelastography in the management of children with snake-bite in southern Africa. Trans R Soc Trop Med Hyg. 1999 Mar-Apr;93(2):177-9.

[91] Davis CL, Chandler W Davis CL, Chandler WL.L. Thromb Davis CL, Chandler WL. Thromboelastography for the prediction of bleeding after transplant renal biopsy. J Am Soc Nephrol. 1995 Oct;6(4):1250-5

[92] S.V. Mallett and M.Platt. Role of thromboelastography in bleeding diatheses and regional anaesrhesia. THE LANCET. VOL 338: SEPT 21, 1991: 765-767.

[93] Elisabeth Mahla, Thomas Long, Martin N. Vicenzi, Georg Werkgartner, Robert maier, Claudia Probst and Helfred Metzler. Thromboelastography for Monitoring Prolonged Hypercoagulability After Major Abdominal Surgery. Anesth Analg 2001; 92: 572-577.

[94] N.M. Gibbs, G.P.M. Crawford, N. Michalopoulos. Thromboelastographic patterns following Abdominal Aortic Surgery. Anaesth Intens Care 1994; 22: 534-538.

[95] Abrahams JM, Torchia MB, McGarvey M, Putt M, Baranov D, Sinson GP. Perioperative assessment of coagulability in neurosurgical patients using thromboelastography. Surg Neurol. 2002 Jul; 58(1): 5-11; discussion 11-2.

[96] Shore-Lesserson L, manspeizer HE, Deperio M,Francis S, Vela cantos F, Ergin MA. Thromboelastography –guided transfusion algorithm reduces transfusions in complex cardiac surgery. Anaesth Analg 1999; 88: 312-9.

[97] Martin P, Greenstein D, Gupta NK, Walker DR, Kester RC. Systemic heparinization during peripheral vascular surgery: thromboelastographic, activated coagulation time, and heparin titration monitoring. J Cardiothorac Vasc Anesth. 1994 Apr; 8(2): 150-2.

[98] Bowbrick VA, Mikhailidis DP, Stansby G. Value of thromboelastography in the assessment of platelet function. Clin Appl Thromb Hemost. 2003 Apr;9(2): 137-42.

[99] Tuman KJ, Spiess BD, McCarthy RJ, Ivankovich AD. Effects of progressive blood loss on coagulation as measured by thrombelastography.

Anesth Analg. 1987 Sep;66(9):856-63.

[100] Nielsen VG. The detection of changes in heparin activity in the rabbit: a comparison of anti-xa activity, thrombelastography, activated partial thromboplastin time, and activated coagulation time. Anesth Analg. 2002 Dec;95(6):1503-6.

[101] Pleym H, Wahba A, Videm V, Asberg A, Lydersen S, Bjella L, Dale O, Stenseth R. Increased fibrinolysis and platelet activation in elderly patients undergoing coronary bypass surgery. Anesth Analg. 2006 Mar; 102(3):660-667.

[102] Boldt J, Haisch G, Suttner S, Kumle B, Schellhase F. Are lactated Ringer's solution and normal saline solution equal with regard to coagulation? Anesth Analg. 2002 Feb;94(2):378-84, table of contents.

[103] Camenzind V, Bombeli T, Seifert B, Jamnicki M, Popovic D, Pasch T, Spahn DR. Citrate storage affects Thrombelastograph analysis. Anesthesiology. 2000 May; 92(5): 1242-9.

[104] Spiess BD, Wall MH, Gillies BS, Fitch JC, Soltow LO, Chandler WL. A comparison of thromboelastography with heparinase or protamine sulfate

added in vitro during heparinized cardiopulmonary bypass. Thromb Haemost. 1997 Aug; 78(2): 820-6.

[105] Nielsen VG. The detection of changes in heparin activity in the rabbit: a comparison of anti-xa activity, thrombelastography, activated partial thromboplastin time, and activated coagulation time. Anesth Analg. 2002 Dec; 95(6): 1503-6.

[106] Tuman KJ, McCarthy RJ, Djuric M, Rizzo V, Ivankovich AD. Evaluation of coagulation during cardiopulmonary bypass with a heparinase-modified thromboelastographic assay. J Cardiothorac Vasc Anesth. 1994 Apr; 8(2): 144-9.

[107] Manspeizer HE, Imai M, Frumento RJ, Parides MK, Mets B, Bennett-Guerrero E. Arterial and venous Thrombelastograph variables differ during cardiac surgery. Anesth Analg. 2001 Aug;93 (2):277-81, 1st contents page.

[108] Spiess BD, Wall MH, Gillies BS, Fitch JC, Soltow LO, Chandler WL. A comparison of thromboelastography with heparinase or protamine sulfate added in vitro during heparinized cardiopulmonary bypass. Thromb Haemost. 1997 Aug; 78(2): 820-6.

[109] Camenzind V, Bombeli T, Seifert B, Jamnicki M, Popovic D, Pasch T, Spahn DR. Citrate storage affects Thrombelastograph analysis. Citrate storage affects Thrombelastograph analysis.

[110] Hsu HW, Huang CH, Chang Y, Shih RL, Chen LK, Chen TL. Perioperative alterations of the thromboelastography in patients receiving one-stage bilateral total knee arthroplasty. Acta Anaesthesiol Sin. 1996 Sep;34(3):129-34.

[111] Pleym H, Wahba A, Videm V, Asberg A, Lydersen S, Bjella L, Dale O, Stenseth R. Increased fibrinolysis and platelet activation in elderly patients undergoing coronary bypass surgery. Anesth Analg. 2006 Mar; 102(3):660-7.

[112] Boldt J, Haisch G, Suttner S, Kumle B, Schellhase F. Are lactated Ringer's solution and normal saline solution equal with regard to coagulation? Anesth Analg. 2002 Feb;94(2):378-84, table of contents.

[113] Camenzind V, Bombeli T, Seifert B, Jamnicki M, Popovic D, Pasch T, Spahn DR. Citrate storage affects Thrombelastograph analysis. Anesthesiology. 2000 May; 92(5): 1242-9.

[114] Mahla E, Lang T, Vicenzi MN, Werkgartner G, Maier R, Probst C, Metzler H. Thromboelastography for monitoring prolonged hypercoagulability after major

abdominal surgery. Anesth Analg. 2001 Mar;92(3):572-7.

[115] McCrath DJ, Cerboni E, Frumento RJ, Hirsh AL, Bennett-Guerrero E. Thromboelastography maximum amplitude predicts postoperative thrombotic complications including myocardial infarction. Anesth Analg. 2005 Jun;100(6):1576-83.

[116] Butler MJ. Thrombelastography during and after elective abdominal surgery. Thromb Haemost. 1978 Apr 30;39(2):488-95.

[117] Gorton H, Lyons G, Manraj P. Preparation for regional anaesthesia induces changes in thrombelastography. Br J Anaesth. 2000 Mar;84(3):403-4.

[118] Ruttmann TG, James MF, Finlayson J. Effects on coagulation of intravenous crystalloid or colloid in patients undergoing peripheral vascular surgery. Br J Anaesth. 2002 Aug;89(2):226-30.

[119] Sharma SK, Philip J. The effect of anesthetic techniques on blood coagulability in parturients as measured by thromboelastography. Anesth Analg. 1997 Jul;85(1):82-6.

[120] Modrau II, Iversen LL, Thorlacius-Ussing OO. Hemostatic alterations in patients with benign and malignant colorectal disease during major abdominal surgery. Thromb Res. 2001 Dec 1;104(5):309-15.

[121] Gorton HJ, Warren ER, Simpson NA, Lyons GR, Columb MO. Thromboelastography identifies sex-related differences in coagulation. Anesth Analg. 2000 Nov;91(5):1279-81.

[122] Bauer KA, Weiss LM, Sparrow D, Vokonas PS, Rosenberg RD. Aging-associated changes in indices of thrombin generation and protein C activation in humans. Normative Aging Study. J Clin Invest. 1987 Dec;80(6):1527-34.

[123] Ofosu FA, Craven S, Dewar L, Anvari N, Andrew M, Blajchman MA. Age-related changes in factor VII proteolysis in vivo. Br J Haematol. 1996 Aug;94(2):407-12.

[124] Boldt J, Haisch G, Kumle B, Brosch C, Lehmann A, Werling C. Does coagulation differ between elderly and younger patients undergoing cardiac surgery? Intensive Care Med. 2002 Apr;28(4):466-71. Epub 2002 Mar 6.

[125] Boldt J, Hüttner I, Suttner S, Kumle B, Piper SN, Berchthold G. Changes of haemostasis in patients undergoing major abdominal surgery--is there a

difference between elderly and younger patients? Br J Anaesth. 2001 Sep;87(3):435-40.

[126] Yamamoto K, Takeshita K, Kojima T, Takamatsu J, Saito H. Aging and plasminogen activator inhibitor-1 (PAI-1) regulation: implication in the pathogenesis of thrombotic disorders in the elderly. Cardiovasc Res. 2005 May 1;66(2):276-85. Epub 2004 Dec 8.

[127] Davì G, Patrono C. Platelet activation and atherothrombosis. N Engl J Med. 2007 Dec 13;357(24):2482-94.

[128] Ekseth K, Abildgaard L, Vegfors M, Berg-Johnsen J, Engdahl O. The in vitro effects of crystalloids and colloids on coagulation. Anaesthesia. 2002 Nov;57(11):1102-8.

[129] Gorton HJ, Warren ER, Simpson NA, Lyons GR, Columb MO. Thromboelastography identifies sex-related differences in coagulation. Anesth Analg. 2000 Nov;91(5):1279-81.

[130] Schreiber MA, Differding J, Thorborg P, Mayberry JC, Mullins RJ. Hypercoagulability is most prevalent early after injury and in female patients. J Trauma. 2005 Mar;58(3):475-80; discussion 480-1.

[131] Mercuro G, Zoncu S, Dragoni F. Gender differences in cardiovascular risk factors. Ital Heart J. 2003 Jun;4(6):363-6.

[132] Koch CG, Nussmeier NA. Gender and cardiac surgery. Anesthesiol Clin North America. 2003 Sep;21(3):675-89.

[133] American Heart Association. Heart Disease and Stroke Statistics; 2003 Update. American heart Association, Dallas, Tx. 2002.

[134] Miller VM, Jayachandran M, Owen WG. Ageing, oestrogen, platelets and thrombotic risk. Clin Exp Pharmacol Physiol. 2007 Aug;34(8):814-21.

[135] Meerarani P, Moreno PR, Cimmino G, Badimon JJ. Atherothrombosis: role of tissue factor; link between diabetes, obesity and inflammation. Indian J Exp Biol. 2007 Jan;45(1):103-10.

[136] Darvall KA, Sam RC, Silverman SH, Bradbury AW, Adam DJ. Obesity and thrombosis. Eur J Vasc Endovasc Surg. 2007 Feb;33(2):223-33. Epub 2006 Dec 20.

[137] Ruttmann TG, James MF, Viljoen JF. Haemodilution induces a hypercoagulable state. Br J Anaesth. 1996 Mar;76(3):412-4.

[138] Ruttmann TG, James MF, Aronson I. In vivo investigation into the effects of haemodilution with hydroxyethyl starch (200/0.5) and normal saline on coagulation. Br J Anaesth. 1998 May;80(5):612-6.

[139] Ruttmann TG, James MF. Pro-coagulant effect of in vitro haemodilution is not inhibited by aspirin. Br J Anaesth. 1999 Aug;83(2):330-2.

[140] Boldt J, Haisch G, Suttner S, Kumle B, Schellhase F. Are lactated Ringer's solution and normal saline solution equal with regard to coagulation? Anesth Analg. 2002 Feb;94(2):378-84, table of contents.

[141] Ng KF, Lam CC, Chan LC. In vivo effect of haemodilution with saline on coagulation: a randomized controlled trial. Br J Anaesth. 2002 Apr;88(4):475-80.

[142] Butwick A, Carvalho B. The effect of colloid and crystalloid preloading on thromboelastography prior to Cesarean delivery. Can J Anaesth. 2007 Mar;54(3):190-5.

[143] Ekseth K, Abildgaard L, Vegfors M, Berg-Johnsen J, Engdahl O. The in vitro effects of crystalloids and colloids on coagulation. Anaesthesia. 2002 Nov;57(11):1102-8.

[144] Sharma SK, Philip J. The effect of anesthetic techniques on blood coagulability in parturients as measured by thromboelastography. Anesth Analg. 1997 Jul;85(1):82-6.

[145] Parker SD, Breslow MJ, Frank SM, Rosenfeld BA, Norris EJ, Christopherson R, Rock P, Gottlieb SO, Raff H, Perler BA, et al. Catecholamine and cortisol responses to lower extremity revascularization:correlation with outcome variables. Perioperative Ischemia Randomized Anesthesia Trial Study Group. Crit Care Med. 1995 Dec;23(12):1954-61.

[146] Naesh O, Hindberg I, Friis J, Christiansen C, Pedersen T, Trap-Jensen J, Lund JO. Platelet activation in major surgical stress: influence of combined epidural and general anaesthesia. Acta Anaesthesiol Scand. 1994 Nov;38(8):820-5.

[147] Leonard SA, Walsh M, Lydon A, O'Hare B, Shorten GD. Evaluation of the effects of levobupivacaine on clotting and fibrinolysis using thromboelastography. Eur J Anaesthesiol. 2000 Jun;17(6):373-8.

[148] Kohrs R, Hoenemann CW, Feirer N, Durieux ME. Bupivacaine inhibits whole blood coagulation in vitro. Reg Anesth Pain Med. 1999 Jul-Aug;24(4):326-30.

[149] Siau C, Ng HP, Tan GM, Ho BS, Pua HL. In vitro effects of local anaesthetics on the thromboelastographic profile of parturients. Br J Anaesth. 2005 Jan;94(1):117-20. Epub 2004 Oct 29.

[150] Benzon HT, Wong CA, Wong HY, Brooke C, Wade L. The effect of low-dose bupivacaine on postoperative epidural fentanyl analgesia and thrombelastography. Anesth Analg. 1994 Nov;79(5):911-7.

[151] Gibbs NM, Sear JW. Effect of ketorolac, bupivacaine and low-dose heparin on thrombelastographic variables in vitro. Br J Anaesth. 1995 Jul;75(1):27-30.

[152] Niemi TT, Kuitunen AH, Vahtera EM, Rosenberg PH. Haemostatic changes caused by i.v. regional anaesthesia with lignocaine. Br J Anaesth. 1996 Jun;76(6):822-8.

[153] Kohro S, Yamakage M, Omote T, Namiki A. In vitro effects of propofol on blood coagulability and fibrinolysis by the use of thromboelastograph technique. Acta Anaesthesiol Scand. 1999 Feb;43(2):217-9.

[154] Law NL, Ng KF, Irwin MG, Man JS. Comparison of coagulation and blood loss during anaesthesia with inhaled isoflurane or intravenous propofol. Br J Anaesth. 2001 Jan;86(1):94-8.

[155] Sharma SK, Philip J. The effect of anesthetic techniques on blood coagulability in parturients as measured by thromboelastography. Anesth Analg. 1997 Jul; 85(1):82-6.

[156] Diamantis T, Tsiminikakis N, Skordylaki A, Samiotaki F, Vernadakis S, BongiorniC, Tsagarakis N, Marikakis F, Bramis I, Bastounis E. Alterations of hemostasis after laparoscopic and open surgery. Hematology. 2007 Dec;12(6):561-70.

[157] Borris LC, Breindahl M, Ryge C, Sommer HM, Lassen MR; uF1+2 study group. Prothrombin fragment 1+2 in urine as an indicator of sustained coagulation activation after total hip arthroplasty. Thromb Res. 2007;121(3):369-76. Epub 2007 Jun 27.

[158] Pleym H, Wahba A, Videm V, Asberg A, Lydersen S, Bjella L, Dale O, Stenseth R. Increased fibrinolysis and platelet activation in elderly patients undergoing coronary bypass surgery. Anesth Analg. 2006 Mar;102(3):660-7.

[159] Schreiber MA, Differding J, Thorborg P, Mayberry JC, Mullins RJ. Hypercoagulability is most prevalent early after injury and in female patients. J Trauma. 2005 Mar;58(3):475-80; discussion 480-1.

[160] Marakis G, Pavlidis TE, Ballas K, Karvounaris D, Rafailidis S, Sakantamis AK. Changes in coagulation and fibrinolysis during laparoscopic cholecystectomy. J Laparoendosc Adv Surg Tech A. 2006 Dec;16(6):582-6.

[161] Modrau II, Iversen LL, Thorlacius-Ussing OO. Hemostatic alterations in patients with benign and malignant colorectal disease during major abdominal surgery. Thromb Res. 2001 Dec 1;104(5):309-15.

[162] Mahla E, Lang T, Vicenzi MN, Werkgartner G, Maier R, Probst C, Metzler H. Thromboelastography for monitoring prolonged hypercoagulability after major abdominal surgery. Anesth Analg. 2001 Mar;92(3):572-7.

[163] Gibbs NM, Crawford GP, Michalopoulos N. Thrombelastographic patterns following abdominal aortic surgery. Anaesth Intensive Care. 1994 Oct; 22(5):534-8.

[164]Thromboelastograph® Haemostasis Analyser, Haemoscope Corporation, Niles IL USA.

[165] Cassar.K, Bachoo.P, Ford.I, Greaves.M, and Brittenden.J. Platelet activation is increased in peripheral arterial disease. J Vasc Surg 2003; 38:99-103.

[166] Reininger C.B, Graf J, Reininger A.J, Spannagl M, Steckmeier B and Schweiberer L. Increased Platelet and Coagulatory activity indicate ongoing thrombogenesis in peripheral Arterial disease. Thrombosis Research, Vol. 82,No. 6, pp. 523-532, 1996.

[167] Boneu. B, Leger. P and Arnaud C. Haemostatic system activation and prediction of vascular events in patients presenting with stable peripheral arterial disease of moderate severity. Blood Coagulation and Fibrinolysis 1998, 9:129-135.

[168] Speiser.W, Speiser.P, Minar.E, Korninger.C, Neissner.H, Huber.K, Schernthaner.G, Ehringer.H, and Lechner.K. Activation of coagulation and fibrinolysis in patients with arteriosclerosis: Relation to localization of vessel disease and risk factors. Thrombosis Research 59; 77-88, 1990.

[169] Banerjee.A.K, Pearson.J, Gilliland.D, Lewis .J.D, Stirling.Y and Meade T.W. A Six-Year Prospective Study of Fibrinogen and other Risk factors Associated with Mortality in Stable Claudicants. Thrombosis and Haemostasis (Stuttgart) 68(3) 261-263 (1992).

[170] Cortellaro M, Boschetti C, Cofranesco E, Zanbussi C, Catalano M, Gaetano G, gabrielli L, Lombardi B, Specchia G, Tavazi L, tremoli E, della Volpe A and the PLAT Study Group. The PLAT Study: Haemostatic function

in relation to atherothrombotic ischaemic events in vascular disease patients: principal results. Arterioscler Thromb. 1992; 12:1063-1070.

[171] Lee AJ, Fowkes FG, Lowe GD, Rumley A. Fibrin D-dimer, haemostatic factors and peripheral vascular disease. Thromb Haemost 1995; 74:828-32.

[172] Van der Bom J.G, Bots M.L, Haverkate F, Meijer P, Hofman A, Kluft C and Grobbee D.E. Activation Products of the Haemostatic System in Coronary, Cerebrovascular and Peripheral Arterial Disease. Thromb Haemost 2001; 85; 233-9.

[173] Meade T W, Ruddock V, Stirling Y, Chakrabarti R and Miller GJ. Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. The Lancet 1993; 342; 1076-79.

[174] Thompson S.G, Kienast.J, Pyke.S.D.M, Haverkate.F and van de Loo.C.J.W., FOR THE EUROPEAN CONCERTED ACTION ON THROMBOSIS AND DISABILITIES ANGINA PECTORIS STUDY GROUP.Haemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. N Engl J med 1995;332:635-41.

[175] Lassila R, Peltonen S, Lepatalo M, Saarien O, Kauhanen P and Manninen V. Severity of peripheral atherosclerosis is associated with fibrinogen and degradation of cross-linked fibrin. Atheroscler Thromb. 1993; 13:1412-1417.

[176] Woodburn KR, Lowe GD, Rumley A, Love J, Pollock JG. Relation of haemostatic, fibrinolytic and rheological variables to the angiographic extent of peripheral arterial occlusive disease. Int Angiol 1995: 14:346-52.

[177] Sobel M, Gervin CA, Qureshi DG, Greenfield LJ. Coagulation responses to heparin in the ischaemic limb: assessment of thrombin and platelet activation during vascular surgery. Circulation. 1987Sep: 76 (3 pt 2): Ш8-13.

[178] Magruder C. Donaldson MD; Eileen T Matthews; Jane Hadjimichael; Fredercik and R.Rickles. Markers of thrombotic activity in arterial disease. Arch Surg 1987; 122:897-900.

[179] Vance G. Nielson. The Detection of Changes in Heparin Activity in the Rabbit; A Comparison of Anti-Xa Activity, Thromboelastography®, Activated partial Thromboplastin Time, and Activated coagulation time. Anaesth Analg 2002; 95:1503-1506.

[180] Essell.J.H, Martin.T.J, Salinas J, Thompson.J.M, and Smith.V.C. Comparison of Thromboelastography to Bleeding Time and Standard Coagulation Tests in Patients After Cardiopulmonary Bypass. Journal of cardiothoracic and Vascular Anaesthesia, Vol 7, No 4(August), 1993: pp410-415.

[181] Zuckerman.L, Cohen.E, Vagher J.P, Woodward E and Caprini.J.A. Comparison of Thromboelasrography® with Common Coagulation Tests. Thromb Haemostas (Stuttgart) 46(4) 752-756.

[182] Shear- induced platelet aggregation. J.R. O`Brien. Lancet 1990;335:711-713.

[183] AjvW, an Anti-vWF Monoclonal antibody, Inhibits enhanced platelet aggregation induced by high shear stress in platelet- rich plasma from patients with Acute coronary syndromes.

[184] Shear-induced platelet aggregation increases in patients with proximal and severe coronary artery stenosis. Kawano K, Yoshino H, Aoki N, Udagawa H, Watanuki A, Hioki Y, Haumura Y, yasumura T, Homori M, Murata M, Ikeda Y, Ishikawa K. Clin Cardiol. 2002 Apr;25(4): 154-60.

[185] Chronos NA, Goodall AH, Wilson DJ, Sigwart U, Buller NP. Profound platelet degranulation is an important side effect of some types of contrast

media used in interventional cardiology. Circulation. 1993 Nov; 88(5 Pt 1): 2035-44.

[186] Grabowski EF. Effects of contrast media on endothelial cell monolayers under controlled flow conditions. Am J Cardiol. 1989 Sep 5; 64(9): 10 E-15E.

[187] Stormorken H, Skalpe IO, Testart MC. Effect of various contrast media on coagulation, fibrinolysis, and platelet function. An in vitro and in vivo study. Invest Radiol. 1986 Apr; 21(4):348-54.

[188] Melton LG, Dehmer GJ, Gabriel DA. Alterations in fibrin detected in coronary sinus blood after heparin and coronary angiography with a nonionic contrast agent (iohexol). Am J Cardiol. 1998 Aug 1;82(3):295-8.

[189] Hoffmann JJ, Tielbeek AV, Krause W. Haemostatic effects of low osmolar non-ionic and ionic contrast media: a double-blind comparative study. Br J Radiol. 2000 Mar; 73(867): 248-55.

[190] Karch LA, Mattos MA, Henretta JP, McLafferty RB, Ramsey DE, Hodgson KJ. Clinical failure after percutaneous transluminal angioplasty of the superficial femoral and popliteal arteries. J Vasc Surg. 2000 May;31(5):880-7.

[191] Grines C.L, Diaz C,Mickelson J. Acute Thrombosis in a canine model of arterial injury: effect of ionic versus nonionic contrast media. Circulation 1989 Vol 80;(4):Supplement II- 411.

[192] Hwang MH, Piao ZE, Murdock DK, Messmore HL, Giardina JJ, Scanlon PJ. Risk of thromboembolism during diagnostic and interventional cardiac procedures with nonionic contrast media. Radiology. 1990 Feb;174(2):453-7.

[193] Kopko PM, Smith DC, Bull BS. Thrombin generation in nonclottable mixtures of blood and nonionic contrast agents. Radiology. 1990 Feb;174(2):459-61.

[194] Robertson HJ. Blood clot formation in angiographic syringes containing nonionic contrast media. Radiology. 1987 Mar;162(3):621-2.

[195] Grollman JH Jr, Liu CK, Astone RA, Lurie MD. Thromboembolic complications in coronary angiography associated with the use of nonionic contrast medium. Cathet Cardiovasc Diagn. 1988;14(3):159-64.

[196] Fareed J, Walenga JM, Saravia GE, Moncada RM. Thrombogenic potential of nonionic contrast media? Radiology. 1990 Feb;174(2):321-5.

[197] Iqbal O, Jeske W, Hoppensteadt D, Koza M, Walenga JM, Fareed J, Moncada R. Procoagulant and anticoagulant effects of intravascular contrast

media as assessed by thromboelastography. Semin Thromb Hemost. 1995;21 Suppl 4:86-90.

[198] Gasperetti CM, Feldman MD, Burwell LR, Angello DA, Haugh KH, Owen RM, Powers ER. Influence of contrast media on thrombus formation during coronary angioplasty. J Am Coll Cardiol. 1991 Aug;18(2):443-50.

[199] Davidson CJ, Mark DB, Pieper KS, Kisslo KB, Hlatky MA, Gabriel DA, Bashore TM. Thrombotic and cardiovascular complications related to nonionic contrast media during cardiac catheterization: analysis of 8,517 patients. Am J Cardiol. 1990 Jun 15;65(22):1481-4.

[200] Mukherjee M, Scully MF, Thomas M, Jewitt D, Kakkar VV. The potential thrombogenic action of a nonionic radiographic contrast medium used during coronary angiography is offset by heparin during coronary angioplasty. Thromb Haemost. 1996 Nov;76(5):679-81.

[201] Wiesel ML, Zupan M, Wolff F, Grunebaum L, Brechenmacher C, Cazenave JP. Potential thrombogenicity of angiographic contrast agents. Thromb Res. 1991 Oct 15;64(2):291-4.

[202] Grabowski EF, Rodriguez M, McDonnell SL. Platelet adhesion/aggregation in an in vitro model of coronary artery stenosis. Cathet Cardiovasc Diagn. 1993 Jan;28(1):65-71.

[203] Heptinstall S, White A, Edwards N, Pascoe J, Sanderson HM, Fox SC, Henderson RA. Differential effects of three radiographic contrast media on platelet aggregation and degranulation: implications for clinical practice? Br J Haematol. 1998 Dec;103(4):1023-30.

[204] Brosstad F, Buchmann M, Schilvold A. Roentgen contrast media and fibrinolysis. Acta Radiol Suppl. 1995;399:185-7.

[205] Engelberg H. Heparin and atherosclerosis. A review of old and recent findings. Am Heart J. 1980 Mar; 99(3): 359-72.

[206] Engelberg H. Low-dose intermittent heparin therapy decreases plasma fibrinogen levels. Semin Thromb Hemost. 1991; 17 Suppl 2:219-23.

[207] Andreozzi GM, Signorelli SS, Cacciaguerra G, Di Pino L, Martini R, Monaco S, Butto G, Sardina M. Three-month therapy with calcium-heparin in comparison with ticlopidine in patients with peripheral arterial occlusive disease at Leriche-Fontaine IIb class. Angiology. 1993 Apr; 44(4): 307-13.

[208] Calabro A, Piarulli F, Milan D, Rossi A, Coscetti G, Crepaldi G. Clinical assessment of low molecular weight heparin effects in peripheral vascular disease. Angiology. 1993 Mar; 44(3):188-95.

[209] Mallett SV, Cox DJ. Thromboelastography. Br. J Anaesth 1992; 69:307-13.

[210] Camenzind V, Bombeli T, Seifert B, Jamnicki M, Popovic D, Pasch T, Spahn DR. Citrate storage affects Thrombelastograph analysis. Anesthesiology. 2000 May; 92(5): 1242-9.

[211] Tuman KJ, McCarthy RJ, Djuric M, Rizzo V, Ivankovich AD. Evaluation of coagulation during cardiopulmonary bypass with a heparinase-modified thromboelastographic assay. J Cardiothorac Vasc Anesth. 1994 Apr; 8(2): 144-9.

[212] Murata K, Yokoyama Y. Acidic glycosaminoglycan, lipid and water contents in human coronary arterial branches. Atherosclerosis. 1982 Oct; 45(1): 53-65.

[213] Stary HC, Blankenhorn DH, Chandler AB, Glagov S, Insull W Jr, Richardson M, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, et al. A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association.Circulation. 1992 Jan; 85(1): 391-405.

[214] Shriver Z, Sundaram M, Venkataraman G, Fareed J, Linhardt R, Biemann K, Sasisekharan R. Cleavage of the antithrombin III binding site in heparin by heparinases and its implication in the generation of low molecular weight heparin. Proc Natl Acad Sci U S A. 2000 Sep 12; 97(19):10365-70.

[215] Simionescu M, Simionescu N. Functions of the endothelial cell surface. Annu Rev Physiol. 1986; 48:279-93.

[216] Marcum JA, Rosenberg RD. Role of endothelial cell surface heparin-like polysaccharides. Ann N Y Acad Sci. 1989; 556:81-94.

[217] Pillarisetti S, Paka L, Obunike JC, Berglund L, Goldberg IJ. Subendothelial retention of lipoprotein (a). Evidence that reduced heparan sulfate promotes lipoprotein binding to subendothelial matrix. J Clin Invest. 1997 Aug 15; 100(4): 867-74.

[218] Corti R, Fuster V, Badimon JJ. Pathogenetic concepts of acute coronary syndromes. J Am Coll Cardiol. 2003 Feb 19; 41(4 Suppl S): 7S-14S.

[219] Hawiger J. Formation and regulation of platelet and fibrin hemostatic plug. Hum Pathol. 1987 Feb; 18 (2): 111-22.

[220] Griffin MJ, Rinder HM, Smith BR, Tracey JB, Kriz NS, Li CK, Rinder CS. The effects of heparin, protamine, and heparin/protamine reversal on platelet function under conditions of arterial shear stress. Anesth Analg. 2001 Jul; 93(1):20-7.

[221] Sternbergh WC 3rd, Makhoul RG, Adelman B. Heparin prevents postischemic endothelial cell dysfunction by a mechanism independent of its anticoagulant activity. J Vasc Surg. 1993 Feb;17(2):318-27.

[222] Koksch M, Zeiger F, Wittig K, Pfeiffer D, Ruehlmann C. Haemostatic derangement in advanced peripheral occlusive arterial disease. Int Angiol. 1999 Dec; 18(4): 256-62.

[223] Koksch M, Zeiger F, Wittig K, Siegemund A, Reininger CB, Pfeiffer D, Ruehlmann C. Coagulation, fibrinolysis and platelet P-selectin expression in peripheral vascular disease. Eur J Vasc Endovasc Surg. 2001 Feb; 21(2): 147-54.

[224] Gibbs NM, Crawford GP, Michalopoulos N. A comparison of postoperative thrombotic potential following abdominal aortic surgery, carotid endarterectomy, and femoro-popliteal bypass. Anaesth Intensive Care. 1996 Feb; 24(1): 11-4.

[225] Collins GJ Jr, Heymann RL, Zajtchuk R. Hypercoagulability in patients with peripheral vascular disease. Am J Surg. 1975 Jul; 130(1): 2-6.

[226] Samama CM, Thiry D, Elalamy I, Diaby M, Guillosson JJ, Kieffer E, Coriat P. Perioperative activation of hemostasis in vascular surgery patients. Anesthesiology. 2001 Jan; 94(1): 74-8.

[227] Majack RA, Clowes AW. Inhibition of vascular smooth muscle cell migration by heparin-like glycosaminoglycans. J Cell Physiol. 1984 Mar; 118(3): 253-6.

[228] Edelman ER, Pukac LA, Karnovsky MJ. Protamine and protamine-insulins exacerbate the vascular response to injury. J Clin Invest. 1993 May; 91(5): 2308-13.

[229] Varty K, Allen KE, Jones L, Sayers RD, Ratliff DA, Bell PR, London NJ. The influence of low molecular weight heparin on neointimal proliferation in cultured human saphenous vein. Eur J Vasc Surg. 1994 Mar; 8(2): 174-8.

[230] Haruguchi H, Teraoka S. Intimal hyperplasia and hemodynamic factors in arterial bypass and arteriovenous grafts: a review. J Artif Organs. 2003;6(4):227-35.

[231] Calabro A, Piarulli F, Milan D, Rossi A, Coscetti G, Crepaldi G. Clinical assessment of low molecular weight heparin effects in peripheral vascular disease. Angiology. 1993 Mar; 44(3):188-95.

[232] Andreozzi GM, Signorelli SS, Cacciaguerra G, Di Pino L, Martini R, Monaco S, Butto G, Sardina M. Three-month therapy with calcium-heparin in comparison with ticlopidine in patients with peripheral arterial occlusive disease at Leriche-Fontaine IIb class. Angiology. 1993 Apr; 44(4): 307-13.

[233] Allegra C, Pollari G, Carioti B, Sardina M. Thrombin and platelet inhibition with low-dose calcium-heparin in comparison with ASA in patients with peripheral arterial occlusive disease at Leriche-Fontaine IIb class. Int J Clin Pharmacol Ther. 1994 Dec;32(12):646-51.

[234] Bradbury A, Adam D, Garrioch M, Brittenden J, Gillies T, Ruckley CV. Changes in platelet count, coagulation and fibrinogen associated with elective repair of asymptomatic abdominal aortic aneurysm and aortic reconstruction for occlusive disease. Eur J Vasc Endovasc Surg. 1997 Apr;13 (4):375-80

[235] Gibbs NM, Crawford GP, Michalopoulos N. Postoperative changes in coagulant and anticoagulant factors following abdominal aortic surgery. J Cardiothorac Vasc Anesth. 1992 Dec;6(6):680-5

[236] Holmberg A, Siegbahn A, Westman B, Bergqvist D. Ischaemia and reperfusion during open abdominal aortic aneurysm surgery induce extensive thrombin generation and activity. Eur J Vasc Endovasc Surg. 1999 Jul;18(1):11-6

[237] Holmberg A, Bergqvist D, Siegbahn A. Coagulation and fibrinolysis after open infrarenal abdominal aortic aneurysm repair in a long-term perspective. Thromb Res. 1999 Oct 15;96(2):99-105.

[238] Adam DJ, Ludlam CA, Ruckley CV, Bradbury AW. Coagulation and fibrinolysis in patients undergoing operation for ruptured and nonruptured infrarenal abdominal aortic aneurysms. Annals of Vascular Surgery Volume 18, Number 5 / September, 2004; 1615-5947.

[239] Skagius E, Siegbahn A, Bergqvist D, Henriksson AE. Fibrinolysis in patients with an abdominal aortic aneurysm with special emphasis on rupture and shock. J Thromb Haemost. 2008 Jan;6(1): 147-50. Epub 2007 Oct 8

[240] Bown MJ, Nicholson ML, Bell PR, Sayers RD. The systemic inflammatory response syndrome, organ failure, and mortality after abdominal aortic aneurysm repair. J Vasc Surg. 2003 Mar;37(3):600-6.

[241] Wallinder J, Bergqvist D, Henriksson AE. Haemostatic markers in patients with abdominal aortic aneurysm and the impact of aneurysm size. Thromb Res. 2009 Sep;124(4):423-6. Epub 2009 Mar 6

[242] Gibbs NM, Crawford GP, Michalopoulos N. A comparison of postoperative thrombotic potential following abdominal aortic surgery, carotid endarterectomy, and femoro-popliteal bypass. Anaesth Intensive Care. 1996 Feb;24(1):11-4.

[243] Thompson JF, Mullee MA, Bell PR, Campbell WB, Chant AD, Darke SG, Jamieson CW, Murie J, Parvin SD, Perry M, Ruckley CV, Wolfe JN, Clyne CA. Joint Vascular Research Group. Intraoperative heparinisation, blood loss and myocardial infarction during aortic aneurysm surgery:. Eur J Vasc Endovasc Surg. 1997 Apr;13(4):425.

[244] Brothers TE, Wakefield TW, McLaren ID, Bockenstedt P, Greenfield LJ. Coagulation status during aortic aneurysm surgery: comparison of thrombelastography with standard tests. J Invest Surg. 1993 Nov-Dec;6(6):527-34

[245] Tuman KJ, Spiess BD, McCarthy RJ, Ivankovich AD. Effects of progressive blood loss on coagulation as measured by thrombelastography. Anesth Analg. 1987 Sep;66(9):856-63.

[246] Gibbs NM, Crawford GP, Michalopoulos N. A comparison of postoperative thrombotic potential following abdominal aortic surgery, carotid endarterectomy, and femoro-popliteal bypass. Anaesth Intensive Care. 1996 Feb;24(1):11-4.

[247] Mahla E, Lang T, Vicenzi MN, Werkgartner G, Maier R, Probst C, Metzler H. Thromboelastography for monitoring prolonged hypercoagulability after major abdominal surgery.

[248] Gibbs NM, Bell R. The effect of low-dose heparin on hypercoagulability following abdominal aortic surgery. Anaesth Intensive Care. 1998 Oct;26(5):503-8.

[249] Kalra, M., Charlesworth, D., Morris, JA and Al-Khaffaf, H. Myocardial infarction after reconstruction of the abdominal aorta. *Br J Surg* 1993 80: pp. 28-31.

[250] Mamode, N., Scott, RN, McLaughlin, SC, McLelland, A. and Pollock, JG Perioperative myocardial infarction in peripheral vascular surgery. [see comments]. *Br Med J* 1996 312 , pp. 1396-1397.

[251]Hobbs, SD, Yapanis, M., Burns, PJ, Wilmink, AB, Bradbury, AW and Adam, DJ Peri-operative myocardial injury in patients undergoing surgery for critical limb ischaemia. *Eur J Vasc Endovasc surg* 2005 29 , pp. 301-304.

[252] Cassar.K, Bachoo.P, Ford.I, Greaves.M, and Brittenden.J. Platelet activation is increased in peripheral arterial disease. J Vasc Surg 2003; 38:99-103.

[253] Cassar, K., Bachoo, P. and Brittenden, J. The role of platelets in peripheral vascular disease. *Eur J Vasc Endovasc Surg* 2003 25:1 pp. 6-15.

[254] Robless, PA, Okonko, D., Lintott, P., Mansfield, AO, Mikhailidis, DP and Stansby, GP Increased platelet aggregation and activation in peripheral arterial disease. *Eur J Vasc Endovasc Surg* 2003 25:1 , pp. 16-22.

[255] Lip, GTH and Blann, AD Von Willebrand factor and its relevance to cardiovascular disorders. *Br Heart J* 1995 74:25, pp. 580-583.

[256] Cassar, K., Bachoo, P., Ford, I., Greaves, M. and Brittenden, J. Markers of coagulation activation, endothelial stimulation and inflammation in patients with peripheral arterial disease. *Eur J Vasc Endovasc surg* 2005 29:2 , pp. 171-176.

[257] Gibbs, NM, Crawford, GP and Michalopoulos, N. A comparison of postoperative thrombotic potential following abdominal aortic surgery, carotid endarterectomy, and femoro-popliteal bypass. *Anaesthesia Intensive Care* 1996 24:1, pp. 11-14.

[258] Tuman, KJ, McCarthy, RJ, March, RJ, DeLaria, GA, Patel, RV and Ivankovich, AD Effects of epidural anesthesia and analgesia on coagulation

and outcome after major vascular surgery. *Anesthesia Analgesia* 1991 73:6 , pp. 696-704.

[259] Reininger C.B, Graf J, Reininger A.J, Spannagl M, Steckmeier B and Schweiberer L. Increased Platelet and Coagulatory activity indicate ongoing thrombogenesis in peripheral Arterial disease. Thrombosis Research, Vol. 82,No. 6, pp. 523-532, 1996.

[260] Boneu. B, Leger. P and Arnaud C. Haemostatic system activation and prediction of vascular events in patients presenting with stable peripheral arterial disease of moderate severity. Blood Coagulation and Fibrinolysis 1998, 9:129-135.

[261] Speiser.W, Speiser.P, Minar.E, Korninger.C, Neissner.H, Huber.K, Schernthaner.G, Ehringer.H, and Lechner.K. Activation of coagulation and fibrinolysis in patients with arteriosclerosis: Relation to localization of vessel disease and risk factors. Thrombosis Research 59; 77-88, 1990.

[262] Banerjee.A.K, Pearson.J, Gilliland.D, Lewis .J.D, Stirling.Y and Meade T.W. A Six Year Prospective Study of Fibrinogen and other Risk factors Associated with Mortality in Stable Claudicants. Thrombosis and Haemostasis (Stuttgart) 68(3) 261-263 (1992).

[263] Cortellaro M, Boschetti C, Cofranesco E, Zanbussi C, Catalano M, Gaetano G, gabrielli L, Lombardi B, Specchia G, Tavazi L, tremoli E, della

Volpe A and the PLAT Study Group. The PLAT Study: Haemostatic function in relation to atherothrombotic ischaemic events in vascular disease patients: principal results. Arterioscler Thromb. 1992; 12:1063-1070.

[264] Lee AJ, Fowkes FG, Lowe GD, Rumley A. Fibrin D-dimer, haemostatic factors and peripheral vascular disease. Thromb Haemost 1995; 74:828-32.

[265] Van der Bom J.G, Bots M.L, Haverkate F, Meijer P, Hofman A, Kluft C and Grobbee D.E. Activation Products of the Haemostatic System in Coronary, Cerebrovascular and Peripheral Arterial Disease. Thromb Haemost 2001; 85; 233-9.

[266] Meade T W, Ruddock V, Stirling Y, Chakrabarti R and Miller GJ. Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. The Lancet 1993; 342; 1076-79.

[267] Thompson S.G, Kienast.J, Pyke.S.D.M, Haverkate.F and van de Loo.C.J.W., FOR THE EUROPEAN CONCERTED ACTION ON THROMBOSIS AND DISABILITIES ANGINA PECTORIS STUDY GROUP.Haemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. N Engl J med 1995; 332:635-41.