

**THE SHORT TERM EFFECTS OF ENDOVASCULAR ANEURYSM
REPAIR (EVAR) ON COAGULATION AND CARDIOVASCULAR
MORBIDITY AND MORTALITY IN PATIENTS WITH INFRA-RENAL
ABDOMINAL AORTIC ANEURYSMS**

BY

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A thesis presented for the degree of Doctor of Medicine,

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*‘If we knew what it was we were doing, it would not be called research,
would it? ‘*

Albert Einstein

(Died from a ruptured abdominal aortic aneurysm 18th April, 1955)

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ABSTRACT

OBJECTIVE:

Patients undergoing open repair of asymptomatic abdominal aortic aneurysms (AAA) demonstrate a prothrombotic state that initially deteriorates in the peri-operative period before improving beyond the pre-operative state. We hypothesised that a similar haemostatic improvement occurs following endovascular AAA repair (EVAR) and that the initial prothrombotic derangement may increase the risk of myocardial injury.

METHODS:

60 patients [57 men; median (IQR) age, 77 (72-82) years] underwent EVAR. Patients were assessed at baseline, 24-hours and 1-month post-procedure. Thrombin-antithrombin III-complex (TAT), tissue-plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1), and soluble (s) P-selectin levels were assessed as biomarkers of coagulation, fibrinolysis and platelet activity, respectively. Cardiac Troponin T (cTnT) levels were assessed as a biomarker of myocardial injury.

RESULTS:

An increase in sP-selectin levels occurred between baseline [median (IQR), 80.5(68-128) ng/ml], 24-hours [median (IQR), 89.5(73-112) ng/ml; $p=0.003$] and 1-month [median (IQR), 110(89-143) ng/ml; $p<0.0001$] post-EVAR. There was a trend towards increased TAT levels at 24-hours [median (IQR), 21.65(13-33.1) $\mu\text{g/l}$; $p=0.069$] compared to pre-operation [median (IQR), 7.15(4.7-31.3) $\mu\text{g/l}$] followed by a significant decrease at 1-month [median (IQR), 8.1

(5.4-14.85) $\mu\text{g/l}$; $p < 0.0001$]. cTnT levels were raised ($>0.03\text{ng/ml}$) in 16% of patients. There was a positive correlation between cTnT and TAT levels at 24 hours post-EVAR ($r=0.38$, $p = 0.039$, Kendall tau B = 0.26)

CONCLUSION:

These novel data suggest that the peri-operative pro-thrombotic state following EVAR may be associated with an increased risk of myocardial injury.

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Short Term Platelet Activity and Fibrinolysis in Patients Undergoing Endovascular Abdominal Aortic Aneurysm Repair

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LIST OF ABBREVIATIONS

AAA: Abdominal aortic aneurysm

AB: Antibody

ADP: Adenosine diphosphate

APC: Activated protein C

CENTRAL: Cochrane Central Register of Controlled Trials

CTa: Computed tomographic aortography

cTn: Cardiac troponin

cTnT: Cardiac troponin-T

DREAM: Dutch Randomised Endovascular Aneurysm Management

ECG: Electrocardiography

EVAR: Endovascular aneurysm repair

EVAR-1/ EVAR-2: EndoVascular Aneurysm Repair Trial 1 and 2

F1+2: Prothrombin fragment 1 +2

FM-F: Fibrin-monomer-fibrinogen complex

GP-1b: Glycoprotein-1b

HBV: Hepatitis B virus

HIV: Human immunodeficiency virus

IL: Interleukin

IQR: Inter-quartile range

ITP: Idiopathic thrombocytopenic purpura

LMWH: Low molecular weight heparin

MMP-3: Metalloproteinase-3

MMP-9: Metalloproteinase-9

Ng/ml: Nanogram per millilitre

OS: Open surgical

PAI-1: Plasminogen Activator Inhibitor-1

PCI: Protein-C inhibitor

sP-Selectin: Soluble P-selectin

t-PA: Tissue Plasminogen Activator

TAFI: Thrombin Activatable Fibrinolysis Inhibitor

TAT: Thrombin-antithrombin III-complex

TNF: Tumour necrosis factor

TxA₂: Thromboxane-A₂

USS: Ultrasound scan

vWF: von Willebrand Factor

CHAPTER 1

THE SHORT TERM EFFECTS OF ENDOVASCULAR ANEURYSM REPAIR (EVAR) ON
COAGULATION AND CARDIOVASCULAR MORBIDITY AND MORTALITY IN PATIENTS
WITH INFRA-RENAL ABDOMINAL AORTIC ANEURYSMS

INTRODUCTION

1.1 BACKGROUND

The incidence of abdominal aortic aneurysm (AAA), defined as an enlargement of at least 3cm, increases with age and affects approximately 4% of the elderly general population (≥ 65 years) within the United Kingdom. The age-specific prevalence is six times greater in men than women with an estimated prevalence of 5-8% for men aged 65-80 years old.^{1 2 3} The majority of abdominal aortic aneurysms affect the infra-renal aorta and 75% are asymptomatic at the time of presentation having been identified incidentally during routine health checks or investigations for other pathologies.

The natural history of AAA is that of gradual accelerating, asymptomatic expansion until rupture occurs. The best-known predictor of rupture rate is the maximum AAA diameter; the annual risk of rupture for an aneurysm with a diameter greater than 6cm may be as high as 25%. (See table 1.1)

DIAMETER	5-YEAR RISK OF RUPTURE
<5 cm	5%
5-6 cm	25%
6-7 cm	35%
>7 cm	75%

Table 1.1: AAA rupture rates based on maximum transverse diameter⁴

Approximately 2500 men aged ≥ 60 years die as a result of a ruptured AAA in England and Wales per year; 2.5% of all deaths in males aged ≥ 60 years. Of these deaths approximately half occur prior to hospital treatment.^{1 5} For patients who survive to reach hospital alive, despite advances in peri-operative management, in-hospital mortality rates following emergency open surgery for ruptured AAA remain devastatingly high. In our experience, open repair of rAAA is associated with an in-hospital mortality rate of 40%.⁶ Therefore in the United Kingdom elective repair of an infrarenal abdominal aortic aneurysm, in a suitably fit patient, is recommended when the aneurysm measures ≥ 5.5 cm, or greater than 4.5cm with a growth rate >0.5 cm/six months.⁴

1.2 ENDOVASCULAR INFRA-RENAL ABDOMINAL AORTIC ANEURYSM REPAIR (EVAR)

Following the first reported endovascular infra-renal abdominal aortic aneurysm repair (EVAR) by Juan Parodi in 1990, EVAR has been widely promoted as a less invasive and safer alternative treatment option to conventional open AAA repair, especially in the high-risk patient. It is now well established following the publications of the UK EndoVascular Aneurysm Repair Trials 1 and 2 (EVAR-1 & EVAR-2) and the Dutch Randomised Endovascular Aneurysm Management (DREAM) trial that elective endovascular repair of an AAA reduces peri-operative mortality by approximately 3% compared with open surgical repair in the fit and anatomically suitable patient.^{7 8 9 10} However exponents have argued that these initial operative mortality benefits are offset by the long-term economic cost due to late graft-related complications.¹³

Endoleak is a complication unique to EVAR and results in perfusion of the aneurysm sac and subsequent risk of rupture. (See appendix 1) The EVAR-1 trial reported 22% (118 cases) of EVARs were complicated by endoleak and 35% require a secondary procedure to maintain complete aneurysm exclusion within 3 years of the procedure.⁷ A systematic review by Drury et al reported 17.5% and 21.3% of all EVARs demonstrate endoleak at 30 days and 12 months respectively.¹¹ However, the endoleak rate is influenced by a variety of factors including pre-procedural planning, type of stent graft deployed, aortic morphology and technical expertise.¹² For these reasons, and the risk of other stent-related complications including stent migration, regular outpatient follow-up with computed tomographic aortography (CTA) is necessary to identify aneurysm exclusion failure. Thus, the short-term peri-operative mortality/morbidity benefits of EVAR may be outweighed by its long-term morbidity in terms of re-intervention as well as the associated economic burden.^{11 13}

Currently a number of different approaches have been instigated to reduce the economic cost of EVAR in order to maximise its short-term benefit. A number of institutions have adopted serial USS and plain radiographs instead of computed tomography as a means of graft surveillance. This requires dedicated ultrasonographers thereby potentially restricting follow-up to a specific centres, and continues to pose a significant long-term burden on an institution's resources. Thus alternative, less onerous, methods of identifying stent-graft failure, and specifically endoleak, would be desirable.

1.3 ENDOVASCULAR ANEURYSM REPAIR AND HAEMOSTASIS

It is reported that patients with asymptomatic AAA demonstrate a hypercoagulable and hypofibrinolytic state.²⁸ It is speculated that the pre-operative haemostatic derangement and subsequent improvement following open repair represents activation of the coagulation system by intraluminal aneurysmal sac thrombus. This would correlate with reports of a positive relationship between the size of the aneurysm sac thrombus and the extent of the haemostatic derangement.¹⁴ Previous work by our research group has demonstrated an attenuation of the prothrombotic diathesis evident in patients affected by AAA follows open repair; Adam et al reported that elective open repair of AAA is associated with intense thrombin generation and inhibition of systemic fibrinolysis that is present pre-operatively and persists until day-2 peri-operatively where upon it largely resolves.²⁹ There are currently no robust studies investigating the effect of EVAR on the hypercoagulable and hypofibrinolytic state observed in these patients.

Recently there has been interest in utilising changes in circulating levels of biomarkers as an alternative means of identifying stent-graft failure following EVAR. Two separate studies report circulating levels of metalloproteinase-3 and -9 (MMP-3 & MMP-9) as being significantly elevated in patients affected by AAA and successful EVAR has been shown to be associated with a decrease in MMP levels.^{15 16} The presence of an endoleak and aneurysm sac pressurisation may cause an increase in MMP levels. To date only one study has investigated a biomarker of thrombin generation and degradation as a marker of stent-graft failure. Serino et al reported elevated levels of D-dimer in patients with type-1 endoleaks *and* stable/increasing sac diameters compared with patients who had endoleaks *and* decreasing sac diameters.¹⁷ However, this study has limited value due to methodology deficiencies and lack of study power. No studies have investigated the use of modern biomarkers of coagulation,

fibrinolysis and platelet function as an alternative/complementary method of identifying patients with endoleak.

1.4 ENDOVASCULAR ANEURYSM REPAIR AND MYOCARDIAL INJURY

Elective open infrarenal AAA repair is associated with a peri-operative mortality rate of 3-10%.^{18 19 20 21 22} Previous work from this institution has demonstrated that over one-quarter of patients undergoing elective open AAA repair suffer peri-operative myocardial injury as determined by raised cardiac troponin (cTn) levels.²³ Furthermore, similar haemostatic derangements to those reported by Adam et al are known to be independently associated with myocardial injury, stroke and multiple organ failure.^{24 25 26 27} Thus, the majority of peri-operative mortality following elective open AAA repair may be secondary to micro- and macro-vascular thrombosis causing myocardial injury, thromboembolism and multiple organ failure.^{28 29} While a positive association between the peri-operative hypofibrinolytic state and myocardial injury has been demonstrated in patients undergoing emergency open repair of ruptured AAA, to date, the relationship between coagulation and fibrinolysis and myocardial injury after EVAR has not been studied.³⁰

1.5 HYPOTHESIS AND AIMS

We hypothesise that following successful aneurysm sac exclusion by EVAR the underlying hypercoagulable state witnessed in these patients will be attenuated as a result of exclusion of the intraluminal thrombus from the systemic circulation. Furthermore, we speculate the re-establishment of aneurysm sac perfusion secondary to an endoleak results in the re-establishment of the pre-operative prothrombotic state secondary to exposure of the sac thrombus to the systemic circulation. We speculate that the novel use of biomarkers of haemostasis can provide endoleaks in patients with EVAR.

The aims of this thesis are to:

1. Establish if intra-luminal thrombus volume is related to the pro-thrombotic diathesis evident in patients affected by AAA.
2. Investigate changes to the resting pro-coagulant and hypofibrinolytic state following elective endovascular aneurysm repair for an asymptomatic infra-renal AAA.
3. Investigate and correlate any peri-operative haemostatic changes with peri-operative myocardial injury.
4. Accumulate pilot data on which to base future studies pertaining to the use of haemostatic biomarkers as a method of identifying endoleak.

1.3 SUMMARY

The proposed study is, to the best of our knowledge, the first to investigate changes to coagulation, fibrinolysis and platelet activity following EVAR for infra-renal AAA. The study will not only attempt to corroborate previously published findings regarding open AAA repair, namely that successful aneurysm sac exclusion attenuates the resting hypercoagulable state in patients with AAA, but will extend our investigation to patients that suffer endoleaks. The proposed study will, therefore, attempt to determine if EVAR attenuates the hypercoagulable state in patients with AAA thereby providing a scientific foundation to base future investigation into whether or not an endoleak results in the re-establishment of this pro-thrombotic diathesis.

CHAPTER 2

AN OVERVIEW OF THE NORMAL COAGULATION AND FIBRINOLYTIC SYSTEMS

2.1 PLATELET ACTIVATION AND THE COAGULATION CASCADE

Normal haemostasis represents a complex interaction between the damaged vessel wall, circulating cells (platelets and leukocytes) and circulating proteins/zymogens (coagulation factors). Injury to the vessel wall is the major stimulus for coagulation and results in the activation of endothelial cells and exposure of sub-endothelial collagen to circulating platelets. The activated endothelial cells express P-selectin on their surfaces and release von Willebrand factor (vWF) which aids in the localisation of thrombus formation at the site of injury.³¹ Tissue factor (TF) is also released from the endothelium and plays a vital role in the activation of the coagulation cascade through the extrinsic pathway. Platelets bind to the exposed sub-endothelial collagen resulting in their activation and creation of a platelet plug. This is mediated by vWF which binds to both platelet membrane glycoprotein (Gp) Iba receptor and sub-endothelial collagen.³² Fibrinogen and vWF binds to Gp-IIb and Gp-IIIa receptors on adjacent platelets creating a lattice (aggregation) of platelets.³³ Activated platelets shed the contents of their stored α -granules and dense bodies (platelet granules), which include vWF, TF, adenosine diphosphate (ADP) and coagulation zymogens receptors. Activated platelets also undergo a morphological change from spherical to stellate that assists in the assembly of coagulation factors on their surface. (See figure 2.1.1)

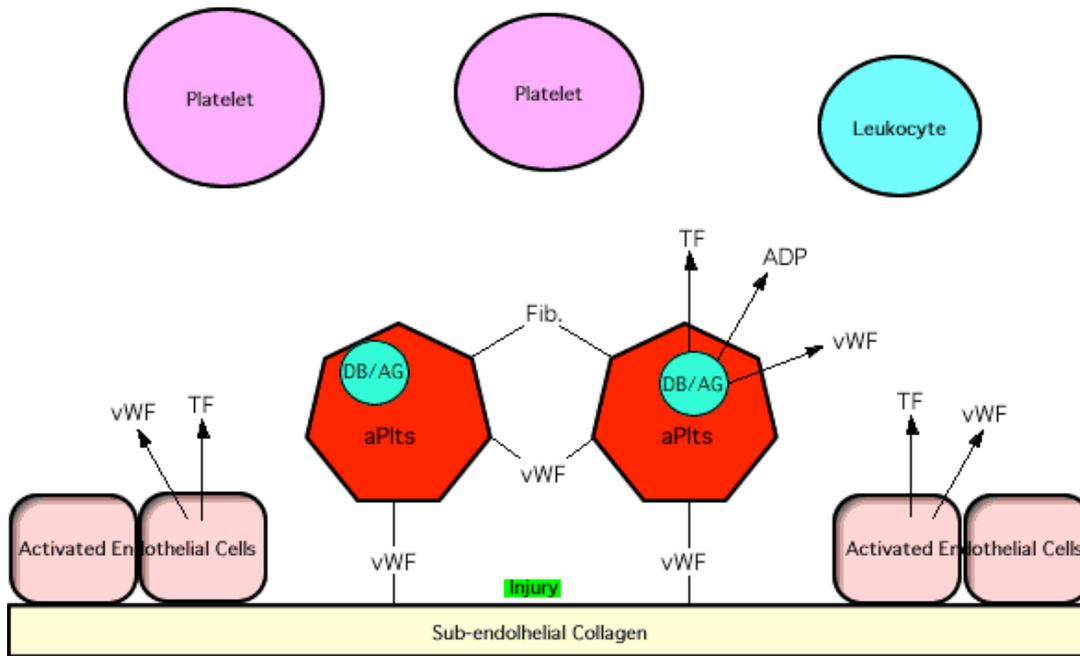


Figure 2.1.1: Platelet activation and aggregation at the site of endothelial cell injury. (TF = tissue factor, ADP = adenosine diphosphate, vWF = von Willebrand factor, Fib. = fibrinogen)

The formation of thrombin (Factor IIa) is vital to ensure stability of the initial platelet plug through its ability to convert fibrinogen to fibrin.³⁴ The formation of fibrin is achieved through activation of the coagulation cascade predominantly through the extrinsic pathway by the release of TF from endothelial cells and activated platelets. TF complexes and activates the zymogen factor VII (TF-FVIIa). This greatly enhances the catalytic ability of FVII which activates factors IX and X. Through a cascade effect that occurs on the surface of the activated platelets, the production of thrombin (factor IIa) occurs (See figure 2.1.2). Initially only trace amounts of thrombin are generated, however through a process of feedback amplification exponentially increasing amounts of thrombin are generated with each feedback loop.³⁵ Thrombin acts upon fibrinogen to create fibrin monomers, which in the presence of FXIIIa undergo polymerisation to form fibrin that strengthens and stabilises the plug.³⁶

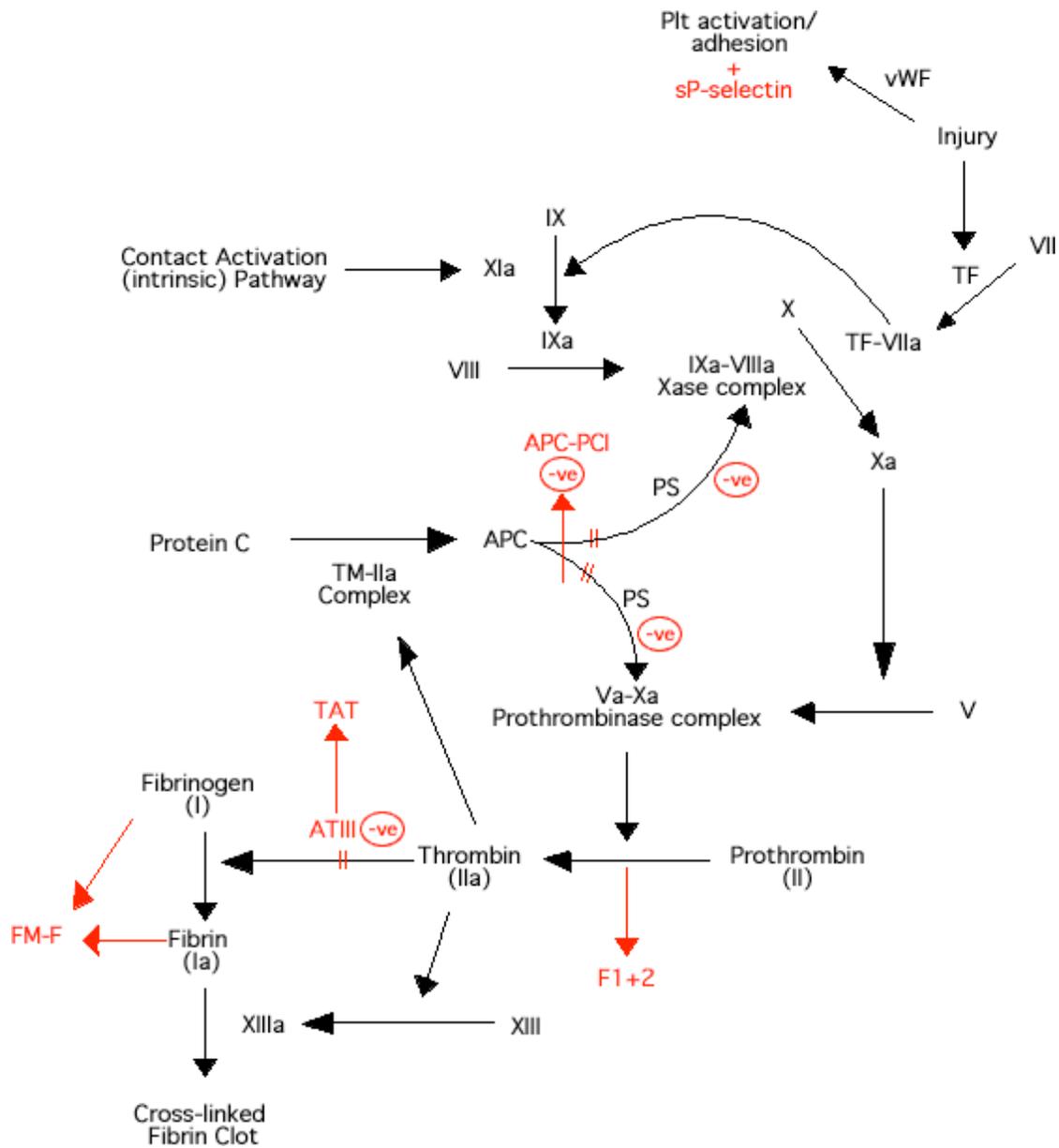


Figure 2.1.2: Coagulation Cascade. Biomarkers described in chapter 3 are highlighted in red. (Plt= platelets, sP-selectin= soluble P-selectin, vWF= von Willebrand factor, TF= tissue factor, APC-PCI=activated protein C-protein C inhibitor complex, PS= protein s, APC= activated protein C, TM-IIa= thrombomodulin-thrombin complex, TAT= Thrombin-antithrombin complex, ATIII= antithrombin III, F1+2= prothrombin fragments 1 & 2, FM-F= fibrin monomer=fibrinogen complex)

2.2 ANTICOAGULANTS AND FIBRINOLYSIS

Diffuse intravascular thrombosis is prevented by the actions of localised and systemic anticoagulants and fibrinolytic mechanisms:

2.2.1 Anticoagulants

The two main natural anticoagulants are Antithrombin (AT) III and activated protein C (APC). AT III is the central physiological anticoagulant and prevents the coagulation cascade amplificatory effects of thrombin; these include the cleavage of fibrinogen to form fibrin, the activation of factors V and VIII and the mediation of platelet activation and aggregation. Thrombin binds to a receptor (thrombomodulin) presented on the surface of intact endothelium to form a thrombin-thrombomodulin complex that activates protein C as well as inhibiting the effects of thrombin. APC in the presence of its co-factor protein S acts as an anticoagulant through its ability to inactivate Factors Va and VIIIa.^{37 38} In addition APC heightens fibrinolysis through two major mechanisms: APC forms a tight complex with plasminogen activator inhibitor type-1 (PAI-1) thereby preventing its inhibitory effect on tissue plasminogen activator (t-PA), secondly through its ability to limit thrombin generation it reduces the activation of thrombin activatable fibrinolysis inhibitor (TAFI).^{39 40} Tissue factor pathway inhibitor (TFPI)/lipoprotein-associated coagulation inhibitor also acts as a reversible anticoagulant through its ability to complex to and inactivate factor Xa. TFPI-Xa complex also has the ability to inhibit factor TF-VIIa complex thereby arresting the extrinsic pathway.⁴¹

2.2.2 Fibrinolysis

Plasmin is the main fibrinolytic enzyme formed by the activation of the zymogen plasminogen through a variety of mechanisms. The main activator of plasminogen is t-PA which is produced by the vascular endothelium in response to thrombin. When incorporated into a forming clot t-PA is a potent catalyst for conversion of plasminogen to plasmin.⁴² (See figure 2.2.1)

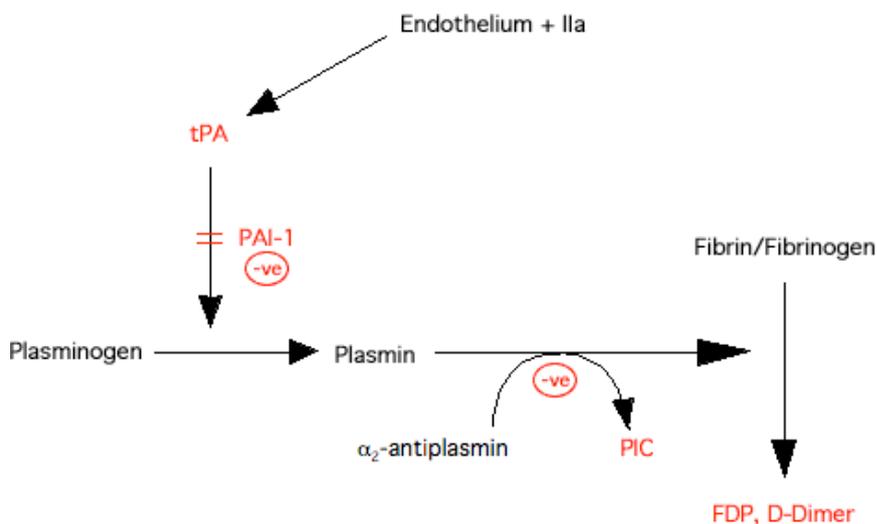


Figure 2.2.1: Diagrammatic representation of the fibrinolytic pathway. Biomarkers described in chapter 3 are highlighted in red. (t-PA= tissue plasminogen activator, PAI-1= plasminogen activator inhibitor type-1, FDP= fibrinogen degradation product, PIC= α_2 -plasmin inhibitor complex, IIa= Thrombin)

Urokinase-type plasminogen activator, APC and factors XIa, XIIa and Kallikrein can also independently activate plasminogen. Plasmin degrades polymerised fibrin to form fibrin degradation products (fragment E and D-Dimer). The activation of plasminogen and fibrin degradation by plasmin is finely balanced by PAI-1 and TAFI. PAI-1 is released by the vascular endothelium in response to thrombin and inhibits t-PA. TAFI eliminates plasminogen receptor sites on partially degraded fibrin and thus slows the binding and activation of plasminogen and thus fibrinolysis.⁴² α -Antiplasmin is also an inhibitor of plasmin.

2.3 SUMMARY

Haemostasis requires the interaction of platelets, coagulation factors and fibrinolytic factors. Platelet and endothelial cell activation at the site of injury initiates clot formation. The coagulation cascade is activated predominantly through the extrinsic pathway to form fibrin, which is vital in creating a stable platelet plug. The fibrinolytic and anticoagulant systems ensure thrombus formation is limited to the site of vascular injury.

CHAPTER 3

THE IMPACT ON BLOOD COAGULATION, FIBRINOLYSIS AND PLATELET
ACTIVATION OF OPEN SURGICAL AND ENDOVASCULAR ANEURYSM REPAIR
(EVAR) IN PATIENTS WITH INFRA-RENAL ABDOMINAL AORTIC ANEURYSMS

A REVIEW OF THE LITERATURE

3.1 INTRODUCTION

Major surgery causes a pro-thrombotic derangement in the peri-operative period with elevated levels of Factor VIII, Fibrinogen, Thrombin-antithrombin III-complex (TAT), von Willebrand Factor (vWF), deranged fibrinolysis and platelet hyperactivity.^{43 44 45 46 47 48 49}

Patients undergoing elective open surgical infra-renal AAA repair have an associated operative mortality rate of 3-10%.^{19 18 20 21 22} The majority of deaths are due to tissue damage and ischaemic-reperfusion injury, which result in an extensive and uncontrolled inflammatory response with micro- and macro- vascular thrombosis that may cause myocardial injury, thromboembolism and multiple organ failure.^{29 50}

Endovascular abdominal aortic aneurysm repair (EVAR) provides a less invasive and safer alternative treatment to open surgical repair in the fit and anatomically suitable patient. The EVAR-1, EVAR-2 and DREAM trials reported EVAR was associated with a 60% reduction in peri-operative mortality when compared to open surgical repair.^{7 8 9 10} These improvements may relate to an attenuation of the inflammatory response and pro-thrombotic diathesis associated with open repair through a reduction in tissue damage and ischaemic-reperfusion injury. However Swartbol et al have reported EVAR as promoting a systemic inflammatory response equal to if not in excess of that witnessed after open repair.⁵¹ This has been hypothesised to be secondary to cytokine release from the aneurysm sac thrombus as a result of introducer and catheter manipulation.^{51 52} The use of contrast media has also been reported to induce arterial endothelial damage.^{53 54}

3.2 AIMS

The aim of this chapter is to review and compare the effects of abdominal aortic aneurysm, open surgical repair and EVAR on coagulation, fibrinolysis and platelet activation as reported in the English-language scientific literature at the time of starting this thesis.

3.3 METHODS

We performed a MEDLINE, EMBASE and Cochrane Central Register of Controlled Trials (CENTRAL) databases search looking for English-language articles between January 1970 and August 2006 relating to abdominal aortic aneurysm, open surgical repair and EVAR, and their effects on haemostatic mechanisms. The terms coagulation, clotting, fibrinolysis, thrombosis and platelets were included amongst others. These were linked with terms such as abdominal aortic aneurysm, open repair, endovascular aneurysm repair and EVAR. Further articles were identified by following MEDLINE links, by cross-referencing from the reference lists of major articles and by following citations for these studies. Studies were specifically rejected if a) patient cohort was less than seven and b) values of assessed biomarkers of haemostasis were not included in the presented results. The studies were then graded and prioritised according to the level of the evidence presented.

3.4 RESULTS

Studies examining the effect of AAA and open surgical or EVAR on blood coagulation, fibrinolysis and platelet activity are summarised in Tables 3.4.1-5. The coagulation cascade and fibrinolysis pathways are summarised in figures 3.4.1 and 3.4.2

3.4.1 Effect of abdominal aortic aneurysm on haemostasis

AAA represents a chronic inflammatory pathology and is usually characterised by the presence of mural thrombus directly proportional to the maximum AAA diameter.⁵⁵ In contrast to atherosclerotic occlusive disease, blood flow is maintained through the mural thrombus thereby providing an interface for exchange between the systemic circulation and thrombus. The structure of the thrombus is highly complex with a network of inter-connecting canaliculi capable of delivering macromolecules between the intra-luminal and thrombus-arterial wall surfaces.⁵⁶ The canaliculi often contain cellular infiltrates including neutrophils, macrophages and platelets, often in state of degranulation. This may lead to consumption of platelets and coagulation factors such that in this group of patients a sub-clinical state of disseminated intravascular coagulation (DIC) may exist.⁵⁷ Thus the mural thrombus represents a biologically active entity with the ability to trap polymorphonuclear leukocytes, absorb circulating plasma components and aggregate platelets as well as being a source of proteolysis and fibrinolytic activity thought to be implicit in AAA progression.⁵⁶⁵⁸

⁵⁹ Several prospective comparative studies have examined the effects of AAA on direct and indirect biomarkers of thrombin generation, fibrinolysis and platelet activity. (See tables 3.4.1-3).

Study	Cases	Controls	Results Format	Fibrinogen			Fibrinolysis								
							D-Dimer			tPA ag			PAI-1		
				case	controls	P-value	case	controls	P-value	case	controls	P-value	case	controls	P-value
Adam et al ⁷¹	7 *	o **	median (range)	4.85 (1.61-7.9) g/l	1.5 - 4.0 g/l	0.033	1633 (753-3014) ng/ml	630-850 ng/ml	0.005	1.7 (0.75-3.2) IU/ml ***	0.2-2.0 IU/ml	0.023	6.3 (3.2-15.4) AU/ml	<15 AU/ml	0.005
Adam et al ²⁹	9	o **	median (range)	2.80 (1.59-.02) g/l	1.5 - 4.0 g/l	N/A				0.49 (0.14-3.2) IU/ml ***	0.2-2.0 IU/ml	N/A	8.2 (3.2-21.7)	<15 AU/ml	N/A
Blann et al ⁶⁷	21	42	mean +/- SD	3.6 +/-1.2 g/l	3.3 +/-0.9	0.185									
Bradbury et al ¹⁸	23	0 **	median (range)	5.16 (2.61-4.3) g/l	1.5-4.0 g/l	n/s									
Fowkes et al ⁶⁵	89	98	median (interquartile range)	3.5 (2.9-4.1) g/l	3.1 (2.7-3.6) g/l	0.02	441.5 (198.8-771.0) ng/ml	93.0 (57.8-158.8) ng/ml	<0.001	7.9 (6.0-11.1) ng/ml	8.6 (6.8-11.5) ng/ml	0.64			
Fisher et al ⁷⁴	22	0 **	Mean	365 mg/dl	150-400 mg/dl	n/s									
Holmberg et al ⁶⁵	23	o **	median (range)	3.6 (1.9-6.3) g/l	2.0-3.6 g/l	n/s									
Holmberg et al ⁷⁷	23	20	median (range)				571 (60-1515) µg/L	32 (10-536) µg/L	<0.01	10.2 (4.9-24.2) µg/ml	9.4 (2.9-19.0) µg/ml	>0.05	3.5 (2.0-33.1) IU/ml	4.7 (2.0-30.9) IU/ml	>0.05
Hosaka et al ⁷⁰	49	o **	mean +/- SD	336 +/- 85 mg/dl	160-350 mg/dl	N/A	8.5 +/- 6.7 µg/ml	<1.0 µg/ml	N/A						
Ihara et al ⁷⁸	22	26	mean +/- SD				732.6 +/- 858.6 ng/ml	125.7 +/- 46.1 ng/ml	<0.0001						
Jelenska et al ⁷³	20	22	mean +/- SD	4.2 +/- .79 mg/dl	313 +/- .55 mg/dl	<0.001	778 +/-311 ng/ml	362 +/- 242 ng/ml	<0.001						
Lee et al ⁷²	40	200	median (interquartile range)	3.05 (2.61-3.57) g/l	2.62 (2.21-3.08) g/l	≤0.001	142 (84-209.5) ng/ml	83 (67.5-129) ng/ml	≤0.001	8.5 (7-10.5) ng/ml	7.9 (5.8-10.1) ng/ml	NS			
Shindo et al ⁶⁹	43	o **	mean +/- SEM	300.2 +/- 15.5 mg/dl	150-330 mg/dl	N/A	10.6 +/- 2.0 µg/ml	<0.8 µg/ml	N/A						
Sofi et al ⁷⁹	438	438	mean +/- SD										28.6 +/- 21.6 mg/dl	17.8 +/- 12.6 mg/dl	<0.0001
Singh et al ⁶⁸	263 ♂	2699 ♂	mean +/- SD	3.72 (0.91) mmol/l	3.32 (0.88) mmol/l	<0.001									
	74 ♀	3350 ♀	mean +/- SD	3.77 (0.68) mmol/l	3.43 (0.80) mmol/l	<0.001									
Yamazumi et al ¹⁴	36	25	mean +/- SD	326 +/- 77 mg/dl	298 +/- 63 mg/dl	0.21	7.7 +/- 6.7 µg/ml	1.0 +/- 1.2 µg/ml	<0.01						

Table 3.4.1: Summary of studies investigating the association between AAA and levels of fibrinogen and biomarkers of fibrinolysis. (* symptomatic unruptured AAA, ** manufacturer's range, *** t-PA activity)

Study	Cases	Controls	Results Format	Thrombin Generation											
				TAT			F1+2			FDP			APC-PCI		
				case	controls	P-value	case	controls	P-value	case	controls	P-value	case	controls	P-value
Adam et al ⁷¹	7 *	0**	median (range)				2.1(1.1-5.2) nmol/l	0.4-1.1 nmol/l	0.001						
Adam et al ²⁹	9	0**	median (range)	21.6 (6.6-180.4) µg/L	1.0-4.0 µg/L	N/A	2.2 (0.7-7.1) nmol/l	0.4-1.1 nmol/l	N/A						
Holmberg et al ⁷⁷	23	20	median (range)	11.5 (2.6-30.3) µg/L	2.6 (2.0-5.6) µg/L	<0.01	2.2 (0.9-4.6) nM	1.2 (0.5-3.1) nM	>0.05						
Hosaka et al ⁷⁰	49	0**	mean +/- SD	11.5 +/- 11.3 ng/ml	<3.5 ng/ml	N/A									
Ihara et al ⁷⁸	22	26	mean +/- SD	16.4 +/- 16/9 ng/ml	2.6 +/- 1.3 ng/ml	<0.0001									
Jelenska et al ⁷³	20	22	mean +/- SD				1.17 +/- 0.36nM	0.99 +/- 0.28 nM	<0.001						
Kolbel et al ⁸⁵	78	121	Median (range; 10th -90th centile)									0.45 (0.24-1.47) µg/l	0.15 (0.10-0.23) µg/l	<0.0001	
Shindo et al ⁶⁹	43	0**	mean +/- SEM							718.6 +/- 138.2 ng/ml	<100 ng/ml	N/A			
Yamazumi et al ¹⁴	36	25	mean +/- SD	17.4 +/- 13.6 ng/ml	3.8 +/- 2.2 ng/ml	<0.01				11.6 +/- 12.0 µg/ml	3.6 +/- 2.0 µg/ml	<0.01			

Table 3.4.2: Summary of studies investigating the association between AAA and biomarkers of thrombin generation. (* symptomatic unruptured AAA, ** manufacturer's range)

Study	Cases	Controls	Results Format	vWF			Platelet count			sP-selectin		
				case	controls	P-value	case	controls	P-value	case	controls	P-value
Adam et al ⁷¹	7 *	0 **	median (range)				183 (75-292) x10 ⁹ /l	150-350 x10 ⁹ /l	<0.05			
Blann et al ⁶⁷	21	42	mean +/- SD	123 +/-37 IU/dl	113 +/- 32 IU/dl	<0.001				325 (155-4253) ng/ml	202 (80-440) ng/ml	<0.001
Bradbury et al ¹⁸	23	0 **	median (range)				204 (140-293) x10 ⁹ /l	150-350 x10 ⁹ /l	>0.05			
Fowkes et al ⁶⁵	89	98	median (interquartile range)	122.5 (98.0-150.2) IU/dl	123.0 (101.0-152.0) IU/dl	0.55						
Milne et al ⁸⁶	105	32	mean +/- SD				215+/- 47.5 x 10 ⁹ /l	269+/-57 x 10 ⁹ /l	<0.0001			
Shindo et al ⁶⁹	43	0 **	mean +/- SEM				17.9 +/- 5.7 x 10 ³ /mm ³	15.7-34.2 x 10 ³ /mm ³	N/A			
Yamazumi et al ¹⁴	36	25	mean +/- SD				19.6 +/- 5.4 x 10 ⁴ /μL	23.7 +/- 6.1 x 10 ⁴ /μL	<0.05			

Table 3.4.3: Summary of studies investigating the association between AAA and vWF, Platelet count and sP-Selectin. (* symptomatic unruptured AAA, ** manufacturer's range)

Biomarker	Study	Maximum diameter of AAA	Worst angle along length of AAA	Maximum thickness of intraluminal AAA thrombus	Total Intra-luminal AAA thrombus volume
APC-PCI	Kolbel et al ⁸⁵	r=0.22, p=0.001			r=0.123, p=0.142
D-Dimer	Shindo et al ⁶⁹	r=0.208, p=NS			r=0.208, p=NS
	Yamazumi et al ¹⁴	r=0.644, p=0.0001	r=-0.411, p=0.009	r=0.650, p=0.0001	
F-TFPI	Yamazumi et al ¹⁴	r=0.408, p=0.016	r=-0.583, p=0.0006		
FDP	Shindo et al ⁶⁹	r=0.208, p=NS			r=0.171, p=NS
	Yamazumi et al ¹⁴	r=0.561, p=0.0009		r=0.513, p=0.0024	
FM-FC	Hosaka et al ⁷⁰	r=0.128, p=0.381		r=0.233, p=0.125	
PIC	Yamazumi et al ¹⁴	r=0.413, p=0.0146		r=0.484, p=0.042	
TAT	Yamazumi et al ¹⁴	r=0.566, p=0.001	r=-0.366, p=0.0305	r=0.677, p=<0.0001	

Table 3.4.4: Summary of studies investigating the association between AAA morphology and biomarkers of haemostasis.

Biomarker	Study	Controls (units)	Pre-operative	24 hours		1 week		3 months		>12 months	
			Value	Value	p-value	Value	p-value	Value	p-value	Value	p-value
D-dimer	Holmberg et al ⁶⁶	32 (10-536) µg/L	511 (60-1275)							39 (23-326)	<0.05
	Yamazumi et al ¹⁴	1.0 +/- 1.2 µg/ml	7.7+/-6.7					4.6+/-3.5	<0.01		
F1+2	Holmberg et al ⁷⁷	1.2 (0.5-3.1) nM	2.2 (0.9-4.6)			2.7 (1.5-5.7)	NS				
	Holmberg et al ⁶⁶	1.2 (0.5-3.1) nM	1.4 (0.0-4.6)							1.2 (0.8-3.0) **	NS
FDP	Yamazumi et al ¹⁴	3.6 +/- 2.0 µg/ml	11.6 +/- 12.0					7.6+/-4.6	0.17		
Fibrinogen	Holmberg et al ⁶⁶	2.0-3.6 g/l *	3.6 (1.9-6.3)			5.6 (3.3-8.4)	<0.001				
	Yamazumi et al ¹⁴	298 +/- 63 mg/dl	326 +/- 77					331+/-55	0.75		
PAI ag	Holmberg et al ⁶⁶	4.7 (2.0-30.9) IU/ml	5.6 (2.0-29.3)							9.9 (2.0-54.5)	NS
Platelet Count	Bradbury et al ¹⁸	150-350 x10 ⁹ /l *	292 (179-51) x 10 ⁹ /L	187 (103-364) x10 ⁹ /l	<0.001						
	Yamazumi et al ¹⁴	23.7 +/- 6.1x10 ⁴ /µL	19.6 +/- 5.4 x 10 ⁴					21.2+/-5.0 x 10 ⁴	<0.05		
TAT	Holmberg et al ⁷⁷	2.6 (2.0-5.6) µg/L	11.5 (2.6-30.3)			11.8 (4.4+/-31.3)	NS				
	Holmberg et al ⁶⁶	2.6 (2.0-5.6) µg/L	11.5 (2.6-26.1)							3.8 (2.7-16.2)**	<0.05
	Yamazumi et al ¹⁴	3.8+/-2.2 ng/ml	17.4 +/-13.6					10.4+/-4.5	<0.01		
tPA ag	Holmberg et al ⁶⁶	9.4 (2.9-19.0) µg/ml	10.3 (7.6-15.3)							12.5(6.6-14.9)	NS

Table 3.4.5: Summary of studies investigating the effects of open surgery on biomarkers of haemostasis. [* Manufacturer's range, **

mean follow up of 26 month (19-37)]

An elevated level of plasma fibrinogen is an independent risk factor for stroke and myocardial infarction as well as cardiovascular mortality.^{60 61 62} An association between elevated fibrinogen and atherosclerotic peripheral vascular disease has been widely reported and elevated levels of plasma fibrinogen are found in patients who subsequently develop peripheral artery disease.^{24 61 63 64} The association between non-ruptured AAA and plasma fibrinogen levels have been extensively investigated.^{14 18 65 66 67 68 69 70 71 72 73 74} (See table 3.4.1) Six from twelve studies have reported significantly elevated levels of fibrinogen in patients with asymptomatic AAA. Singh et al reported significantly increased levels of plasma fibrinogen in patients with AAA.⁶⁸ On multivariate analysis Lee et al reported an independent association between AAA and plasma fibrinogen ($p=0.05$, $OR=1.51$).⁷² While Singh et al reported similar findings but only in the studies male cohort; male ($p < 0.001$, $OR = 1.42$) vs. females ($p = 0.18$, $OR = 1.23$).⁶⁸

Hosaka et al reported elevated levels of both fibrinogen and fibrin-monomer-fibrinogen (FM-F) complex in patients with AAA indicating increased thrombin activity. During the cleavage of fibrinogen by thrombin fibrinopeptide A and B are released to create fibrin monomer (FM) that undergoes polymerisation –catalysed by factor XIIIa- to form insoluble fibrin clots.⁷⁵ When a pro-thrombotic state exists and thrombin generation is high FM forms soluble complexes with fibrinogen (FM-F complex) due to excess plasma fibrinogen.⁷⁶ Thus elevated levels of FM-F represent heightened thrombin activity. Furthermore, a sufficiently elevated concentration of FM-F may itself add to the pro-thrombotic diathesis as it precipitates from plasma.⁷⁶

Adam et al reported significantly elevated levels of plasma fibrinogen in patients with symptomatic, but non-ruptured AAAs.⁷¹ In a separate study, the same group reported no difference in pre-operative plasma fibrinogen levels between those patients with ruptured

AAA and asymptomatic, non-ruptured AAA (median (range), 2.27 (0.86-3.75) vs. 2.8 (1.59-6.02) g/l, $p = \text{NS}$).²⁹ However, these studies involved small numbers of patients.

Ten studies were identified investigating the association between AAA and fibrinolysis.^{14 65 69 70 71 72 73 77 78 79} (See table 3.4.1) Nine studies assessed the impact of AAA on plasma D-dimer levels. The degradation of fibrin by plasmin ultimately results in the formation of two fragment-D molecules that covalently link to form a dimer: D-dimer. Thus the presence of D-dimer in the circulation represents ongoing clot formation and fibrinolysis. All studies report elevated levels of plasma D-dimer in patients with AAA indicating ongoing clot formation and fibrinolysis. On multivariate analysis Lee et al ($p = \leq 0.001$, OR = 3.75) reported an independent association between circulating D-dimer levels and AAA.⁷²

Tissue Plasminogen Activator (t-PA) is released from vascular endothelium in response to thrombin generation and becomes incorporated into the forming fibrin clot. When bound to fibrin t-PA is a potent activator of plasminogen and is therefore a marker of fibrinolysis. PAI-1 is also released from the vascular endothelium in the presence of thrombin and acts to maintain a balance between clot formation and lysis by inhibiting t-PA. Four studies have reported the effect of AAA on both D-dimer levels and t-PA and/or Plasminogen Activator Inhibitor-1 (PAI-1).^{65 71 72 77} All three studies reporting t-PA antigen levels found no difference in circulating levels in patients with or without an AAA. However, t-PA antigen levels reflect both the unbound, active t-PA and the bound, inactive t-PA/PAI-1 complexes. Therefore it is important to measure t-PA activity as opposed to t-PA antigen to avoid the paradox in which elevated levels of t-PA antigen are associated with a pro-coagulant state due to increase PAI-1 activity.²⁹ Only one study has analysed both t-PA antigen and PAI-1 activity levels and reports no significant difference compared to their control populations although elevated D-dimer levels in the AAA cohort was found.⁷⁷ Adam et al are the only group to report on t-PA

activity levels that were found to be comparable to the manufactures normal reference range as was PAI-1 activity in symptomatic, non-ruptured AAA.⁷¹

Thrombin plays an intricate part in coagulation initiation and platelet activation. More recently thrombin has been implicated in smooth muscle cell mitogenesis, with intimal vascular smooth muscle cells showing increased thrombin receptor expression.⁸⁰ The conversion of prothrombin to thrombin by prothrombinase complex results in the production of a degradation product with a half life of <90 mins; prothrombin fragment 1+2 (F1+2).⁸¹ Thrombin released through this enzymatic process is inactivated by antithrombin-III to form a Thrombin-antithrombin III-complex (TAT).⁸² Thus, F1+2 and TAT are biomarkers of coagulation activation and thrombin generation. This review identified six studies that reported on the effect of AAA on the levels of one or both of these biomarkers.^{14 70 71 73 77 78} (See table 3.4.2) All studies reporting TAT found elevated circulating levels in patients affected by AAA. Holmberg et al report elevated levels of TAT, but normal levels of F1+2.⁷⁷ This may be explained by the short half-life of F1+2; elevated TAT levels are more indicative of ongoing thrombosis whereas F1+2 may be more reflective of a single acute thrombotic event.

Activated protein-C (APC) is a natural anticoagulant and is produced on the surface of intact endothelium following the binding of thrombin to the endothelial cell cofactor: thrombomodulin.⁸³ APC in the presence of protein-S cofactor reduces Xase and prothrombinase activity through its ability to cleave factors Va and VIIIa, thereby limiting thrombin production to the site of endothelial injury.^{37 38} APC is inactivated through binding to protein-C inhibitor (PCI) to form APC-PCI complex. This complex is detectable in the blood for <20 minutes after formation and is an indicator of thrombin generation.⁸⁴ Kolbel et al report a three-fold increase in median APC-PCI complex levels in patients with AAA when

compared to healthy controls ($p = <0.0001$), indicating that patients with AAA would appear to undergo periods of acute thrombus formation on background of chronically increased thrombin generation.⁸⁵

Adhesion of platelets to exposed sub-endothelial collagen leads to platelet activation and aggregation. Von Willebrand Factor (vWF) through its platelet receptor Glycoprotein-1b (GP-1B) mediates this interaction.³² These activated platelets release prothrombotic mediators form dense bodies and alpha-granules that include vWF, adenosine diphosphate (ADP) and Thromboxane-A₂ (TxA₂). They are also rich in FVa and FVIIa receptors and undergo aggregation by the binding of fibrinogen to platelet GP-IIb/-IIIa receptors on adjacent platelets.³³ Thus, the central role of platelet-vessel wall interaction is the initiation and progression of thrombosis. Six studies report on the effects of AAA on platelets.^{14 67 69 71 73 86}

Three of six studies reports significantly lower levels of platelets in patients with AAA. Milne et al, in addition to finding a lower a platelet count, reported elevated levels of Glycocalicin, a biomarker for GP-1B levels and suggested that this represented increased activation and destruction of platelets as a result of adherence and incorporation into the thrombus mass.⁸⁶

This would appear to be supported by Mukaiyama et al who demonstrated uptake of radiolabelled platelets by the AAA lumen.⁸⁷ Platelets activated through exposure to sub-endothelial collagen without concomitant aggregation at the site of injury are removed from the circulation by the reticulo-endothelial system irrespective of platelet age.⁸⁸ Thus the aneurysm thrombus mass may also induce platelet activation without platelet adhesion leading to their subsequent destruction and GP-1B release. Blann et al demonstrated increased levels of soluble P-selectin, a marker of platelet activation, and vWF in AAA patients, which would lend support to the theory of increased platelet activation.⁶⁷

3.4.2 Association between AAA morphology and haemostasis

Three studies have reported on the correlation between AAA maximum diameter and thrombus volume, and markers of haemostasis.^{14 69 70} (See table 3.4.4). AAA size and total volume/maximum thrombus thickness appear to correlate with the aforementioned changes in thrombin generation and fibrinolysis. Yamazumi et al reported a correlation between AAA tortuosity and markers of thrombosis.⁸⁹ This association may represent the association between blood flow velocity changes, particularly turbulent flow, and red blood cell (RBC) activation. Shindo et al reported a correlation between RBC counts and the AAA lumen volume.⁶⁹ Activated RBCs release ADP resulting in platelet aggregation and activation.⁹⁰ Thus the consumptive coagulopathy, resulting from the thrombus mass and the abnormal flow field in a tortuous lumen may both contribute to the haemostatic derangement reported in patients with AAA.

3.4.3 Effect of open surgical repair on biomarkers of haemostasis

Major surgery is known to produce a pro-thrombotic derangement in the peri-operative period with raised levels of FVIII, Fibrinogen, TAT, vWF, platelet hyper-activity, and evidence of deranged fibrinolysis.^{43 44 46 47 48 49 91} Patients undergoing major lower limb arterial reconstructive surgery exhibit defective endogenous fibrinolytic activity: increased levels of PAI-1 and t-PA antigen that return to pre-operative levels by one week post-procedure suggestive of a consumptive hypofibrinolytic state.^{92 93 94}

Studies reporting the effect of open surgical AAA repair on haemostasis are summarised in table 3.4.5. Yamazumi et al reported a significant decrease in circulating levels of TAT and D-dimer at 3-months post repair when compared to pre-operative levels ($p = <0.01$).¹⁴ However, the levels remained elevated when compared to those of the control group ($p = <0.01$) suggestive of ongoing up-regulation of thrombin generation and lysis. Holmberg et al reported similar findings with significantly elevated levels of TAT and F1+2 reported peri-operatively which returned to pre-operative levels by one-week post surgery, but once again remained elevated compared to age-matched controls.⁷⁷ In a separate study the same group reported open surgical repair attenuating the pre-operative thrombotic derangement in the long term with a reduction in TAT and D-dimer plasma levels at a median follow up of 26 months.⁶⁶ However, the values remained slightly higher than the normal healthy reference ranges suggesting that there was ongoing haemostatic derangement despite thrombus volume reduction.

3.4.4 Effect of EVAR on biomarkers of haemostasis

To date only two studies have investigated the effect of EVAR on haemostasis.^{17 95} Serino et al report the short-term effects of EVAR on circulating D-dimer levels in nine patients assessed pre-operatively and on day four post-operatively. D-dimer levels were elevated in seven patients and decreased in two patients. The median level for the entire patient cohort did not demonstrate a statistically significant difference between baseline and day four post-operation [563±521 ng/ml vs. 799±443 ng/ml ($p = 0.19$)]. This lack of statistical significance may represent a type-II error due to the study's lack of power. Odegard et al reported increased platelet activation following the administration of contrast media intra-operatively and this remained increased during variable lengths of hospital stays.⁹⁵

3.5 DISCUSSION

AAA is associated with increased thrombin generation, activity and fibrin turnover as demonstrated by increased plasma levels of TAT, APC-PCI, FM-F, F1+2, fibrinogen and D-dimer. Increased fibrin turnover occurs on the surface of intra-luminal AAA thrombus. Several authors have reported a positive correlation between the extent of haemostatic derangement and AAA size and thrombus load.^{14 85} Thus the pro-thrombotic diathesis witnessed in this patient population may be secondary to continuous intra-luminal thrombus remodelling.

Open surgical repair causes significant haemostatic derangement in the peri-operative period with increased thrombin generation and activity, and increased fibrin turnover. By 3-months post-repair, the pro-thrombotic diathesis is similar to if not improved compared to the pre-operative state. However, open surgical repair does not tend to complete resolution of the pre-operative haemostatic derangement. Plasma levels of TAT and D-dimer remain elevated after surgery compared to healthy controls suggestive that the surgical prosthesis may be capable of inducing a prothrombotic derangement. Other factors that may contribute to these ongoing haemostatic derangements include atherosclerotic disease affecting other vascular beds with a number of studies reporting a positive correlation between the extent of atherosclerotic vessel disease and a pro-thrombotic state.^{96 97 98} Red blood cell concentrates have been shown to have high levels of cytokines including IL-1, IL-6, and TNF which are potent coagulation stimulators and thus the volume of blood transfusion may contribute to the peri-operative coagulation derangement witnessed after aneurysm repair.^{66 99} Increasing age has also been reported to influence biomarkers of coagulation and fibrinolysis.^{100 101}

Only one study has studied the effect EVAR on coagulation/fibrinolysis in the peri-operative period. The small patient numbers and lack statistical power did not allow any firm

conclusions to be drawn from this study. However, the majority of patients demonstrated increased fibrin turnover with elevated levels of circulating D-dimer in the peri-operative phase. This may occur due to aneurysm sac exclusion with sac thrombosis resulting in increased coagulation and fibrinolysis activation, and platelet consumption; contrast media induced endothelial cell injury and platelet activation; or endothelial damage due to the passage of guide-wires and catheters as has been demonstrated after coronary and peripheral angioplasty.^{53 54 102 103 104}

3.6 SUMMARY

AAA is associated with a pro-thrombotic diathesis proportional to the volume of intra-luminal thrombus. The pro-coagulant state is exaggerated in the immediate peri-operative period following open repair but is attenuated at medium-term follow-up although not normalised. These changes may account for the high level of thrombotic complications observed in this cohort of patients. There are currently no robust studies investigating the effect of EVAR on the underlying pro-thrombotic diathesis evident in AAA patients.

CHAPTER 4

THE SHORT TERM EFFECTS OF ENDOVASCULAR ANEURYSM REPAIR (EVAR) ON
COAGULATION AND CARDIOVASCULAR MORBIDITY AND MORTALITY IN PATIENTS
WITH INFRA-RENAL ABDOMINAL AORTIC ANEURYSMS

METHODOLOGY

4.1 INTRODUCTION

This chapter describes the methodology involved in the investigation of the hypotheses described in chapter 1. The premise for this study is that the AAA intra-luminal thrombus load causes a pro-thrombotic diathesis that is attenuated upon exclusion of the aneurysm through endovascular stent-graft repair (EVAR).

4.2 ETHICAL APPROVAL

Ethical approval was granted by Birmingham East, North and Solihull Research Ethics Committee and South Birmingham Research Ethics Committee for all aspects of work contained within this thesis (reference: 06/Q2703/44). The study was named Coagulation, Endoleak and Myocardial Injury after EVAR (CEMIE) study.

4.3 EXPERIMENTAL DESIGN

4.3.1 Study Design

This is a prospective, non-randomized, multi-center study designed to investigate the short-term haemostatic derangement in patients with abdominal aortic aneurysms undergoing elective EVAR. AAA—for this protocol- is defined as an asymptomatic infra-renal aortic aneurysm with a maximum anterior-posterior diameter measuring ≥ 5.5 cm on ultrasonography or computed tomographic angiography (CTA).

4.3.2 Patient recruitment

Initially all patients presenting to the Vascular Surgery Units of the Heart of England NHS Foundation Trust and University Hospital Birmingham NHS Trust between April 2006 and October 2007 and undergoing EVAR were considered for inclusion in the study. However, due to a freezer malfunction the majority of samples/results collected from this patient cohort were lost or invalidated. (See appendix 2) As a result a second cohort of patients using the same methods and protocols for enrolment was recruited between January 2008 and December 2009. Thus this thesis presents two equal cohorts of patients as separate datasets: cohort one (April 2006- October 2007) and cohort two (January 2008- December 2009).

Patients were primarily identified through a) liaising with lead clinicians in their respective vascular surgery departments and radiology departments, b) attendance at weekly vascular multidisciplinary team meetings, and c) attendance at outpatient clinics in the aforementioned hospitals.

Following identification of a suitable study patient (see inclusion and exclusion criteria below) the patient was interviewed by the lead investigator (RSM Davies) and provided with a comprehensive CEMIE study information leaflet. (See appendix 3) The patient was then allowed at least 24 hours to decide whether or not they wished to participate in the study. Those patients willing to participate underwent a subsequent written fully informed consent process with a concomitant study participation notice forwarded to the patients' general practitioner. (See appendices 4 & 5)

4.3.3 Patient Inclusion/Exclusion Criteria

a) Inclusion

All patients met the following criteria:

1. Males and females 50-90 years of age inclusive
2. Asymptomatic AAA
3. Radiological (USS/CTA) diagnosis of an infra-renal AAA ≥ 5.5 cm
4. Suitable for EVAR
5. Smoking status stable for ≥ 3 months
6. Patient was committed and able to follow the protocol requirements as evidenced by written informed consent

b) Exclusion

Patients were excluded if they met any of the following criteria:

1. A non-cardiac endovascular intervention procedure other than a diagnostic percutaneous trans-femoral/-brachial digital subtraction angiography within the previous 3 months.
2. Patients with concomitant thoracic or suprarenal aortic aneurysm or dissection
3. Patients with significant lower limb peripheral vascular disease (Fontaine Classification IIb-IV)
3. Presence of the following conditions that could confound coagulation studies:
 - History of malignancy and/or chemotherapy within the last 5 years
 - Coagulation disorders:
 - Hereditary coagulopathy e.g. Haemophilia, von Willebrand disease
 - Acquired e.g. liver disease, malabsorption syndromes
 - Thrombocytopenia e.g. bone marrow failure, idiopathic thrombocytopenic purpura (ITP), hypersplenism
 - Kidney Disease Outcomes Quality Initiative (KDOQI) Stage 3-5 chronic renal failure
 - History of deep venous thrombosis and/or venous ulceration within last 3 months
 - Evidence of sepsis or systemic inflammatory response syndrome
 - Patients with immuno-compromised conditions, organ transplant recipients, or those being treated with immunosuppressive pharmacotherapy

4. Patients who have undergone previous surgery within the last 6 months
5. Patients who have undergone coronary artery angiography/stent insertion within the last 3 months
6. Patients who have been on treatment dose anti-coagulation pharmacotherapy within the last 6 months e.g. Heparin, low molecular weight heparin (LMWH), warfarin
7. Patients with known blood borne infections e.g. Hepatitis B virus (HBV), Human immunodeficiency virus (HIV)
8. Patients who have undergone a blood transfusion within the last month

4.3.4 Data collection

The following patient clinico-pathological data were collected prospectively and stored in an encrypted database: patient age and sex; co-morbidities and medications; pre-operative imaging investigations; procedural details; post-operative data including imaging investigations, re-interventions, complications and mortality. Specifically cardiovascular morbidity and mortality at each study time point was recorded and all patients underwent ECG pre-operatively and at 24-hours post-operatively.

Blood Sample Collection

All patients had a resting venous blood sample drawn from an antecubital fossa vein without tourniquet at the following time points for haemostatic assays: pre-procedure, 24-hours post-procedure, and 1-month post-procedure. Blood for the assay of myocardial injury was drawn pre-procedure, and 24 hours post-procedure. Post-procedure is defined as the time following the successful completion of EVAR.

Blood was drawn into a standard syringe utilising a 21g hypodermic needle and then immediately transferred to specific tubes; a 2.7 ml sample was collected into EDTA anticoagulant (1.6mg/ml), a 3ml sample into sodium citrate anticoagulant (0.106 mol/l), a 9ml sample into a tube containing Z-serum clot activator. Samples were placed immediately on ice and transferred to the laboratory within 30 minutes of collection. Plasma was separated by centrifugation at 3,000 revolutions per minute for 30 minutes at a temperature of 4° C (equivalent to 1400g). Plasma and serum were separated utilising a standard 1 ml graduated pasteur pipette and stored in cryogenic vials at - 80 C for subsequent batch analysis.

4.3.5 Assay Methodology

a) Markers of Haemostasis

Commercially available assays were used to measure circulating levels of Thrombin-antithrombin III-complex (TAT), Plasminogen activator inhibitor 1 (PAI-1) antigen, tissue plasminogen activator (t-PA) activity and soluble P-selectin (sP-Selectin).



Figure 4.3.1: Triturus® (*Grifols*) fully automated enzyme immunoassay analyser

All assays for biomarkers of coagulation, fibrinolysis and platelet activity were batch analysed utilising an open system Triturus® (*Grifols*) fully automated enzyme immunoassay analyser. (See figure 4.3.1) The following plasma circulating biomarkers were analysed at the aforementioned time points:

1) Marker of Coagulation

Name: Thrombin-antithrombin III (TAT) Complex

Biomarker of: Thrombin generation

Information: Thrombin plays an intricate part in coagulation initiation and platelet activation. Prothrombinase complex (Va-Xa) in the presence of Ca^{2+} acts as a catalyst converting prothrombin (II) into Thrombin (IIa). Thrombin released through this enzymatic process is inactivated by antithrombin III to form a Thrombin-antithrombin III-complex (TAT).⁸² Thus circulating plasma levels of TAT reflect thrombin generation.

Test Kit: AssayMax Human Thrombin-antithrombin (TAT) Complexes ELISA Kit

Company: Assaypro®

Principal of assay: This assay employs a quantitative sandwich enzyme immunoassay technique utilising an immobilised monoclonal antibody (AB) specific for antithrombin and a polyclonal AB -with a covalently attached biotin tag (biotinylated) - specific for thrombin. TAT complexes are sandwiched by the immobilized AB and biotinylated polyclonal AB that is recognized by an enzyme-bound streptavidin (streptavidin-peroxidase). Unbound material is then washed away and a peroxidase enzyme substrate catalyst is added to generate a coloured product whose intensity is measured.

2) Markers of Fibrinolysis

Name: Plasminogen Activator Inhibitor Type-1 (PAI-1) antigen

Biomarker of: Fibrinolysis

Information: PAI-1 is released from the vascular endothelium in the presence of thrombin and is the principal inhibitor of tissue plasminogen activator (t-PA). Through its ability to inhibit the activators of plasminogen, PAI-1 acts to maintain balance between clot formation and lysis and thus is a primary regulator of fibrinolysis.

Test Kit: Spectrolyse ®PAI-1

Company: American Diagnostica Inc. ®

Healthy Range: 7-43 ng/ml

Principal of assay: This is a two-stage, indirect chromogenic assay based upon the work by Chmielewska and Eriksson.^{105 106} The first stage consists of adding a known amount of t-PA (40 IU/ml) to the plasma allowing reaction with PAI-1. The second stage involves calculating the residual t-PA activity. This is achieved through the addition of human glu-plasminogen, poly-D-lysine and a chromogenic substrate for plasmin. The residual t-PA activity catalyses the conversion of plasminogen to plasmin stimulated by poly-D-lysine. The conversion of plasminogen to plasmin hydrolyses the chromogenic substrate causing a colour reaction that is proportional to PAI-1 content.

Name: Tissue plasminogen activator (t-PA) activity

Biomarker of: Fibrinolysis

Information: t-PA is a 70,000 Dalton glycoprotein released from the vascular endothelium in response to thrombin generation. It is highly fibrin specific and when bound efficiently cleaves Glu-plasminogen to form plasmin and, therefore, serves as the major activator of fibrinolysis. The majority of circulating t-PA is complexed to PAI-1 and thus inactive. Therefore the active, non-complexed t-PA reflects true t-PA activity in the calculation.

Test Kit: t-PA actibind ELISA kit

Company: Technoclone. ®

Healthy Range: 0 U/ml

Principal of assay: An AB that does not interfere with t-PA functional activity is coated onto a microtitre plate and used to bind t-PA contained in the test plasma sample to the plate surface. Following an incubation period, non-bound plasma components are washed away. Active non-complexed t-PA is photometrically quantified through the addition and incubation of an activity substrate solution containing Glu-plasminogen, cyanogen bromide fragments of fibrinogen and a chromogenic plasmin. The AB bound t-PA activates Glu-plasminogen to yield plasmin that interacts with the chromogenic plasmin substrate to release a coloured product. The concentration of the coloured product is proportional to the amount of non-complexed, active t-PA in the test sample.

3) Marker of Platelet Function

Name: Soluble P-Selectin (sP-selectin)

Biomarker of: Platelet activation

Information: Plasma sP-selectin is an adhesion molecule predominantly found in α -granules of unstimulated platelets. Upon platelet activation, sP-selectin is expressed on the platelet surface and subsequently shed by cleavage over a short period of time.^{107 108} Circulating levels of sP-selectin are not influenced by different anticoagulants, although levels are influenced by anti-platelet agents.¹⁰⁹ This, in combination with its ability to resist ex-vivo activation enables it to act as a more reliable marker of in-vivo platelet activation than alternative methods in patients affected by thrombotic conditions, including light transmittance aggregometry.

Test Kit: Human sP-selectin ELISA

Company: Immuno-Biological Laboratories, Inc ®

Healthy Range: 92-212 ng/ml

Principal of assay: This is a chromogenic assay utilising an AB sandwich immunoassay technique. Anti-human sP-selectin AB is immobilised onto microwells. The plasma sample is then added with the sP-selectin binding to the immobilised antibodies. Horseradish peroxidase (HRP) conjugated anti-human sP-selectin AB is then added sandwiching the bound sP-selectin. Unbound HRP-conjugated anti-human sP-selectin is then removed and tetramethylbenzidine is added that reacts with HRP to form a coloured product proportional to the sP-selectin levels present in the sample.

b) Marker of Myocardial injury

Name: Cardiac troponin T (cTnT)

Biomarker of: Micro-myocardial infarction

Information: Troponins are normal regulatory muscle proteins involved in the calcium regulated actin-myosin interactions. Three sub-types exist (T, I and C) of which Troponin T binds to tropomyosin attaching the troponin complex to the thin filament.¹¹⁰ Troponin T exists as a distinct cardiac structural protein subtype (cTnT) that is released following cardiac muscle cell necrosis; numerous studies have demonstrated cTnT to be a highly sensitive and specific biomarker for micro-myocardial injury.^{110 111 112} Our research group has previously reported that over a third of patients undergoing surgery for critical lower limb ischaemia sustain peri-operative micro-myocardial injury as evidenced by elevated plasma cardiac troponin levels.¹¹³ Furthermore, none of these patients demonstrated any clinical evidence of cardiac injury or had evidence of myocardial ischaemia on ECG. cTnT is detected 3-6 hours after myocardial damage and reaches peak level at 12-48 hours.

Test Kit: Cardiac troponin T enzyme immunoassay kit using the ES300 fully automated multichannel immuno-assay analyzer

Company: Boehringer Mannheim

Lower limit of detection: ≤ 0.01 ng/ml

Principal of assay: This test employs a standard sandwich immunoassay technique with streavidin-coated tubes and two monoclonal antibodies; one AB acts as the capture AB and binds to the streavidin-coated test tube, the second AB act as the signal AB and is labelled with horseradish peroxidase.

4.3.6 Assessment of thrombus load

All computed tomographic aortograms (CTA) were performed according to a standard acquisition protocol using an Aquilion system (Toshiba). Images were uploaded to a Vitrea Fx (Vital Images) workstation for post-processing thrombus load calculation. The Vitrea Fx workstation has a semi-automated thrombus load estimation function that estimates total aneurysm, lumen and thrombus volumes through a process of interpolation. The start and endpoints were standardised to the aorta immediately distal to the lowest renal artery and the aortic bifurcation, respectively. (See figures 4.3.2) If the software incorrectly identified thrombus as lumen and vice versa, thrombus/lumen contours were manually corrected prior to thrombus calculation. (4.3.3) Thrombus volume is measured in cubic centimetres (CC).

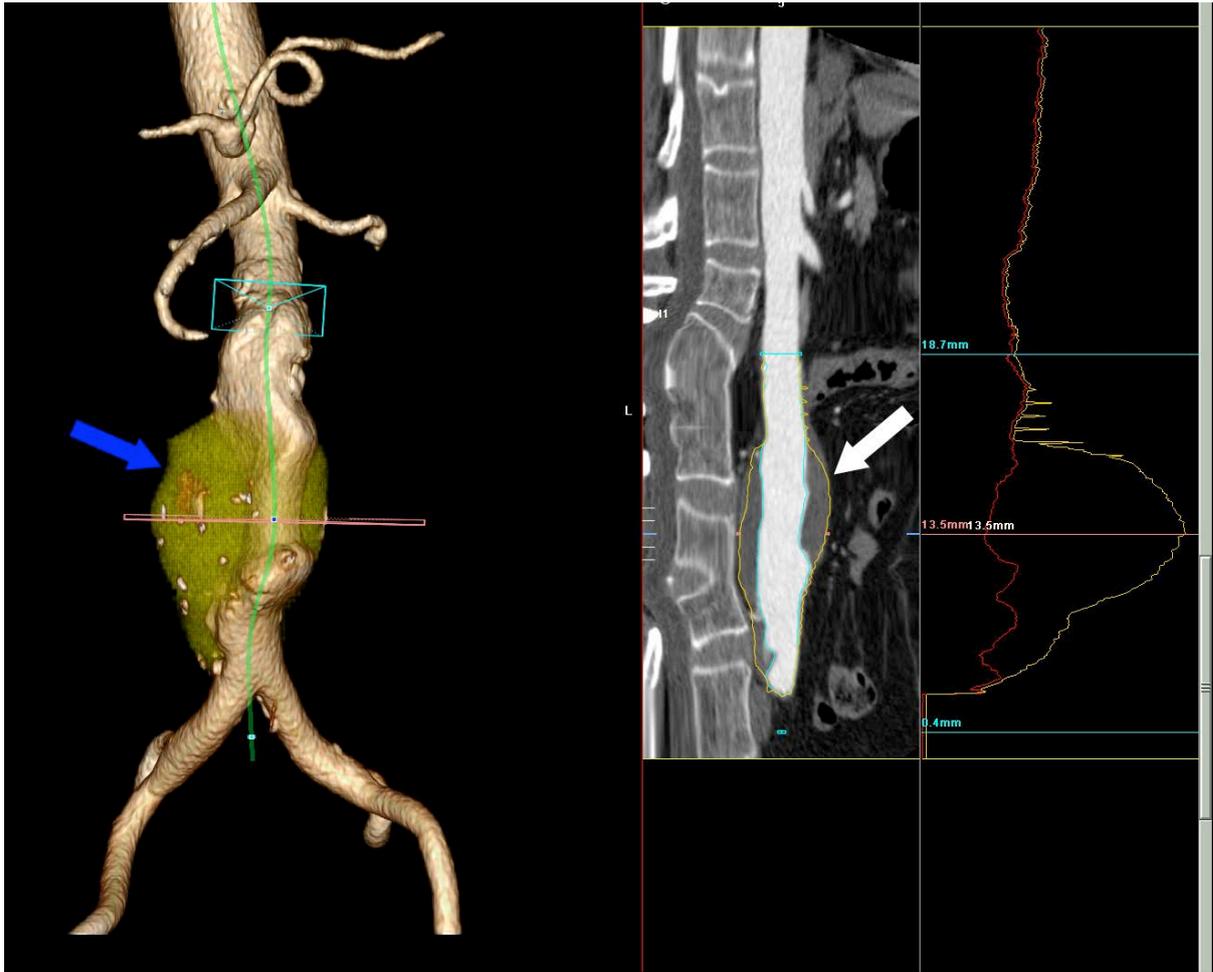


Figure 4.3.2: Snapshot from Vitrea Fx demonstrating 3D thrombus mapping (blue arrow) and curvilinear projection with thrombus outlined (white arrow).

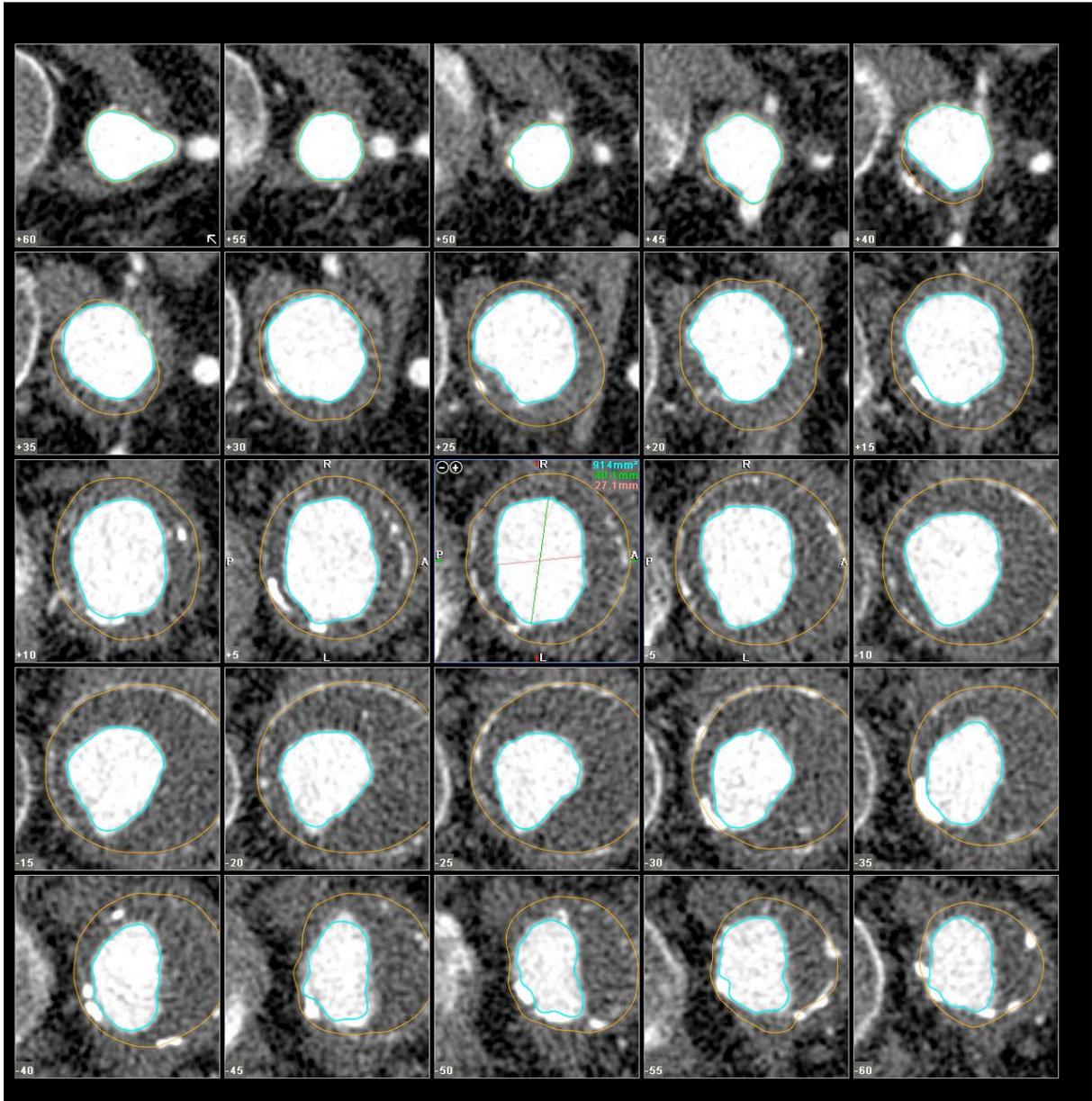


Figure 4.3.3: Manual remapping of lumen (white line) and thrombus (green line) contours using Vitrea Fx workstation.

4.4 POWER CALCULATION AND STATISTICAL ANALYSIS

Statistical advice was provided by Mr. Peter Nightingale (Statistician, University Hospital Birmingham). A priori power analysis was performed based upon previously published data by our research group utilising techniques described by Gottfried E Noether. A minimum sample size of 17 patients was calculated as being required to demonstrate a similar degree of haemostatic improvement following EVAR as open surgical repair with a power of 80% (β -error =0.8) and a significance of <0.05 (α -error=0.05).¹¹⁴ Based on this calculation, in combination with an expected drop out rate of 30% (based on our units experience in running research studies in similar patient populations), we estimated a minimum cohort recruitment of 25 patients was required.

All analyses were carried out using StatsDirect version 2.7.2 (StatsDirect Ltd, Cheshire, UK). Although data sets were expected to show non-Gaussian/normal distribution, all data were assessed for Gaussian/normal distribution utilising the Shapiro-Wilk W test; p-value <0.05 for W rejects the assumption of normality.¹¹⁵

Differences between patient characteristics were assessed using Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables. The effect of surgical treatment was analysed with Wilcoxon signed-ranked test. Correlation analyses were performed using the Spearman rank test. Where levels of biomarkers were below the limit of detection of the assay, the minimum detection concentration was assigned to that sample and statistical analysis performed using this definition. Data are presented as median (inter-quartile range) unless stated otherwise. A probability value of less than 0.05 was considered statistically significant.

CHAPTER 5

PRE-OPERATIVE MARKERS OF COAGULATION AND FIBRINOLYSIS IN PATIENTS WITH ASYMPTOMATIC INFRA-RENAL ABDOMINAL AORTIC ANEURYSM UNDERGOING ENDOVASCULAR ANEURYSM REPAIR

“Blood alone moves the wheels of history.”

Martin Luther King

5.1 INTRODUCTION

Abdominal aortic aneurysms (AAA) represent a chronic inflammatory pathology and is usually characterised by the presence of biologically active mural thrombus directly proportional to the maximum AAA diameter.⁵⁵ This mural thrombus is thought to cause systemic haemostatic derangement characterised by increased thrombin production and reduced fibrinolysis.²⁹ The extent of this prothrombotic diathesis is reported to correlate with the aneurysm sac thrombus volume.^{14 70 85} At the time of starting this thesis only one study had investigated the effect of infra-renal AAA on plasma levels of sP-selectin, a biomarker of platelet activity and no study has attempted to correlate plasma sP-selectin levels with aneurysm sac thrombus volume.⁶⁷

5.2 AIMS

The aims of this study are to 1) report the effect of the presence of an infra-renal AAA on the circulating plasma levels of novel biomarkers of coagulation, fibrinolysis and platelet activity, and 2) analyse whether any haemostatic derangement including platelet activation is related to AAA diameter or sac thrombus volume.

5.3 METHODS

Patients presenting to the Vascular Surgery Units of the Heart of England NHS Foundation Trust and University Hospital Birmingham NHS Trust between April 2006 and October 2007 (cohort 1) and January 2008 and December 2009 (cohort 2) with an infra-renal AAA measuring ≥ 5.5 cm in maximum diameter *and* due to undergo endovascular AAA repair were assessed for study inclusion suitability. (See chapter 4.3.3)

Blood sample collection

A resting venous blood sample was drawn from an antecubital fossa vein 24 hours prior to EVAR. The samples were placed immediately on ice and centrifuged within 30 minutes of collection at 3,000 revolutions per minute for 30 minutes at a temperature of 4° C (equivalent to 1400g). Plasma was separated and stored at - 80 C for later batch analysis.

Markers of Thrombin generation, Fibrinolysis and Platelet activity

Plasma thrombin antithrombin III-complex (TAT) (healthy range, <4.2 $\mu\text{g/L}$) was assayed as a marker of thrombin generation. Plasma plasminogen activator inhibitor 1 (PAI-1) (healthy range, 7-43 ng/ml) and tissue plasminogen activator (t-PA) activity (healthy range, 0 U/ml) were assayed as markers of fibrinolysis. Soluble P-selectin (sP-selectin) (healthy range, 92-212 ng/ml) was assayed as a marker of platelet activity. The assay's manufacturer determined the healthy range for each haemostatic marker. (For further details of these commercially available assays please see chapter 4.3.5)

Assessment of thrombus load

AAA thrombus volume, measured in cubic centimetres (CC), was calculated utilising the Vitrea Fx post-processing workstation. (See chapter 4.3.6)

Statistics

Differences between patient characteristics were assessed using Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables. Correlation analyses were performed using the Spearman rank test. A probability value of less than .05 was regarded as statistically significant. Data are presented as median (inter-quartile range). All analyses were carried out using StatsDirect version 2.7.2 (StatsDirect Ltd, Cheshire, UK).

5.4 RESULTS

Patient Cohort 1

30 patients (28 men, 2 women of median (IQR) age 77.5 (72-82) years) who underwent elective EVAR for infra-renal AAA were studied. Patient co-morbidities are shown in table 5.4.1. The median (IQR) pre-operative plasma levels of PAI-1 were significantly higher than the normal range: 51 (12.7-51) ng/ml vs. 7-43 ng/ml; $p=0.05$. There was no correlation between pre-operative anti-platelet ($p = 0.71$) or statin ($p = 0.59$) pharmacotherapy and PAI-1 levels.

Median (IQR) maximum aneurysm diameter was 62.5 (59-69) mms. Median (IQR) total aneurysm volume and aneurysm lumen volume were 210 (164-235) cc and 101 (77-125) cc. Median (IQR) aneurysm sac thrombus volume was 112 (95-148) cc. There was no correlation between aneurysm thrombus volume and maximum aneurysm diameter: $r = -0.03$, $p = 0.89$. There was no correlation between aneurysm thrombus volume and PAI-1 plasma levels: $r = 0.211$, $p = 0.41$.

	Patient cohort		p-value
	1 (n = 30)	2 (n = 30)	
<u>Co-morbidities</u>			
Renal failure (CKD Stage = <3)	6	4	0.51
Diabetes	7	4	0.34
Hypercholesterolaemia	7	2	0.09
Ischaemic Heart Disease	10	10	>0.99
Smoker	5	1	0.11
Peripheral vascular disease (fontaine = <IIb)	11	1	0.0013
<u>Medication</u>			
Antiplatelet	16	16	>0.99
Aspirin	16	14	0.62
Clopidogrel	0	1	0.5
Dual therapy	0	1	0.5
Statin	19	16	0.45

Table 5.4.1: Patient pre-operative co-morbidities and anti-platelet/statin status.

Patient Cohort 2

30 patients (29 men, 1 women of median (IQR) age 75 (71-82) years) who underwent elective EVAR for infra-renal AAA were prospectively studied. The pre-operative plasma levels of PAI-1 (median (IQR) = 20.9 (8.4-50.7) ng/ml) and t-PA activity (median (IQR) = 0 (0-0) U/ml) were within the normal values.

The pre-operative plasma levels of sP-selectin were significantly lower than the normal range: median (IQR) = 80.5 (68-128) ng/ml, $p = <0.0001$. The pre-operative use of a statin (median (IQR); 72 (66-81) vs. 84 (80-117) ng/ml, $p = 0.0165$) resulted in lower plasma sP-selectin levels. The pre-operative use of an anti-platelet (median (IQR); 73 (66-81) ng/ml vs. 84 (76-99) ng/ml, $p = 0.076$) tended to result in lower sP-selectin levels.

Assay	Normal Range	Pre-operative Values (median (IQR))	
		Cohort 1	Cohort 2
PAI-1	7-43 ng/ml	51 (12.7-51) ↑	20.9 (8.4-50.7)
t-PA	0 U/ml	NA	0 (0-0)
sP-Selectin	92-212 ng/ml	NA	80.5 (68-128) ↓
TAT	<4.2 µg/L	NA	7.15 (4.7-31.3) ↑

Table 5.4.2: Pre-operative values of markers of haemostasis. The arrows indicate comparisons to normal reference ranges.

The pre-operative plasma levels of TAT were significantly higher than the normal reference range: median (IQR) = 7.15 (4.7-31.3) $\mu\text{g/L}$, $P = <0.001$. There was no correlation between pre-operative anti-platelet ($p = 0.13$) or statin ($p = 0.21$) pharmacotherapy and TAT levels.

(See table 5.4.2)

Median (IQR) maximum aneurysm diameter was 66.5 (57.3-70.3) mms. Median (IQR) total aneurysm volume and aneurysm lumen volume were 188 (147-247) cc and 80 (54.3-107) cc. Median (IQR) aneurysm sac thrombus volume was 97.6 (63-127) cc. There was no correlation between aneurysm thrombus volume and maximum aneurysm diameter: $r = 0.45$, $p = 0.15$. There was no correlation between aneurysm thrombus volume and PAI-1 ($r = -0.25$, $p = 0.47$), sP-Selectin ($r = 0.26$, $p = 0.43$) or TAT plasma levels ($r = -0.21$, $p = 0.54$).

5.5 DISCUSSION

Several prospective studies report the effects of AAA on a variety of different biomarkers of coagulation and fibrinolysis.

To date only thirteen studies in the English language literature report the association between AAA and fibrinolysis.^{14 65 69 71 70 72 73 77 78 79 116 117 118} (See tables 3.4.1 & 5.5.1) Nine studies report often-contradictory effects of AAA on levels of PAI-1 and/or t-PA antigen.^{65 29 71 72 77 79 118 116 117} The majority of studies report no difference in circulating t-PA antigen levels in patients with AAA compared to control populations. The one exception is Wanhainen et al who report elevated levels of t-PA antigen in patients with screen detected AAA suggestive of increased fibrinolysis in this cohort of patients.¹¹⁷ Sofi et al report the largest series analysing the effects of AAA on PAI-1 levels and describes a significant elevation in those affected with AAA compared to a control population. The majority of our study's patients have either normal or slightly increased levels of PAI-1 when compared to the normal reference range. Only three studies have analysed both t-PA antigen and PAI-1 levels and all three studies report no significant difference compared to their control populations.^{77 116 118} We analysed t-PA activity levels as a true reflection of systemic fibrinolysis and found comparable results in that levels were analogous to the normal range. Thus despite PAI-1 levels being elevated in some patients overall systemic fibrinolysis was normal.

Study	Cases	Controls	Results Format	Fibrinolysis									Thrombin Generation		
				D-Dimer			tPA ag			PAI-1			TAT		
				case	controls	P-value	case	controls	P-value	case	controls	P-value	case	controls	P-value
Parry et al ¹¹⁸	75 males	90	variable	346.7 (288.5-427.5) ng/ml	120.2 (106.9-134.2) ng/ml	<0.001	9.12 (8.03-10.50) ng/ml	8.80 (8.14-9.51) ng/ml	0.719	17.9 (15.4-20.4) ng/ml	16.2 (14.4-17.9) ng/ml	0.619	4.57 (2.68-8.7) ng/ml	1.89 (1.48-2.49) ng/ml	<0.001
Wallinder et al ¹¹⁶	40	41	median (interquartile range)	625 (460-1437) ng/ml	86 (38-176) ng/ml	<0.001	10.5 (8.6 - 14.8) ng/ml	11.1 (8.5-15.2)ng/ml	>0.05	4.0 (1.9-8.7) IU/ml	2.7 (1.9-7.9) IU/ml	>0.05	6.0 (3.8-9.1) µg/l	2.9 (2.1-3.9) µg/l	<0.001
Wanhainen et al ¹¹⁷	42	100	mean +/- SD				13.6 +/- 4.7 µg/ml	11.4 +/- 4.3 µg/ml	0.016						

Table 5.5.1: Latest studies investigating the association between AAA and levels of fibrinogen and biomarkers of fibrinolysis. and thrombin generation (* symptomatic unruptured AAA, ** manufacturer's range, *** t-PA activity)

In our study patients with AAA demonstrated increased thrombin generation as evidenced by elevated levels of TAT. Similar findings have been reported in five of seven English language studies.^{14 29 70 71 77 78 116 118} (See table 3.4.2 & 5.5.1) The largest study reported elevated levels of TAT and F1+2 on both univariate and multivariate analysis in men with small AAA: median (range) maximum diameter 41mm (30-55 mm).¹¹⁸ In our patient cohort TAT levels were elevated independent of anti-platelet or statin pharmacotherapy.

To date only one study has reported on the effects of AAA on platelet activation through the measurement of plasma sP-selectin.⁶⁷ P-selectin is an adhesion molecule and is expressed on the cell surface upon activation; plasma sP-selectin derives predominantly from platelets and is an accurate marker of in vivo platelet activation.¹¹⁹ Blann et al reported elevated levels of sP-selectin in 21 patients with uncomplicated AAA implying increased platelet activation. The current study appears to contradict this finding in that patients with AAA had reduced plasma sP-selectin levels and thus depressed platelet activity. This may reflect differences in the proportion of patients with hypercholesterolaemia and/or treated with a statin; the majority of patient cohort reported by Blann et al demonstrated cholesterol levels in excess of 5.8 mmol/l. Patients with hypercholesterolaemia have increased platelet cell-membrane cholesterol concentrations which results in a heightened sensitivity to platelet-aggregation inducing agents with increased plasma levels of P-selectin and other platelet derived micro-particles reported.¹²⁰ Statins inhibit platelet activation and aggregation through both cholesterol dependent and cholesterol independent pathways.¹²¹ By reducing plasma cholesterol concentration statins disrupt platelet cell membrane phospholipid and attenuates ADP-induced platelet aggregation. Statins have also been reported to inhibit platelet activation through interactions with the RhoA-Rho kinase pathway.¹²² We found a direct correlation between statin pharmacotherapy and platelet activation.

To date six studies have investigated the effects of AAA thrombus volume/maximum thickness on haemostasis, often with contradictory results.^{14 69 70 123 124 125} (See table 5.5.2)

Both Yamazumi et al and Aho et al reported a correlation between AAA total thrombus volume/maximum thrombus thickness and changes in biomarkers of systemic fibrinolysis. However, only Yamazumi et al reported a positive correlation between maximum thickness of intraluminal AAA thrombus and heightened thrombin generation.¹⁴

In direct contradiction, Kolbel et al, using APC-PCI as a marker of thrombin generation, reported no correlation between thrombus volume or thrombus intra-luminal surface area and the underlying pro-coagulant state.¹²⁴ The present study failed to show a correlation between systemic thrombin generation and total aneurysm thrombus volume. These discrepancies may reflect differing methods of thrombus volume measurement. We utilised a semi-automated interpolation method using a Vitrea Fx post-processing workstation. This compares to Kolbel et al who estimated thrombus volume using the formula for measuring the volume of a cylindrical tube, and Yamazumi who utilised 2D computed tomography. Thus we believe our methodology to better reflect the true thrombus volume as it allows for varying vessel morphologies in the calculation.

In conclusion, the present study confirms that patients with AAA demonstrate haemostatic derangement, but the extent of the haemostatic derangement does not correlate with the volume of intra-luminal aneurysm thrombus.

CHAPTER 6

THE SHORT TERM EFFECTS OF ENDOVASCULAR ANEURYSM REPAIR ON MARKERS
OF COAGULATION, FIBRINOLYSIS AND PLATELET ACTIVITY IN PATIENTS WITH
ASYMPTOMATIC INFRA-RENAL ABDOMINAL AORTIC ANEURYSMS

6.1 INTRODUCTION

Elective EVAR for asymptomatic AAA is associated with a 60% reduction in peri-operative mortality when compared to open surgical repair in the fit and anatomically suitable patient.⁷¹²⁶ However, exponents of EVAR argue that these initial short-term benefits are offset by the long-term propensity for stent-related complications, in particular endoleak.¹³ Endoleak is a complication unique to EVAR and results in aneurysm sac reperfusion. The EVAR-1 trial reported one quarter of EVARs were complicated by endoleak, with one third requiring a secondary procedure to maintain complete aneurysm exclusion within 3 years of the index procedure.⁷ Thus it is necessary for all patients to be entered into a stent-graft surveillance program to identify stent-graft failure prior to aneurysm sac rupture. Despite imaging protocols undergoing considerable evolution this need for long-term follow-up poses a significant socio-economic burden on the treating institution.¹¹

Major surgery is known to produce a pro-thrombotic derangement with elevated plasma biomarkers of thrombosis reported in the post-operative period.^{43 44 46 47 48 49 127} Patients undergoing major lower limb arterial reconstructive surgery exhibit defective endogenous fibrinolytic activity; increased levels of PAI-1 and t-PA antigen that return to pre-operative levels by one week post-procedure.^{92 93 94}

Open surgical repair of an infra-renal AAA causes significant haemostatic derangement in the peri-operative period with increased thrombin generation and activity, and increased fibrin turnover reported during the first week. This pro-coagulant state would appear to attenuate thereafter such that by 3-months post-repair the pro-thrombotic diathesis is similar to, if not improved, compared to the pre-operative haemostatic state. However, open surgical repair does not lead to resolution of the pre-operative derangement.

Careful review of the literature has revealed no studies investigating the effect of EVAR on biomarkers of coagulation, fibrinolysis and platelet activity. We hypothesised that 1) similar changes to systemic coagulation, fibrinolysis, and platelet activity would occur following EVAR and 2) failure to exclude the aneurysm sac from the systemic circulation, in the form of an endoleak, would result in the re-establishment of the heightened pre-operative thrombotic state.

6.2 AIMS

The aim of this chapter is to 1) investigate peri-operative changes in plasma levels of biomarkers of coagulation, fibrinolysis and platelet activity following EVAR for asymptomatic AAA, and 2) examine the relationship between peri- and post-operative changes in coagulation, fibrinolysis and platelet function following the development of endoleak after EVAR.

6.3 METHODS

Patients presenting to the Vascular Surgery Units of the Heart of England NHS Foundation Trust and University Hospital Birmingham NHS Trust between April 2006 and October 2007 (cohort 1), and January 2008 and December 2009 (cohort 2) with an infra-renal AAA measuring ≥ 5.5 cm in maximum diameter *and* due to undergo endovascular AAA repair were assessed for study inclusion suitability. (For inclusion and exclusion criteria see chapter 4.3.3)

Blood sample collection

A resting venous blood sample was drawn from an antecubital fossa vein 24 hours prior to, 24-hours after and 1-month following EVAR. The samples were placed immediately on ice and centrifuged within 30 minutes of collection at 3,000 revolutions per minute for 30 minutes at a temperature of 4° C (equivalent to 1400g). Plasma was separated and stored at -80 C for later batch analysis.

Markers of Thrombin generation, fibrinolysis and Platelet activity

Plasma thrombin antithrombin III-complex (TAT) (healthy range, <4.2 $\mu\text{g/L}$) was assayed as a marker of thrombin generation. Plasma plasminogen activator inhibitor 1 (PAI-1) (healthy range, 7-43 ng/ml) and tissue plasminogen activator (t-PA) activity (healthy range, 0 U/ml) were assayed as a marker of fibrinolysis. Soluble P-selectin (sP-selectin) (healthy range, 92-212 ng/ml) was assayed as a marker of platelet activity. The assay's manufacturer determined the healthy range for each haemostatic marker. (For further details of these commercially available assays please see chapter 4.3.5)

Assessment for Endoleak

All patients underwent computed tomography angiography assessment at 24 hours and 1-month post-EVAR to assess for endoleak.

Statistics

Differences between patient characteristics were assessed using Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables. Wilcoxon signed-ranked test was used to assess the effects of EVAR at different time points. Correlation analyses were performed using the Spearman rank test. Where levels of assays were below the lower limit of assay detection, this level was assigned for statistical analysis. A probability value of less than .05 was regarded as statistically significant. Data are presented as median (inter-quartile range). All analyses were carried out using StatsDirect version 2.7.2 (StatsDirect Ltd, Cheshire, UK).

6.4 RESULTS

Patient Cohort 1

30 patients (28 men 2 women of median (IQR) age 77.5 (72-82) years) underwent EVAR. 22 patients underwent insertion of a Zenith Endovascular stent-graft (Cook, Bloomington, Indiana) under loco-regional anaesthesia and 8 patients underwent insertion of a Gore Excluder stent-graft (W. L. Gore & Associates, Flagstaff, Arizona) under general anaesthesia. EVAR was successfully completed in all patients and there were no in-hospital deaths.

Median (IQR) levels of PAI-1 pre-operative, 24-hours post-operation and 1-month post-operation were 51 (12.7-51) ng/ml, 43.8 (22.2-51) ng/ml and 36 (31.7-45.1) ng/ml. There was no significant difference in plasma levels of PAI-1 between pre-operation and 24-hours post-operation ($p = 0.23$), pre-operation and 1-month post-operation ($p = 0.67$) or 24-hours post-operation and 1-month post-operation ($p = 0.37$). (See figure 6.4.1).

Median (IQR) levels of PAI-1 pre-operative, 24-hours post-operation and 1-month post-operation for patients undergoing EVAR under general anaesthesia/Gore Excluder were 20.3 (9.2-50.2) ng/ml, 45 (22.6-51) ng/ml and 33.8 (31.2-44.9) ng/ml, respectively. Median (IQR) levels of PAI-1 pre-operative, 24-hours post-operation and 1-month post-operation for patients undergoing EVAR under loco-regional anaesthesia (Zenith stent) were 51 (40.6-51) ng/ml, 42 (13.8-51) ng/ml and 36.9 (31.7-47.7) ng/ml, respectively. There was no significant difference in changes to plasma PAI-1 levels at 24-hours ($p = 0.47$) or 1-month ($p = 0.21$) post-operation when stratified for those EVARs using Zenith or Gore Excluder stent-grafts.

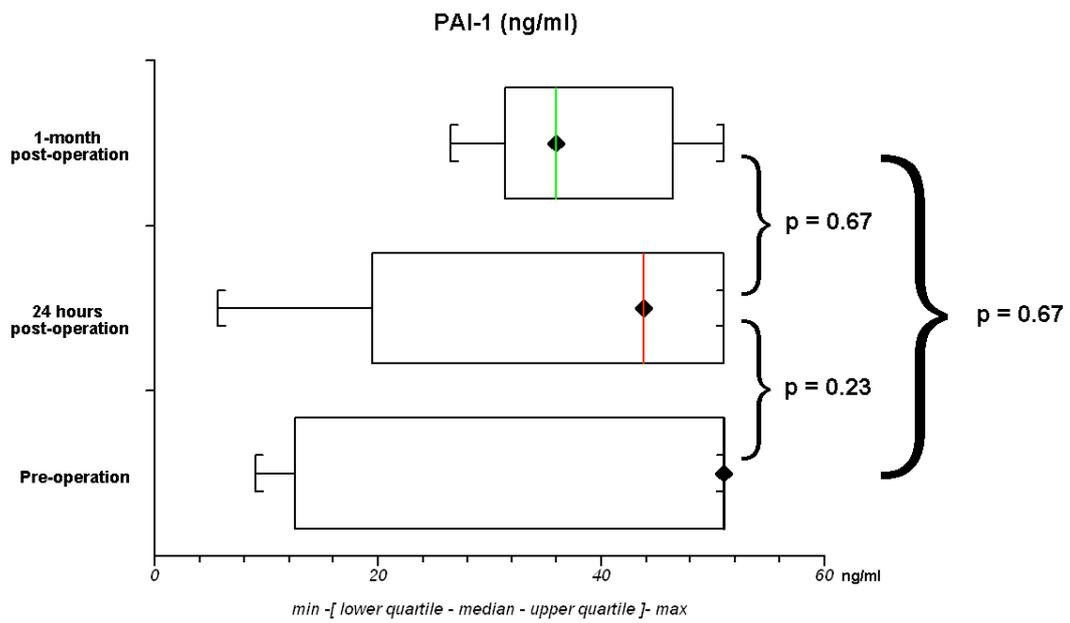


Figure 6.4.1: Comparison of plasma levels of PAI-1 at pre-operation, 24 hours post-operation and 1-month post-operation.

Effect of Endoleak

Seven patients demonstrated evidence of an endoleak at 1-month follow-up post-EVAR: type-2 = 6, type-1 = 1. Median (IQR) difference in plasma levels of PAI-1 between baseline and 1-month post-EVAR for patients with and without endoleak were -13.1 (-25-14.1) ng/ml and 12.3 (0-17.3) and ng/ml, respectively ($p = 0.043$). (See figure 6.4.2) Two patients with endoleaks had positive differences (+14.1 ng/ml and +1.1 ng/ml) between PAI-1 levels at 1-month and baseline

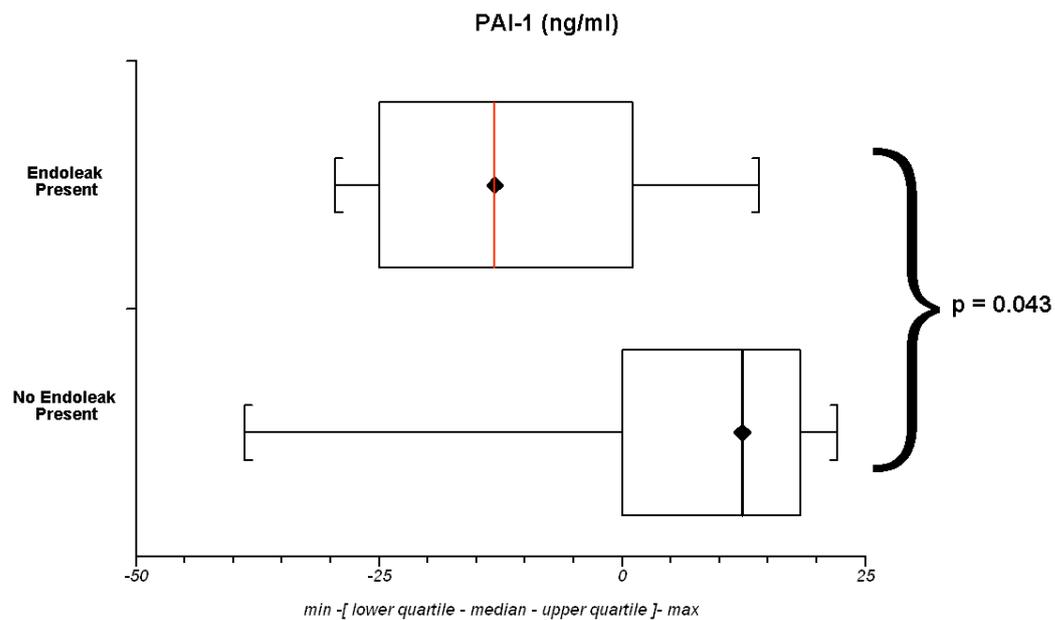


Figure 6.4.2: Median (IQR) differences of PA-1 levels between baseline and 1-month post-EVAR in patients with or without endoleak.

Patient Cohort 2

30 patients (29 men and 1 women of median (IQR) age 75 (71-82) years) underwent EVAR. All EVARs were performed using a Zenith Endovascular stent-graft (Cook) and were performed under loco-regional anaesthesia. There were no in-hospital deaths and EVAR was successfully completed in all patients. Changes in coagulation, fibrinolysis and platelet activity parameters are illustrated in table 6.4.1

BIOMARKERS	NORMAL RANGE	PRE-OPERATIVE VALUES	POST-OPERATIVE VALUES	
			24 HOURS	1-MONTH
PAI-1(ng/ml)	7-43 ng/ml	20.9 (8.4-50.7)	30.75 (19.8-46.8)	30.25 (15.8-56.9)
t-PA (U/ml)	0 U/ml	0 (0-0)	0 (0-0)	0 (0-0.06)
sP-Selectin (ng/ml)	92-212 ng/ml	80.5 (68-128)	89.5 (73-112)	110 (89-143)
TAT (µg /ml)	<4.2 µg/L	7.15 (4.7-31.3)	21.65 (13-33.1)	8.1 (5.4-14.85)

Table 6.4.1: Effect of EVAR on coagulation, fibrinolysis and platelet activity parameters.

We noted no significant differences between pre-operative, 24-hours post-operative and 1-month post-operative levels of PAI-1. A significant increase in sP-selectin levels occurred between pre-operation and 24-hours post-operation ($p = 0.003$), pre-operation and 1-month post-operation ($p = <0.0001$) and 24-hours and 1-month post-operation ($p = 0.0003$). There was increased t-PA activity at 1-month post-operation compared to pre-operation ($p = 0.0012$) and 24-hours post-operation ($p = 0.0012$). There was a trend towards increased thrombin generation at 24 hours compared to pre-operation ($p = 0.069$) followed by a significant decrease at 1-month ($p = <0.0001$) such that thrombin generation returned to pre-operative levels by 1-month post-EVAR. (See figures 6.4.3 – 6.4.6).

Effect of Endoleak

No endoleaks occurred in this patient cohort at 1-month post-procedure. Combining the results from patient cohorts 1 and 2 median (IQR) difference in plasma levels of PAI-1 between baseline and 1-month post-EVAR for patients with and without endoleak were -13.1 (-25-14.1) ng/ml and 2.8 (-9.4-16) ng/ml, respectively ($p = 0.079$).

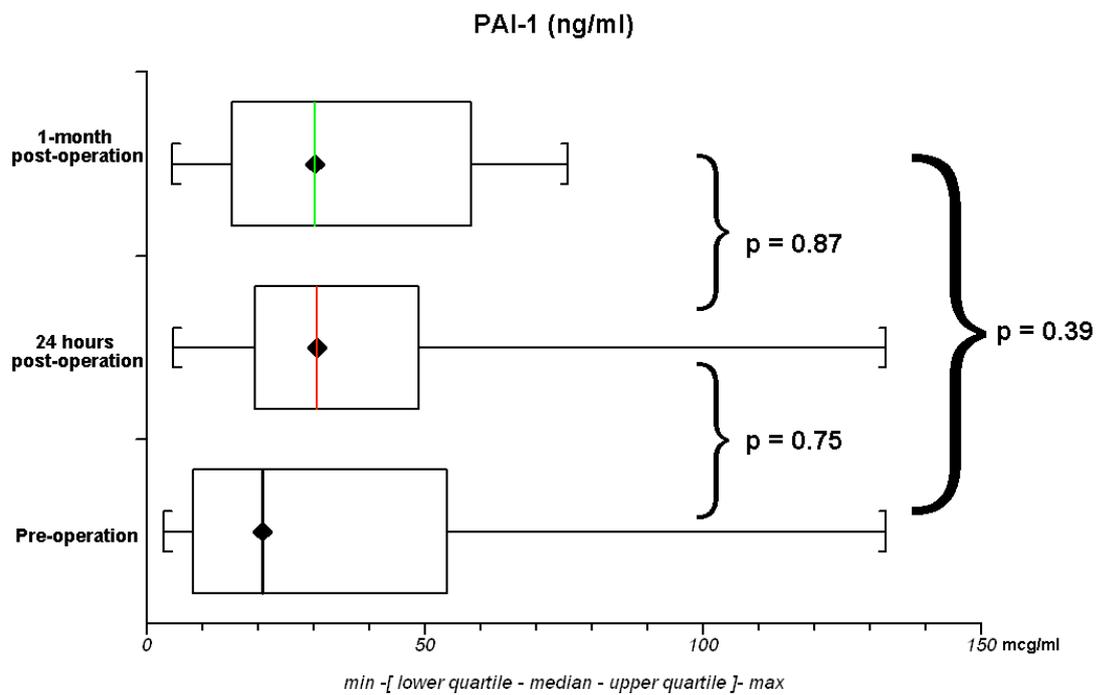


Figure 6.4.3: Comparison of plasma levels of PAI-1 at pre-operation, 24 hours post-operation and 1-month post-operation.

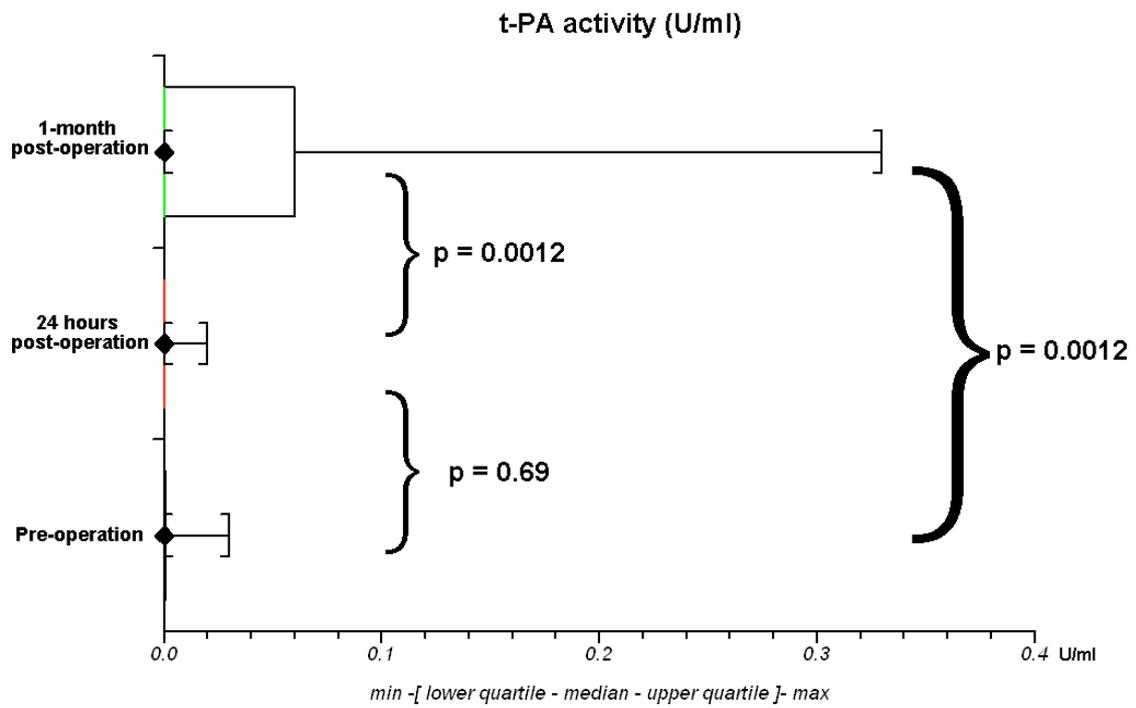


Figure 6.4.4: Comparison of plasma levels of t-PA activity at pre-operation, 24 hours post-operation and 1-month post-operation.

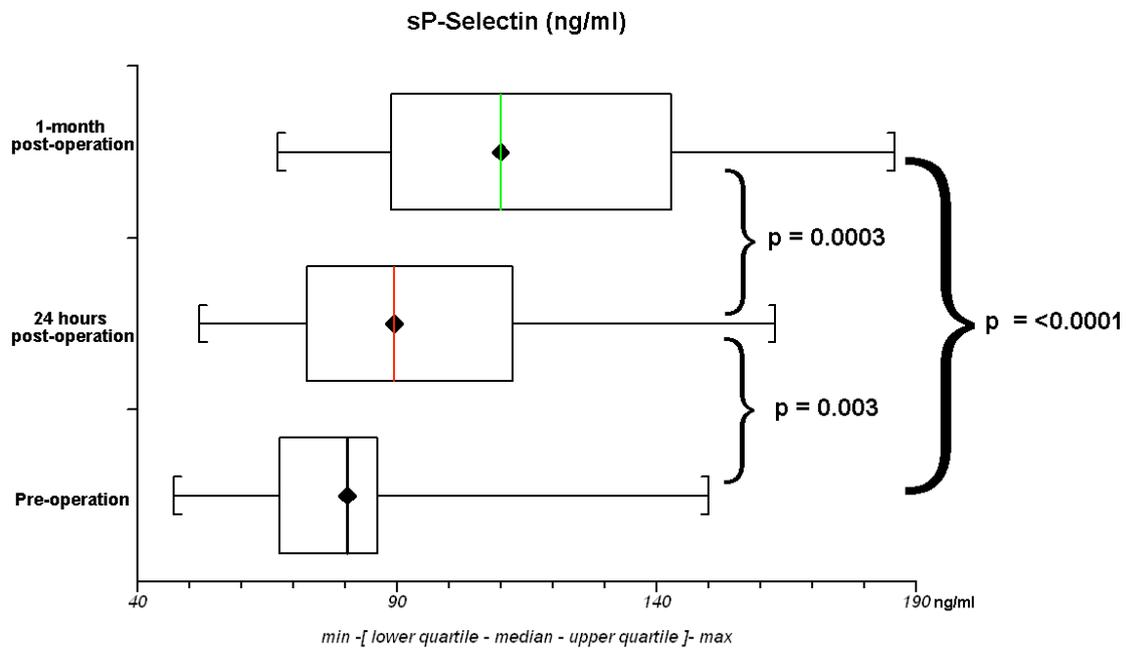


Figure 6.4.5: Comparison of plasma levels of sP-selectin at pre-operation, 24 hours post-operation and 1-month post-operation.

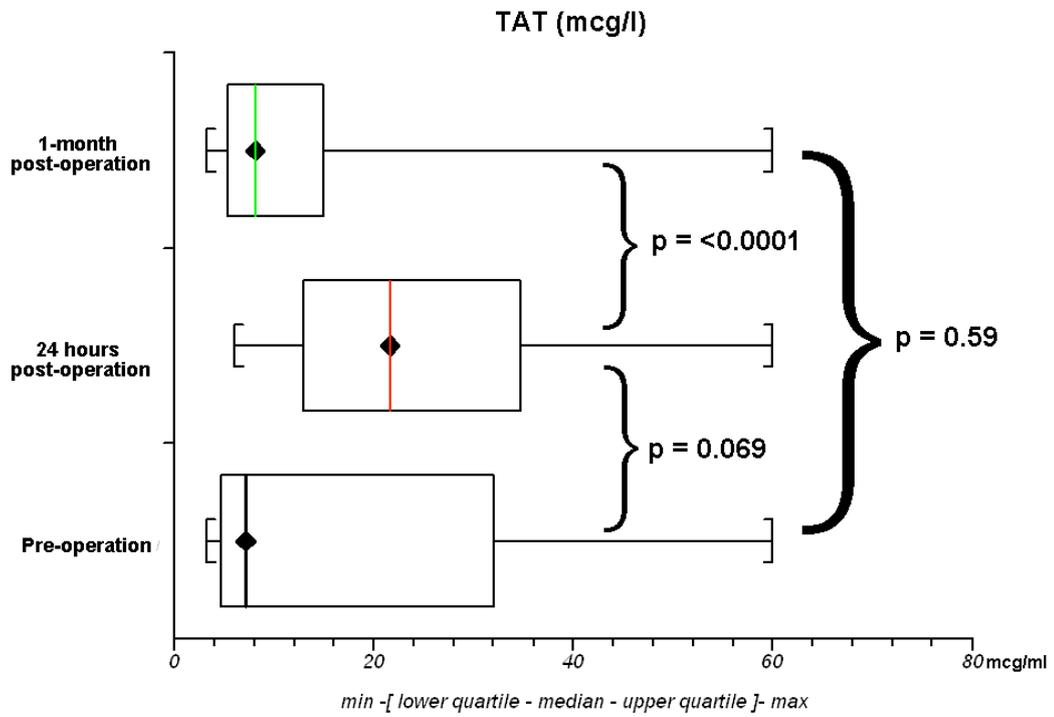


Figure 6.4.6: Comparison of plasma levels of TAT at pre-operation, 24 hours post-operation and 1-month post-operation.

6.5 DISCUSSION

Only five studies to date have examined the effects of EVAR on coagulation and fibrinolysis and platelet activity.^{17 93 125 128 129}

(Studies reporting the effects of EVAR on coagulation, fibrinolysis and platelet levels are summarised in table 6.5.1)

Aho et al investigated the impact of EVAR on haemostasis in seventeen patients with AAA.¹²⁵ They reported a three-fold increase in TAT levels at 24 hours followed by a gradual decline over the ensuing week. A similar transient increase was demonstrated for prothrombin fragments 1 & 2 (F 1+2), a marker of factor Xa activity and thus thrombin generation. Levels of both TAT and F 1+2 returned to pre-operative values by 1-week post-EVAR, however levels remained elevated when compared to the normal ranges. The current study reports similar findings with intense thrombin generation occurring within 24-hours of EVAR followed by a return to up-regulated pre-operative levels by 1-month. Engleberger et al demonstrated similar effects with elevated levels of plasma markers of thrombin generation (TAT) and activity (fibrin monomer (FM) and fibrinopeptide A (FPA)) occurring in the peri-operative period.¹²⁹ Maximum levels of TAT and FPA were found at the end of surgery whereas FM levels were at their highest at 24 hours post-EVAR. This may reflect the differing plasma kinetics of these markers; TAT and FPA reflect acute thrombin generation and activity due to their short half-life (TAT $t_{1/2}$ = 15 minutes, FPA $t_{1/2}$ = 3-5 minutes) whereas FM reflects the overall coagulation process due to its much longer half-life ($t_{1/2}$ \geq 3 hours).

Serino et al reported the short-term effects of EVAR on circulating D-dimer levels in nine patients assessed pre-operatively and day four post-operatively.¹⁷ D-dimer levels were elevated in seven patients and decreased in two patients; the median level for the entire patient cohort did not demonstrate a statistically significant difference between baseline and day four post-operation; this may reflect the study's lack of statistical power.

Monaco et al reported a significant decrease in levels of platelets, fibrinogen, plasminogen and prothrombin activity in patients undergoing EVAR during the first 10 days post-procedure.¹²⁸ This was coupled with increased D-dimer and FDP levels suggestive of a coagulation factor/platelet consumption coupled with systemic hyper-fibrinolysis during the peri-operative period in patients undergoing EVAR. By one-month post-EVAR all biomarkers returned to pre-operative levels with the exception of fibrinogen, which peaked at one-month and remained significantly elevated at six-month post-EVAR.

To date only Aho et al have reported the effect of EVAR on systemic fibrinolysis through the investigation of t-PA antigen and PAI-1 antigen levels. This group reported elevated levels of both t-PA antigen and PAI-1 antigen during the first 24-hours post-operatively compared to pre-operatively, thereafter returning to baseline levels by 1-week. However, t-PA antigen and PAI-1 antigen levels remained within the normal reference ranges throughout. The current study is the first to investigate t-PA activity as a marker of fibrinolysis following EVAR. t-PA activity better reflects the true state of systemic fibrinolysis compared to t-PA antigen which reflects the level of inactive circulating t-PA/PAI complexes. Thus despite normal levels of PAI-1 antigen we report a proportion of patients demonstrate heightened systemic fibrinolysis at one month post-operation as evidenced by elevated levels of t-PA activity. This finding is consistent with elevated levels of D-dimer reported at 3-months by Aho et al despite PAI-I antigen and t-PA antigen levels returning to baseline values at 1-week post-EVAR.¹²⁵

Only one previous study has reported the effects of EVAR on platelet activity utilising novel markers of platelet activity. Aho et al reported P-selectin levels as reducing during the first three days post-operative and then returning to baseline levels by one-week. P-selectin levels measured at 3-months were elevated in the EVAR group.¹²⁵ We report elevated platelet activity as evidenced by increased levels of sP-selectin at both 24-hours and 1-month post-operation. However, at both time points levels remained within normal reference ranges.

The Perioperative Ischemia Randomized Anesthesia Trial demonstrated that patients undergoing lower limb revascularisation under epidural anaesthetic did not show any change between pre- and peri-operative PAI-1 levels and suffered fewer post-operative graft thromboses.¹³⁰ This was in comparison to patients operated under general anaesthesia where PAI-1 levels were significantly higher in the peri-operative period as was the early graft thrombosis rate. Other studies have shown fewer post-operative thrombotic complications in patients undergoing major surgery under loco-regional anaesthesia.¹³¹ The ability of loco-regional anaesthesia to attenuate the catecholamine and cortisol biochemical response to trauma may account for the reported haemostatic benefits when compared to general anaesthesia. However, this apparent benefit has not been uniformly reported; Parrson et al report elevated levels of PAI-1 in patients undergoing infra-inguinal vascular reconstruction under epidural anaesthesia in the first 48 hours post-operative.¹³² The current study is the first to report the effects of differing methods of anaesthesia on haemostasis in patients undergoing EVAR. We found no significant differences in peri-operative plasma PAI-1 levels between patients undergoing EVAR under loco-regional or general anaesthesia. A number of confounding factors may account for this lack of difference including differing peri-operative analgesic regimes and type of stent-graft deployed. Furthermore the study was not powered to detect such differences.

To date, radiological imaging is required to identify endoleak and other stent related complications. There has been interest in utilising changes in circulating levels of biomarkers as an alternative means of identifying endoleak following EVAR. Sangiorgi et al and Lorelli et al have both reported elevated levels of circulating metalloproteinase-3 and -9 (MMP-3 & MMP-9) in patients affected by AAA.^{15 16} Furthermore, successful endovascular repair causes a decrease in MMP levels, whereas the presence of an endoleak post-EVAR appears to attenuate this decrease. Only one study has investigated the effects of endoleak on levels of a haemostatic biomarker in patients undergoing EVAR.¹⁷ Serino et al reported patients with a type-1 endoleak and stable/increasing aneurysm sac diameter demonstrated heightened circulating D-dimer levels compared to patients with an endoleak with a decreasing aneurysm sac diameter. The current study suggests that patients undergoing EVAR complicated by endoleak demonstrate a relative increase in systemic fibrinolysis as evidenced by a reduction in PAI-1 plasma levels at 1-month compared to pre-operative levels. Further research is required in larger groups of patients.

Biomarker	Study	Type of repair	Controls (units)	Pre-operative Value	P-value	24 hours		72 hours		5-7 days Post-op		3 months	
						Value	P-value	Value	P-value	Value	P-value	Value	P-value
D-dimer	Monaco et al ¹²⁸	endo	<450ng/ml*	278+/-93.3		428.44+/-147.3	<0.01			420.5+/-149.8	<0.05		
	Serino et al ¹⁷	endo	238 +/- 180 ng/ml	563+/-521 ng/ml						799+/-443	0.19		
F1+2	Aho et al ¹²⁵	endo	0.4-1.1 nmol/ml *	1.4+/-0.4	NS	2.0+/-1.0	NS	2.3+/-0.9	<0.05	2.0+/-0.7	NS	1.7+/-0.6	NS
FDP	Monaco et al ¹²⁸	endo	<100ng/ml *	6.3 +/- 1.3		11.2 +/- 3.8	<0.01			12.0+/-3.4	<0.01		
Fibrinogen	Aho et al ¹²⁵	endo	1.7-4 g/l *	3.7+/-0.6	NS	3.5+/-0.8	NS	6.1+/-1.6	<0.05	6.1+/-2.1	NS	3.6+/-0.7	NS
	Odegard et al ⁹⁵	endo	N/A g/l							5.8 (5.0-6.7)	p<0.001		
	Monaco et al ¹²⁸	endo	160-350 mg/dl *	309.6+/-50.6		159.4+/-44.9	<0.01			187.3+/-44.7	<0.01		
PAI ag	Aho et al ¹²⁵	endo	4-43 ng/ml *	16.5+/-6.4	NS	24.5+/-8.5	<0.05	21.3+/-8.2	NS	25.3+/-8.0	NS	18.6+/-6.4	NS
Platelet Count	Englberger et al ¹²⁹	endo	N/a x 10 ⁹ /l	226+/-46	0.82	165+/-56				229+/-74			
	Monaco et al ¹²⁸	endo	130-340 x10 ³ /dl *	233.2+/-52.9		190.9+/-48.4	<0.05			170.6+/-34.7	<0.001		
tPA ag	Aho et al ¹²⁵	endo	1-20 ng/ml *	8.5+/-3.1	NS	11.4+/-4.1	<0.05	9.9+/-4.3	NS	10.3+/-4.9	NS	8.7+/-3.2	NS

Table 6.5.1: Summary of studies investigating the effects of EVAR on biomarkers of haemostasis (* manufacturer's range)

In conclusion, the principal finding of this study is that EVAR causes significant haemostatic derangement in the peri-operative period with increased platelet activity and thrombin generation (as evidenced by increased sP-selectin and TAT levels) demonstrated at 24-hours post-operation. At 1 month the amplified thrombin generation has largely resolved with TAT levels returning to pre-operative levels, although remaining elevated above the normal healthy range. Conversely, platelet activity continues to increase during the first month post-operation. Furthermore, a proportion of patients demonstrate increased systemic fibrinolysis at 1-month post-EVAR as evidenced by elevated levels of t-PA activity.

CHAPTER 7

PERI-OPERATIVE MYOCARDIAL INJURY AND HAEMOSTASIS IN PATIENTS
UNDERGOING ENDOVASCULAR ANEURYSM REPAIR FOR ASYMPTOMATIC INFRA-
RENAL ABDOMINAL AORTIC ANEURYSM

7.1 INTRODUCTION

Cardiac related complications, including myocardial ischaemic injury, are reported to occur in 5% of patients in the peri-operative period following EVAR.¹¹ This may represent a significant under-estimation of the true rate of myocardial injury due to sub-clinical events remaining undiagnosed.^{113 133 134 135}

To date no study has reported on the routine use of cardiac troponins as a method for identifying patients with sub-clinical myocardial injury following EVAR, or attempted to correlate cTn levels with the haemostatic derangement that can occur as a result of EVAR.

7.2 AIMS

The aim of this study is to 1) investigate the incidence of myocardial injury in patients undergoing EVAR through the routine measurement of peri-operative cTn, and 2) investigate and correlate any increase in cTn with concomitant haemostatic derangement.

7.3 METHODS

All patients presenting to the Vascular Surgery Units of the Heart of England NHS Foundation Trust and University Hospital Birmingham NHS Trust between January 2007 and January 2009 with an infra-renal AAA measuring ≥ 5.5 cm in maximum diameter *and* due to undergo EVAR were assessed for study inclusion suitability. (For study inclusion and exclusion criteria see chapter 4.3.3)

Assessment for Myocardial Injury

Patients were assessed pre-operatively for cardiovascular disease including a full history and examination. Pre-operative electrocardiograms (ECG's) were performed. Post-operative complications including myocardial infarction, cardiac failure, unstable angina and cardiac dysrhythmias were assessed for clinically. All patients underwent routine ECG at 24 hours and severe ischaemia was defined as ST-elevation/depression >2 mm in two or more leads.

Blood sample collection

A resting venous blood sample was drawn from an antecubital fossa vein 24 hours prior to EVAR and 24 hours post EVAR. The samples were placed immediately on ice and centrifuged within 30 minutes of collection at 3,000 revolutions per minute for 30 minutes at a temperature of 4° C (equivalent to 1400g). Plasma was separated and stored at - 80 C for later batch analysis.

Cardiac troponin-T levels

All patients underwent routine cardiac troponin T (cTnT) measurement 24 hours post-procedure using an ES300 fully automated second-generation multichannel immuno-assay analyzer (Boehringer Mannheim). This second-generation test does not detect cross-reacting isoforms from skeletal muscle. The lower limit for cTnT detection using this analyzer is <0.01 ng/ml; cTnT values equal to or greater than 0.03 ng/ml were considered positive for myocardial injury.¹³⁶

Markers of Thrombin generation, fibrinolysis and Platelet activity

Plasma thrombin antithrombin III-complex (TAT) (healthy range, <4.2 µg/L) was assayed as a marker of thrombin generation. Plasma plasminogen activator inhibitor 1 (PAI-1) (healthy range, 7-43ng/ml) and tissue plasminogen activator (t-PA) activity (healthy range, 0 U/ml) were assayed as markers of fibrinolysis. Soluble P-selectin (sP-selectin) (healthy range, 92-212 ng/ml) was assayed as a marker of platelet activity. The assay manufacturer determined the healthy range for each haemostatic marker. (For further details of these commercially available assays please see chapter 4.3.5)

Statistics

Differences between patient characteristics were assessed using Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables. Correlation analyses were performed using the Spearman rank test to test the null hypothesis and Kendall rank correlation to test the strength of dependence between two variables if the null hypothesis was rejected. Where levels of cTnT were below the limit of detection of the assay, a value of 0.01 ng/ml was assigned. A probability value (p-value) of less or equal to 0.05 was regarded as statistically significant. Data are presented as median (inter-quartile range). All analyses were carried out using StatsDirect version 2.7.2 (StatsDirect Ltd, Cheshire, UK).

7.4 RESULTS

General

30 patients (29 men and 1 women of median (IQR) age 75 (71-82) years) who underwent elective EVAR for infra-renal AAA were prospectively studied. Ten patients had ischaemic heart disease co-morbidity, four patients suffered with diabetes, four patients suffered with chronic renal failure (Stage <3), one patient was an active smoker and one patient suffered with peripheral vascular disease (Fontaine Classification < IIb). Sixteen patients were being treated with an anti-platelet agent (aspirin = 14, clopidogrel = 1, aspirin & clopidogrel = 1); sixteen patients were being treated with a statin.

Myocardial Injury

Two patients (7%) suffered with significant cardiac complication within 24 hours of EVAR. One patient suffered with central chest pain and hypotension accompanied by ischaemic ECG changes (ST-depression) and an elevated cTnT level (0.042 ng/ml). He was successfully treated for acute coronary syndrome with a glyceryl trinitrate (GTN) infusion for 24 hours. One patient developed fast atrial fibrillation requiring rate control pharmacotherapy; cTnT at 24 hours post-procedure was significantly elevated; cTnT = 0.178 ng/ml. Three patients without clinical or ECG evidence of myocardial injury had cTnT levels >0.03 ng/ml; patient 2 cTnT = 0.04 ng/ml, patient 8 cTnT = 0.134 ng/ml, patient 25 cTnT = 0.058 ng/ml. Six patients without clinical or ECG evidence of myocardial injury had cTnT levels >0.01 ng/ml; patient 7 cTnT = 0.023 ng/ml, patient 11 cTnT = 0.012 ng/ml, patient 18 cTnT = 0.019 ng/ml, patient 19 cTnT = 0.021 ng/ml, patient 27 cTnT = 0.021 ng/ml, patient 29 cTnT = 0.021 ng/ml. (See figure 7.4.1) There was no association between pre-operative antiplatelet or statin usage and myocardial injury (p = NS)

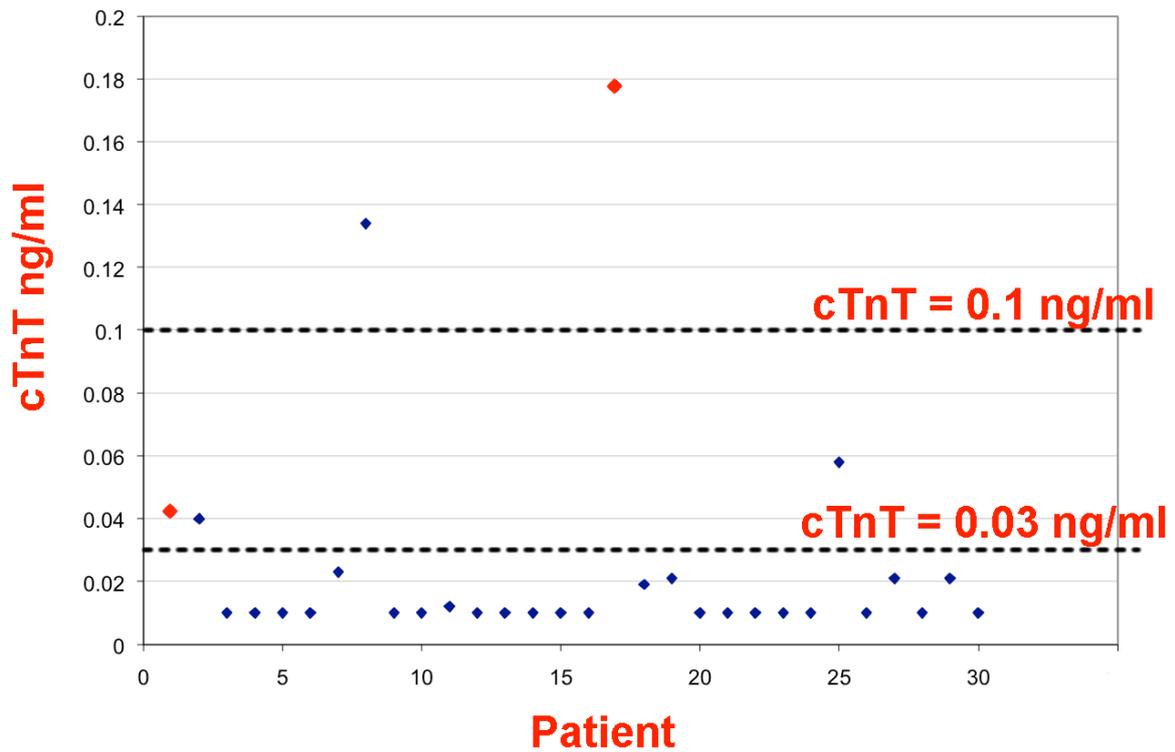


Figure 7.4.1: Individual patient data points for cTnT. The red points represent the two patients with clinical/ECG signs of cardiac injury.

Relationship between haemostatic markers and cTnT

Median (IQR) plasma levels of sP-selectin were 80.5 (68-86) ng/ml pre-operatively and 89.5 (73-112) ng/ml 24 hours post-EVAR ($p = 0.0028$). There was no correlation between raised cTnT levels and pre-operative ($r = -0.06$, $p = 0.76$) or 24 hours post-EVAR ($r = 0.23$, $p = 0.22$) levels of sP-selectin.

Median (IQR) plasma levels of PAI-I were 20.9 (8.4-50.7) ng/ml pre-operatively and 30.75 (19.8-46.8) ng/ml 24 hours post-EVAR ($p = 0.75$). There was no correlation between raised cTnT levels and pre-operative ($r = 0.03$, $p = 0.87$) or 24 hours post-EVAR ($r = 0.12$, $p = 0.54$) levels of PAI-I. Median (IQR) plasma levels of t-PA were 0 (0-0) U/ml pre-operatively and 0 (0-0) U/ml 24 hours post-EVAR ($p = 0.6875$).

Median (IQR) plasma levels of TAT were 7.15 (4.7-31.3) $\mu\text{g/l}$ pre-operatively and 21.65 (13-33.1) $\mu\text{g/l}$ 24 hours post-EVAR ($p = 0.069$). There was no correlation between raised cTnT levels and pre-operative ($r = 0.11$, $p = 0.56$) levels of TAT. There was a positive correlation between cTnT and TAT levels at 24 hours post-EVAR ($r=0.38$, $p = 0.039$, Kendall tau B = 0.26) (see figure 7.4.2)

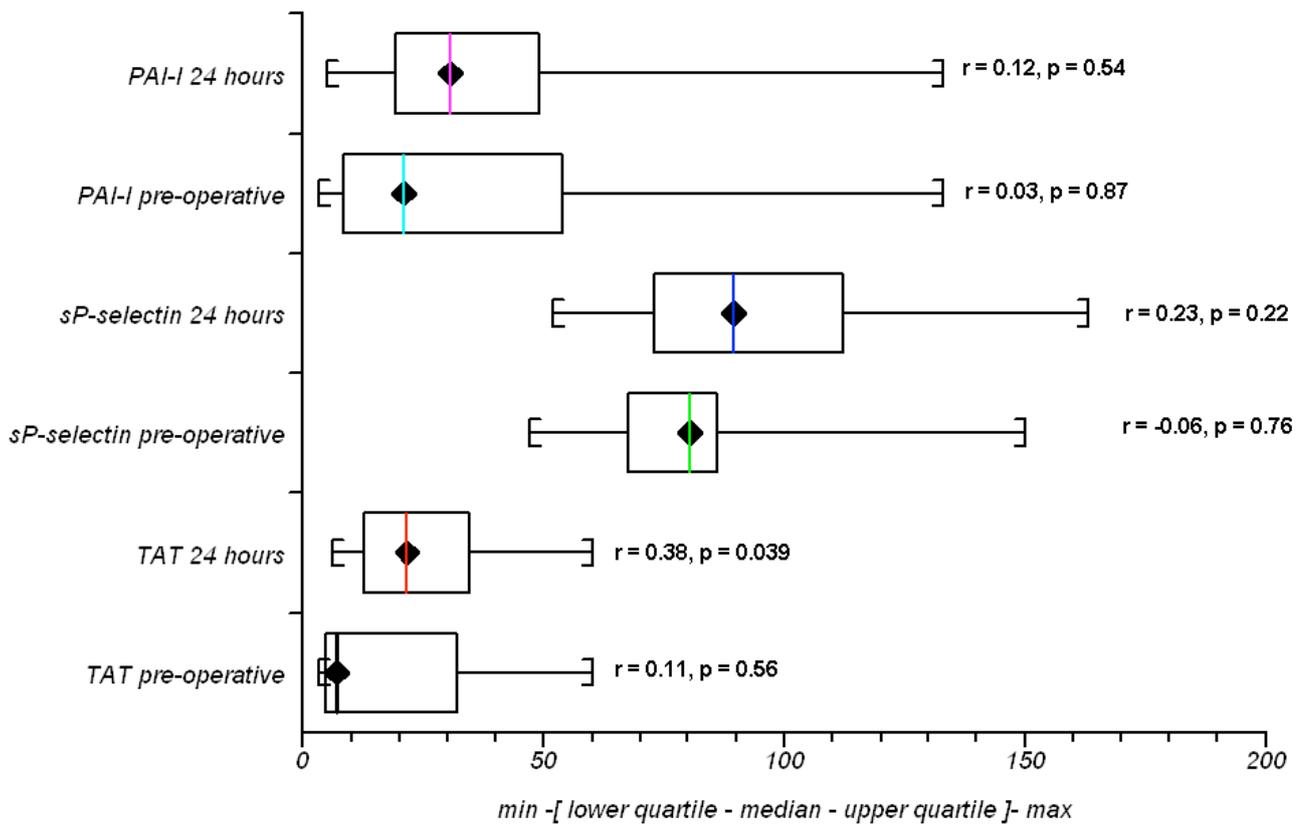


Figure 7.4.2: Box and Whisker plot of haemostatic variables pre- and 24 hours post-EVAR. Spearman's rank correlation coefficient (r) and p -value for each haemostatic variable vs. cTnT is shown.

7.5 DISCUSSION

This is the first study to investigate the routine use of cTnT as a marker of myocardial injury and its association with altered peri-operative haemostasis following EVAR. The principle findings of the present study are a) EVAR is associated with a significant risk of myocardial injury [5 of 30 patients (16%)], as demonstrated by elevated levels of cTnT, and b) the pro-thrombotic state that occurs in the peri-operative period after EVAR is associated with myocardial injury.

Cardiac troponins (cTn) are highly sensitive and specific markers of myocardial necrosis. An elevated peri-operative cTnT is an independent predictor of peri-operative and long-term mortality.^{30 136 137 134} Kertai et al reported peri-operative cTnT levels >0.1 ng/ml were independently associated with a two-fold increased risk of long-term mortality following successful major vascular surgery.³⁰ Landesberg et demonstrated that even minor elevations in post-operative cTnT levels was a predictor of increased mortality; cTnT levels >0.1 ng/ml and >0.03ng/ml independently predicted a 2.06 and 1.89 fold increase in mortality following major vascular surgery, respectively.¹³⁶ In our study, utilising 0.03ng/ml as the cut-off level for myocardial injury, 5 patients suffered peri-operative myocardial injury as opposed to 2 patients if the conventional cut-off of 0.1 ng/ml was utilised. This is considerably higher than the 5% peri-operative cardiovascular morbidity reported by Drury et al in their Cochrane review of endovascular aneurysm repair.

A number of studies have shown that routine cTnT surveillance in the peri-operative period identifies a significant number of patients with sub-clinical myocardial injury following major vascular surgery.^{113 133 134 135} Hobbs et al reported 38% of patients undergoing surgery for critical limb ischaemia had evidence of peri-operative myocardial injury as evidenced by raised cardiac troponin levels; only one third of these cases were clinically apparent.¹¹³

Haggart et al reported that more than one quarter of patients undergoing elective open surgical AAA repair suffer peri-operative myocardial injury.²³ However, 50% of these events remained sub-clinical, identified only through routine monitoring of cardiac troponins.²³ In our study only 2 of 5 patients with peri-operative levels of cTnT >0.03 ng/ml demonstrated clinical evidence of cardiac dysfunction. Thus 10% of patients with peri-operative myocardial injury would have gone undetected if routine surveillance with cTnT had not been undertaken.

The present study demonstrates a correlation between myocardial injury and increased peri-operative thrombin generation. Gibbs et al reported an association between the peri-operative hypercoagulable state following open abdominal aortic surgery and peri-operative myocardial infarction.¹³⁸ However, this study was limited to Q-wave infarcts whereas the majority of peri-operative MI are non-Q-wave.¹³³ In the present study there were no Q-wave infarcts, but the association between myocardial injury as defined by cTnT >0.03 ng/ml and a hypercoagulable state was maintained. This may suggest that the elevated cTnT levels, with the exception of the patient who developed atrial fibrillation, are secondary to a true thrombotic myocardial injury. However, platelet activation and aggregation is the initial step in the formation of clot and the present study did not demonstrate any correlation between elevated cTnT and peri-operative platelet activation. The cTnT elevation witnessed in the peri-operative phase of the present study may represent myocardial stress from causes other than thrombosis.

In conclusion, EVAR is associated with a significant risk of peri-operative myocardial injury that is under-detected clinically. The resultant pro-coagulopathic state following EVAR may be associated with an increased risk of myocardial injury. The routine use peri-operative cTnT as marker of myocardial injury testing may allow for the identification of patients at increased risk of mortality and the judicious use of peri-operative anti-coagulation may help prevent myocardial injury.

CHAPTER 8

AREAS OF FUTURE RESEARCH

8.1 THE RELATIONSHIP BETWEEN MURAL THROMBUS AND HAEMOSTASIS

The current study has not demonstrated the expected correlation between aneurysm thrombus volume and biomarkers of haemostasis. However, these results do not necessarily exclude the thrombus from being of importance in the prothrombotic state we report in patients with AAA. Vascular surgeons operating on AAA will note that the thrombus composition and density is often considerably different between patients. On occasion the thrombus can be removed en masse with little fracturing, whereas in other patients the thrombus is very fragile and semi-viscous in consistency. Microscopic examination of the mural thrombus reveals a highly complex network of inter-connecting canaliculi. These exponentially increase the surface area in direct contact with the luminal contents in a similar fashion to the villi and microvilli of the gastrointestinal tract. Thus, the mural thrombus is a biologically active entity that is not uniform in architecture, composition or density at a macro- or microscopic level. These differences may directly impact the level of haemostatic derangement an AAA exerts on the systemic circulation and may account for the conflicting studies reported in the English literature. (see table 3.4.4)

To date only Kolbel *et al* have investigated the relationship between thrombin activation and thrombus luminal surface area (TLS).⁸⁵ This group reported no correlation between the APC-PCI levels and surface area in 130 patients. The study was limited by its methodology in that it did not quantify the additional surface area contributed by macroscopic thrombus surface irregularities and microscopic canaliculi thereby grossly underestimating the TLS. In order to investigate this relationship comprehensively an accurate method for measuring true TLS is required. This is extremely difficult and is akin to measuring the surface area of grass on a football field. Currently, to the best of our knowledge, no non-invasive/radiographical method is available to measure the surface area of the thrombus with the canaliculi taken into account.

One potential solution is the utilisation of microscopic optical planimetry as described by Spring et al.¹³⁹ The technique utilises optical cross-sectional imaging in multiple planes at 1µm intervals through a tissue sample allowing an accurate computer 3D- reconstruction. From this 3D- reconstruction surface area and volume can be extrapolated. In the case of mural thrombus the luminal surface area of a known volume of thrombus biopsied during open repair can be utilised to estimate the luminal surface area for the total volume of thrombus. Furthermore, the method of calculating thrombus volume described in Chapter 3 could be validated by the direct measurement of thrombus volume removed at open operation. The current study did not investigate the differing thrombus biological qualities/variables and in particular the TLS. We speculate that the TLS may demonstrate correlation with markers of haemostasis if assessed appropriately and that further investigation is required to establish/refute any such relationship.

8.2 BIOMARKER OF ENDOLEAK

The current study investigated the novel use of biomarkers of haemostasis as a potential marker of endoleak as a secondary outcome. However, the results from this secondary outcome are of limited clinical value due to a) the study was not powered to investigate this relationship, and b) the lack of endoleak occurring in the second cohort of patients.

We report endoleak occurring in 12% of patients within one month post-procedure. This compares to the reported incidence of 22% in the EVAR-1 trial.⁷ However, it is the clinically significant endoleaks, those associated with sac expansion, that we wish to identify and only one (2%) patient from the current study required re-intervention secondary to a type-1 endoleak. In order for a study to be sufficiently powered to investigate a relationship between clinically significant endoleaks and novel markers of haemostasis we estimate a patient cohort of >1000 would be required. Thus, a multi-centre, multi-regional study is required to investigate this relationship further.

8.3 MYOCARDIAL INJURY AND EVAR

Perhaps the most clinically relevant finding of the current study is patients undergoing EVAR are at significant risk of peri-operative myocardial injury of which significant proportions are clinically occult. It is established that patients with peri-operative cardiac troponin elevation with or without clinical evidence of myocardial injury demonstrate adverse cardiovascular short- and medium term-outcomes. Therefore the ability to pre-operatively identify individuals at increased risk of peri-operative myocardial injury may allow targeted optimisation to occur.

B-type natriuretic peptide (BNP) and its pro-hormone N-terminal fragment pro-BNP (NT-pro-BNP) have recently been investigated as a potential pre-operative predictor of peri-operative myocardial injury.^{140 141 142} Rajagopalan *et al* demonstrated a pre-operative cut-off value for NT-pro-BNP of 308 pg/ml correctly indentified patients at heightened risk of peri-operative myocardial injury as identified by a cardiac troponin rise.¹⁴³ Feringa *et al* reported a pre-operative NT-pro-BNP value of >533 pg/ml was independently associated and more accurate than pre-operative dobutamine stress echocardiography in predicting early post-operative adverse cardiac events.¹⁴² To date no study has utilised NT-pro-BNP as a pre-operative risk stratification method for peri-operative cardiac injury in patients undergoing EVAR.

The best means of pre-operative cardiac optimisation in patients at risk for peri-operative adverse cardiac events remains unknown. A number of studies have investigated the use of peri-operative β -blockers as a cardio-protective pharmacotherapy in patients undergoing non-cardiac surgery. The PeriOperative ISschemic Evaluation (POISE) study reported a reduction in peri-operative cardiovascular death following the administration of metoprolol in patients with or at risk of atherosclerotic disease and undergoing non-cardiac surgery.¹⁴⁴ However, the study protocol did not include routine peri-operative cardiac troponin measurement and

was underpowered to assess for sub-group analysis i.e. patients undergoing major vascular surgery. The Dutch Echocardiographic Cardiac Risk Evaluation Applying Stress Echocardiography Study group (DECREASE) trial 1 reported a significant reduction in major cardiovascular events following the administration of peri-operative bisoprolol.¹⁴⁵ The study is limited through the utilisation of CK-MB as the marker of myocardial injury as opposed to cardiac troponin resulting in a likely underestimation of the incidence of myocardial injury. The only trial to employ routine peri-operative cardiac troponin assessment following major infra-renal vascular surgery is the Perioperative β -Blockade (POBBLE) trial.¹⁴⁶ This trial reported one third of patients suffered peri-operative myocardial ischaemia and that peri-operative β -blockade did not reduce the 30-day major cardiovascular event rate. The study protocol required a combination of cTnT >0.1 ng/ml and persistent ECG ischaemic changes before the diagnosis of myocardial injury could be inferred. Landesberg *et al* demonstrated that even isolated minor elevations in post-operative cTnT (>0.03 ng/ml) levels independently predicted a two-fold increase in mortality following major vascular surgery.¹³⁶ Thus, the POBBLE trial may be underestimating the actual incidence of clinically significant myocardial injury in the study population. Only Rajagopalan *et al* have reported the relationship between peri-operative β -blockade and isolated peri-operative cTnI levels in patients undergoing major vascular surgery.¹⁴³ This longitudinal observational study reported the incidence of peri-operative cardiac troponin rise was not affected by peri-operative β -Blockade.

The aetiology of peri-operative myocardial injury is complex. Traditionally it has been thought that prolonged myocardial stress in the setting of coronary artery atherosclerotic disease combined with plaque rupture account for the majority of peri-operative myocardial ischaemic events. However, as demonstrated in the current study, the resultant peri-operative

hypercoagulable state following major vascular surgery may also contribute to the development of myocardial injury in the peri-operative period. This may partially explain why pre-operative stress testing is relatively inaccurate for predicting post-operative myocardial ischaemic injury.¹⁴⁷

During open infra-renal AAA repair cross-clamping of the aorta places considerable strain on the myocardium by dramatically increasing systemic vascular resistance and arterial pressure.¹⁴⁸ It is thought β -blockade attenuates this physiological response by lowering the heart rate and improving coronary artery diastolic perfusion thereby reducing myocardial stress and improving myocardial oxygenation. During EVAR the infra-renal aorta is rarely totally occluded, thus any benefit inferred by the peri-operative use of β -blockade during open AAA repair may be negated in patients undergoing EVAR. To date no study has investigated the effects of peri-operative β -blockade on myocardial injury as defined by elevated post-operative cardiac troponin levels in patients undergoing EVAR.

APPENDICES

APPENDIX 1

Classification of Endoleak

Type-I: Failure of stent-graft to seal against the aortic wall proximally (type-Ia) or against the iliac arteries distally (type-Ib)

Type-II: Retrograde blood flow through one (type-IIa) or more (type-IIb) normal branches of the excluded aortic segment e.g. inferior mesenteric artery or lumbar arteries.

Type-III: Structural failure of the stent-graft secondary to fabric disruption (type-IIIa), separation of modular components (type-IIIb), suture holes in fabric (type-IIIc)

Type-IV: Caused by graft porosity typically identified at the time of stent-graft deployment.

APPENDIX 2: LOST SAMPLES



A



B

Unfortunately due to the departments research freezer being turned-off by a member of the domestic services department the majority of the initial patient cohort plasma samples (figures A & B) were invalidated prior to batch analysis.

APPENDIX 3: PATIENT INFORMATION LEAFLET



University Hospital Birmingham **NHS**
NHS Foundation Trust

Heart of England **NHS**
NHS Foundation Trust

	<h2>Coagulation, Endoleak and Myocardial Injury after EVAR (CEMIE Study)</h2>
<p>THE CEMIE STUDY</p>	
<p>Researchers:</p>	
<p>Mr RSM Davies Mr. DJ Adam Mr. RK Vohra</p>	<p>Vascular Research Fellow Senior Lecturer Vascular Surgery Consultant Vascular Surgeon</p>
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<p>E-mail: RobertM.Davies@UHB.NHS.UK</p>	



Thank you for reading this leaflet. This hospital is involved in a study to try and find out if patients with Abdominal Aortic Aneurysms (AAA) undergoing an endovascular repair (EVAR) demonstrate an increased/decreased tendency to form blood clots (thrombosis) compared to open surgical repair. This is an invitation to patients such as yourself to consider joining this study. If there is anything you do not understand, or if you have any other questions, your doctor or research investigator will be able to discuss this with you or you can contact us directly (for details see below).

Background Information

What is an Abdominal Aortic Aneurysm (AAA)?

AAA is a swelling of the main artery in your abdomen usually as a result of an underlying weakness in the artery. The prevalence of AAAs is greater in males and increases with age, with the average age at diagnosis being 65-70.

What are its symptoms?

Most AAA remain asymptomatic. However as the aneurysm continues to enlarge the risk of rupture increases. If rupture does occur the patient will classically collapse complaining of pain in the lower back. If this occurs an emergency life saving operation is required.

How is it treated?

We know that once an abdominal aortic aneurysm reaches 5.5cm in diameter the risk of rupture per year begins to increase exponentially. Therefore at this size elective repair in order to reduce the risk of future rupture is indicated.

How do we repair it?

There are two main techniques—open surgical repair (traditional) and endovascular repair (latest technology)

What is an endovascular repair (EVAR) ?

EVAR involves placing a metallic stent within the aorta under x-ray guidance to exclude the AAA from the circulation and thus prevent rupture

Are there any benefits of EVAR over Open repair?

Studies have reported that patients undergoing EVAR show reduced mortality associated with the procedure compared to open surgical repair.

Are there any drawbacks of EVAR over Open repair?

Patients undergoing EVAR require close surveillance with regular scans to detect complications associated with the stent. These may require further procedures at a latter date thus adding to the economic burden of this technique.

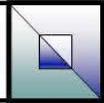
The CEMIE study

What is the Research Study about?

We know that patients with AAA have blood that is more sticky than those without an AAA. Previous work has shown that this stickiness is attenuated following open surgical repair thus theoretically reducing the long term risk of an heart attack. Currently we do not know what happens to this sticky blood following EVAR.

Where is it being performed?

Heart of England NHS Foundation trust and University Hospital Birmingham NHS trust.

***What will I have to do?***

To carry out the study we need individuals with AAA who are due to undergo an EVAR. We would require you to undergo investigations within the trust as listed below:

1. ECG

This is a trace of your heart rhythm carried out using probes on your skin. We would like to perform this 4 times (once before and three times after the procedure).

2. Blood test

a) During your Hospital Stay (when you have your procedure performed)

- We would like to take a small quantity of blood (about 5 teaspoons full) from your arm vein 4 times.

b) During the follow up at 1 month

- We would like to take a small amount of blood from your arm.

What are the benefits?

Although this study may be of no personal benefit to you, we hope to gain a better understanding of the changes to an individuals blood properties following EVAR. This would in turn provide the groundwork for future studies investigating ways at limiting these changes and associated risks.

What are the risks?

The blood tests can result in the formation of a small bruise when they are taken.

What if I do not want to take part?

If you decide not to participate in the study, this will not affect any treatment you will receive and all patients will get the best possible treatment we can give.

What Happens to the Information?

The identity of the volunteers in the study will be kept strictly confidential. Only after statistical analysis will we be able to say if we have identified differences between differing groups of patients.

What happens at the end of the research study?

We hope to publish the results in medical journals to enable other doctors to learn from our findings. This may help the treatment of others in the future. Your identity will remain anonymous in these publications.

What happens if I have more questions or do not understand something?

If you would like further information, please ask to speak to one of the doctors listed below

What happens now if I decide to take part?

If you would like to take part please let any of the doctors looking after you know and someone will come and answer any further questions you may have before signing a consent form.

What happens if I change my mind during the study?

You can withdraw from the study at any time and this will not affect your future management in any way. If you wish to withdraw please contact one of the doctors listed below.

**Your Decision**

You may want to think a little bit longer and discuss it with a relative before deciding whether or not to take part in the study. If you do decide to join the study we will inform your GP and send them information about the study.

Investigators**Mr. Robert Davies**

Vascular Research Fellow

Tel. 0121-678669

Mr. D Adam

Senior Lecturer Vascular Surgery

Heart of England NHS trust

Tel. 0121-4241633

Mr. R Vohra

Consultant Vascular Surgery

University Hospital Birmingham NHS Trust

Tel. 0121-6278669

APPENDIX 4: PATIENT CONSENT FORM

Coagulation, Endoleak and Myocardial Injury after EVAR (CEMIE Study)

Consent Form

Authorisation and Signatures

I, _____, have been invited to participate in a study (The CEMIE study) investigating the short term changes to coagulation (clotting) following my endovascular aneurysm repair under the direction of Mr DJ Adam, in which I voluntarily consent to participate.

a. The implications of my voluntary participation in this study, its nature, the duration, the purpose, the methods and means by which it is to be conducted, and the hazards which may be expected have been thoroughly explained to me by

Mr Robert Davies

b. I have read and understand all the written materials that have been provided to me describing this study and its potential risks and benefits.

c. I have been given an opportunity to ask any questions I wish concerning the procedures, and all such questions have been answered to my complete satisfaction. I understand that my participation in this evaluation can be terminated at any time upon my request.

d. I agree to allow authorised personnel to examine my medical Records and understand that this will be done in the strictest confidence.

Date _____/_____/_____
 Day Month Year

 Patient signature

 Patient name printed

 Doctor Signature

 Doctor name printed

APPENDIX 5: GP LETTER

University Hospital Birmingham 
NHS Foundation Trust

Heart of England 
NHS Foundation Trust

*Patient Sticker
Here*

Dear Sir/Madam

I am writing to inform you that your patient _____ has agreed to participate in the following study:

Coagulation, Endoleak and Myocardial Injury after EVAR (CEMIE Study)

The study involves obtaining peri-procedural blood samples (10-20ml) to assess altered haemostasis and myocardial injury. The study **does not** involve any new therapeutic pharmacotherapy or modality and **will not** influence the clinical treatment of your patient. Your patient will be followed up during their routine clinic appointments where possible.

I have enclosed an information sheet providing additional information. Should you have any queries please feel free to contact me.

Kind regards

Mr. Robert Davies

Vascular Research Fellow

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